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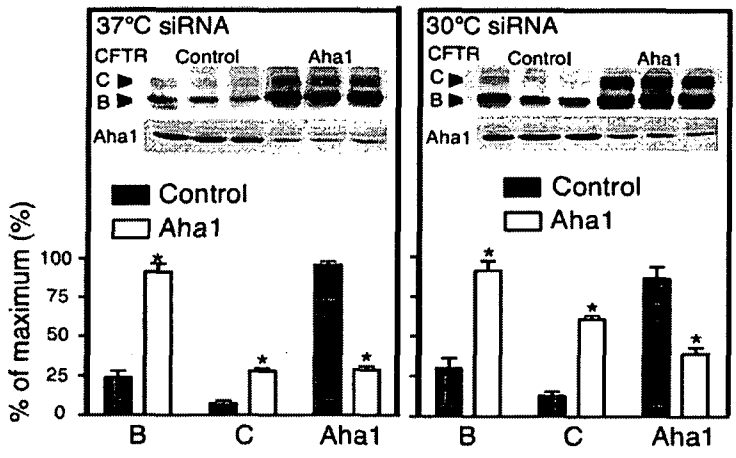
(54) Title: TREATMENT OF PROTEIN MISFOLDING



(57) Abstract: The present invention is directed to preventing the consequences of the misfolding of proteins, such as those associated with protein folding diseases. Provided are methods of treatment that involve administering an agent that decreases the level of the heat shock protein ATPase Aha1 and/or related molecules with similar function. Such methods can result in the rescue of folding, trafficking, and function of proteins with suboptimal folding kinetics. Also provided are screening methods to identify agents for the treatment of protein misfolding diseases.

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TREATMENT OF PROTEIN MISFOLDING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Serial No. 60/801,840 filed on May 19, 2006, U.S. Provisional Application Serial No. 60/815,494 filed on June 21, 2006, and U.S. Provisional Application Serial No. 60/859,890 filed on November 17, 2006, each of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made in part with Government support under National Institutes of Health Grants GM42336 and GM45678/NIH RR11823. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ON A COMPACT DISC

[0003] The Sequence Listing, which is a part of the present disclosure, includes a computer file "Sequence Listing_ST25.TXT" generated by U.S. Patent & Trademark Office PatentIn Version 3.4 software comprising nucleotide and/or amino acid sequences of the present invention. The subject matter of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD

[0004] The present invention generally relates to methods for treatment of protein misfolding diseases. In particular, the present invention concerns methods of treatment using modulators of the gene Activator of Heat Shock Protein 90 ATPase (Aha). More specifically, the invention concerns methods of treating disorders associated with undesired Aha activity, by administering short interfering RNA which down-regulate the expression of Aha, and agents useful therein.

INTRODUCTION

[0005] The endoplasmic reticulum (ER) is a specialized folding environment in which nearly one-third of the proteins encoded by a eukaryotic genome are translocated and folded as either lumenal secreted proteins or transmembrane proteins. Proteins are exported from the ER by the concatamer complex II (COPII) machinery which generates transport vesicles for delivery of cargo to the Golgi (Lee et al., Annu. Rev Cell Dev. Biol. 20,

87 (2004)). The ER-associated folding (ERAF) pathways are also coordinated with ER-associated degradation (ERAD) pathways whereby misfolded proteins are targeted for translocation to the cytosolic proteasome system (Wegele et al., Rev Physiol Biochem Pharmacol 151, 1 (2004); Young et al., Trends Biochem. Sci. 28, 541 (2003)).

[0006] Numerous misfolding diseases occur in which variants of either lumenal or transmembrane cargo do not fold properly, fail to engage the COPII export machinery and are degraded in the ER resulting in loss of function phenotype. Cystic fibrosis (CF) is an inherited childhood disease primarily triggered by defective folding and export of CF transmembrane conductance regulator (CFTR; a multi-domain cAMP-regulated chloride channel found in the apical membrane of polarized epithelia lining many tissues) from the ER (Riordan, Annu. Rev. Physiol. 67, 701 (2005)). CFTR consists of two transmembrane domains (TMD1 and 2), separated biosynthetically by cytosol oriented N- and C-terminal domains, and the NBD1, R and NBD2 domains that regulate channel conductance. Transport of CFTR involves chaperones directing folding and export from the ER (Amaral, J. Mol. Neurosci. 23, 41 (2004); Wang et al., J. Struct. Biol. 146, 44 (2004)) as well as adaptor proteins that direct trafficking from the trans Golgi (Cheng et al., J. Biol. Chem. 280, 3731 (2005)) and recycling through endocytic pathways to maintain the proper level of chloride channel activity at the cell surface (Gentzsch et al., Mol. Biol. Cell 15, 2684 (2004); Swiatecka-Urban et al., J. Biol. Chem. 280, 36762 (2005)).

[0007] Over 90% of CF patients carry at least one allele of the Phe 508 deletion (ΔF508) in the cytosolic NBD1 ATP-binding domain of CFTR leading to severe forms of disease. ΔF508 disrupts the folding of CFTR in the ER (Qu et al., J. Bioenerg. Biomembr. 29, 483 (1997); Riordan, supra). The folding of ΔF508 NBD1 is reported to be kinetically impaired (Qu et al., supra; Qu et al., J. Biol. Chem. 272, 15739 (1997); Qu and Thomas, J. Biol. Chem. 271, 7261 (1996)). As a consequence of this energetic defect in folding, ΔF508 fails to achieve a wild-type fold in the ER, fails to engage the COPII ER export machinery (Wang et al., supra) and is targeted for ER-associated degradation (ERAD) (Nishikawa et al., J Biochem (Tokyo) 137, 551 (2005)). Thus, it would be desirable to provide some means for preventing the consequences of the misfolding of ΔF508 CFTR in the treatment of CF.

[0008] Activator of Heat Shock Protein 90 ATPase 1 (Aha1) is an activator of the ATPase-activity of Hsp90 and is able to stimulate the inherent activity of yeast Hsp90 by 12-fold and human Hsp90 by 50-fold (Panaretou, B., et al., Mol. Cell 2002, 10:1307–1318). Biochemical studies have shown that Aha1 binds to the middle region of Hsp90 (Panaretou et al., 2002, *supra*, Lotz, G. P., et al., J. Biol. Chem. 2003, 278:17228–17235), and recent structural studies of the Aha1-Hsp90 core complex suggest that the co-chaperone promotes

a conformational switch in the middle segment catalytic loop (370–390) of Hsp90 that releases the catalytic Arg380 and facilitates its interaction with ATP in the N-terminal nucleotide-binding domain (Meyer, P., et al., EMBO J. 2004, 23:511–519).

[0009] The molecular chaperone Heat shock protein 90 (Hsp90) is responsible for the in vivo activation or maturation of specific client proteins (Picard, D., Cell Mol. Life Sci. 2002, 59:1640–1648; Pearl, L. H., and Prodromou, C., Adv. Protein Chem. 2002, 59:157–185; Pratt, W. B., and Toft, D. O., Exp. Biol. Med. 2003, 228:111–133; Prodromou, C., and Pearl, L. H., Curr. Cancer Drug Targets 2003, 3:301–323). Crucial to such activation is the essential ATPase activity of Hsp90 (Panaretou, B., et al., EMBO J. 1998, 17:4829–4836), which drives a conformational cycle involving transient association of the N-terminal nucleotide-binding domains within the Hsp90 dimer (Prodromou, C., et al., EMBO J. 2000, 19:4383–4392).

[0010] As a molecular chaperone, HSP90 promotes the maturation and maintains the stability of a large number of conformationally labile client proteins, most of which are involved in biologic processes that are often deranged within tumor cells, such as signal transduction, cell-cycle progression and apoptosis. As a result, and in contrast to other molecular targeted therapeutics, inhibitors of HSP90 achieve promising anticancer activity through simultaneous disruption of many oncogenic substrates within cancer cells (Whitesell L, and Dai C., Future Oncol. 2005; 1:529-540; WO 03/067262). Furthermore, HSP90 has been implicated in the degradation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Mutations in the CFTR gene lead to defective folding and ubiquination of the protein as a consequence of HSP90 ATPase activity. Following ubiquitination, CFTR is degraded before it can reach its site of activity. Lack of active CFTR then leads to the development of cystic fibrosis in human subjects having such mutation. Therefore, the inhibition of HSP90 activity may be beneficial for subjects suffering from cancer or Cystic Fibrosis.

[0011] Hsp90 constitutes about 1-2% of total cellular protein (Pratt, W. B., Annu. Rev. Pharmacol. Toxicol. 1997, 37:297-326), and the inhibition of such large amounts of protein by means of an antagonist or inhibitor would potentially require the introduction of excessive amounts of the inhibitor or antagonist into a cell. An alternative approach is the inhibition of activators of HSP90's ATPase activity, such as Aha1, which are present in smaller amounts. By downregulating the amount of Aha1 present in the cell, the activity of HSP90 may be lowered substantially.

[0012] Significant sequence homology exists between Homo sapiens (NM_012111.1), Mus musculus (NM_146036.1) and Pan troglodytes (XM_510094.1) Aha 1.

A clear rattus norvegicus homologue of Aha 1 has not been identified; however, there is a Rattus norvegicus (XM_223680.3) gene which has been termed activator of heat shock protein ATPase homolog 2 (Ahsa 2) on the basis of its sequence homology to yeast Ahsa 2. Its sequence is homologous to mus musculus RIKEN cDNA 1110064P04 gene (NM_172391.3), which is in turn similar in sequence to Aus musculus Aha 1 except for N-terminal truncation. A homo sapiens Ahsa 2 (NM_152392.1) has also been predicted, but sequence homology is limited. The functions of these latter three genes have not been sufficiently elucidated. However, there exists one region in which all of the above sequences are identical, and which may be used as the target for RNAi agents. It may be advantageous to inhibit the activity of more than one Aha gene.

[0013] Recently, double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., *Curr. Biol.* (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). This natural mechanism has now become the focus for the development of a new class of pharmaceutical agents for treating disorders that are caused by the aberrant or unwanted regulation of a gene.

[0014] Despite significant advances in the field of RNAi and advances in the treatment of pathological processes mediated by HSP90, there remains a need for agents that can selectively and efficiently attenuate HSP90 ATPase activity, for example, by using the cell's own RNAi machinery. Such agents shall possess both high biological activity and in vivo stability, and shall effectively inhibit expression of a target Aha gene, such as Aha1, for use in treating pathological processes mediated directly or indirectly by Aha expression, e.g. Aha1 expression.

SUMMARY

[0015] Accordingly, the present inventors have succeeded in discovering that decreasing levels of functional Aha1, a heat shock protein (Hsp) co-chaperone and ATPase activator, can result in energetic stabilization of the ΔF508 variant of CFTR, associated with CF. This results in rescue of folding, trafficking, and function of ΔF508.

[0016] Thus, the present invention includes compositions and methods for treating a disease resulting from protein misfolding. The compositions can generally comprise a

dsRNA, vector, short hairpin RNA (shRNA), small molecule, antibody, antisense nucleic acid, aptamer, ribozyme, and any combination thereof for inhibiting functional Aha protein expression in a cell.

[0017] For example, the dsRNA can comprise a sense strand and an antisense strand, wherein said antisense strand comprises a region of complementarity having a sequence substantially complementary to an Aha target sequence, wherein said target sequence is less than 30 nucleotides in length, wherein said sense strand is substantially complimentary to said antisense strand, and wherein said dsRNA, upon contact with a cell expressing functional Aha protein, inhibits functional Aha protein expression by at least 20%. In various aspects, the Aha target sequence can comprise a sequence selected from the group consisting of SEQ ID NOs: 12-56. In another aspect, the dsRNA can comprises a sense strand having a sequence selected from the group consisting of SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID NO: 123, SEQ ID NO: 125, SEQ ID NO: 127, SEQ ID NO: 129, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 139, SEQ ID NO: 141, SEQ ID NO: 143, and SEQ ID NO: 145; and an antisense strand complementary to the sense strand having a sequence selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 140, SEQ ID NO: 142, SEQ ID NO: 144, and SEQ ID NO: 146.

[0018] In another example, the vector for expressing a shRNA for inhibiting functional Aha1 expression in a cell can comprise a sense strand, a hairpin linker, and an antisense strand. In various aspects, the sense strand can comprise a region of complementarity having a sequence substantially complementary to an Aha target

sequence, wherein said target sequence is less than 30 nucleotides in length, the antisense strand can be substantially complimentary to said sense strand, and the dsRNA, upon contact with a cell expressing functional Aha protein, can inhibit functional Aha protein expression by at least 20%. In various aspects, the Aha target sequence can comprise a sequence selected from the group consisting of SEQ ID NOs: 12-56. In another aspect, the vector can comprise a sense strand having a sequence selected from the group consisting of SEQ ID NO: 147, SEQ ID NO: 149, and SEQ ID NO: 151; and an antisense strand having a sequence selected from the group consisting of SEQ ID NO: 148, SEQ ID NO: 150, and SEQ ID NO: 152.

[0019] In another example, the shRNA for inhibiting functional Aha1 protein expression in a cell, can comprise a region of complementarity having a sequence substantially complementary to an Aha target sequence, wherein said target sequence is less than 30 nucleotides in length, and wherein said shRNA, upon contact with a cell expressing functional Aha protein, inhibits functional Aha protein expression by at least 20%. In various aspects, the Aha target sequence can comprise a sequence selected from the group consisting of SEQ ID NOs: 12-56. In another aspect, the shRNA can comprise a sequence selected from the group consisting of SEQ ID NO: 153, SEQ ID NO: 154, and SEQ ID NO: 155;

[0020] The invention also provides a cell or cell population comprising the dsRNA, vector and/or shRNA.

[0021] In another example, the antibody can specifically bind functional Aha1, the Hsp90 ATPase binding site for functional Aha1, and/or the functional Aha1-Hsp90 ATPase complex.

[0022] In yet another example, the agent can include any combination of a small molecule, an antibody, an antisense nucleic acid, an aptamer, a dsRNA, and a ribozyme.

[0023] The invention also provides a method of treating a disease associated with misfolding of a protein. The method can comprise administering to a subject in need thereof a therapeutically effective amount of at least one agent that decreases intracellular levels of functional Aha1 protein. In various aspects, the agent can be selected from the group consisting of a small molecule, an antibody, an antisense nucleic acid, an aptamer, an siRNA, a ribozyme, and combinations thereof. In various aspects, the disease can include cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease. In another aspect, the misfolded protein can be a misfolded CFTR. In yet another aspect, the misfolded protein can be a ΔF508 protein.

[0024] The method can also include administering to a subject in need thereof a therapeutically effective amount of at least one dsRNA inhibitor of functional Aha1 expression, said dsRNA comprising a sense strand and an antisense strand. In various aspects, the antisense strand can comprise a region of complementarity having a sequence substantially complementary to an Aha target sequence, wherein said target sequence is less than 30 nucleotides in length, the sense strand is substantially complimentary to said antisense strand, and the dsRNA, upon contact with a cell expressing functional Aha protein, inhibits functional Aha protein expression by at least 20%. In various aspects, the disease can include cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease. In another aspect, the misfolded protein can be a misfolded CFTR. In yet another aspect, the misfolded protein can be a ΔF508 protein.

[0025] The method can also include administering to a subject in need thereof a therapeutically effective amount of at least one dsRNA inhibitor of functional Aha1 expression. In various aspects, the dsRNA inhibitor can comprise a sequence selected on the basis of a) the dsRNA comprising a sense strand sequence of about 19 nucleotides to about 25 nucleotides and an antisense strand sequence of about 19 nucleotides to about 25 nucleotides; and b) the sense strand sequence or antisense strand sequence comprises no more than 15 contiguous nucleotides identical to a contiguous sequence comprised by a 5' untranslated region, a 3' untranslated region, an intron or an exon of any gene or mRNA other than functional Aha1. In various aspects, the disease can include cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease. In another aspect, the misfolded protein can be a misfolded CFTR. In yet another aspect, the misfolded protein can be a ΔF508 protein.

[0026] A method of the invention can also include screening an agent for treating a disease associated with misfolding of a protein. In various aspects, the method can comprise providing a cell or cell population expressing functional Aha1; administering a candidate agent to the cell or cell population; quantifying functional Aha1 activity in the cell or cell population; and determining whether the candidate agent decreases functional Aha1 activity in the cell or cell population, whereby a decrease in functional Aha1 activity is indicative of reducing misfolding of the protein. In various aspects, the candidate agent can be a dsRNA which inhibits functional Aha1 expression. In another aspect, the dsRNA can comprise a) a sequence of from about 19 nucleotides to about 25 nucleotides, and b) the sequence comprises no more than 15 contiguous nucleotides identical to a contiguous

sequence comprised by a 5' untranslated region, a 3' untranslated region, an intron or an exon of any gene or mRNA other than an Aha gene or mRNA. In various aspects, the Aha gene or mRNA is a human Aha gene or mRNA. In another aspect, the disease can be selected from the group consisting of cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease. In another aspect, the misfolded protein can be selected from the group consisting of a misfolded CFTR, a misfolded fibrillin, a misfolded alpha galactosidase, a misfolded beta glucocerebrosidase, a misfolded rhodopsin, aggregated an amyloid beta and tau, an aggregated amylin, an aggregated alpha synuclein and an aggregated prion. In yet another aspect, the misfolded protein can be a misfolded CFTR. And in another aspect, the misfolded protein can be a ΔF508 protein.

[0027] The screening method can also comprise providing a cell or cell population which expresses functional Aha1; administering a candidate agent to the cell or cell population; quantifying Hsp90/ADP complex, Hsp90/ATP complex or a combination thereof in the cell or cell population; and determining whether the candidate agent decreases the quantity of Hsp90/ADP complex, Hsp90/ATP complex or the combination thereof in the cell or cell population, whereby a decrease in quantity of Hsp90/ADP complex or Hsp90/ATP complex is indicative of decreasing misfolding of the protein. In various aspects, the candidate agent can be a dsRNA which inhibits functional Aha1 expression. In another aspect, the dsRNA can comprises a) a sequence of from about 19 nucleotides to about 25 nucleotides, and b) the sequence comprises no more than 15 contiguous nucleotides identical to a contiguous sequence comprised by a 5' untranslated region, a 3' untranslated region, an intron or an exon of any gene or mRNA other than an Aha gene or mRNA. In yet another aspect, the Aha gene or mRNA can be a human Aha gene or mRNA. In various aspects, the disease can be selected from the group consisting of cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease. In another aspect, the misfolded protein can be selected from the group consisting of a misfolded CFTR, a misfolded fibrillin, a misfolded alpha galactosidase, a misfolded beta glucocerebrosidase, a misfolded rhodopsin, aggregated an amyloid beta and tau, an aggregated amylin, an aggregated alpha synuclein and an aggregated prion. In yet another aspect, the misfolded protein can be a misfolded CFTR. In another aspect, the misfolded protein can be a ΔF508 protein.

[0028] The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of an Aha gene in a cell or

mammal using such dsRNA. The invention also provides compositions and methods for treating pathological conditions and diseases mediated by the expression of an Aha gene, such as in cancer or cystic fibrosis. The dsRNA of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an mRNA transcript of an Aha gene.

[0029] In one aspect, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an Aha gene. The dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence. The antisense strand comprises a nucleotide sequence which is substantially complementary to at least part of an mRNA encoding an Aha gene, and the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length. The dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177,

SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339 (see Table 1 and Table 2). The Aha gene is preferably an Aha1 gene, and more preferably a Homo sapiens Aha1 gene. The dsRNA, upon contacting with a cell expressing the Aha gene, may inhibit the expression of the Aha gene in said cell by at least 20%, or at least 25%, 30%, 35%, 40%, 45%, 50%, 55% 60%, 65%, 70%, 85%, 90% or 95%, e.g. in HeLa and/or MLE 12 cells. The dsRNA may be different from said second dsRNA, but may have at least 5, at least 10, at least 15, at least 18, or at least 20 contiguous nucleotides per strand in common with one of the above named nucleotide sequences.

[0030] Preferably, the second dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-

DP-9287, AL-DP-9288, and AL-DP-9289 (see Table 1 and Table 2).

[0031] Alternatively, the dsRNA itself may be chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289 (see Table 1 and Table 2).

[0032] The dsRNA may comprise at least one modified nucleotide. Preferably, the modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. Alternatively, the modified nucleotide is chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

[0033] In another aspect, the invention provides an isolated cell comprising one of the dsRNAs of the invention. The cell is generally a mammalian cell, such as a human cell. Other embodiments of the cell comprising a dsRNA of the invention are as provided for other aspects of the invention above.

[0034] In yet another aspect, a pharmaceutical composition for inhibiting the expression of an Aha gene in an organism is provided, comprising a dsRNA and a pharmaceutically acceptable carrier, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding an Aha gene, and wherein said region of complementarity is less than 30 nucleotides in length, and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of

SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO:

331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339 (see Table 1 and Table 2). Therein, the Aha gene may be an Aha1 gene, and preferably a Homo sapiens Aha1 gene. The dsRNA comprised in the pharmaceutical composition may, upon contact with a cell expressing said Aha gene, inhibit the expression of said Aha gene in said cell by at least 20%, or at least 25%, 30%, 35%, 40%, 45%, 50%, 55% 60%, 65%, 70%, 85%, 90% or 95%, e.g. in HeLa and/or MLE 12 cells. The dsRNA may be different from said second dsRNA, but may have at least 5, at least 10, at least 15, at least 18, or at least 20 contiguous nucleotides per strand in common with one of the above named nucleotide sequences.

[0035] Preferably, the second dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289 (see Table 5 and Table 6).

[0036] Alternatively, the dsRNA comprised in the pharmaceutical composition itself may be chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289

(see Table 5 and Table 6).

[0037] The dsRNA comprised in the pharmaceutical composition may comprise at least one modified nucleotide. Preferably, said modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. Alternatively, said modified nucleotide is chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

[0038] In yet another aspect, a method for inhibiting the expression of an Aha gene in a cell is provded, the method comprising:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding Aha1, and wherein said region of complementarity is less than 30 nucleotides in length and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID

NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an Aha gene, thereby inhibiting expression of an Aha gene in the cell. The Aha gene is preferably an Aha1 gene, and more preferably a homo sapiens Aha1 gene. The dsRNA may be different from said second dsRNA, but may have at least 5, at least 10, at least 15, at least 18, or at least 20 contiguous nucleotides per strand in common with one of the above named nucleotide sequences.

[0039] Preferably, the second dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-

DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289 (see Table 5 and Table 6).

[0040] Alternatively, the dsRNA itself is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289. Preferably, the method is performed in vitro. Other embodiments of the method for inhibiting the expression of an Aha gene in a cell are as provided for other aspects of the invention above.

[0041] In yet another aspect, a method of treating, preventing or managing pathological processes mediated by Aha expression is provided, comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a dsRNA, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding Aha1, and wherein said region of complementarity is less than 30 nucleotides in length and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222,

SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339. The dsRNA may be different from said second dsRNA, but may have at least 5, at least 10, at least 15, at least 18, or at least 20 contiguous nucleotides per strand in common with one of the above named nucleotide sequences.

[0042] Preferably, the second dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326,

AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289 (see Table 5 and Table 6).

[0043] Alternatively, the dsRNA itself is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289. Other embodiments of the method comprising administering a dsRNA of the invention are as provided for other aspects of the invention above.

[0044] In yet another aspect, a vector for inhibiting the expression of an Aha gene in a cell is provided, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA, wherein one of the strands of said dsRNA is substantially complementary to at least a part of a mRNA encoding Aha1 and wherein said dsRNA is less than 30 base pairs in length and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184,

SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339. The dsRNA may be different from said second dsRNA, but may have at least 5, at least 10, at least 15, at least 18, or at least 20

contiguous nucleotides per strand in common with one of the above named nucleotide sequences.

[0045] Preferably, the second dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289 (see Table 5 and Table 6). Other embodiments of the vector of the invention are as provided for other aspects of the invention above.

[0046] In yet another aspect, an isolated cell comprising the above vector is provided. Other embodiments of the cell comprising a vector of the invention are as provided for other aspects of the invention above.

[0047] These and other features, aspects and advantages of the present teachings will become better understood with reference to the following description, examples and appended claims.

DRAWINGS

[0048] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0049] Figure 1. Depiction of the CFTR interactome.

[0050] Figure 2. (A) Depiction of the ER folding network, and (B) immunoblot depicting protein expression levels in WT and ΔF508 expressing cells.

[0051] Figure 3. Series of bar graphs depicting the effect of the Hsp90 co-chaperone p23 on folding and export of ΔF508 from the ER.

[0052] Figure 4. Series of bar graphs depicting the effect of the Hsp90 co-chaperone FKBP8 on folding and export of ΔF508 from the ER.

[0053] Figure 5. Series of bar graphs depicting the effect of the Hsp90 co-

chaperone HOP on folding and export of ΔF508 from the ER.

[0054] Figure 6. Series of bar graphs illustrating that ΔF508 export to the cell surface can be rescued by downregulation of functional Aha1.

[0055] Figure 7. Line and scatter plot and a bar graph showing the effect of dsRNA Aha1 on iodide efflux by the CFBE41o- cell line.

[0056] Figure 8. Series of depictions of Hsp90 chaperone/co-chaperone interactions directing CFTR folding.

[0057] Figure 9. Illustration (using immunoblot) of effects of dsRNA Aha1 on Hsp90.

DETAILED DESCRIPTION

[0058] Abbreviations and Definitions

[0059] To facilitate understanding of the invention, a number of terms and abbreviations as used herein are defined below as follows:

[0060] "G," "C," "A", "T" and "U" (irrespective of whether written in capital or small letters) each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, thymine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

[0061] The terms "functional Aha1 protein" or "functional Aha1" as used herein are intended to include a human Aha1 polypeptide (SEQ ID NO: 4) having heat shock protein ATPase activator activity as well as molecules related to Aha1 having heat shock protein ATPase activator activity. Such molecules related to human Aha1 include polypeptides having heat shock protein ATPase activator activity and at least 80% homology to functional Aha1. For example, related molecules can have 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homology to human Aha1 and can have heat shock protein ATPase activator activity. Such molecules can include, for example, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; and SEQ ID NO: 8.

In addition, such molecules related to human Aha1 include polypeptides having longer or shorter amino acid sequences and having heat shock protein ATPase activator activity.

[0062] Heat shock protein ATPase activator activity may be determined using standard assays, for example, by determining the production of inorganic phosphate (P_i) by Hsp90. P_i production may be determined, for example, by measuring or determining the generation or depletion of a reporter molecule. One such method utilizes a regenerating ATPase assay using a pyruvate kinase/lactate dehydrogenase linked assay in which the generation of P_i can be measured spectrophotometrically (Ali et al., Biochemistry (1993) 32:2717-2724). Other spectrophotometric methods include those described by Lanzetta et al. (1979) Anal. Biochem. 100, 95-97; Lill et al., (1990) Cell 60, 271-280; and Cogan et al., Anal. Biochem. (1999) 271:29-35. Those of skill in the art will recognize other methods of measuring heat shock protein ATPase activator activity.

[0063] As used herein, “Aha gene” refers to an Activator of Heat Shock Protein 90 ATPase genes that can express a functional Aha1 protein. "Aha1" refers to Activator of Heat Shock Protein 90 ATPase 1 genes, non-exhaustive examples of which are found under Genbank accession numbers NM_012111.1 (Homo sapiens), NM_146036.1 (Mus musculus), and XM_510094.1 (Pan troglodytes). "Aha2" refers to putative Activator of Heat Shock Protein 90 ATPase 2 genes, also known Ahsa2, non-exhaustive examples of which may be found under Genbank accession numbers NM_172391.3 (Mus musculus) and XM_223680.3 (Rattus norvegicus).

[0064] As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an Aha gene, including mRNA that is a product of RNA processing of a primary transcription product. The target sequence of any given RNAi agent of the invention means an mRNA-sequence of X nucleotides that is targeted by the RNAi agent by virtue of the complementarity of the antisense strand of the RNAi agent to such sequence and to which the antisense strand may hybridize when brought into contact with the mRNA, wherein X is the number of nucleotides in the antisense strand plus the number of nucleotides in a single-stranded overhang of the sense strand, if any.

[0065] As used herein, the term “strand comprising a sequence” refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

[0066] As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first

nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

[0067] This includes base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence. Such sequences can be referred to as “fully complementary” with respect to each other herein. However, where a first sequence is referred to as “substantially complementary” with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but generally not more than 4, **3** or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes of the invention.

[0068] “Complementary” sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled.

[0069] The terms “complementary”, “fully complementary” and “substantially complementary” herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

[0070] As used herein, a polynucleotide which is “substantially complementary to at least part of” a messenger RNA (mRNA) refers to a polynucleotide which is substantially

complementary to a contiguous portion of the mRNA of interest (e.g., encoding Aha1). For example, a polynucleotide is complementary to at least a part of an Aha1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding Aha1.

[0071] The term "double-stranded RNA" or "dsRNA", as used herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined above, nucleic acid strands. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop" and the entire structure is referred to as a "short hairpin RNA" or "shRNA". Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker". In various aspects, the linker can include the sequences AUG, CCC, UUCG, CCACC, CTCGAG, AAGCUU, CCACACC, and UUCAAGAGA. The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs.

[0072] As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that has no nucleotide overhang at either end of the molecule.

[0073] The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, *e.g.*, within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus. Most preferably, the mismatches are located within 6, 5, 4, 3, or 2 nucleotides of the 5'

terminus of the antisense strand and/or the 3' terminus of the sense strand.

[0074] The term “sense strand,” as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand.

[0075] “Introducing into a cell”, when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells in vitro; a dsRNA may also be "introduced into a cell", wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for in vivo delivery, dsRNA can be injected into a tissue site or administered systemically. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection.

[0076] The terms “decrease, decreased or decreasing levels” as used herein are intended to include inhibiting Aha1 heat shock protein ATPase activator activity and reducing the amount of functional Aha1 protein in a cell. For example, an antibody or dsRNA can decrease the level of functional Aha1 protein by interfering with or silencing heat shock protein ATPase activator activity without removing the Aha1 protein from the cell. In another example, a ribozyme can cleave the functional Aha1 protein to reduce the amount of whole Aha1 protein in the cell. In another example, a dsRNA can silence the expression an Aha gene, e.g. an Aha1 gene, to reduce the amount of mRNA transcribed from the Aha gene.

[0077] The terms “silence” and “inhibit the expression of”, in as far as they refer to an Aha gene, e.g. an Aha1 gene, herein refer to the at least partial suppression of the expression of an Aha gene, e.g. an Aha1 gene, as manifested by a reduction of the amount of mRNA transcribed from an Aha gene which may be isolated from a first cell or group of cells in which an Aha gene is transcribed and which has or have been treated such that the expression of an Aha gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). Preferably, the cells are HeLa or MLE 12 cells. The degree of inhibition is usually expressed in terms of

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

[0078] Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to Aha gene transcription, e.g. the amount of protein encoded by an Aha gene which is secreted by a cell, or found in solution after lysis of such

cells, or the number of cells displaying a certain phenotype, e.g. apoptosis or cell surface CFTR. In principle, Aha gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given dsRNA inhibits the expression of an Aha gene by a certain degree and therefore is encompassed by the instant invention, the assays provided in the Examples below shall serve as such reference.

[0079] For example, in certain instances, expression of an Aha gene, e.g. an Aha1 gene, is suppressed by at least about **20%**, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49% or 50% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, an Aha gene, e.g. an Aha1 gene, is suppressed by at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79% or 80% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, an Aha gene, e.g. an Aha1 gene, is suppressed by at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% by administration of the double-stranded oligonucleotide of the invention. Table 15 provides values for inhibition of Aha1 expression using various dsRNA molecules of the invention.

[0080] As used herein in the context of Aha expression, e.g. Aha1 expression, the terms "treat", "treatment", and the like, refer to relief from or alleviation of pathological processes mediated by Aha expression. In the context of the present invention insofar as it relates to any of the other conditions recited herein below (other than pathological processes mediated by Aha expression), the terms "treat", "treatment", and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition.

[0081] As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pathological processes mediated by Aha expression or an overt symptom of pathological processes mediated by Aha expression. The specific amount that is therapeutically effective can be readily determined by ordinary medical practitioner, and may vary depending on factors known in the art, such as, e.g. the type of pathological processes mediated by Aha expression, the patient's history and age, the stage of pathological processes mediated by Aha expression, and the administration of other anti-pathological processes mediated by Aha expression agents.

[0082] As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 25% reduction in that parameter.

[0083] The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

[0084] As used herein, a "transformed cell" is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

[0085] Treatment of Protein Misfolding

[0086] The present invention provides methods for the treatment of misfolding diseases by decreasing levels of functional Aha1 protein (e.g., SEQ ID NO: 4) and/or other related molecules with similar function, as well as methods for the screening of agents useful for treatment of protein misfolding diseases.

[0087] The technology described herein is based in part on the observation that decreased levels of functional Aha1, an Hsp90 ATPase activator, can markedly stabilize ΔF508 (ΔF508 polypeptide of SEQ ID NO: 3), a mutant of CFTR (CFTR mRNA of SEQ ID NO: 1; CFTR polypeptide of SEQ ID NO: 2) characterized by a phenylalanine deletion at 508, in a folded state that is accessible to the COPII export machinery for transport to the cell surface. Various therapeutic strategies described herein are directed to downregulation

of functional Aha1 and/or other related molecules with similar function, salvage of mutant CFTR misfolding, rescue of Hsp90-mediated trafficking to the cell surface, and at least partially restoration of channel functions in a subject.

[0088] Agents that Decrease Functional Aha1

[0089] Agents that decrease levels of functional Aha1 and/or other related molecules with similar function, can target functional Aha1 and/or Hsp90 ATPase such that binding to one component or both of the components by the agent effects a decrease in heat shock protein ATPase activator activity, the activation state of Hsp90 ATPase, and/or the activty level of activated Hsp90 ATPase, consequently resulting in stabilization of misfolded proteins. A crystal structure of the complex between Hsp90 and Aha1 has been reported (Meyer et al. (2004) EMBO J. 23, 511-519). Given a structural and mechanistic understanding of Aha1, Hsp90 ATPase, and the binding complex formed between the two, it is within the skill of the art to design agents that bind, for example ionically or covalently, to one or both components and thereby reduce the activation of Hsp90 ATPase by functional Aha1.

[0090] The various classes of agents for use herein as agents that decrease levels of functional Aha1 and/or related molecules with similar function, generally include, but are not limited to, RNA interference molecules, antibodies, small inorganic molecules, antisense oligonucleotides, and aptamers.

[0091] RNA interference (RNAi) can be used to decrease the levels of functional Aha1 (and/or other related molecules with similar functions) (see e.g., Examples 8-10). RNAi methods can utilize double stranded RNAs, for example, small interfering RNAs (siRNA), short hairpin RNA (shRNA), and micro RNAs (miRNA). The following discussion will focus on dsRNA generally, but one skilled in the art will recognize many approaches are available for other RNAi molecules, such as shRNA and miRNA. RNAi molecules, specific for functional Aha1 and/or other related molecules of similar function, are also commercially available from a variety of sources (e.g., Silencer® In Vivo Ready dsRNAs, Aha1 dsRNA ID#s 1136422, 136423, 36424, 19683, 19588, 19773, Ambion, TX; Sigma Aldrich, MO; Invitrogen).

[0092] Several dsRNA molecule design programs using a variety of algorithms are known to the art (see e.g., Cenix algorithm, Ambion; BLOCK-iT™ RNAi Designer, Invitrogen; dsRNA Whitehead Institute Design Tools, Bioinofrmatics & Research Computing). Traits influential in defining optimal dsRNA sequences include G/C content at the termini of the dsRNAs, T_m of specific internal domains of the dsRNA, dsRNA length, position of the target

sequence within the CDS (coding region), and nucleotide content of the 3' overhangs.

[0093] Administration of dsRNA molecules specific for functional Aha1, and/or other related molecules with similar functions, can effect the RNAi-mediated degradation of the target (Aha1) mRNA. For example, a therapeutically effective amount of dsRNA specific for Aha1 can be adminstered to patient in need thereof to treat a protein misfolding disease. In one embodiment, the dsRNA that effects decreased levels of functional Aha1 has a nucleotide sequence including SEQ ID NOs: 57-146 (see Tables 2, 5 and 6 below).

[0094] Generally, an effective amount of dsRNA molecule comprises an intercellular concentration at or near the site of misfolding from about 1 nanomolar (nM) to about 100 nM, preferably from about 2 nM to about 50 nM, more preferably from about 2.5 nM to about 10 nM. It is contemplated that greater or lesser amounts of dsRNA can be administered.

[0095] The dsRNA can be administered to the subject by any means suitable for delivering the RNAi molecules to the cells of interest. For example, dsRNA molecules can be administered by gene gun, electroporation, or by other suitable parenteral or enteral administration routes, such as intravitreous injection. RNAi molecules can also be administered locally (lung tissue) or systemically (circulatory system) via pulmonary delivery. A variety of pulmonary delivery devices can be effective at delivering functional Aha1-specific RNAi molecules to a subject (see below). RNAi molecules can be used in conjunction with a variety of delivery and targeting systems, as described in further detail below. For example, dsRNA can be encapsulated into targeted polymeric delivery systems designed to promote payload internalization.

[0096] The dsRNA can be targeted to any stretch of less than 30 contiguous nucleotides, generally about 19-25 contiguous nucleotides, in the functional Aha1 (or other related molecule with similar function) mRNA target sequences, e.g. SEQ ID NOs: 12-56 (see Table 1 below). Searches of the human genome database (BLAST) can be carried out to ensure that selected dsRNA sequence will not target other gene transcripts. Techniques for selecting target sequences for dsRNA are known in the art (see e.g., Reynolds et al. (2004) Nature Biotechnology 22(3), 326 – 330). Thus, the sense strand of the present dsRNA can comprise a nucleotide sequence identical to any contiguous stretch of about 19 to about 25 nucleotides in the target mRNA of functional Aha1 (or related molecule with similar function). Generally, a target sequence on the target mRNA can be selected from a given cDNA sequence corresponding to the target mRNA, preferably beginning 50 to 100 nt downstream (i.e., in the 3' direction) from the start codon. The target sequence can, however, be located in the 5' or 3' untranslated regions, or in the region nearby the start

codon.

[0097] The dsRNA of the invention can comprise an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-25 nucleotides in length, and is substantially complementary to at least part of an mRNA transcript of an Aha gene. The use of these dsRNAs enables the targeted degradation of mRNAs of genes that are implicated in replication and or maintenance of cancer cells in mammals, and/or in the degradation of misfolded Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Using cell-based and animal assays, very low dosages of these dsRNA can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of an Aha gene. Thus, the methods and compositions of the invention comprising these dsRNAs are useful for treating pathological processes mediated by Aha expression, e.g. protein misfolding, including cancer and/or cystic fibrosis, by targeting a gene involved in protein degradation.

[0098] The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of an Aha gene, as well as compositions and methods for treating diseases and disorders caused by the expression of an Aha gene, such as cancer and/or cystic fibrosis. The pharmaceutical compositions of the invention comprise a dsRNA having an antisense strand comprising a region of complementarity which is less than 30 nucleotides in length, generally 19-25 nucleotides in length, and is substantially complementary to at least part of an RNA transcript of an Aha gene, together with a pharmaceutically acceptable carrier.

[0099] Accordingly, certain aspects of the invention provide pharmaceutical compositions comprising the dsRNA of the invention together with a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of an Aha gene, and methods of using the pharmaceutical compositions to treat diseases caused by expression of an Aha gene.

[0100] In one embodiment, the invention provides dsRNA molecules for inhibiting the expression of an Aha gene, e.g. an Aha1 gene, in a cell or mammal, wherein the dsRNA comprises an antisense strand comprising a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an Aha gene, e.g. an Aha1 gene, and wherein the region of complementarity is less than 30 nucleotides in length, generally 19-25 nucleotides in length. The dsRNA may be identical to one of the dsRNAs shown in Tables 2, 5 or 6, or it may effect cleavage of an mRNA encoding an Aha gene within the target sequence of one of the dsRNAs shown in Tables 2, 5 or 6.

Table 1. Homo sapiens Aha1 mRNA Target Sequences (Sequence Position Based on Coding Sequence of GenBank Accession No. NM_012111.1 (SEQ ID NO: 11; Ensembl Gene Report No. ENSG00000100591))

1. mRNA Target Sequence Based on Aha Gene Sequence AAATTGGTCCACGGATAAGCT:
AAAUUGGUCCACGGAUAAGCU (SEQ ID NO: 12)
Position in gene sequence: 99

2. mRNA Target Sequence Based on Aha Gene Sequence AAGCTGAAAACACTGTTCCTG:
AAGCUGAAAACACUGUUCCUG (SEQ ID NO: 13)
Position in gene sequence: 115

3. mRNA Target Sequence Based on Aha Gene Sequence AAAACACTGTTCCTGGCAGTG:
AAAACACUGUUCCUGGCAGUG (SEQ ID NO: 14)
Position in gene sequence: 121

4. mRNA Target Sequence Based on Aha Gene Sequence AAAATGAAGAAGGCAAGTGTG:
AAAAUGAAGAAGGCAAGUGUG (SEQ ID NO: 15)
Position in gene sequence: 149

5. mRNA Target Sequence Based on Aha Gene Sequence AATGAAGAAGGCAAGTGTGAG:
AAUGAAGAAGGCAAGUGUGAG (SEQ ID NO: 16)
Position in gene sequence: 151

6. mRNA Target Sequence Based on Aha Gene Sequence AAGAAGGCAAGTGTGAGGTGA:
AAGAAGGCAAGUGUGAGGUGA (SEQ ID NO: 17)
Position in gene sequence: 155

7. mRNA Target Sequence Based on Aha Gene Sequence AAGTGAGTAAGCTTGATGGAG:
AAGUGAGUAAGCUUGAUGGAG (SEQ ID NO: 18)
Position in gene sequence: 179

8. mRNA Target Sequence Based on Aha Gene Sequence AACAATCGCAAAGGGAAACTT:
AACAAUCGCAAAGGGAAACUU (SEQ ID NO: 19)
Position in gene sequence: 211

9. mRNA Target Sequence Based on Aha Gene Sequence AATCGCAAAGGGAAACTTATC:
AAUCGCAAAGGGAAACUUAUC (SEQ ID NO: 20)
Position in gene sequence: 214

10. mRNA Target Sequence Based on Aha Gene Sequence AAAGGGAAACTTATCTTCTTT:

AAAGGGAAACUUAUCUUCUUU (SEQ ID NO: 21)
Position in gene sequence: 220

11. mRNA Target Sequence Based on Aha Gene Sequence AAACTTATCTTCTTTTATGAA:
AAACUUAUCUUCUUUUAUGAA (SEQ ID NO: 22)
Position in gene sequence: 226

12. mRNA Target Sequence Based on Aha Gene Sequence AATGGAGCGTCAAACTAAACT:
AAUGGAGCGUCAAACUAAACU (SEQ ID NO: 23)
Position in gene sequence: 245

13. mRNA Target Sequence Based on Aha Gene Sequence AAACTAAACTGGACAGGTACT:
AAACUAAACUGGACAGGUACU (SEQ ID NO: 24)
Position in gene sequence: 256

14. mRNA Target Sequence Based on Aha Gene Sequence AAACTGGACAGGTACTTCTAA:
AAACUGGACAGGUACUUCUAA (SEQ ID NO: 25)
Position in gene sequence: 261

15. mRNA Target Sequence Based on Aha Gene Sequence AAGTCAGGAGTACAATACAAA:
AAGUCAGGAGUACAAUACAAA (SEQ ID NO: 26)
Position in gene sequence: 280

16. mRNA Target Sequence Based on Aha Gene Sequence AATACAAAGGACATGTGGAGA:
AAUACAAAGGACAUGUGGAGA (SEQ ID NO: 27)
Position in gene sequence: 293

17. mRNA Target Sequence Based on Aha Gene Sequence AATTTGTCTGATGAAAACAGC:
AAUUUGUCUGAUGAAAACAGC (SEQ ID NO: 28)
Position in gene sequence: 319

18. mRNA Target Sequence Based on Aha Gene Sequence AAAACAGCGTGGATGAAGTGG:
AAAACAGCGUGGAUGAAGUGG (SEQ ID NO: 29)
Position in gene sequence: 332

19. mRNA Target Sequence Based on Aha Gene Sequence AAGTGGAGATTAGTGTGAGCC:
AAGUGGAGAUUAGUGUGAGCC (SEQ ID NO: 30)
Position in gene sequence: 347

20. mRNA Target Sequence Based on Aha Gene Sequence AAAGATGAGCCTGACACAAAT:
AAAGAUGAGCCUGACACAAAU (SEQ ID NO: 31)
Position in gene sequence: 373

21. mRNA Target Sequence Based on Aha Gene Sequence AAATCTCGTGGCCTTAATGAA:
AAAUCUCGUGGCCUUAAUGAA (SEQ ID NO: 32)
Position in gene sequence: 390

22. mRNA Target Sequence Based on Aha Gene Sequence AATGAAGGAAGAAGGGGTGAA:
AAUGAAGGAAGAAGGGGUGAA (SEQ ID NO: 33)
Position in gene sequence: 405

23. mRNA Target Sequence Based on Aha Gene Sequence AAGGAAGAAGGGGTGAAACTT:
AAGGAAGAAGGGGUGAAACUU (SEQ ID NO: 34)
Position in gene sequence: 409

24. mRNA Target Sequence Based on Aha Gene Sequence AAGAAGGGGTGAAACTTCTAA:
AAGAAGGGGUGAAACUUCUAA (SEQ ID NO: 35)
Position in gene sequence: 413

25. mRNA Target Sequence Based on Aha Gene Sequence AAGGGGTGAAACTTCTAAGAG:
AAGGGGUGAAACUUCUAAGAG (SEQ ID NO: 36)
Position in gene sequence: 416

26. mRNA Target Sequence Based on Aha Gene Sequence AAACTTCTAAGAGAAGCAATG:
AAACUUCUAAGAGAAGCAAUG (SEQ ID NO: 37)
Position in gene sequence: 424

27. mRNA Target Sequence Based on Aha Gene Sequence AAGAGAAGCAATGGGAATTTA:
AAGAGAAGCAAUGGGAAUUUA (SEQ ID NO: 38)
Position in gene sequence: 432

28. mRNA Target Sequence Based on Aha Gene Sequence AAGCAATGGGAATTTACATCA:
AAGCAAUGGGAAUUUACAUCA (SEQ ID NO: 39)
Position in gene sequence: 437

29. mRNA Target Sequence Based on Aha Gene Sequence AATGGGAATTTACATCAGCAC:
AAUGGGAAUUUACAUCAGCAC (SEQ ID NO: 40)
Position in gene sequence: 441

30. mRNA Target Sequence Based on Aha Gene Sequence AATTTACATCAGCACCCTCAA:
AAUUUACAUCAGCACCCUCAA (SEQ ID NO: 41)
Position in gene sequence: 447

31. mRNA Target Sequence Based on Aha Gene Sequence

AATGAATGGAGAGTCAGTAGA:
AAUGAAUGGAGAGUCAGUAGA (SEQ ID NO: 42)
Position in gene sequence: 501

32. mRNA Target Sequence Based on Aha Gene Sequence
AATGGAGAGTCAGTAGACCCA:
AAUGGAGAGUCAGUAGACCCA (SEQ ID NO: 43)
Position in gene sequence: 505

33. mRNA Target Sequence Based on Aha Gene Sequence
AAGCCTGCTCCTTCAAAAACC:
AAGCCUGCUCCUUCAAAAACC (SEQ ID NO: 44)
Position in gene sequence: 565

34. mRNA Target Sequence Based on Aha Gene Sequence
AAAATCCCCACTTGTAAGATC:
AAAAUCCCCACUUGUAAGAUC (SEQ ID NO: 45)
Position in gene sequence: 607

35. mRNA Target Sequence Based on Aha Gene Sequence
AATCCCCACTTGTAAGATCAC:
AAUCCCCACUUGUAAGAUCAC (SEQ ID NO: 46)
Position in gene sequence: 609

36. mRNA Target Sequence Based on Aha Gene Sequence
AAGATCACTCTTAAGGAAACC:
AAGAUCACUCUUAAGGAAACC (SEQ ID NO: 47)
Position in gene sequence: 622

37. mRNA Target Sequence Based on Aha Gene Sequence
AAGGAAACCTTCCTGACGTCA:
AAGGAAACCUUCCUGACGUCA (SEQ ID NO: 48)
Position in gene sequence: 634

38. mRNA Target Sequence Based on Aha Gene Sequence
AACATTAGAAGCAGACAGAGG:
AACAUUAGAAGCAGACAGAGG (SEQ ID NO: 49)
Position in gene sequence: 720

39. mRNA Target Sequence Based on Aha Gene Sequence
AAGCAGACAGAGGTGGAAAGT:
AAGCAGACAGAGGUGGAAAGU (SEQ ID NO: 50)
Position in gene sequence: 728

40. mRNA Target Sequence Based on Aha Gene Sequence
AAAGTTCCACATGGTAGATGG:
AAAGUUCCACAUGGUAGAUGG (SEQ ID NO: 51)
Position in gene sequence: 744

41. mRNA Target Sequence Based on Aha Gene Sequence
AACGTCTCTGGGGAATTTACT:
AACGUCUCUGGGGAAUUUACU (SEQ ID NO: 52)

Position in gene sequence: 766

42. mRNA Target Sequence Based on Aha Gene Sequence AATTTACTGATCTGGTCCCTG:
AAUUUACUGAUCUGGUCCCUG (SEQ ID NO: 53)
Position in gene sequence: 779

43. mRNA Target Sequence Based on Aha Gene Sequence AAACATATTGTGATGAAGTGG:
AAACAUAUUGUGAUGAAGUGG (SEQ ID NO: 54)
Position in gene sequence: 802

44. mRNA Target Sequence Based on Aha Gene Sequence AAGTGGAGGTTTAAATCTTGG:
AAGUGGAGGUUUAAAUCUUGG (SEQ ID NO: 55)
Position in gene sequence: 817

45. mRNA Target Sequence Based on Aha Gene Sequence AAACAGACCTTTGGCTATGGC:
AAACAGACCUUUGGCUAUGGC (SEQ ID NO: 56)
Position in gene sequence: 982

Table 2. dsRNA agents for the down-regulation of Homo sapiens Functional Aha Protein Expression

1. dsRNA based on Aha Gene Target Sequence 1
Sense strand dsRNA: AUUGGUCCACGGAUAAGCU (SEQ ID NO: 57)
Antisense strand dsRNA: AGCUUAUCCGUGGACCAAU (SEQ ID NO: 58)

2. dsRNA based on Aha Gene Target Sequence 2
Sense strand dsRNA: GCUGAAAACACUGUUCCUG (SEQ ID NO: 59)
Antisense strand dsRNA: CAGGAACAGUGUUUUCAGC (SEQ ID NO: 60)

3. dsRNA based on Aha Gene Target Sequence 3
Sense strand dsRNA: AACACUGUUCCUGGCAGUG (SEQ ID NO: 61)
Antisense strand dsRNA: CACUGCCAGGAACAGUGUU (SEQ ID NO: 62)

4. dsRNA based on Aha Gene Target Sequence 4
Sense strand dsRNA: AAUGAAGAAGGCAAGUGUG (SEQ ID NO: 63)
Antisense strand dsRNA: CACACUUGCCUUCUUCAUU (SEQ ID NO: 64)

5. dsRNA based on Aha Gene Target Sequence 5
Sense strand dsRNA: UGAAGAAGGCAAGUGUGAG (SEQ ID NO: 65)
Antisense strand dsRNA: CUCACACUUGCCUUCUUCA (SEQ ID NO: 66)

6. dsRNA based on Aha Gene Target Sequence 6
Sense strand dsRNA: GAAGGCAAGUGUGAGGUGA (SEQ ID NO: 67)
Antisense strand dsRNA: UCACCUCACACUUGCCUUC (SEQ ID NO: 68)

7. dsRNA based on Aha Gene Target Sequence 7
Sense strand dsRNA: GUGAGUAAGCUUGAUGGAG (SEQ ID NO: 69)

Antisense strand dsRNA: CUCCAUCAAGCUUACUCAC (SEQ ID NO: 70)

8. dsRNA based on Aha Gene Target Sequence 8
   Sense strand dsRNA: CAAUCGCAAAGGGAAACUU (SEQ ID NO: 71)
   Antisense strand dsRNA: AAGUUUCCCUUUGCGAUUG (SEQ ID NO: 72)

9. dsRNA based on Aha Gene Target Sequence 9
   Sense strand dsRNA: UCGCAAAGGGAAACUUAUC (SEQ ID NO: 73)
   Antisense strand dsRNA: GAUAAGUUUCCCUUUGCGA (SEQ ID NO: 74)

10. dsRNA based on Aha Gene Target Sequence 10
   Sense strand dsRNA: AGGGAAACUUAUCUUCUUU (SEQ ID NO: 75)
   Antisense strand dsRNA: AAAGAAGAUAAGUUUCCCU (SEQ ID NO: 76)

11. dsRNA based on Aha Gene Target Sequence 11
   Sense strand dsRNA: ACUUAUCUUCUUUUAUGAA (SEQ ID NO: 77)
   Antisense strand dsRNA: UUCAUAAAAGAAGAUAAGU (SEQ ID NO: 78)

12. dsRNA based on Aha Gene Target Sequence 12
   Sense strand dsRNA: UGGAGCGUCAAACUAAACU (SEQ ID NO: 79)
   Antisense strand dsRNA: AGUUUAGUUUGACGCUCCA (SEQ ID NO: 80)

13. dsRNA based on Aha Gene Target Sequence 13
   Sense strand dsRNA: ACUAAACUGGACAGGUACU (SEQ ID NO: 81)
   Antisense strand dsRNA: AGUACCUGUCCAGUUUAGU (SEQ ID NO: 82)

14. dsRNA based on Aha Gene Target Sequence 14
   Sense strand dsRNA: ACUGGACAGGUACUUCUAA (SEQ ID NO: 83)
   Antisense strand dsRNA: UUAGAAGUACCUGUCCAGU (SEQ ID NO: 84)

15. dsRNA based on Aha Gene Target Sequence 15
   Sense strand dsRNA: GUCAGGAGUACAAUACAAA (SEQ ID NO: 85)
   Antisense strand dsRNA: UUUGUAUUGUACUCCUGAC (SEQ ID NO: 86)

16. dsRNA based on Aha Gene Target Sequence 16
   Sense strand dsRNA: UACAAAGGACAUGUGGAGA (SEQ ID NO: 87)
   Antisense strand dsRNA: UCUCCACAUGUCCUUUGUA (SEQ ID NO: 88)

17. dsRNA based on Aha Gene Target Sequence 17
   Sense strand dsRNA: UUUGUCUGAUGAAAACAGC (SEQ ID NO: 89)
   Antisense strand dsRNA: GCUGUUUUCAUCAGACAAA (SEQ ID NO: 90)

18. dsRNA based on Aha Gene Target Sequence 18
   Sense strand dsRNA: AACAGCGUGGAUGAAGUGG (SEQ ID NO: 91)
   Antisense strand dsRNA: CCACUUCAUCCACGCUGUU (SEQ ID NO: 92)

19. dsRNA based on Aha Gene Target Sequence 19
   Sense strand dsRNA: GUGGAGAUUAGUGUGAGCC (SEQ ID NO: 93)
   Antisense strand dsRNA: GGCUCACACUAAUCUCCAC (SEQ ID NO: 94)

20. dsRNA based on Aha Gene Target Sequence 20
   Sense strand dsRNA: AGAUGAGCCUGACACAAAU (SEQ ID NO: 95)

Antisense strand dsRNA: AUUUGUGUCAGGCUCAUCU (SEQ ID NO: 96)

21. dsRNA based on Aha Gene Target Sequence 21
    Sense strand dsRNA: AUCUCGUGGCCUUAAUGAA (SEQ ID NO: 97)
    Antisense strand dsRNA: UUCAUUAAGGCCACGAGAU (SEQ ID NO: 98)

22. dsRNA based on Aha Gene Target Sequence 22
    Sense strand dsRNA: UGAAGGAAGAAGGGGUGAA (SEQ ID NO: 99)
    Antisense strand dsRNA: UUCACCCCUUCUUCCUUCA (SEQ ID NO: 100)

23. dsRNA based on Aha Gene Target Sequence 23
    Sense strand dsRNA: GGAAGAAGGGGUGAAACUU (SEQ ID NO: 101)
    Antisense strand dsRNA: AAGUUUCACCCCUUCUUCC (SEQ ID NO: 102)

24. dsRNA based on Aha Gene Target Sequence 24
    Sense strand dsRNA: GAAGGGGUGAAACUUCUAA (SEQ ID NO: 103)
    Antisense strand dsRNA: UUAGAAGUUUCACCCCUUC (SEQ ID NO: 104)

25. dsRNA based on Aha Gene Target Sequence 25
    Sense strand dsRNA: GGGGUGAAACUUCUAAGAG (SEQ ID NO: 105)
    Antisense strand dsRNA: CUCUUAGAAGUUUCACCCC (SEQ ID NO: 106)

26. dsRNA based on Aha Gene Target Sequence 26
    Sense strand dsRNA: ACUUCUAAGAGAAGCAAUG (SEQ ID NO: 107)
    Antisense strand dsRNA: CAUUGCUUCUCUUAGAAGU (SEQ ID NO: 108)

27. dsRNA based on Aha Gene Target Sequence 27
    Sense strand dsRNA: GAGAAGCAAUGGGAAUUUA (SEQ ID NO: 109)
    Antisense strand dsRNA: UAAAUUCCCAUUGCUUCUC (SEQ ID NO: 110)

28. dsRNA based on Aha Gene Target Sequence 28
    Sense strand dsRNA: GCAAUGGGAAUUUACAUCA (SEQ ID NO: 111)
    Antisense strand dsRNA: UGAUGUAAAUUCCCAUUGC (SEQ ID NO: 112)

29. dsRNA based on Aha Gene Target Sequence 29
    Sense strand dsRNA: UGGGAAUUUACAUCAGCAC (SEQ ID NO: 113)
    Antisense strand dsRNA: GUGCUGAUGUAAAUUCCCA (SEQ ID NO: 114)

30. dsRNA based on Aha Gene Target Sequence 30
    Sense strand dsRNA: UUUACAUCAGCACCCUCAA (SEQ ID NO: 115)
    Antisense strand dsRNA: UUGAGGGUGCUGAUGUAAA (SEQ ID NO: 116)

31. dsRNA based on Aha Gene Target Sequence 31
    Sense strand dsRNA: UGAAUGGAGAGUCAGUAGA (SEQ ID NO: 117)
    Antisense strand dsRNA: UCUACUGACUCUCCAUUCA (SEQ ID NO: 118)

32. dsRNA based on Aha Gene Target Sequence 32
    Sense strand dsRNA: UGGAGAGUCAGUAGACCCA (SEQ ID NO: 119)
    Antisense strand dsRNA: UGGGUCUACUGACUCUCCA (SEQ ID NO: 120)

33. dsRNA based on Aha Gene Target Sequence 33
    Sense strand dsRNA: GCCUGCUCCUUCAAAAACC (SEQ ID NO: 121)

Antisense strand dsRNA: GGUUUUUGAAGGAGCAGGC (SEQ ID NO: 122)

34. dsRNA based on Aha Gene Target Sequence 34
    Sense strand dsRNA: AAUCCCCACUUGUAAGAUC (SEQ ID NO: 123)
    Antisense strand dsRNA: GAUCUUACAAGUGGGGAUU (SEQ ID NO: 124)

35. dsRNA based on Aha Gene Target Sequence 35
    Sense strand dsRNA: UCCCCACUUGUAAGAUCAC (SEQ ID NO: 125)
    Antisense strand dsRNA: GUGAUCUUACAAGUGGGGA (SEQ ID NO: 126)

36. dsRNA based on Aha Gene Target Sequence 36
    Sense strand dsRNA: GAUCACUCUUAAGGAAACC (SEQ ID NO: 127)
    Antisense strand dsRNA: GGUUUCCUUAAGAGUGAUC (SEQ ID NO: 128)

37. dsRNA based on Aha Gene Target Sequence 37
    Sense strand dsRNA: GGAAACCUUCCUGACGUCA (SEQ ID NO: 129)
    Antisense strand dsRNA: UGACGUCAGGAAGGUUUCC (SEQ ID NO: 130)

38. dsRNA based on Aha Gene Target Sequence 38
    Sense strand dsRNA: CAUUAGAAGCAGACAGAGG (SEQ ID NO: 131)
    Antisense strand dsRNA: CCUCUGUCUGCUUCUAAUG (SEQ ID NO: 132)

39. dsRNA based on Aha Gene Target Sequence 39
    Sense strand dsRNA: GCAGACAGAGGUGGAAAGU (SEQ ID NO: 133)
    Antisense strand dsRNA: ACUUUCCACCUCUGUCUGC (SEQ ID NO: 134)

40. dsRNA based on Aha Gene Target Sequence 40
    Sense strand dsRNA: AGUUCCACAUGGUAGAUGG (SEQ ID NO: 135)
    Antisense strand dsRNA: CCAUCUACCAUGUGGAACU (SEQ ID NO: 136)

41. dsRNA based on Aha Gene Target Sequence 41
    Sense strand dsRNA: CGUCUCUGGGGAAUUUACU (SEQ ID NO: 137)
    Antisense strand dsRNA: AGUAAAUUCCCCAGAGACG (SEQ ID NO: 138)

42. dsRNA based on Aha Gene Target Sequence 42
    Sense strand dsRNA: UUUACUGAUCUGGUCCCUG (SEQ ID NO: 139)
    Antisense strand dsRNA: CAGGGACCAGAUCAGUAAA (SEQ ID NO: 140)

43. dsRNA based on Aha Gene Target Sequence 43
    Sense strand dsRNA: ACAUAUUGUGAUGAAGUGG (SEQ ID NO: 141)
    Antisense strand dsRNA: CCACUUCAUCACAAUAUGU (SEQ ID NO: 142)

44. dsRNA based on Aha Gene Target Sequence 44
    Sense strand dsRNA: GUGGAGGUUUAAAUCUUGG (SEQ ID NO: 143)
    Antisense strand dsRNA: CCAAGAUUUAAACCUCCAC (SEQ ID NO: 144)

45. dsRNA based on Aha Gene Target Sequence 45
    Sense strand dsRNA: ACAGACCUUUGGCUAUGGC (SEQ ID NO: 145)
    Antisense strand dsRNA: GCCAUAGCCAAAGGUCUGU (SEQ ID NO: 146)

Table 3. Primers for Vector Transcribing shRNA for the down-regulation of Homo sapiens Functional Aha Protein Expression

1. Primer based on Aha Gene Target Sequence 1 (SEQ ID NO: 12)
   Sense strand:
   GATCCATTGGTCCACGGATAAGCTTTCAAGAGAAGCTTATCCGTG GACCAATTTTTTTGGAAA (SEQ ID NO: 147)
   Antisense strand:
   AGCTTTTCCAAAAAAATTGGTCCACGGATAAGCTTCTCTTGAAAG CTTATCCGTGGACCAATG (SEQ ID NO: 148)

2. Primer based on Aha Gene Target Sequence 7 (SEQ ID NO: 18)
   Sense strand:
   GATCCGTGAGTAAGCTTGATGGAGTTCAAGAGACTCCATCAAGC TTACTCACTTTTTTGGAAA (SEQ ID NO: 149)
   Antisense strand:
   AGCTTTTCCAAAAAAGTGAGTAAGCTTGATGGAGTCTCTTGAACT CCATCAAGCTTACTCACG (SEQ ID NO: 150)

3. Primer based on Aha Gene Target Sequence 13 (SEQ ID NO: 24)
   Sense strand:
   GATCCACTAAACTGGACAGGTACTTTCAAGAGAAGTACCTGTCCA GTTTAGTTTTTTTGGAAA (SEQ ID NO: 151)
   Antisense strand:
   AGCTTTTCCAAAAAAACTAAACTGGACAGGTACTTCTCTTGAAAG TACCTGTCCAGTTTAGTG (SEQ ID NO: 152)

Table 4. shRNA Sequences Transcribed by Encoding Vector

1. GAUCCAUUGGUCCACGGAUAAGCUUUCAAGAGAAGCUUAUCCGUGGACCA AUUUUUUUGGAAA (SEQ ID NO: 153)

2. GAUCCGUGAGUAAGCUUGAUGGAGUUCAAGAGACUCCAUCAAGCUUACUC ACUUUUUUGGAAA (SEQ ID NO: 154)

3. GAUCCACUAAACUGGACAGGUACUUUCAAGAGAAGUACCUGUCCAGUUUA GUUUUUUUGGAAA (SEQ ID NO: 155)

Table 5: RNAi agents for the down-regulation of homo sapiens (NM_012111.1), mus musculus (NM_146036.1) and pan troglodytes (XM_510094.1) Aha 1, and minimal off-target interactions in rat cells; AL-DP-7561, AL-DP-7562, AL-DP-7563 and AL-DP-7564 are additionally cross-reactive to mus musculus (NM_172391.3) and rattus norvegicus (XM_223680.3) Aha 2

Duplex identifier	Sense strand sequence[1]	SEQ ID NO:	Antisense strand sequence[1]	SEQ ID NO:
AL-DP-7299	auugguccacggauaagcuTT	156	agcuuauccguggaccaauTT	157
AL-DP-7300	gugaguaagcuugauggagTT	158	cuccaucaagcuuacucacTT	159
AL-DP-7301	agucaaaaucccccacuuguTT	160	acaaaguggggauuuugacuTT	161
AL-DP-7302	aaaucucguggccuuaaugTT	162	cauuaaggccacgagauuuTT	163
AL-DP-7303	gagauuagugugagccuugTT	164	caaggcucacacuaaucucTT	165
AL-DP-7304	aaucucguggccuuaaugaTT	166	ucauuaaggccacgagauuTT	167
AL-DP-7305	agauuagugugagccuugcTT	168	gcaaggcucacacuaaucuTT	169
AL-DP-7306	cgggcggacgccaccaacgTT	170	cguugguggcguccgcccgTT	171
AL-DP-7307	ggcggacgccaccaacgucTT	172	gacguugguggcguccgccTT	173
AL-DP-7308	gggcggacgccaccaacguTT	174	acguugguggcguccgcccTT	175
AL-DP-7309	caacgucaacaacuggcacTT	176	gugccaguuguugacguugTT	177
AL-DP-7310	gcgggcggacgccaccaacTT	178	guugguggcguccgcccgcTT	179
AL-DP-7311	aucucguggccuuaaugaaTT	180	uucauuaaggccacgagauTT	181
AL-DP-7312	acgucaacaacuggcacugTT	182	cagugccaguuguugacguTT	183
AL-DP-7313	accaacgucaacaacuggcTT	184	gccaguuguugacguugguTT	185
AL-DP-7314	acgcuggaucguggaggagTT	186	cuccuccacgauccagcguTT	187
AL-DP-7315	agacccacgcuggaucgugTT	188	cacgauccagcgugggucuTT	189
AL-DP-7316	gacccacgcuggaucguggTT	190	ccacgauccagcgugggucTT	191
AL-DP-7317	gaauuuacaucagcacccuTT	192	agggugcugauguaaauucTT	193
AL-DP-7318	gggaauuuacaucagcaccTT	194	ggugcugauguaaauucccTT	195
AL-DP-7319	ugggaauuuacaucagcacTT	196	gugcugauguaaauucccaTT	197
AL-DP-7320	ccaacgucaacaacuggcaTT	198	ugccaguuguugacguuggTT	199
AL-DP-7321	aaguggggugagggagaccTT	200	ggucucccucaccccacuuTT	201
AL-DP-7322	acacaaaucucguggccuuTT	202	aaggccacgagauuuguguTT	203
AL-DP-7323	acccacgcuggaucguggaTT	204	uccacgauccagcguggguTT	205
AL-DP-7324	gagucaaaaucccccacuugTT	206	caaguggggauuuugacucTT	207
AL-DP-7325	gagcucuauagaguguuuaTT	208	uaaacacucuauagagcucTT	209
AL-DP-7326	ggcagcgguacuacuuugaTT	210	ucaaaguaguaccgcugccTT	211
AL-DP-7327	gacacaaaucucguggccuTT	212	aggccacgagauuugugucTT	213
AL-DP-7328	agcgggcggacgccaccaaTT	214	uugguggcguccgcccgcuTT	215
AL-DP-7329	caaaaucccccacuuguaagTT	216	cuuacaaguggggauuuugTT	217
AL-DP-7330	gagacccacgcuggaucguTT	218	acgauccagcgugggucucTT	219
AL-DP-7331	gagccuugccaaagaugagTT	220	cucaucuuuggcaaggcucTT	221
AL-DP-7332	ugacacaaaucucguggccTT	222	ggccacgagauuugugucaTT	223
AL-DP-7333	ggagcucuauagaguguuuTT	224	aaacacucuauagagcuccTT	225
AL-DP-7334	cccacgcuggaucguggagTT	226	cuccacgauccagcgugggTT	227
AL-DP-7335	gaucccccaauuugucugauTT	228	aucagacaaauuggggaucTT	229
AL-DP-7336	gagaucccccaauuugucugTT	230	cagacaaauuggggaucucTT	231
AL-DP-7337	agccugacacaaaucucguTT	232	acgagauuugugucaggcuTT	233
AL-DP-7338	agaucccccaauuugucugaTT	234	ucagacaaauuggggaucuTT	235
AL-DP-7339	agggagacccacgcuggauTT	236	auccagcgugggucucccuTT	237
AL-DP-7340	gagggagacccacgcuggaTT	238	uccagcgugggucucccucTT	239

Duplex identifier	Sense strand sequence[1]	SEQ ID NO:	Antisense strand sequence[1]	SEQ ID NO:
AL-DP-7341	gccaaguggggugagggagTT	240	cucccucaccccacuuggcTT	241
AL-DP-7342	uggcagcgguacuacuuugTT	242	caaaguaguaccgcugccaTT	243
AL-DP-7343	ugagggagacccacgcuggTT	244	ccagcgugggucucccucaTT	245
AL-DP-7344	aguggagauuagugugagcTT	246	gcucacacuaaucuccacuTT	247
AL-DP-7345	aggagcucuauagaguguuTT	248	aacacucuauagagcuccuTT	249
AL-DP-7346	agcgguacuacuuugagggTT	250	cccucaaaguaguaccgcuTT	251
AL-DP-7561	cgcuggaucguggaggagcTT	252	gcuccuccacgauccagcgTT	253
AL-DP-7562	gcuggaucguggaggagcgTT	254	cgcuccuccacgauccagcTT	255
AL-DP-7563	cuggaucguggaggagcggTT	256	ccgcuccuccacgauccagTT	257
AL-DP-7564	uggaucguggaggagcgggTT	258	cccgcuccuccacgauccaTT	259

[1] Capital letters = desoxyribonucleotides; small letters = ribonucleotides

Table 6: RNAi agents for the down-regulation of homo sapiens (NM_012111.1), mus musculus (NM_146036.1) and pan troglodytes (XM_510094.1) Aha 1, and minimal off-target interactions in human cells

Duplex identifier	Sense strand sequence[1]	SEQ ID NO:	Antisense strand sequence[1]	SEQ ID NO:
AL-DP-9250	gccugacacaaaucucgugTT	260	cacgagauuugugucaggcTT	261
AL-DP-9251	ccugacacaaaucucguggTT	262	ccacgagauuugugucaggTT	263
AL-DP-9252	acgccaccaacgucaacaaTT	264	uuguugacguugguggcguTT	265
AL-DP-9253	agcucuauagaguguuuacTT	266	guaaacacucuauagagcuTT	267
AL-DP-9254	gggcuggcagcgguacuacTT	268	guaguaccgcugccagcccTT	269
AL-DP-9255	cuggcagcgguacuacuuuTT	270	aaaguaguaccgcugccagTT	271
AL-DP-9256	ggaugaaguggagauuaguTT	272	acuaaucuccacuucauccTT	273
AL-DP-9257	accagaggagcucuauagaTT	274	ucuauagagcuccucugguTT	275
AL-DP-9258	aaguggagauuagugugagTT	276	cucacacuaaucuccacuuTT	277
AL-DP-9259	gaggagcucuauagaguguTT	278	acacucuauagagcuccucTT	279
AL-DP-9260	gggagacccacgcuggaucTT	280	gauccagcgugggucucccTT	281
AL-DP-9261	ugagccugacacaaaucucTT	282	gagauuugugucaggcucaTT	283
AL-DP-9262	gcggacgccaccaacgucaTT	284	ugacguugguggcguccgcTT	285
AL-DP-9263	cggacgccaccaacgucaaTT	286	uugacguugguggcguccgTT	287
AL-DP-9264	gaaguggagauuagugugaTT	288	ucacacuaaucuccacuucTT	289
AL-DP-9265	cucguggccuuaaugaaggTT	290	ccuucauuaaggccacgagTT	291
AL-DP-9266	ucguggccuuaaugaaggaTT	292	uccuucauuaaggccacgaTT	293
AL-DP-9267	aaugggaauuuacaucagcTT	294	gcugauguaaauucccauuTT	295
AL-DP-9268	ggaauuuacaucagcacccTT	296	gggugcugauguaaauuccTT	297
AL-DP-9269	ggagauuagugugagccuuTT	298	aaggcucacacuaaucuccTT	299
AL-DP-9270	cacaaaucucguggccuuaTT	300	uaaggccacgagauuugugTT	301
AL-DP-9271	acaaaucucguggccuuaaTT	302	uuaaggccacgagauuuguTT	303
AL-DP-9272	ggagacccacgcuggaucgTT	304	cgauccagcgugggucuccTT	305

Duplex identifier	Sense strand sequence[1]	SEQ ID NO:	Antisense strand sequence[1]	SEQ ID NO:
AL-DP-9273	ggacgccaccaacgucaacTT	306	guugacguugguggcguccTT	307
AL-DP-9274	gaugaaguggagauuagugTT	308	cacuaaucuccacuucaucTT	309
AL-DP-9275	gugagccuugccaaagaugTT	310	caucuuuggcaaggcucacTT	311
AL-DP-9276	caaugaauggagagucaguTT	312	acugacucuccauucauugTT	313
AL-DP-9277	auuagugugagccuugccaTT	314	uggcaaggcucacacuaauTT	315
AL-DP-9278	agaugagccugacacaaauTT	316	auuugugucaggcucaucuTT	317
AL-DP-9279	uagugugagccuugccaaaTT	318	uuuggcaaggcucacacuaTT	319
AL-DP-9280	uuugccaccaucaccuugaTT	320	ucaaggugaugguggcaaaTT	321
AL-DP-9281	acggagagagaugcuucaaTT	322	uugaagcaucucucuccguTT	323
AL-DP-9282	cggagagagaugcuucaaaTT	324	uuugaagcaucucucuccgTT	325
AL-DP-9283	aaaaucccccacuuguaagaTT	326	ucuuacaaguggggauuuuTT	327
AL-DP-9284	aucccccaauuugucugaugTT	328	caucagacaaauuggggauTT	329
AL-DP-9285	ucaaaaucccccacuuguaaTT	330	uuacaaguggggauuuugaTT	331
AL-DP-9286	aaaucccccacuuguaagauTT	332	aucuuacaaguggggauuuTT	333
AL-DP-9287	uccccaauuugucugaugaTT	334	ucaucagacaaauuggggaTT	335
AL-DP-9288	auggccaaguggggugaggTT	336	ccucaccccacuuggccauTT	337
AL-DP-9289	ggagucaaaaucccccacuuTT	338	aaguggggauuuugacuccTT	339

[1] Capital letters = desoxyribonucleotides; small letters = ribonucleotides

[0101] Preferably, the dsRNA has at least 5, at least 10, at least 15, at least 18, or at least 20 contiguous nucleotides per strand in common with at least one strand, but preferably both strands, of one of the dsRNAs shown in Tables 2, 5 and 6. Alternative dsRNAs that target elsewhere in the target sequence of one of the dsRNAs provided in Tables 2, 5 and 6 can readily be determined using the target sequence and the flanking Aha1 sequence.

[0102] The dsRNA comprises two RNA strands that are complementary to hybridize to form a duplex structure. One strand of the dsRNA (the antisense strand) comprises a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of an Aha gene, the other strand (the sense strand) comprises a region which is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 base pairs in length. Similarly, the region of complementarity to the target sequence is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 nucleotides in length. The dsRNA of the invention may further comprise one or more single-stranded nucleotide overhang(s).). For example, deoxyribonucleotide sequence “tt” or ribonucleotide sequence “UU” can be connected to the 3’-end of both sense and antisense strands to form overhangs. The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc. In a preferred embodiment, an Aha gene is the human Aha1 gene.

[0103] In other embodiments, the dsRNA comprises at least two sequences selected from this group, wherein one of the at least two sequences is complementary to another of the at least two sequences, and one of the at least two sequences is substantially complementary to a sequence of an mRNA generated in the expression of an Aha gene, e.g. an Aha1 gene.

[0104] The skilled person is well aware that dsRNAs comprising a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., EMBO 2001, 20:6877-6888). However, others have found that shorter or longer dsRNAs can be effective as well.

[0105] The RNAi agents provided in Tables 2, 5 and 6 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an

mRNA encoding an Aha gene. For example, the 3'-most 15 nucleotides of the target sequence of AL-DP-7299 combined with the next 6 nucleotides from the target Aha1 gene produces a single strand agent of 21 nucleotides that is based on one of the sequences provided in Tables 2, 5 and 6.

[0106] Preferably, the second dsRNA is chosen from the group of dsRNAs having a certain activity in inhibiting the expression of an Aha gene in a suitable assay, such as the assays described herein. Consequently, in certain preferred ambodiments, the second dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7311, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7319, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9278, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289.

[0107] The dsRNA of the invention can contain one or more mismatches to the target sequence. In a preferred embodiment, the dsRNA of the invention contains no more than 3 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located in the center of the region of complementarity. If the antisense strand of the dsRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to 5 nucleotides from either end, for example 5, 4, 3, 2, or 1 nucleotide from either the 5' or 3' end of the region of complementarity, and preferably from the 5'-end. For example, for a 23 nucleotide dsRNA strand which is complementary to a region of an Aha gene, the dsRNA generally does not contain any mismatch within the central 13 nucleotides. In another embodiment, the antisense strand of the dsRNA does not contain any mismatch in the region from positions 1, or 2, to positions 9, or 10, of the antisense strand (counting 5'-3'). The methods described within the invention can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of an Aha gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of an Aha gene is important,

especially if the particular region of complementarity in an Aha gene is known to have polymorphic sequence variation within the population.

[0108] In one embodiment, at least one end of the dsRNA has a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties than their blunt-ended counterparts. Moreover, the present inventors have discovered that the presence of only one nucleotide overhang strengthens the interference activity of the dsRNA, without affecting its overall stability. dsRNA having only one overhang has proven particularly stable and effective in vivo, as well as in a variety of cells, cell culture mediums, blood, and serum. Generally, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3‘-terminal end of the sense strand. The dsRNA may also have a blunt end, generally located at the 5’-end of the antisense strand. Such dsRNAs have improved stability and inhibitory activity, thus allowing administration at low dosages, i.e., less than 5 mg/kg body weight of the recipient per day. Generally, the antisense strand of the dsRNA has a nucleotide overhang at the 3’-end, and the 5’-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

[0109] In yet another embodiment, the dsRNA is chemically modified to enhance stability. The nucleic acids of the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry", Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Specific examples of preferred dsRNA compounds useful in this invention include dsRNAs containing modified backbones or no natural internucleoside linkages. As defined in this specification, dsRNAs having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified dsRNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[0110] Preferred modified dsRNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the

adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[0111] Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference

[0112] Preferred modified dsRNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[0113] Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, each of which is herein incorporated by reference.

[0114] In other preferred dsRNA mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an dsRNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an dsRNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et

al., Science, 1991, 254, 1497-1500.

[0115] Most preferred embodiments of the invention are dsRNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular -CH_2-NH-CH_2-, -CH_2-N(CH_3)-O-CH_2-[known as a methylene (methylimino) or MMI backbone], -CH_2-O-N(CH_3)-CH_2-, -CH_2-N(CH_3)-N(CH_3)-CH_2- and -N(CH_3)-CH_2-CH_2- [wherein the native phosphodiester backbone is represented as -O-P-O-CH_2-] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. Also preferred are dsRNAs having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[0116] Modified dsRNAs may also contain one or more substituted sugar moieties. Preferred dsRNAs comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred dsRNAs comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an dsRNA, or a group for improving the pharmacodynamic properties of an dsRNA, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) i.e., an alkoxy-alkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-CH_2-O-CH_2-$N(CH_2)_2$, also described in examples hereinbelow.

[0117] Other preferred modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the dsRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. DsRNAs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957;

5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

[0118] DsRNAs may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, DsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2.degree. C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., DsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[0119] Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and

5,681,941, each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, also herein incorporated by reference.

[0120] Another modification of the dsRNAs of the invention involves chemically linking to the dsRNA one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the dsRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acid. Sci. USA, 199, 86, 6553-6556), cholic acid (Manoharan et al., Biorg. Med. Chem. Let., 1994 4 1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Biorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J, 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

[0121] Representative U.S. patents that teach the preparation of such dsRNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

[0122] It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an dsRNA. The present invention also includes dsRNA compounds which are chimeric compounds. "Chimeric" dsRNA compounds or "chimeras," in the context of this invention, are dsRNA

compounds, particularly dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an dsRNA compound. These dsRNAs typically contain at least one region wherein the dsRNA is modified so as to confer upon the dsRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the dsRNA may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNAduplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of dsRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter dsRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxydsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

[0123] In certain instances, the dsRNA may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to dsRNAs in order to enhance the activity, cellular distribution or cellular uptake of the dsRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3:2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10:111; Kabanov et al., FEBS Lett., 1990, 259:327; Svinarchuk et al., Biochimie, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651; Shea et al., Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such dsRNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of dsRNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being

conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the dsRNA still bound to the solid support or following cleavage of the dsRNA in solution phase. Purification of the dsRNA conjugate by HPLC typically affords the pure conjugate.

[0124] Vector encoded RNAi agents

[0125] The dsRNA of the invention can also be expressed from recombinant viral vectors intracellularly in vivo. The recombinant viral vectors of the invention comprise sequences encoding the dsRNA of the invention and any suitable promoter for expressing the dsRNA sequences. Suitable promoters include, for example, the U6 or H1 RNA pol III promoter sequences and the cytomegalovirus promoter. Selection of other suitable promoters is within the skill in the art. The recombinant viral vectors of the invention can also comprise inducible or regulatable promoters for expression of the dsRNA in a particular tissue or in a particular intracellular environment. The use of recombinant viral vectors to deliver dsRNA of the invention to cells in vivo is discussed in more detail below.

[0126] dsRNA of the invention can be expressed from a recombinant viral vector either as two separate, complementary RNA molecules, or as a single RNA molecule with two complementary regions.

[0127] Any viral vector capable of accepting the coding sequences for the dsRNA molecule(s) to be expressed can be used, for example vectors derived from adenovirus (AV); adeno-associated virus (AAV); retroviruses (e.g, lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

[0128] For example, lentiviral vectors of the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors of the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. For example, an AAV vector expressing a serotype 2 capsid on a serotype 2 genome is called AAV 2/2. This serotype 2 capsid gene in the AAV 2/2 vector can be replaced by a serotype 5 capsid gene to produce an AAV 2/5 vector. Techniques for constructing AAV vectors which express different capsid protein serotypes are within the skill in the art; see, e.g., Rabinowitz J E et al. (2002), J Virol 76:791-801, the entire disclosure of which is herein incorporated by reference.

[0129] Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the dsRNA into the vector, and

methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis M A (1988), Biotechniques 6: 608-614; Miller A D (1990), Hum Gene Therap. 1: 5-14; Anderson W F (1998), Nature 392: 25-30; and Rubinson D A et al., Nat. Genet. 33: 401-406, the entire disclosures of which are herein incorporated by reference.

[0130] Preferred viral vectors are those derived from AV and AAV. In a particularly preferred embodiment, the dsRNA of the invention is expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector comprising, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter.

[0131] A suitable AV vector for expressing the dsRNA of the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H et al. (2002), Nat. Biotech. 20: 1006-1010.

[0132] Suitable AAV vectors for expressing the dsRNA of the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R et al. (1987), J. Virol. 61: 3096-3101; Fisher K J et al. (1996), J. Virol, 70: 520-532; Samulski R et al. (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

[0133] Non-dsRNA Agents

[0134] Antibodies can be used to decrease levels of functional Aha1 and/or other related molecles with similar function. For example, antibodies can decrease levels of functional Aha1 by specifically binding to functional Aha1, the Hsp90 ATPase binding site for functional Aha1, and/or the functional Aha1-Hsp90 ATPase complex. Antibodies within the scope of the invention include, for example, polyclonal antibodies, monoclonal antibodies, antibody fragments, and antibody-based fusion molecules. Engineering, production, screening, purification, fragmentation, and therapeutic use of antibodies are well known in the art (see generally, Carter (2006) Nat Rev Immunol. 6(5), 343-357; Coligan (2005) Short Protocols in Immunology, John Wiley & Sons, ISBN 0471715786); Teillaud (2005) Expert Opin Biol Ther. 5(Supp. 1) S15-27; Subramanian, ed. (2004) Antibodies : Volume 1: Production and Purification, Springer, ISBN 0306482452; Brent et al., ed. (2003) Current Protocols in Molecular Biology, John Wiley & Sons Inc, ISBN 047150338X; Lo, ed. (2003) Antibody Engineering Methods and Protocols, Humana Press, ISBN 1588290921; Ausubel et al., ed. (2002) Short Protocols in Molecular Biology 5th Ed., Current Protocols, ISBN

0471250929). Various types of antibodies specific for functional Aha1 can also be obtained from a variety of commercial sources. The terminal half-life of antibodies in plasma can be tuned over a wide range, for example several minutes to several weeks, to fit clinical goals for treating protein misfolding diseases (see e.g., Carter et al. (2006) Nat Rev Immunol. 6(5), 343-357, 353). Chimeric, humanized, and fully human MAbs can effectively overcome potential limitations on the use of antibodies derived from non-human sources to treat protein misfolding diseases, thus providing decreased immunogenicity with optimized effector functions (see e.g., Teillaud (2005) Expert Opin. Biol. Ther. 5(1), S15-S27; Tomizuka et al. (2000) Proc. Nat. Acad. Sci. USA 97, 722-727; Carter et al. (2006) Nat Rev Immunol. 6(5), 343-357, 346-347). Antibodies can be altered or selected so as to achieve efficient antibody internalization. As such, the antibodies can more effectively interact with target intracellular molecules, such as functional Aha1 and/or related molecules with similar functions, or complexes including such. Further, antibody-drug conjugates can increase the efficiency of antibody internalization. Efficient antibody internalization can be desirable for delivering functional Aha1 specific antibodies to the intracellular environment so as to salvage defective folding and transit of proteins characterized by suboptimal folding energetics. Conjugation of antibodies to a variety of agents that can facilitate cellular internalization of antibodies is known in the art (see generally Wu et al. (2005) Nat Biotechnol. 23(9), 1137-1146; McCarron et al. (2005) Mol Interv 5(6), 368-380; Niemeyer (2004) Bioconjugation Protocols, Strategies and Methods, Humana Press, ISBN 1588290980; Hermanson (1996) Bioconjugate Techniques, Academic Press, ISBN 0123423368).

[0135] Small organic molecules that interact specifically with heat shock protein co-chaperones, such as the Hsp90 co-chaperone Aha1, can be used to decrease the levels of functional Aha1 and/or other related molecules with similar functions. Identification of a pharmaceutical or small molecule inhibitor of functional Aha1 can be readily accomplished through standard high-throughput screening methods. Furthermore, standard medicial chemistry approaches can be applied to these agents to enhance or modify their activity so as to yield additional agents.

[0136] Purified aptamers that specifically recognize and bind to functional Aha1 (or other related molecules with similar function) nucleotides or proteins can be used to decrease the level of functional Aha1 (and/or other related molecules with similar functions). Aptamers are nucliec acids or peptide molecules selected from a large random sequence pool to bind to specific target molecule. The small size of aptamers makes them easier to synthesize and chemically modify and enables them to access epitopes that otherwise might

be blocked or hidden. And aptamers are generally nontoxic and weak antigens because of their close resemblance to endogenous molecules. Generation, selection, and delivery of aptamers is within the skill of the art (see e.g., Lee et al. (2006) Curr Opin Chem Biol. 10, 1-8; Yan et al. (2005) Front Biosci 10, 1802-1827; Hoppe-Seyler and Butz (2000) J Mol Med. 78(8), 426-430). Negative selection procedures can yield aptamers that can finely discriminate between molecular variants. For example, negative selection procedures can yield aptamers that can discriminate between Hsp90/ADP and Hsp90/ATP; or can discriminate between functional Aha1, Hsp90 ATPase, and the functional Aha1-Hsp90 ATPase binding complex. Aptamers can also be used to temporally and spatially regulate protein function (e.g., functional Aha1 function) in cells and organisms. For example, the ligand-regulated peptide (LiRP) system provides a general method where the binding activity of intracellular peptides is controlled by a peptide aptamer in turn regulated by a cell-permeable small molecule (see e.g., Binkowski (2005) Chem & Biol. 12(7), 847-55). Using LiRP or a similar delivery system, the binding activity of functional Aha1 could be controlled by a cell-permeable small molecule that interacts with the introduced intracellular functional Aha1-specific protein aptamer. Thus, aptamers can provide an effective means to decrease functional Aha1 levels by, for example, directly binding the functional Aha1 mRNA, functional Aha1 expressed protein, the Hsp90 ATPase binding site for functional Aha1, and/or the functional Aha1-Hsp90 ATPase complex.

[0137] Purified antisense nucleic acids that specifically recognize and bind to ribonucleotides encoding functional Aha1 (and/or other related molecules with similar function) can be used to decrease the levels of functional Aha1 (and/or other related molecules with similar functions). Antisense nucleic acid molecules within the invention are those that specifically hybridize (e.g., bind) under cellular conditions to cellular mRNA and/or genomic DNA encoding, for example functional Aha1 protein, in a manner that inhibits expression of that protein, e.g., by inhibiting transcription and/or translation. Antisense molecules, effective for decreasing functional Aha1 levels, can be designed, produced, and administered by methods commonly known to the art (see e.g., Chan et al. (2006) Clinical and Experimental Pharmacology and Physiology 33(5-6), 533-540).

[0138] Ribozyme molecules designed to catalytically cleave target mRNA transcripts can also be used to decrease levels of functional Aha1 and/or related molecules with similar activity. Ribozyme molecules specific for functional Aha1 can be designed, produced, and administered by methods commonly known to the art (see e.g., Fanning and Symonds (2006) Handbook Experimental Pharmacology 173, 289-303G, reviewing therapeutic use of hammerhead ribozymes and small hairpin RNA). Triplex-forming

oligonucleotides can also be used to decrease levels of functional Aha1 and/or related molecules with similar activity (see generally, Rogers et al. (2005) Current Medicinal Chemistry 5(4), 319-326).

[0139] Administration

[0140] Agents for use in the methods described herein can be delivered in a variety of means known to the art. The agents can be used therapeutically either as exogenous materials or as endogenous materials. Exogenous agents are those produced or manufactured outside of the body and administered to the body. Endogenous agents are those produced or manufactured inside the body by some type of device (biologic or other) for delivery within or to other organs in the body.

[0141] The agents described herein can be formulated by any conventional manner using one or more pharmaceutically acceptable carriers and/or excipients as described in, for example, Remington's Pharmaceutical Sciences (A.R. Gennaro, Ed.), 21st edition, ISBN: 0781746736 (2005), incorporated herein by reference in its entirety. Such formulations will contain a therapeutically effective amount of the agent, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration. The agents of use with the current invention can be formulated by known methods for administration to a subject using several routes which include, but are not limited to, parenteral, pulmonary, oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, buccal, and rectal. The individual agents may also be administered in combination with one or more additional agents of the present invention and/or together with other biologically active or biologically inert agents. Such biologically active or inert agents may be in fluid or mechanical communication with the agent(s) or attached to the agent(s) by ionic, covalent, Van der Waals, hydrophobic, hydrophillic or other physical forces.

[0142] When used in the methods of the invention, a therapeutically effective amount of one of the agents described herein can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt form and with or without a pharmaceutically acceptable excipient. For example, the agents of the invention can be administered, at a reasonable benefit/risk ratio applicable to any medical treatment, in a sufficient amount sufficient to rescue intracellular and/or extracellular trafficking of a protein characterized by suboptimal folding energetics and/or at least partially restore channel functions in a subject.

[0143] Toxicity and therapeutic efficacy of such agents can be determined by standard pharmaceutical procedures in cell cultures and/or experimental animals for

determining the LD50 (the dose lethal to 50% of the population) and the ED50, (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index that can be expressed as the ratio LD50/ED50, where large therapeutic indices are preferred.

[0144] The amount of an agent that may be combined with a pharmaceutically acceptable carrier to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. It will be appreciated by those skilled in the art that the unit content of agent contained in an individual dose of each dosage form need not in itself constitute a therapeutically effective amount, as the necessary therapeutically effective amount could be reached by administration of a number of individual doses. Agent administration can occur as a single event or over a time course of treatment. For example, an agent can be administered daily, weekly, bi-weekly, or monthly. For some conditions, treatment could extend from several weeks to several months or even a year or more.

[0145] The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific agent employed; the duration of the treatment; drugs used in combination or coincidental with the specific agent employed and like factors well known in the medical arts. It will be understood by a skilled practitioner that the total daily usage of the agents for use in the present invention will be decided by the attending physician within the scope of sound medical judgment.

[0146] Agents that decrease the level of functional Aha1, or other related molecules with similar function, can also be used in combination with other therapeutic modalities. Thus, in addition to the therapies described herein, one may also provide to the subject other therapies known to be efficacious for particular protein misfolding diseases.

[0147] Controlled-release (or sustained-release) preparations may be formulated to extend the activity of the agent and reduce dosage frequency. Controlled-release preparations can also be used to effect the time of onset of action or other characteristics, such as blood levels of the agent, and consequently affect the occurrence of side effects.

[0148] Controlled-release preparations may be designed to initially release an amount of an agent that produces the desired therapeutic effect, and gradually and continually release other amounts of the agent to maintain the level of therapeutic effect over an extended period of time. In order to maintain a near-constant level of an agent in the body, the agent can be released from the dosage form at a rate that will replace the amount

of agent being metabolized and/or excreted from the body. The controlled-release of an agent may be stimulated by various inducers, e.g., change in pH, change in temperature, enzymes, water, or other physiological conditions or molecules.

[0149] Controlled-release systems may include, for example, an infusion pump which may be used to administer the agent in a manner similar to that used for delivering insulin or chemotherapy to specific organs or tumors. Typically, using such a system, the agent is administered in combination with a biodegradable, biocompatible polymeric implant (see below) that releases the agent over a controlled period of time at a selected site. Examples of polymeric materials include polyanhydrides, polyorthoesters, polyglycolic acid, polylactic acid, polyethylene vinyl acetate, and copolymers and combinations thereof. In addition, a controlled release system can be placed in proximity of a therapeutic target, thus requiring only a fraction of a systemic dosage.

[0150] The agents of the invention may be administered by other controlled-release means or delivery devices that are well known to those of ordinary skill in the art. These include, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or the like, or a combination of any of the above to provide the desired release profile in varying proportions (see below). Other methods of controlled-release delivery of agents will be known to the skilled artisan and are within the scope of the invention.

[0151] Agents that decrease levels of functional Aha1 and/or other related molecules with similar functions can be administered through a variety of routes well known in the arts. Examples include methods involving direct injection (e.g., systemic or stereotactic), implantation of cells engineered to secrete the factor of interest, drug-releasing biomaterials, implantable matrix devices, implantable pumps, injectable gels and hydrogels, liposomes, micelles (e.g., up to 30 □m), nanospheres (e.g., less than 1 □m), microspheres (e.g., 1-100 □m), reservoir devices, etc.

[0152] Pulmonary delivery of macromoles and/or drugs, such as the agents described herein, provide for relatively easy, non-invasive administration to the local tissue of the lungs or the circulatory system for systemic circulation (see e.g., Cryan (2004) AAPS J. 7(1) article 4, E20-41, providing a review of pulmonary delivery technology). Advantages of pulmonary delivery include noninvasiveness, large surface area for absorption (~75 m2), thin (~0.1 to 0.5 □m) alveolar epitheliuem permitting rapid absorption, absence of first pass metabolism, decreased proteolytic activity, rapid onset of action, and high bioavailablity. Drug formulations for pulmonary delivery, with or without excipients and/or a dispersible liquid, are known to the art. Carrier-based systems for biomolecule delivery, such as

polymeric delivery systems, liposomes, and micronized carbohydrates, can be used in conjunction with pulmonary delivery. Penetration enhancers (e.g., surfactants, bile salts, cyclodextrins, enzyme inhibitors (e.g., chymostatin, leupeptin, bacitracin), and carriers (e.g., microspheres and liposomes) can be used to enhance uptake across the alveolar epithelial cells for systemic distribution. Various inhalation delivery devices, such as metered-dose inhalers, nebulizers, and dry-powder inhalers, that can be used to deliver the biomolecules described herein are known to the art (e.g., AErx (Aradigm, CA); Respimat (Boehringer, Germany); AeroDose (Aerogen Inc., CA)). As known in the art, device selection can depend upon the state of the biomolecule (e.g., solution or dry powder) to be used, the method and state of storage, the choice of excipients, and the interactions between the formulation and the device. Dry powder inhalation devices are particularly preferred for pulmonary delivery of protein-based agents (e.g., Spinhaler (Fisons Pharmaceuticals, NY); Rotohaler (GSK, NC); Diskhaler (GSK, NC); Spiros (Dura Pharmaceuticals, CA); Nektar (Nektar Pharmaceuticals, CA)). Dry powder formulation of the active biological ingredient to provide good flow, dispersability, and stability is known to those skilled in the art.

[0153] Agents effecting a decrease in levels of functional Aha1 can be encapsualted and administered in a variety of carrier delivery systems. Examples of carrier delivery systems include microspheres, hydrogels, polymeric implants, smart ploymeric carriers, and liposomes. Carrier-based systems for biomolecular agent delivery can: provide for intracellular delivery; tailor biomolecule/agent release rates; increase the proportion of biomolecule that reaches its site of action; improve the transport of the drug to its site of action; allow colocalized deposition with other agents or excipients; improve the stability of the agent in vivo; prolong the residence time of the agent at its site of action by reducing clearance; decrease the nonspecific delivery of the agent to nontarget tissues; decrease irritation caused by the agent; decrease toxicity due to high initial doses of the agent; alter the immunogenicity of the agent; decrease dosage frequency, improve taste of the product; and/or improve shelf life of the product.

[0154] Polymeric microspheres can be produced using naturally occurring or synthetic polymers and are particulate systems in the size range of 0.1 to 500 μm. Polymeric micelles and polymeromes are polymeric delivery vehicles with similar characteristics to microspheres and can also facilitate encapsulation and delivery of the biomolecules described herein. Fabrication, encapsulation, and stabilization of microspheres for a variety of biomolecule payloads are within the skill of the art (see e.g., Varde & Pack (2004) Expert Opin. Biol. 4(1) 35-51). Release rate of microspheres can be tailored by type of polymer, polymer molecular weight, copolymer composition, excipients

added to the microsphere formulation, and microsphere size. Polymer materials useful for forming microspheres include PLA, PLGA, PLGA coated with DPPC, DPPC, DSPC, EVAc, gelatin, albumin, chitosan, dextran, DL-PLG, SDLMs, PEG (e.g., ProMaxx), sodium hyaluronate, diketopiperazine derivatives (e.g., Technosphere), calcium phosphate-PEG particles, and oligosaccharide derivative DPPG (e.g., Solidose). Encapsulation can be accomplished, for example, using a water/oil single emulsion method, a water-oil-water double emulsion method, or lyophilization. Several commercial encapsulation technologies are available (e.g., ProLease®, Alkerme). Microspheres encapsulating the agents described herein can be administered in a variety of means including parenteral, oral, pulmonary, implantation, and pumping device.

[0155] Polymeric hydrogels, composed of hydrophillic polymers such as collagen, fibrin, and alginate, can also be used for the sustained release of agents that decrease levels of functional Aha1 and/or other related molecules with similar function (see generally, Sakiyama et al. (2001) FASEB J. 15, 1300-1302).

[0156] Three-dimensional polymeric implants, on the millimeter to centimeter scale, can be loaded with agents that decrease levels of functional Aha1 and/or other related molecules with similar function (see generally, Teng et al (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 3024-3029). A polymeric implant typically provides a larger depot of the bioactive factor. The implants can also be fabricated into structural supports, tailoring the geometry (e.g., shape, size, porosity) to the application. Implantable matrix-based delivery systems are also commercially available in a variety of sizes and delivery profiles (e.g., Innovative Research of America, Sarasota, FL).

[0157] "Smart" polymeric carriers can be used to administer agents that decrease levels of functional Aha1 and/or other related molecules with similar function (see generally, Stayton et al. (2005) Orthod Craniofacial Res 8, 219-225; Wu et al. (2005) Nature Biotech (2005) 23(9), 1137-1146). Carriers of this type utilize polymers that are hydrophilic and stealth-like at physiological pH, but become hydrophobic and membrane-destabilizing after uptake into the endosomal compartment (i.e., acidic stimuli from endosomal pH gradient) where they enhance the release of the cargo molecule into the cytoplasm. Design of the smart polymeric carrier can incorporate pH-sensing functionalities, hydrophobic membrane-destabilizing groups, versatile conjugation and/or complexation elements to allow the drug incorporation, and an optional cell targeting component. Potential therapeutic macromolecular cargo includes peptides, proteins, antibodies, polynucleotides, plasmid DNA (pDNA), aptamers, antisense oligodeoxynucleotides, silencing RNA, and/or ribozymes that effect a decrease in levels of functional Aha1 and/or related molecules with similar function.

As an example, smart polymeric carriers, internalized through receptor mediated endocytosis, can enhance the cytoplasmic delivery of functional Aha1-targeted dsRNA, and/or other agents described herein. Polymeric carriers include, for example, the family of poly(alkylacrylic acid) polymers, specific examples including poly(methylacrylic acid), poly(ethylacrylic acid) (PEAA), poly(propylacrylic acid) (PPAA), and poly(butylacrylic acid) (PBAA), where the alkyl group progressively increased by one methylene group. Smart polymeric carriers with potent pH-responsive, membrane destabilizing activity can be designed to be below the renal excretion size limit. For example, poly(EAA-co-BA-co-PDSA) and poly(PAA-co-BA-co-PDSA) polymers exhibit high hemolytic/membrane destabilizing activity at the low molecular weights of 9 and 12 kDa, respectively. Various linker chemistries are available to provide degradable conjugation sites for proteins, nucleic acids, and/or targeting moieties. For example, pyridyl disulfide acrylate (PDSA) monomer allow efficient conjugation reactions through disulfide linkages that can be reduced in the cytoplasm after endosomal translocation of the therapeutics.

[0158] Liposomes can be used to administer agents that decrease levels of functional Aha1 and/or other related molecules with similar function. The drug carrying capacity and release rate of liposomes can depend on the lipid composition, size, charge, drug/lipid ratio, and method of delivery. Conventional liposomes are composed of neutral or anionic lipids (natural or synthetic). Commonly used lipids are lecithins such as (phosphatidylcholines), phosphatidylethanolamines (PE), sphingomyelins, phosphatidylserines, phosphatidylglycerols (PG), and phosphatidylinositols (PI). Liposome encapsulation methods are commonly known in the arts (Galovic et al. (2002) Eur. J. Pharm. Sci. 15, 441-448; Wagner et al. (2002) J. Liposome Res. 12, 259-270). Targeted liposomes and reactive liposomes can also be used to deliver the biomolecules of the invention. Targeted liposomes have targeting ligands, such as monoclonal antibodies or lectins, attached to their surface, allowing interaction with specific receptors and/or cell types. Reactive or polymorphic liposomes include a wide range of liposomes, the common property of which is their tendency to change their phase and structure upon a particular interaction (eg, pH-sensitive liposomes) (see e.g., Lasic (1997) Liposomes in Gene Delivery, CRC Press, FL).

[0159] Various other delivery systems are known in the art and can be used to administer the agents of the invention. Moreover, these and other delivery systems may be combined and/or modified to optimize the administration of the agents of the present invention.

[0160] Pharmaceutical compositions comprising dsRNA

[0161] In one embodiment, the invention provides pharmaceutical compositions comprising a dsRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition comprising the dsRNA is useful for treating a disease or disorder associated with the expression or activity of an Aha gene, such as pathological processes mediated by Aha1 expression. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery.

[0162] The pharmaceutical compositions of the invention are administered in dosages sufficient to inhibit expression of an Aha gene. The present inventors have found that, because of their improved efficiency, compositions comprising the dsRNA of the invention can be administered at surprisingly low dosages. A maximum dosage of 5 mg dsRNA per kilogram body weight of recipient per day is sufficient to inhibit or completely suppress expression of an Aha gene.

[0163] In general, a suitable dose of dsRNA will be in the range of 0.01 microgram to 5.0 milligrams per kilogram body weight of the recipient per day, generally in the range of 1 microgram to 1 mg per kilogram body weight per day. The pharmaceutical composition may be administered once daily, or the dsRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the dsRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation which provides sustained release of the dsRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for vaginal delivery of agents, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

[0164] The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and in vivo half-lives for the individual dsRNAs encompassed by the invention can be made using conventional methodologies or on the basis of in vivo testing using an appropriate animal model, as described elsewhere herein.

[0165] Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as pathological processes mediated by Aha expression. Such models are used for in vivo testing of dsRNA, as well as for determining a therapeutically effective dose.

[0166] The present invention also includes pharmaceutical compositions and formulations which include the dsRNA compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

[0167] Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful. Preferred topical formulations include those in which the dsRNAs of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidyl ethanolamine = DOPE, dimyristoylphosphatidyl choline = DMPC, distearolyphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol = DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl = DOTAP and dioleoylphosphatidyl ethanolamine = DOTMA). DsRNAs of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, dsRNAs may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters include but are not limited arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-10} alkyl ester (e.g. isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. patent application Ser. No. 09/315,298 filed on May 20, 1999 which is incorporated herein by reference in its entirety.

[0168] Compositions and formulations for oral administration include powders or

granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which dsRNAs of the invention are administered in conjunction with one or more penetration enhancers, surfactants, and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Preferred fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (e.g. sodium). Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Particularly preferred complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (e.g. p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. application. Ser. No. 08/886,829 (filed Jul. 1, 1997), Ser. No. 09/108,673 (filed Jul. 1, 1998), Ser. No. 09/256,515 (filed Feb. 23, 1999), Ser. No. 09/082,624 (filed May 21, 1998) and Ser. No. 09/315,298 (filed May 20, 1999), each of which is incorporated herein by reference in their entirety.

[0169] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0170] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0171] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0172] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0173] In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. The preparation of such compositions and formulations is generally known to those skilled in the pharmaceutical and formulation arts and may be applied to the formulation of the compositions of the present invention.

[0174] Emulsions

[0175] The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker,

Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

[0176] Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[0177] Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel

Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

[0178] Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

[0179] A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[0180] Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

[0181] Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives

included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

[0182] The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

[0183] In one embodiment of the present invention, the compositions of dsRNAs and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical

Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

[0184] The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

[0185] Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C_8-C_{12}) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C_8-C_{10} glycerides, vegetable oils and silicone oil.

[0186] Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides et al., Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides et al., Pharmaceutical Research, 1994, 11,

1385; Ho et al., J. Pharm. Sci., 1996, 85, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or dsRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of dsRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of dsRNAs and nucleic acids within the gastrointestinal tract, vagina, buccal cavity and other areas of administration.

[0187] Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the dsRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

[0188] Liposomes

[0189] There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

[0190] Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages in vivo.

[0191] In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

[0192] Further advantages of liposomes include; liposomes obtained from natural

phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

[0193] Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

[0194] Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

[0195] Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis

[0196] Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., Biochem. Biophys. Res. Commun., 1987, 147, 980-985).

[0197] Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., Journal of Controlled Release, 1992, 19, 269-274).

[0198] One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

[0199] Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (e.g. as a solution or as an emulsion) were ineffective (Weiner et al., Journal of Drug Targeting, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis et al., Antiviral Research, 1992, 18, 259-265).

[0200] Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome.TM. I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome.TM. II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. S.T.P.Pharma. Sci., 1994, 4, 6, 466).

[0201] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_m1, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., FEBS Letters, 1987, 223, 42; Wu

et al., Cancer Research, 1993, 53, 3765).

[0202] Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (Ann. N.Y. Acad. Sci., 1987, 507, 64) reported the ability of monosialoganglioside G_m1, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_m1 or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al).

[0203] Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, $2C_{1215G}$, that contains a PEG moiety. Illum et al. (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klibanov et al. (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin et al.) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al). U.S. Pat. No. 5,540,935 (Miyazaki et al.) and U.S. Pat. No. 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

[0204] A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry et al. discloses methods for encapsulating high molecular

weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include dsRNA. U.S. Pat. No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising dsRNAs targeted to the raf gene.

[0205] Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g. they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

[0206] Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

[0207] If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

[0208] If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric

acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

[0209] If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

[0210] If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

[0211] The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

[0212] Penetration Enhancers

[0213] In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly dsRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

[0214] Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

[0215] Surfactants: In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of dsRNAs through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and

perfluorochemical emulsions, such as FC-43 (Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

[0216] Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_1 - C_{10} alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., Critical Reviews in Therapeutic Drug Carryier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).

[0217] Bile salts: The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579-583).

[0218] Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of dsRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most

characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur et al., J. Control Rel., 1990, 14, 43-51).

[0219] Non-chelating non-surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of dsRNAs through the alimentary mucosa (Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621-626).

[0220] Agents that enhance uptake of dsRNAs at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs.

[0221] Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

[0222] Carriers

[0223] Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by in vivo processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other

extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao et al., Antisense Res. Dev., 1995, 5, 115-121; Takakura et al., Antisense & Nucl. Acid Drug Dev., 1996, 6, 177-183.

[0224] Excipients

[0225] In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc).

[0226] Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

[0227] Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

[0228] Suitable pharmaceutically acceptable excipients include, but are not limited

to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

[0229] Pharmaceutical compositions for the delivery to the respiratory tract

[0230] Another aspect of the invention provides for the delivery of IRNA agents to the respiratory tract, particularly for the treatment of cystic fibrosis. The respiratory tract includes the upper airways, including the oropharynx and larynx, followed by the lower airways, which include the trachea followed by bifurcations into the bronchi and bronchioli. The upper and lower airways are called the conductive airways. The terminal bronchioli then divide into respiratory bronchioli which then lead to the ultimate respiratory zone, the alveoli, or deep lung. The deep lung, or alveoli, are the primary target of inhaled therapeutic aerosols for systemic delivery of iRNA agents.

[0231] Pulmonary delivery compositions can be delivered by inhalation by the patient of a dispersion so that the composition, preferably the iRNA agent, within the dispersion can reach the lung where it can, for example, be readily absorbed through the alveolar region directly into blood circulation. Pulmonary delivery can be effective both for systemic delivery and for localized delivery to treat diseases of the lungs.

[0232] Pulmonary delivery can be achieved by different approaches, including the use of nebulized, aerosolized, micellular and dry powder-based formulations; administration by inhalation may be oral and/or nasal. Delivery can be achieved with liquid nebulizers, aerosol-based inhalers, and dry powder dispersion devices. Metered-dose devices are preferred. One of the benefits of using an atomizer or inhaler is that the potential for contamination is minimized because the devices are self contained. Dry powder dispersion devices, for example, deliver drugs that may be readily formulated as dry powders. An iRNA composition may be stably stored as lyophilized or spray-dried powders by itself or in combination with suitable powder carriers. The delivery of a composition for inhalation can be mediated by a dosing timing element which can include a timer, a dose counter, time measuring device, or a time indicator which when incorporated into the device enables dose tracking, compliance monitoring, and/or dose triggering to a patient during administration of the aerosol medicament.

[0233] Examples of pharmaceutical devices for aerosol delivery include metered dose inhalers (MDIs), dry powder inhalers (DPIs), and air-jet nebulizers. Exemplary delivery systems by inhalation which can be readily adapted for delivery of the subject iRNA agents are described in, for example, U.S. Pat. Nos. 5,756,353; 5,858,784; and PCT applications

WO98/31346; WO98/10796; WO00/27359; WO01/54664; WO02/060412. Other aerosol formulations that may be used for delivering the iRNA agents are described in U.S. Pat. Nos. 6,294,153; 6,344,194; 6,071,497, and PCT applications WO02/066078; WO02/053190; WO01/60420; WO00/66206. Further, methods for delivering iRNA agents can be adapted from those used in delivering other oligonucleotides (e.g., an antisense oligonucleotide) by inhalation, such as described in Templin et al., Antisense Nucleic Acid Drug Dev, 2000, 10:359-68; Sandrasagra et al., Expert Opin Biol Ther, 2001, 1:979-83; Sandrasagra et al., Antisense Nucleic Acid Drug Dev, 2002, 12:177-81.

[0234] The delivery of the inventive agents may also involve the administration of so called "pro-drugs", i.e. formulations or chemical modifications of a therapeutic substance that require some form of processing or transport by systems innate to the subject organism to release the therapeutic substance, preferably at the site where its action is desired; this latter embodiment may be used in conjunction with delivery of the respiratory tract, but also together with other embodiments of the present invention. For example, the human lungs can remove or rapidly degrade hydrolytically cleavable deposited aerosols over periods ranging from minutes to hours. In the upper airways, ciliated epithelia contribute to the "mucociliary excalator" by which particles are swept from the airways toward the mouth. Pavia, D., "Lung Mucociliary Clearance," in Aerosols and the Lung: Clinical and Experimental Aspects, Clarke, S. W. and Pavia, D., Eds., Butterworths, London, 1984. In the deep lungs, alveolar macrophages are capable of phagocytosing particles soon after their deposition. Warheit et al. Microscopy Res. Tech., 26: 412-422 (1993); and Brain, J. D., "Physiology and Pathophysiology of Pulmonary Macrophages," in The Reticuloendothelial System, S. M. Reichard and J. Filkins, Eds., Plenum, New. York., pp. 315-327, 1985.

[0235] In preferred embodiments, particularly where systemic dosing with the iRNA agent is desired, the aerosoled iRNA agents are formulated as microparticles. Microparticles having a diameter of between 0.5 and ten microns can penetrate the lungs, passing through most of the natural barriers. A diameter of less than ten microns is required to bypass the throat; a diameter of 0.5 microns or greater is required to avoid being exhaled.

[0236] Other Components

[0237] The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional

materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0238] Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0239] Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more dsRNA agents and (b) one or more other chemotherapeutic agents which function by a non-RNA interference mechanism. Examples of such chemotherapeutic agents include but are not limited to daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed. 1987, pp. 1206-1228, Berkow et al., eds., Rahway, N.J. When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-dsRNA

chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

[0240] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit high therapeutic indices are preferred.

[0241] The data obtained from cell culture assays and animal studies can be used in formulation a range of dosage for use in humans. The dosage of compositions of the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (e.g., achieving a decreased concentration of the polypeptide) that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0242] In addition to their administration individually or as a plurality, as discussed above, the dsRNAs of the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by Aha expression. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

[0243] Screening

[0244] Another aspect of the invention is directed to a system for screening candidate agents for actions on functional Aha1 and/or other related molecules with similar functions, which can be useful for the development of compositions for therapeutic or prophylactic treatment of protein folding diseases. Assays can be performed on living mammalian cells, which more closely approximate the effects of a particular serum level of drug in the body. Cell lines expressing a protein with energetically disfavorable folding

characteristics would be useful for evaluating the activity of potential bioactive agents on functional Aha1 and/or other related molecules with similar function, or on extracts prepared from the cultured cell lines. Studies using extracts offer the possibility of a more rigorous determination of direct agent/enzyme interactions.

[0245] Thus, the present invention may provide a method to evaluate a agent to decrease the level of functional Aha1 and/or other related molecules with similar functions, and thus to stabilize the folding of proteins with energetically disfavorable folding characteristics in a mammalian host, preferably a human host. This assay may comprise contacting the misfolded protein-expressing transgenic cell line or an extract thereof with a preselected amount of the agent in a suitable culture medium or buffer, and measuring the level of functional Aha1 and/or other related molecules with similar functions, as compared to a control cell line or portion of extract in the absence of said agent and/or a control cell line expressing a non-misfolded variant of the protein of interest. For example, screening methods can identify agents that decrease levels of functional Aha1, decrease intracellular Aha1 binding to Hsp90, decrease activation of Hsp90 ATPase, decrease intracellular levels of Hsp90/ATP, and/or increase intracellular levels of Hsp90/ADP.

[0246] More specifically, a candidate agent for the treatment of a protein misfolding disease can be screened by providing a cell stably expressing a misfolded protein of interest in a suitable culture medium or buffer, administering the candidate agent to the cell, measuring the levels of functional Aha1 in the cell, and determining whether the candidate agent decreases intracellular functional Aha1 level. Alternatively, a candidate agent for the treatment of a protein misfolding disease can be screened by providing a cell stably expressing a misfolded protein of interest in a suitable culture medium or buffer, administering the candidate agent to the cell, measuring the levels of intracellular Aha1 binding to Hsp90 and/or activation of Hsp90 ATPase, and determining whether the candidate agent decreases such binding and/or activation. Desirable candidates will generally possess the ability to decrease the levels of functional Aha1 in the cell. Provision of a cell stably expressing a misfolded protein is within the skill of the art (see e.g., Examples 1-10).

[0247] Any method suitable for detecting levels of functional Aha1 and/or related molecules with similar function, or complexes formed thereto, may be employed for levels resultant from administration of the candidate agent (see e.g., Examples 5-9). Among the traditional methods which may be employed are co-immunoprecipitation, crosslinking, co-purification through gradients or chromatographic columns, and activity assays related to Aha1 function. Utilizing procedures such as these allows for the identification of the proteins

and/or complexes of interest.

[0248] The agents identified in the screen will generally demonstrate the ability to interact with functional Aha1 and/or related molecules with similar function in such a way as to effect a stabilization of proteins with suboptimal folding kinetics so as to result in increased protein transit. For example, identified agents may decrease levels of functional Aha1, decrease intracellular Aha1 binding to Hsp90, decrease activation of Hsp90 ATPase, decrease intracellular levels of Hsp90/ATP, and/or increase intracellular levels of Hsp90/ADP. These agents can include, but are not limited to, nucleic acids, polypeptides, dsRNAs, antisense molecules, aptamers, ribozymes, triple helices, antibodies, and small inorganic molecules.

[0249] Further, the screening methods described above can employ another cell stably expressing a non-misfolded protein variant. By administering the candidate agent, in a substantially similar fashion as to the other cell (expressing a protein with suboptinmal folding kinetics), and measuring the transit level of the non-misfolded protein, one can determine whether the candidate agent substantially decreases the transit level of the non-misfolded protein. Preferably, identified agents do not substantially interfere with folding and/or transit of the non-misfolded protein. Also, a cell stably expressing other proteins can be used similarly to determine whether the agent affects the folding and/or transit of other related or unrelated proteins. For example, an identified agent that decreases levels of functional Aha1 preferably does not significantly impair transit of other proteins with more energetically stable folds.

[0250] The invention also encompasses methods for identifying agents that specifically bind to functional Aha1 and/or other related molecules with similar function. One such method involves the steps of providing immobilized purified functional Aha1 protein and at least one test agent; contacting the immobilized protein with the test agent; washing away agents not bound to the immobilized protein; and detecting whether or not the test agent is bound to the immobilized protein. Those agents remaining bound to the immobilized protein are those that specifically interact with the functional Aha1 protein.

[0251] The present invention also comprises the use of functional Aha1 (and/or other molecules with similar function) in drug discovery efforts to elucidate relationships that exist between functional Aha1 (and/or other molecules with similar function) and a disease state, phenotype, or condition, such as protein misfolding diseases. These methods include detecting or decreasing levels of Aha1 polynucleotides comprising contacting a sample, tissue, cell, or organism with the agents of the present invention, measuring the nucleic acid or protein level of functional Aha1, and/or a related phenotypic or chemical endpoint at some

time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further agent of the invention. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype.

[0252] Therapeutic Treatment

[0253] One aspect of the invention provides methods of treatment for protein folding diseases. Without being bound by a particular theory, it is possible that decreasing functional Aha1 levels, and hence decreasing Hsp90 ATPase activity, may allow additional time for the kinetically challenged ΔF508 mutant to utilize the rescue chaperome to create a more export competent fold. Protein folding can, therefore, be treated in a subject in need thereof by administering an agent that decreases the level of functional Aha1 and/or other related molecules having similar function.

[0254] Further, and again without being bound by a particular theory, it is possible that the Hsp90-ADP-state favors a link of cargo and ERAD pathways, whereas the Hsp90-ATP-state affords coupling to COPII based on the response to functional Aha1 (see e.g., Fig. 8B, X) or p23 (see e.g., Fig. 8B, Y) given their known biochemical properties. Thus, another approach for prophylactic or therapeutic treatment of a protein misfolding disease can involve administering to a subject in need thereof an agent that decreases binding of a functional Aha1 to Hsp90 ATPase and/or decreases resulting activation levels resulting from binding of functional Aha1 to Hsp90 ATPase.

[0255] Preferably, administration of the agent does not substantially interfere with folding and/or transit of other intracellular proteins. For example, administration of an agent that decrease levels of functional Aha1, decreases binding of functional Aha1 to Hsp90 ATPase, and/or decreases resulting activation levels resulting from binding of functional Aha1 to Hsp90 ATPase to treat a protein misfolding disease preferably does not significantly impair transit of other proteins, for example, other proteins with more energetically stable folds.

[0256] Disease states or conditions indicative of a need for therapy in the context of the present invention, and/or amenable to treatment methodologies described herein, include protein misfolding diseases such as CF, marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease, spongiform encephalopathies such as Creutzfeldt-Jakob disease,

primary systemic amyloidosis, secondary systemic amyloidosis, senile systemic amyloidodis, familial amyloid polyneuropathy I, hereditary cerebral amyloid angiopathy, hemodialysis-related amyloidosis, familial amyloid polyneuropathy III, Finnish heriditary systemic amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary non-neuropathic systemic amyloidosis, injection-localized amyloidosis, and heriditary renal amyloidosis. For example, protein misfolding diseases treatable according to methods described herein include those diseases where misfolded proteins result in decreased protein transit from the ER and increased protein degradation, such as CF, marfan syndrome, Fabry disease, Gaucher's disease, and retinitis pigmentosa 3. As another example, protein misfolding diseases treatable according to methods described herein include those diseases where misfolded proteins result in deposition of insoluble aggregates, such as Alzheimer's disease, Type II diabetes, Parkinson's disease, and spogiform encephalopathies (e.g., Creutzfeldt-Jakob disease). The protein misfolding diseases listed above can be caused, at least in part, by misfolded of CFTR, fibrillin, alpha galactosidase, beta glucocerebrosidase, rhodopsin, amyloid beta and tau (islet amyloid polypeptide), amylin, alpha synuclein, prion, immunoglobulin light chain, serum amyloid A, transthyretin, cystatin C, β2-microglobulin, apolipoprotein A-1, gelsolin, calcitonin, atrial natriuretic factor, lysozyme, insulin, and fibrinogen.

[0257] A determination of the need for treatment will typically be assessed by a history and physical exam consistent with the disease. For example, the diagnosis of CF can involve a combination of clinical criteria and analysis of sweat Cl- values. In addition, DNA analysis for ΔF508 can be performed. Such CF diagnosis is within the skill of the art (see e.g., Cutting (2005) Annu Rev Genomics Hum Genet 6, 237-260, reviewing CF). Subjects with an identified need of therapy include those with a diagnosed protein misfolding disease or indication of a protein misfolding disease amenable to therapeutic treatment described herein and subjects who have been treated, are being treated, or will be treated for a protein misfolding disease. The subject is preferably an animal, including, but not limited to, mammals, reptiles, and avians, more preferably horses, cows, dogs, cats, sheep, pigs, and chickens, and most preferably human.

[0258] Another aspect of the invention is directed toward rescuing a cell from the effects of protein misfolding. Such approach is directed to cellular function and can be performed in vitro, in vivo, or ex vivo. As an example, rescue of a cell from the effect of protein misfolding can occur in a cell from a cultured cell line. As another example, rescue of a cell from the effect of protein misfolding can occur in a cell removed from a subject and then subsequently reintroduced to the subject. As a further example, rescue of a cell from

the effect of protein misfolding can occur in a cell of the subject in situ. Administration of an agent that decreases levels of functional Aha1 and/or related molecules with similar function to a cell wherein protein misfolding occurs can facilitate stabilization of energetically unstable folds, resulting in rescue of impaired intracellular and/or extracellular transit of the protein. For example, administration to a cell expressing misfolded ΔF508 CFTR of an agent that reduces levels of the Hsp90 co-chaperone and functional Aha1 can enhance ΔF508 ER stability, rescue ΔF508 trafficking to the cell surface, increase cell surface ΔF508 availability, and/or at least partially restore channel function (see e.g., Example 10). Preferably, administration of an agent to decrease levels of functional Aha1 to rescue a cell from the effects of protein misfolding preferably does not substantially interfere with folding and/or transit of other intracellular proteins.

[0259] Therapeutic Treatment Using dsRNA

[0260] The invention relates in particular to the use of a dsRNA or a pharmaceutical composition prepared therefrom for the treatment of Cystic Fibrosis. Owing to the inhibitory effect on Aha1 expression, an dsRNA according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life of Cystic Fibrosis patients.

[0261] Furthermore, the invention relates to the use of a dsRNA or a pharmaceutical composition of the invention aimed at the treatment of cancer, e.g., for inhibiting tumor growth and tumor metastasis. For example, the dsRNA or a pharmaceutical composition prepared therefrom may be used for the treatment of solid tumors, like breast cancer, lung cancer, head and neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma and for the treatment of skin cancer, like melanoma, for the treatment of lymphomas and blood cancer. The invention further relates to the use of an dsRNA according to the invention or a pharmaceutical composition prepared therefrom for inhibiting Aha1 expression and/or for inhibiting accumulation of ascites fluid and pleural effusion in different types of cancer, e.g., breast cancer, lung cancer, head cancer, neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma, skin cancer, melanoma, lymphomas and blood cancer. Owing to the inhibitory effect on Aha1 expression, an dsRNA according to the

invention or a pharmaceutical composition prepared therefrom can enhance the quality of life of cancer patients.

[0262] The invention furthermore relates to the use of an dsRNA or a pharmaceutical composition thereof, e.g., for treating Cystic Fibrosis or cancer or for preventing tumor metastasis, in combination with other pharmaceuticals and/or other therapeutic methods, e.g., with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating Cystic Fibrosis or cancer and/or for preventing tumor metastasis. Where the pharmaceutical composition aims for the treatment of Cystic fibrosis, preference is given to a combination with daily chest physiotherapy, orally applied pancreatic enzymes, daily oral or inhaled antibiotics to counter lung infection, inhaled anti-asthma therapy, corticosteroid tablets, dietary vitamin supplements, especially A and D, inhalation of Pulmozyme™, medicines to relieve constipation or to improve the activity of the enzyme supplements, insulin for CF-related diabetes, medication for CF-associated liver disease, and oxygen to help with breathing.

[0263] Where the pharmaceutical composition aims for the treatment of cancer and/or for preventing tumor metastasis, preference is given to a combination with radiation therapy and chemotherapeutic agents, such as cisplatin, cyclophosphamide, 5-fluorouracil, adriamycin, daunorubicin or tamoxifen.

[0264] The invention can also be practiced by including with a specific RNAi agent another anti-cancer chemotherapeutic agent, such as any conventional chemotherapeutic agent. The combination of a specific binding agent with such other agents can potentiate the chemotherapeutic protocol. Numerous chemotherapeutic protocols will present themselves in the mind of the skilled practitioner as being capable of incorporation into the method of the invention. Any chemotherapeutic agent can be used, including alkylating agents, antimetabolites, hormones and antagonists, radioisotopes, as well as natural products. For example, the compound of the invention can be administered with antibiotics such as doxorubicin and other anthracycline analogs, nitrogen mustards such as cyclophosphamide, pyrimidine analogs such as 5-fluorouracil, cisplatin, hydroxyurea, taxol and its natural and synthetic derivatives, and the like. As another example, in the case of mixed tumors, such as adenocarcinoma of the breast, where the tumors include gonadotropin-dependent and gonadotropin-independent cells, the compound can be administered in conjunction with leuprolide or goserelin (synthetic peptide analogs of LH-RH). Other antineoplastic protocols include the use of a tetracycline compound with another treatment modality, e.g., surgery, radiation, etc., also referred to herein as "adjunct antineoplastic modalities." Thus, the method of the invention can be employed with such conventional regimens with the benefit

of reducing side effects and enhancing efficacy.

[0265] Methods for inhibiting expression of an Aha gene

[0266] In yet another aspect, the invention provides a method for inhibiting the expression of an Aha gene in a mammal. The method comprises administering a composition of the invention to the mammal such that expression of the target Aha gene, e.g. Aha1, is silenced. Because of their high specificity, the dsRNAs of the invention specifically target RNAs (primary or processed) of the target Aha gene. Compositions and methods for inhibiting the expression of these Aha genes using dsRNAs can be performed as described elsewhere herein.

[0267] In one embodiment, the method comprises administering a composition comprising a dsRNA, wherein the dsRNA comprises a nucleotide sequence which is complementary to at least a part of an RNA transcript of an Aha gene, e.g. Aha1, of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, vaginal and topical (including buccal and sublingual) administration. In preferred embodiments, the compositions are administered by intravenous infusion or injection.

[0268] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0269] EXAMPLES

[0270] Aspects of the present teachings may be further understood in light of the following examples, which should not be construed as limiting the scope of the present teachings in any way.

[0271] Example 1: CFTR Interactome

[0272] To define global protein interactions involved in CFTR trafficking and

function in the exocytic and endocytic pathways, CFTR-containing protein complexes were immunoisolated from cell lines expressing wild-type CFTR (see e.g., Fig. 1), protease digested, and the composition of the peptide mixture determined using multidimensional protein identification technology (MudPIT) (Lin et al., Biochim Biophys Acta 1646, 1 (2003)).

[0273] CFTR was immunoprecipitated from stable BHK cell lines over-expressing either wild-type or ΔF508 CFTR, or the Calu-3, HT29 and T84 cell lines expressing wild-type CFTR. Baby Hamster Kidney (BHK) cells stably expressing wt or ΔF508 CFTR were maintained in DMEM supplemented with F12, 5% fetal bovine serum (FBS), 100 units/ml each of penicillin and streptomycin (Pen/Strep), and 500 μM methotrexate (Xanodyne Pharmacal, Inc., Florence, KY). Parental BHK cells not expressing CFTR were cultured in the same medium except without methotrexate. Human lung cell line Calu-3, and human intestinal cell lines HT29 and T84, all expressing endogenous wt CFTR were purchased from ATCC and maintained according to manufacturer's instructions.

[0274] CFTR and co-immunoprecipitating proteins in whole cell detergent lysates were bound to Sepharose beads coupled with the anti-CFTR monoclonal antibody M3A7. To capture transient or weak interactions that occur as CFTR transits through different subcellular compartments, immunoprecipitations were carried out in the absence or presence of the cleavable chemical cross-linker dithiobissuccinimidylpropionate (DSP) that was added to intact cells prior to cell lysis. To control for non-specific binding of proteins to beads, several conditions were used to indicate background recoveries including incubation of cell lysates in the presence beads alone, or beads coupled to a monoclonal antibody directed against the VSV-G, a protein only found in cells infected with vesicular stomatitis virus (Calu-3, T84 and H89 datasets). In the case of BHK cells, immunoprecipitates from wild-type and ΔF508 expressing stable cell lines was compared directly to the parent cell line not expressing CFTR. In the datasets provided in the Excel files, recovered proteins from both non-cross-linked and cross-linked methods are pooled.

[0275] Following immunoprecipitation, protein complexes were digested by denaturing the proteins in freshly prepared 8 M guanidine HCl followed by dilution to 2 M. Endoproteinase LysC was used to digest the proteins for 8 hours, followed by dilution to 1 M guanidine HCl and trypsin digestion using PorozymeTM trypsin beads. All digestions are performed at 37°C.

[0276] Protease digested immune complexes were subjected to LC/LC/MS/MS analysis using MudPIT. This approach has been described in detail by several authors (Link et al., 1999 Nat Biotechnol 17, 676-682; MacCoss et al., 2002, Proc Natl Acad Sci U S A 99, 7900-7905; MacCoss et al., 2002, Anal Chem 74, 5593-5599; McDonald et al., 2002 Int J

Mass Spect 219, 245-251; Washburn et al., 2001 Nat Biotechnol 19, 242-247). Briefly, the denatured, reduced and alkylated proteins were split into three fractions and digested over night at 37°C with three different proteases (trypsin, subtylisin and elastase). The resulting peptide mixture was acidified with formic acid (5%). Subsequently, a three phase microcapillary column was constructed by slurry packing ~7 cm of 5-µm Aqua C18 material (Aqua, Phenomimex) into a 100 µm fused silica capillary, which had been previously pulled to a tip diameter of ~ 5 µm using a Sutter Instruments laser puller (Sutter Manufacturing, Novato, CA). Next, 3 cm of 5-µm Partisphere strong cation exchange resin (Partisphere, Whatman) followed by another 3 cm of 5-µm Aqua C18 chromatography material was packed into the column. The column was then equilibrated with 5% acetonitrile / 0.1% formic acid for ~30 min before the peptide mixture was loaded onto the back-end of a triphasic chromatography column using a high pressure cell.

[0277] After loading the peptide digests, the column was placed inline with an Agilent 1100 quaternary HPLC and analyzed using a modified 6-step separation. The buffer solutions were 5% acetonitrile/0.1% formic acid (buffe] A), 80% acetonitrile/0.1% formic acid (buffer B), and 500 mM ammonium acetate/5% acetonitrile/0.1% formic acid (buffer C). Step I consisted of a 100 min gradient from 0-100% buffer B. Steps 2-5 had the following profile: 3 min of 100% buffer A, 2 min of X% buffer C, a 10 min gradient from 0-15% buffer B, and a 97 min gradient from 15-45% buffer B. The 2 min buffer C percentages (X) were 10, 20, 30, 40% respectively for the 6-step analysis. In the final step, the gradient contained: 3 min of 100% buffer A, 20 min of 100% buffer C, a 10 min gradient from 0-15% buffer B, and a 107 min gradient from 15-70% buffer B.

[0278] As peptides eluted from the microcapillary column, they were electrosprayed directly into an LCQ-Deca mass spectrometer with the application of a distal 2.4 kV spray voltage. A cycle of one full-scan mass spectrum (400-1400 m/z) followed by 3 data-dependent MS/MS spectra at a 35% normalized collision energy was repeated continuously throughout each step of the multidimensional separation. Application of mass spectrometer scan functions and HPLC solvent gradients are controlled by the Xcalibur data system.

[0279] Tandem mass spectra were analyzed sequentially using the following protocol. First, a software algorithm (2to3) was used to determine the appropriate charge state (either +2 or +3) from multiple charged peptide mass spectra, and delete spectra of poor quality (Sadygov et al., 2002, J Proteome Res 1, 211-215). The MS/MS spectra after 2to3 was searched using a parallel virtual machine (PVM) version of SEQUEST™ (Yates et al., 1995, Anal Chem 67, 3202-3210; Yates et al., 1995, Anal Chem 67, 1426-1436) running

on a Beowolf computer cluster (~75 cpu's) against a protein database constructed from the combined human, mouse and rat databases (HMR) from Refseq. Database search results were filtered, sorted, and displayed using the DTASelect program (Tabb et al., 2002, J Proteome Res 1, 21-26). Default DTASelect criteria were employed (i.e., +1 1.8, +2 2.5, +3 3.5, ΔCN 0.08, and at least two peptides per locus).

[0280] The protein components identified from replicate immuonprecipitations were merged and the resulting dataset was then annotated with the GO_Annotation using the EASE analysis program supplied by the NIH (Hosack et al., 2003, Genome Biol *4*, R70). From the control-subtracted proteome, proteins which were annotated by GO_Molecular_Function as belonging to the nucleic acid binding, or structural categories which are common contaminants from whole cell proteomic experiments, were eliminated. The resulting list of proteins primarily included cytoplasmic chaperones, endoplasmic reticulum (ER) lumenal proteins, late secretory pathway components, cell surface interactors, and proteosome/ubiquitination components.

[0281] Fig. 1 is a cartoon depicting components comprising the CFTR interactome (light ovals, previously established interactions; dark ovals, new interactions recovered in the current study) as nodes in the network and are divided into subnetworks that potentially facilitate protein folding in the ER (I), ERAD (II), membrane trafficking (III), and post-ER regulators and effectors (IV). Dark lines are edges in the network that show direct or indirect protein interactions between CFTR and the indicated component identified by MudPIT. Light gray lines illustrate edges that define interactions based on the Tmm co-expression database, accessed using the Cytoscape platform. Table 9 shows the results of an array conducted on proteins recovered using multidimensional protein identification technology (MudPIT) in the indicated cell types expressing wild-type CFTR, arranged in the order of fractional sequence coverage by mass spectrometry.

[0282] Results showed that the identified protein generate a network of protein interactions defining the CFTR proteome or interactome (see e.g., Fig. 1). Proteins comprising the CFTR interactome can be divided into subnetworks that collectively define functional groups that include components required for folding and export from the ER (see e.g., Fig. 1-I), that mediate ERAD (see e.g., Fig. 1-II), that direct transport between the exocytic and endocytic compartments (see e.g., Fig. 1-III), and components that are potential binding partners involved in CFTR function and regulation at the cell surface (see e.g., Fig. 1B-IV).

[0283] A number of protein interactions found for mature wild-type CFTR found at the cell surface validate the database (see e.g., Table 10). For example, CFTR is a gated

chloride channel whose activity is regulated by cAMP-dependent protein kinases and protein phosphatases (Guggino and Banks-Schlegel, 2004). Protein phosphatase 2A (PP2A) or PP2C have a role in CFTR dephosphorylation and down-regulation of CFTR activity in a variety of cell types. Although kinases were not detected as a stable interacting partners in any cell line examined, presumably because of their very transient interaction, wild-type CFTR in nearly all cell lines showed strong interaction with PP2A- both the regulatory and catalytic subunits (Thelin et al., 2005; Vastiau et al., 2005). In addition to PP2A, sodium-hydrogen exchanger (NHE) isoform 3 regulators 1 and 3 (NHERF-1/3) (Mohler et al., 1999; Yun et al., 1997) were recovered in the CFTR proteome. NHERFs are localized to the apical surface of lung cells and are well-documented to interact with CFTR through the C-terminal PDZ domains (Guggino and Banks-Schlegel, 2004, Am J Respir Crit Care Med 170, 815-820). A previous unknown interactor includes calgranulin B (S 100-A8), a member of the divergent S 100 family of EF-hand-containing cytosolic Ca" binding proteins (Donato, 2003, Microsc Res Tech 60, 540-551; Heizmann, 2002, Methods Mol Biol 172, 69-80). Calgranulin B has been implicated in CF inflammatory pathways (Fanjul et al., 1995, Am J Physiol 268, C1241-1251; Renaud et al., 1994, Biochem Biophys Res Commun 201, 1518-1525; Xu et al., 2003, J Biol Chem 278, 7674-7682), suggesting a possible modulatory role related to CFTR lung pathophysiology.

[0284] Results also showed that, generally, the identification of multiple endocytic trafficking components illustrate the importance of CFTR internalization and recycling in normal function. A second group of components highlights direct or indirect interactions of wild-type CFTR with the membrane trafficking machinery (see e.g., Table 8). These include sortilinrelated receptor L (SORL1), disabled homolog 2 (Dab2), RalBP1 associated Eps domain containing protein (Reps 1), ARF4, clathrin light chain, vacuolar sorting protein 4 (Vps4p), enthoprotin, and sorting nexins (SNX) 4 and 9. SORL1 has a single transmembrane domain, is localized to recycling endosomes and involved in internalization of multiple ligands (Jacobsen et al., 2001, J Biol Chem 276, 22788-22796). Dab2 functions as a cargo-selective endocytic clathrin adaptor (Bonifacino and Traub, 2003, Annu Rev Biochem 72, 395-447; Mishra et al., 2002, Embo J 21, 4915-4926), whereas Repsl is able to bind to proteins containing the NPF internalization motif found in CFTR (Yamaguchi et al., 1997, J Biol Chem 272, 31230-31234) and couple to the Rab11-FIP2 family of endocytic GTPase regulators (Bilan et al., 2004, J Cell Sci 117, 1923-1935; Cullis et al., 2002, J Biol Chem 277, 49158-49166; Gentzsch et al., 2004, Mol Biol Cell 15, 2684-2696; Swiatecka-Urban et al., 2005, J Biol Chem 280, 36762-36772) by a cargo selection machinery containing AP2, Dab2. ARF4 is a small GTPase implicated in endocytic/recycling

compartments (Donaldson and Honda, 2005, Biochem Soc Trans 33, 639-642; Langhorst et al., 2005, Cell Mol Life Sci 62, 2228-2240; Morrow and Parton, 2005, Traffic 6, 725-740), whereas VPS4 likely functions in the transport of proteins from late endosomal compartments to the lysosome (Bowers et al., 2004, Traffic 5, 194-210; Hislop et al., 2004, J Biol Chem 279, 22522-22531; Scheuring et al., 2001, J Mol Biol 312, 469-480; Scott et al., 2005, Embo J 24, 3658-3669). Enthoprotin interacts with clathrin adaptor API, with the Golgi-localized γ-ear containing, ARF-binding protein 2 (McPherson and Ritter, 2005, Mol Neurobiol 32, 73-87; Wasiak et al., 2003, FEBS Lett 555, 437-442; Wasiak et al., 2002, J Cell Biol 158, 855-862), and through its carboxyl terminal domain, to the terminal domain of clathrin heavy chain to stimulate the formation of clathrin-coated vesicle (Kalthoff et al., 2002, Mol Biol Cell 13, 4060-4073; Wasiak et al., 2003, supra; Wasiak et al., 2002, supra), consistent with the recovery of the clathrin light chain (Ybe et al., 2003, Traffic 4, 850-856) and the established role for clathrin in CFTR recycling (Cheng et al., 2004, J Biol Chem 279, 1892-1898; Hu et al., 2001, Biochem J 354, 561-572; Lukacs et al., 1997, Biochem J 328 (Pt 2), 353-361; Peter et al., 2002, J Biol Chem 277, 49952-49957; Picciano et al., 2003, Am J Physiol Cell Physiol 285, C1009-1018; Weixel and Bradbury, 2000, J Biol Chem 275, 3655-3660; Weixel and Bradbury, 2001, Pflugers Arch 443 Suppl 1, S70-74; Weixel and Bradbury, 2001, J Biol Chem 276, 46251-46259). Additional adaptors identified in the interactome include Snx4 and Snx9 (Carlton et al., 2005, Traffic 6, 75-82; Lundmark and Carlsson, 2003, J Biol Chem 278, 46772-46781; Wasiak et al., 2003, J Cell Biol 158, 855-862). Snx9 binds the β-appendage domain of AP2 and assists AP2 in its function at the plasma membrane in clathrin and dynamin mediated internalization (Lin et al., 2002, supra; Lundmark and Carlsson, 2003, supra; Lundmark and Carlsson, 2004, J Biol Chem 279, 42694-42702; Lundmark and Carlsson, 2005, Methods Enzymol 404, 545-556; Soulet et al., 2005, Mol Biol Cell 16, 2058-2067; Teasdale et al., 2001, Biochem J 358, 7-16). Snx4 has been reported to interact with amphiphysin to facilitate endocytic trafficking of transferrin and other recycling components (Hettema et al., 2003, Embo J 22, 548-557; Leprince et al., 2003, J Cell Sci 116, 1937-1948).

[0285] While the above results focus on effector and trafficking components, both wild-type and ΔF508-CFTR are degraded by ERAD pathways that involve both ubiquitin and proteasome components (Amaral, 2004, J Mol Neurosci 23, 41-48) (see e.g., Table 10). The proteome from both wild-type and ΔF508 CFTR expressing cells contain components involved in ERAD. These include the translocation/dislocation Sec61 channel and VCP/p97/Cdc48, a chaperone directing delivery to the proteasome. The role of the proteasome is indicated by the enrichment in 26S proteasome subunits and components of

the ubiquitination pathway in the interactome (see e.g., Table 10). Interestingly, the ubiquitinating-conjugating protein E3A recovered in the proteome shows interaction with Ubc6, a class of E2 ubiquitin-conjugating enzymes frequently invoked for ERAD, including CFTR (Lenk et al., 2002, J Cell Sci 115, 3007-3014).

[0286] Whether these ligases are only involved in ERAD at the level of the ER or also participate in down-regulation of CFTR at the cell surface through endocytic/lysosomal targeting pathways remains to be determined (Gentzsch et al., 2004, Mol Biol Cell; Sharma et al., 2004, J Cell Biol 164, 923-933; Swiatecka-Urban et al., 2005, supra). In addition to those examples discussed above, additional components are predicted to have direct or indirect interactions with CFTR (see e.g., Tables 9 and 10).

[0287] The above is a systems biology approach aided by the sensitivity of the MudPIT proteomics technology taken to identify transient interactions that contribute to CFTR folding and trafficking pathways, the CFTR interactome. While some proteins that have been shown to interact with CFTR in post-ER compartments were not identified, this could reflect limitations of the mass spectometry technique, the immunoprecipitation conditions optimized for consistency within the study, and the fact that the interactome is likely composed of very dynamic, and therefore, transient interactions that are difficult to capture and highly depended on cell type and growth conditions. The interactome encompassing all known interactions (see e.g., Fig. 1) can provide a new baseline to begin to assess the many different protein complexes necessary for CFTR to achieve and maintain functionally at the apical cell surface.

Table 7. Post-ER CFTR interacting proteins of known function *

Reference Sequence accession code	Protein name	%cov.	unique	total
Cell surface transporters and regulators				
NM_004252	NHERF-1**	20%	5	6
NM_004785	NHERF-2***	14%	4	5
NM_001285	CLCA1	2%	2	2
Components of post-ER trafficking machinery				
NM_003105	SORL1	1%	1	12
NM_016760	clathrin, light chain	13%	3	11
NM_003794	sorting nexin 4	3%	3	5
NM_014666	enthoprotin	9%	4	5
NM_007479	ARF4	16%	2	4
NM_023118	Dab2	5%	3	3
NM_016451	βCOP	5%	2	2
NM_013245	VPS 4A	5%	2	2
NM_009503	VCP	7%	2	2
NM_009048	Reps1	6%	2	2
NM_016224	sorting nexin 9	5%	2	2
NM_008028	flotillin 2	5%	2	2

* Shown are the percentage sequence coverage (% coverage), number of unique peptide spectra (unique), and number of total peptide spectra (total) as identified by mass spectrometry.

** Shown are the peptide data for BHK cells. Those for HT29 are 11%, 2, 2.

*** Shown are peptide data for Calu-3 cells. Those for HT29 are 20%, 7, 10.

Table 8. Degradation proteome associated with CFTR

Reference Sequence accesssion code	Protein name	ΔF508			WT#		
		A*	B*	C*	A*	B*	C*
NM_017314	ubiquitin C	6%	5	117	5%	3	36
NM_011664	ubiquitin B	16%	5	52	12%	3	16
NM_007126	VCP/p97/Cdc48	25%	10	14	7%	2	2
NM_002808	proteasome 26S, non-ATPase, 2	13%	7	8	4%	2	2
NM_008944	proteasome, alpha type 2	17%	2	5			
NM_002795	proteasome, beta type, 3	24%	3	3			
NM_011185	proteasome, beta type 1	23%	3	3			
NM_011967	proteasome, alpha type 5	23%	3	3			
NM_006503	proteasome 26S, ATPase, 4	12%	2	3			
NM_008948	proteasome 26S, ATPase 3	8%	2	3			
NM_002796	proteasome, beta type, 4	16%	2	2			
NM_002815	proteasome 26S, non-ATPase, 11	5%	2	2			
NM_013336	Sec61 alpha subunit isoform 1				11%	4	7
NM_004652	ubiquitin specific protease 9				1%	2	3
NM_000462	ubiquitin protein ligase E3A				6%	2	2
NM_004238	THR interactor 12				2%	2	2
NM_018144	Sec61, alpha subunit 2				11%	2	2

Proteins recovered from BHK(wild-type CFTR), Calu-3, HT29, T84 cell lines

* Shown are the percentage sequence coverage (A), number of unique peptide spectra (B), and number of total peptide spectra (C) as identified by mass spectrometry.

Table 9: CFTR proteome for cell lines expressing wild-type CFTR

			Sequence Coverage (%)			
Protein#	RefSeq AC	gene name	BHK wt	Calu-3	HT29	T84
1	NM_000492	cystic fibrosis transmembrane conductance regulator	0.599	0.526	0.455	0.407
2	NM_008379	karyopherin (importin) beta 1	0.579	0.287	0.068	0.075
3	NM_009037	reticulocalbin	0.495			
4	NM_006597	Hsc70	0.466		0.375	0.354
5	NM_001539	Hsp40-A1 (Hdj2)	0.403	0.081	0.113	
6	NM_006391	importin 7	0.334	0.078	0.046	
7	NM_022310	GRP78	0.328	0.188	0.137	
8	NM_005507	cofilin 1 (non-muscle)	0.307			
9	NM_005880	Hsp40-A2 (Hdj3)	0.282		0.133	
10	NM_002715	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	0.275	0.11		
11	NM_001316	CSE1 chromosome segregation 1-like (yeast)	0.255	0.143	0.048	
12	NM_002717	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	0.248			
14	NM_011992	reticulocalbin 2	0.221			
15	NM_002901	reticulocalbin 1, EF-hand calcium binding domain	0.215	0.145		
16	NM_008302	Hsp90 beta	0.214	0.171	0.076	
17	NM_012030	NHERF-1	0.197		0.112	
18	NM_006098	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1	0.189			
19	NM_002902	reticulocalbin 2, EF-hand calcium binding domain	0.183	0.186	0.123	
20	NM_011313	S100 calcium binding protein A6 (calcyclin)	0.18			
22	NM_018243	hypothetical protein FLJ10849	0.175			
23	NM_009906	ceroid-lipofuscinosis, neuronal 2	0.16			
24	NM_002882	RAN binding protein 1	0.159			
25	NM_007479	ADP-ribosylation factor 4	0.156			
26	NM_003400	exportin 1 (CRM1 homolog, yeast)	0.154	0.093	0.053	0.142
27	NM_002716	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	0.151			
28	NM_001681	SERCA2	0.15			0.092

29	NM_021594	ERM-binding phosphoprotein	0.149			
30	NM_005998	chaperonin containing TCP1, subunit 3 (gamma)	0.14			
31	NM_007637	chaperonin subunit 5 (epsilon)	0.131			
32	NM_011664	ubiquitin B	0.121	0.072		
33	NM_021671	db83	0.117			
34	NM_013336	protein transport protein SEC61 alpha subunit isoform 1	0.111		0.113	0.099
35	NM_028152	MMS19 (MET18 S. cerevisiae)-like	0.11			
36	NM_004282	BAG-2	0.104			
37	NM_001746	calnexin	0.096			0.095
38	NM_012470	transportin-SR	0.094			
39	NM_025291	steroid receptor RNA activator 1	0.091			
40	NM_013686	TCP1	0.09			
41	NM_005345	Hsp70-1A	0.083		0.315	0.083
42	NM_020645	chromosome 11 open reading frame 14	0.083			
43	NM_018307	ras homolog gene family, member T1	0.081			
44	NM_021979	Hsp70-2	0.078	0.128	0.102	0.078
45	NM_001219	calumenin	0.07	0.111		
46	NM_006430	chaperonin containing TCP1, subunit 4 (delta)	0.067			
47	NM_006310	aminopeptidase puromycin sensitive	0.066			
48	NM_006325	RAN, member RAS oncogene family	0.065			
50	NM_005348	Hsp90, alpha	0.061			
51	NM_007995	ficolin A	0.057			
52	NM_019685	RuvB-like protein 1	0.057			
53	NM_004461	phenylalanine-tRNA synthetase-like	0.055			
54	NM_000917	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	0.054			
55	NM_019942	septin 6	0.049			
56	NM_017314	ubiquitin C	0.046	0.027		
57	NM_004522	kinesin family member 5C	0.04			
58	NM_007508	ATPase, H+ transporting, V1 subunit A, isoform 1	0.039			
59	NM_030706	tripartite motif protein 2	0.039			
60	NM_009955	dihydropyrimidinase-like 2	0.037			
61	NM_032069	Glutamate receptor interacting protein	0.036			
62	NM_002155	Hsp70B'	0.034	0.061	0.107	0.034

63	NM_003794	sorting nexin 4	0.033		0.102	
64	NM_033309	hypothetical protein MGC4655	0.032	0.032		
65	NM_016338	importin 11	0.032			
66	NM_004521	kinesin family member 5B	0.028			
67	NM_007054	kinesin family member 3A	0.027			
68	NM_008633	microtubule-associated protein 4	0.025			
69	NM_023115	protocadherin 15	0.024			
70	NM_016448	RA-regulated nuclear matrix-associated protein	0.015			
71	NM_000038	adenomatosis polyposis coli	0.014			
72	NM_004624	vasoactive intestinal peptide receptor 1	0.014			
73	NM_004652	ubiquitin specific protease 9, X chromosome (fat facets-like Drosophila)	0.014			
74	NM_022954	MEGF1	0.012			
75	NM_000296	polycystic kidney disease 1 (autosomal dominant)	0.009			
76	NM_000100	cystatin B (stefin B)		0.337		
77	NM_007108	transcription elongation factor B (SIII), polypeptide 2 (18kDa, elongin B)		0.314	0.398	0.314
78	NM_002965	S100 calcium binding protein A9 (calgranulin B)		0.246		
79	NM_008143	guanine nucleotide binding protein, beta 2, related sequence 1		0.243		
80	NM_007355	heat shock 90kDa protein 1, beta		0.229		
81	NM_002818	proteasome (prosome, macropain) activator subunit 2 (PA28 beta)		0.226		
82	NM_002306	lectin, galactoside-binding, soluble, 3 (galectin 3)		0.212		
83	NM_017147	cofilin 1		0.205		
84	NM_002963	S100 calcium binding protein A7 (psoriasin 1)		0.198	0.198	
85	NM_006070	TRK-fused gene		0.195		
86	NM_016647	mesenchymal stem cell protein DSCD75		0.178	0.178	
87	NM_021199	sulfide quinone reductase-like (yeast)		0.151	0.073	0.151
88	NM_004785	NHERF-2		0.139	0.198	
89	NM_002156	heat shock 60kDa protein 1 (chaperonin)		0.133		
90	NM_005527	heat shock 70kDa protein 1-like		0.131	0.109	
91	NM_014225	protein phosphatase 2 (formerly 2A), regulatory		0.126		

		subunit A (PR 65), alpha isoform				
92	NM_012111	Aha1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)	0.34	0.121		
93	NM_006415	serine palmitoyltransferase, long chain base subunit 1		0.116		
95	NM_004208	programmed cell death 8 (apoptosis-inducing factor)		0.093		
96	NM_022934	DnaJ-like protein		0.081	0.081	
97	NM_021863	testis-specific heat shock protein-related gene hst70		0.062	0.103	0.079
98	NM_019390	lamin A		0.058	0.162	
99	NM_000462	ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)		0.055		
100	NM_002808	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2		0.04		
101	NM_024334	hypothetical protein MGC3222		0.022		
102	NM_004327	breakpoint cluster region		0.017		
103	NM_001035	ryanodine receptor 2 (cardiac)		0.006		
104	NM_005648	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C)			0.357	
106	NM_005389	protein-L-isoaspartate (D-aspartate) O-methyltransferase			0.33	0.617
107	NM_008786	protein-L-isoaspartate (D-aspartate) O-methyltransferase 1			0.291	
108	NM_013232	programmed cell death 6			0.215	
109	NM_010481	GRP75			0.189	0.262
110	NM_000117	emerin (Emery-Dreifuss muscular dystrophy)			0.181	
112	NM_018144	likely ortholog of mouse SEC61, alpha subunit 2 (S. cerevisiae)			0.113	
114	NM_009795	calpain, small subunit 1			0.108	0.134
115	NM_007126	valosin-containing protein			0.096	
116	NM_030971	similar to rat tricarboxylate carrier-like protein			0.093	
117	NM_022314	tropomyosin 3, gamma			0.085	0.349
118	NM_006149	lectin, galactoside-binding, soluble, 4 (galectin 4)			0.056	0.053
119	NM_014612	chromosome 9 open reading frame 10			0.051	

120	NM_016451	coatomer protein complex, subunit beta	0.051	
121	NM_005358	LIM domain only 7	0.05	
122	NM_013245	vacuolar protein sorting 4A (yeast)	0.046	
123	NM_016739	GPI-anchored membrane protein 1	0.046	
124	NM_007245	ataxin 2 related protein	0.044	
125	NM_004238	thyroid hormone receptor interactor 12	0.015	
126	NM_006904	protein kinase, DNA-activated, catalytic polypeptide	0.014	0.013
127	NM_031819	FAT tumor suppressor (Drosophila) homolog	0.004	
128	NM_001540	heat shock 27kDa protein 1		0.346
129	NM_013474	apolipoprotein A-II		0.324
130	NM_000611	CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, EJ16, EJ30, EL32 and G344)		0.297
131	NM_031469	SH3 domain binding glutamic acid-rich protein like 2		0.29
132	NM_023009	MARCKS-like protein		0.251
133	NM_018362	lin-7 homolog C (C. elegans)		0.228
134	NM_006118	HS1 binding protein		0.201
135	NM_014666	enthoprotin		0.174
136	NM_023945	membrane-spanning 4-domains, subfamily A, member 5		0.17
137	NM_002067	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)		0.162
138	NM_001833	clathrin, light polypeptide (Lca)		0.138
139	NM_002354	tumor-associated calcium signal transducer 1		0.131
140	NM_018188	hypothetical protein FLJ10709		0.128
141	NM_017724	leucine rich repeat (in FLII) interacting protein 2		0.118
142	NM_016963	tropomodulin 3		0.116
143	NM_031033	guanine nucleotide-binding protein alpha 11 subunit		0.111
144	NM_004447	epidermal growth factor receptor pathway substrate 8		0.101

145	NM_002070	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	0.082
146	NM_001835	clathrin, heavy polypeptide-like 1	0.077
147	NM_002087	granulin	0.074
148	NM_019653	WD-40-repeat-containing protein with a SOCS box 1	0.074
149	NM_009386	tight junction protein 1	0.073
150	NM_002778	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy)	0.071
151	NM_004360	cadherin 1, type 1, E-cadherin (epithelial)	0.068
152	NM_031922	Reps1	0.062
153	NM_014935	phosphoinositol 3-phosphate-binding protein-3	0.06
154	NM_022098	hypothetical protein LOC63929	0.055
155	NM_001343	Dab2	0.053
156	NM_004475	flotillin 2	0.05
157	NM_016224	sorting nexin 9	0.05
158	NM_014271	interleukin 1 receptor accessory protein-like 1	0.049
159	NM_014812	KARP-1-binding protein	0.049
160	NM_002958	RYK receptor-like tyrosine kinase	0.048
161	NM_033299	phospholipase D gene 2	0.045
162	NM_023063	epithelial protein lost in neoplasm	0.044
163	NM_014428	tight junction protein 3 (zona occludens 3)	0.039
164	NM_031382	testis expressed gene 16	0.037
165	NM_033049	mucin 13, epithelial transmembrane	0.037
166	NM_016745	ATPase, Ca++ transporting, ubiquitous	0.032
167	NM_031823	Wolfram syndrome 1	0.027
168	NM_001115	adenylate cyclase 8 (brain)	0.024
169	NM_007454	AP-1, beta 1 subunit	0.024
170	NM_001285	CLCA1	0.023
171	NM_003253	T-cell lymphoma invasion and metastasis 1	0.023
172	NM_003174	supervillin	0.019
173	NM_015756	shroom	0.018
174	NM_003105	SORL1	0.01

Table 10: Comparison of wild-type and ΔF508 CFTR BHK proteome

Protein #	Refseq AC	gene name	Sequence Coverage (%) BHK D508	BHK wt
1	NM_000492	cystic fibrosis transmembrane conductance regulator	0.569	0.599
2	NM_008379	karyopherin (importin) beta 1	0.209	0.579
3	NM_009037	reticulocalbin	0.274	0.495
4	NM_006597	Hsc70	0.582	0.466
5	NM_001539	Hsp40-A1 (Hdj2)	0.307	0.403
6	NM_006391	importin 7	0.043	0.334
7	NM_022310	GRP78	0.397	0.328
8	NM_005507	cofilin 1 (non-muscle)	0.337	0.307
9	NM_005880	Hsp40-A2 (Hdj3)	0.318	0.282
10	NM_002715	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	0.084	0.275
11	NM_001316	CSE1 chromosome segregation 1-like (yeast)	0.045	0.255
12	NM_002717	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	0.235	0.248
13	NM_023565	chromosome segregation 1-like (S. cerevisiae)	0.045	0.238
14	NM_011992	reticulocalbin 2	0.087	0.221
15	NM_002901	reticulocalbin 1, EF-hand calcium binding domain	0.224	0.215
16	NM_008302	Hsp90, beta	0.358	0.214
17	NM_012030	NHERF-1	0.152	0.197
18	NM_006098	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1		0.189
19	NM_002902	reticulocalbin 2, EF-hand calcium binding domain		0.183
20	NM_011313	S100 calcium binding protein A6 (calcyclin)		0.18
22	NM_018243	hypothetical protein FLJ10849	0.184	0.175
23	NM_009906	ceroid-lipofuscinosis, neuronal 2		0.16
24	NM_002882	RAN binding protein 1		0.159
25	NM_007479	ADP-ribosylation factor 4		0.156
26	NM_003400	exportin 1 (CRM1 homolog, yeast)		0.154
27	NM_002716	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	0.201	0.151
28	NM_001681	SERCA2	0.085	0.15
29	NM_021594	ERM-binding phosphoprotein	0.104	0.149
30	NM_005998	chaperonin containing TCP1, subunit 3 (gamma)		0.14
31	NM_007637	chaperonin subunit 5 (epsilon)		0.131
32	NM_011664	ubiquitin B	0.161	0.121

33	NM_021671	db83		0.117
34	NM_013336	protein transport protein SEC61 alpha subunit isoform 1		0.111
35	NM_028152	MMS19 (MET18 S. cerevisiae)-like		0.11
36	NM_004282	BAG-2	0.28	0.104
37	NM_001746	calnexin	0.164	0.096
38	NM_012470	transportin-SR		0.094
39	NM_025291	steroid receptor RNA activator 1		0.091
40	NM_013686	TCP1	0.095	0.09
41	NM_005345	Hsp70-1A	0.193	0.083
42	NM_020645	chromosome 11 open reading frame 14		0.083
43	NM_018307	ras homolog gene family, member T1		0.081
44	NM_021979	Hsp70-2	0.156	0.078
45	NM_001219	calumenin		0.07
46	NM_006430	chaperonin containing TCP1, subunit 4 (delta)		0.067
47	NM_006310	aminopeptidase puromycin sensitive		0.066
48	NM_006325	RAN, member RAS oncogene family		0.065
50	NM_005348	Hsp90, alpha	0.392	0.061
51	NM_007995	ficolin A		0.057
52	NM_019685	RuvB-like protein 1	0.143	0.057
53	NM_004461	phenylalanine-tRNA synthetase-like		0.055
54	NM_000917	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I		0.054
55	NM_019942	septin 6		0.049
56	NM_017314	ubiquitin C	0.06	0.046
57	NM_004522	kinesin family member 5C	0.061	0.04
58	NM_007508	ATPase, H+ transporting, V1 subunit A, isoform 1		0.039
59	NM_030706	tripartite motif protein 2		0.039
60	NM_009955	dihydropyrimidinase-like 2		0.037
61	NM_032069	Glutamate receptor interacting protein		0.036
62	NM_002155	Hsp70B'	0.096	0.034
63	NM_003794	sorting nexin 4		0.033
64	NM_033309	hypothetical protein MGC4655	0.032	0.032
65	NM_016338	importin 11	0.045	0.032
66	NM_004521	kinesin family member 5B	0.038	0.028
67	NM_007054	kinesin family member 3A		0.027
68	NM_008633	microtubule-associated protein 4		0.025
69	NM_023115	protocadherin 15		0.024
70	NM_016448	RA-regulated nuclear matrix-associated protein	0.015	0.015
71	NM_000038	adenomatosis polyposis coli	0.013	0.014
72	NM_004624	vasoactive intestinal peptide receptor 1		0.014
73	NM_004652	ubiquitin specific protease 9, X chromosome (fat facets-like		0.014

		Drosophila)		
74	NM_022954	MEGF1		0.012
75	NM_000296	polycystic kidney disease 1 (autosomal dominant)	0.031	0.009
76	NM_014225	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform	0.413	
78	NM_022934	DnaJ-like protein	0.34	
79	NM_010481	GRP75	0.337	
80	NM_007175	chromosome 8 open reading frame 2	0.324	
81	NM_002965	S100 calcium binding protein A9 (calgranulin B)	0.263	
82	NM_007126	valosin-containing protein	0.246	
83	NM_002795	proteasome (prosome, macropain) subunit, beta type, 3	0.239	
84	NM_005866	type I sigma receptor	0.229	
85	NM_011185	proteasome (prosome, macropain) subunit, beta type 1	0.229	
86	NM_002793	proteasome (prosome, macropain) subunit, beta type, 1	0.228	
87	NM_011967	proteasome (prosome, macropain) subunit, alpha type 5	0.228	
88	NM_006459	similar to Caenorhabditis elegans protein C42C1.9	0.171	
89	NM_008944	proteasome (prosome, macropain) subunit, alpha type 2	0.171	
90	NM_007688	cofilin 2, muscle	0.169	
91	NM_010223	FKBP8	0.166	
92	NM_011971	proteasome (prosome, macropain) subunit, beta type 3	0.166	
93	NM_024661	hypothetical protein FLJ12436	0.162	
94	NM_002796	proteasome (prosome, macropain) subunit, beta type, 4	0.159	
95	NM_006601	p23	0.156	
96	NM_019766	telomerase binding protein, p23	0.156	
97	NM_025736	RIKEN cDNA 4921531G14 gene	0.145	
98	NM_008143	guanine nucleotide binding protein, beta 2, related sequence 1	0.142	
99	NM_000942	cyclophilin B	0.13	
100	NM_002808	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	0.129	
101	NM_006503	proteasome (prosome, macropain) 26S subunit, ATPase, 4	0.124	
102	NM_013863	BAG-3	0.121	
103	NM_016737	Hop	0.114	
104	NM_013559	Hsp105	0.095	
105	NM_016127	hypothetical protein MGC8721	0.088	
106	NM_017374	protein phosphatase 2a, catalytic subunit, beta isoform	0.084	
107	NM_011889	septin 3	0.082	

108	NM_014673	KIAA0103 gene product	0.081	
110	NM_016742	Cdc37	0.079	
111	NM_008948	proteasome (prosome, macropain) 26S subunit, ATPase 3	0.077	
112	NM_018085	importin 9	0.077	
113	NM_025754	RIKEN cDNA 4933425L11 gene	0.074	
114	NM_018448	TBP-interacting protein	0.072	
115	NM_002271	karyopherin (importin) beta 3	0.063	
116	NM_004576	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta isoform	0.063	
117	NM_015129	septin 6	0.062	
118	NM_011304	RuvB-like protein 2	0.06	
119	NM_016395	butyrate-induced transcript 1	0.056	
120	NM_009864	cadherin 1	0.055	
121	NM_015292	likely ortholog of mouse membrane bound C2 domain containing protein	0.053	
122	NM_002815	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	0.05	
123	NM_018695	erbb2 interacting protein	0.048	
124	NM_008803	phosphodiesterase 8A	0.045	
125	NM_004734	doublecortin and CaM kinase-like 1	0.044	
126	NM_008450	kinesin 2	0.044	
127	NM_006640	MLL septin-like fusion	0.042	
128	NM_031508	Glutamate receptor, ionotropic, kainate 5	0.042	
129	NM_019548	trophinin	0.041	
130	NM_004320	SERCA1	0.033	
131	NM_017249	membrane bound C2 domain containing protein	0.026	
132	NM_000014	alpha-2-macroglobulin	0.023	
133	NM_019120	protocadherin beta 8	0.022	
134	NM_004274	A kinase (PRKA) anchor protein 6	0.019	
135	NM_005120	trinucleotide repeat containing 11 (THR-associated protein, 230kDa subunit)	0.018	
136	NM_019226	dynein, cytoplasmic, heavy chain 1	0.015	
137	NM_031819	FAT tumor suppressor (Drosophila) homolog	0.008	
138	NM_001036	ryanodine receptor 3	0.005	
139	NM_012111	Aha1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)	0.15	0.34

[0288] Example 2: CFTR Spectra Linkage

[0289] From the wealth of interactions observed in the interactome (*see* Example 1), the basis for the loss of export of ΔF508 from the ER was examined as a means of understanding the most common form of CF. A change in protein folding energetics (Sekijima et al., 2005, Cell 121, 73-85; Strickland and Thomas, 1997, J Biol Chem 272, 25421-25424) in response to the Phe 508 deletion results in failure of ΔF508 CFTR to couple to the COPII budding machinery (Wang et al., 1998, FEBS Lett 427, 103), resulting in ER-associated degradation (ERAD) (Nishikawa 2005, J Biochem (Tokyo) 137, 551). Chaperone components that are currently thought to significantly affect CFTR folding through ERAD (Sekijima et al., 2005, supra) pathways include calnexin (Farinha and Amaral, 2005, Mol Cell Biol 25, 5242; Okiyoneda et al., 2004, Mol Biol Cell 15, 563; Pind et al., 1994, J Biol Chem 269, 12784) found in the lumen of the ER, as well as the cytosolic chaperone complexes Hsc-Hsp70/40 and Hsp90 (Albert et al., 2004, Mol Biol Cell 15, 4003; Amaral, 2004, supra; Loo et al., 1998, Embo J 17, 6879; Meacham et al., 1999, Embo J 18, 1492; Meacham et al., 2001, Nat Cell Biol 3, 100; Strickland et al., 1997, J Biol Chem 272, 25421; Younger et al., 2004, supra).

[0290] Consistent with these results, the proteomes of wild-type CFTR expressing cells (*see e.g.*, Fig. 1) showed robust linkage based on total spectra recovered (*see e.g.*, Table 9) to calnexin, Hsc-Hsp70/40 and Hsp90 cytosolic chaperones. These chaperone components likely define core machineries (Fig. 2) facilitating folding of wild-type CFTR as has been observed for other proteins (McClellan et al., 2005, Nat Cell Biol 7, 736-741).

[0291] Results showed that the proteomes of wild-type CFTR expressing cells (Fig. 1) showed robust linkage based on total spectra recovered (*see e.g.*, Table 9) to calnexin, Hsc-Hsp70/40 and Hsp90 cytosolic chaperones. These results are consistent with reports that chaperone components currently thought to significantly affect CFTR folding through ERAF (Sekijima et al., 2005, supra) pathways include calnexin (Farinha and Amaral, 2005, supra; Okiyoneda et al., 2004, supra; Pind et al., 1994, J Biol Chem 269, 12784-12788) found in the lumen of the ER, as well as the cytosolic chaperone complexes Hsc-Hsp70/40 and Hsp90 (Albert et al., 2004, supra; Amaral, 2004, supra; Loo et al., 1998, supra; Meacham et al., 1999, supra; Meacham et al., 2001, supra; Strickland et al., 1997, supra; Younger et al., 2004, supra).

[0292] These chaperone components likely define core machineries (*see e.g.*, Fig. 2A) facilitating folding of wild-type CFTR, as has been observed for other proteins (McClellan et al., 2005, supra).

Table 11. CFTR ER-associated folding proteome *

RefSeq AC	Protein Name	ΔF508 CFTR % sequence coverage A	ΔF508 CFTR unique B	ΔF508 CFTR total C	wt CFTR % sequence coverage A	wt CFTR unique B	wt CFTR total C
NM_000492	CFTR	57	229	2481	60	333	4172
NM_024351	Hsc70	60	66	369	48	39	132
NM_022310	GRP78#	40	30	85	33	17	37
NM_021979	Hsp70-2	16	23	57	8	7	17
NM_001746	Calnexin#	16	13	52	10	4	7
NM_008302	Hsp90β	36	24	49	21	11	18
NM_010481	GRP75	34	23	40			
NM_005348	Hsp90□	39	24	37	6	4	5
NM_022934	DnaJ-like protein	34	11	35			
NM_001539	Hsp40-A1 (Hdj2)	31	10	33	40	13	25
NM_005345	Hsp70-1A	19	12	20	8	4	6
NM_004282	BAG-2	28	7	20	10	2	2
NM_005880	Hsp40-A2 (Hdj3)	32	9	19	28	6	16
NM_002155	Hsp70B'	10	8	14	3	3	5
NM_013559	Hsp105	10	5	6			
NM_013686	TCP1	10	3	5	9	3	5
NM_010223	FKBP38	17	4	5			
NM_013863	BAG-3	12	4	5			
NM_016737	Hop	11	4	4			
NM_016742	Cdc37	8	2	3			
NM_000942	cyclophilin B#	13	2	2			
NM_006601	p23	16	2	2			
NM_012111	Aha1	15	7	15	34	10	20
NM_009037	Reticulocalbin#	27	5	8	50	14	19
NM_011992	Reticulocalbin 2#	9	3	4	22	6	12
NM_001219	Calumenin#				7	3	3

*Indicated are the interacting proteins in BHK cells, their percentage sequence coverage (A), number of unique spectra (B) and number of total spectra (C) as detected by mass spectrometry in cell lines examined (Fig. 1). #ER luminal chaperones.

[0293] Example 3: CFTR and ΔF508 Localization and Interactions

[0294] To identify components in the interactome that may be involved in the failure of ΔF508 to couple to the ER export machinery, the proteomes of wild-type and ΔF508 CFTR immunoprecipitated from BHK cells were compared. The parent BHK cell line not expressing CFTR was used as a negative control for non-specific interactions (*see e.g.*, Fig. 2).

[0295] Fig. 2 is a series of depictions of the ER folding network. Table 10 shows the results of an array of proteins recovered using MudPIT in BHK cells not expressing CFTR (control), or those expressing either ΔF508 or wild-type CFTR, arranged in the order of fractional sequence covergage by mass spectrometry.

[0296] Fig. 2A is a cartoon depicting a composite view of the network comprising the CFTR ER folding and degradation proteomes. Light gray edges indicate potential direct or indirect interactions with CFTR; dark edges indicate known physical interactions between components based on data from HPRD, BIND, and IntAct protein interaction databases. Light green circle indicates core folding chaperones; the light pink circle indicates regulatory co-chaperones and ERAD components. Fig. 2B is an image of an SDS-PAGE immunoblot showing the typical steady-state levels of bands B and C observed in wild-type and ΔF508 CFTR expressing cells. For further methodology information, see Example 1.

[0297] For SDS-PAGE and immunoblotting, cells were washed twice with 500 μl of ice cold PBS and lysed by addition of 45 μl of freshly prepared TBS (50 mM Tris-HCl pH 7.0, 150 mM NaCl) supplemented with 1% Triton X-100 and protease inhibitor cocktail (Pierce) at 2 mg/ml of lysis buffer and incubated on ice for 30 min with occasional agitation. The lysates were collected and spun at 16,000 x g for 20 min at 4°C and the supernatants were collected and analyzed for protein concentration. The lysates (25 μg of total protein per lane) were separated by SDS-PAGE and transferred to nitrocellulose for Western blot analysis. Immunoblotting for actin (Chemicon, Temecula, CA) was used as an additional internal control for consistency of sample loading (not shown). CFTR was detected with a monoclonal antibody (M3A7 ascites) against an epitope at the C-terminal end of the second nucleotide binding domain (Kartner et al., 1992). p23 was detected with p23 ascites (JJ3, Abcam, Cambridge, MA), HOP with a rabbit polyclonal serum, FKBP8 with a rabbit polyclonal serum, and Aha1 with a rabbit Aha1 polyclonal serum. Also used was a monoclonal antibody (P5D4) against the C-terminal cytoplasmic tail of the vesicular stomatitis virus glycoprotein (VSV-G). The amount of each protein of interest was quantified by densitometry using an AlphaInnotech Fluorochem SP (AlphaInnotech, San Leandro, CA). Experiments were conducted in triplicates, and mean and standard error of the mean

determined using an unpaired two-tailed t-test.

[0298] Results showed that, at physiological temperature (37°C), wild-type CFTR is principally (>80-90%) in the band C Golgi processed glycoform found at the cell surface, with the remaining CFTR detected in the band B ER-associated core glycosylated glycoform (*see e.g.*, Fig. 2B). In contrast, in cells expressing ΔF508 at 37°C, generally only 5-20% of the protein (reflecting cell type and growth conditions) can be detected in band C due to significantly reduced stability and folding for export. In this case, the protein is largely restricted to the immature core glycosylated band B ER glycoform (*see e.g.*, Fig. 2B) where it is targeted for ERAD (Jensen et al., 1995, Cell 83, 129-135; Ward and Kopito, 1994, J Biol Chem 269, 25710-25718; Ward et al., 1995, Cell 83, 121-127). In addition to components likely involved in ERAD (Table 8), the ER folding interactome (*see e.g.*, Fig. 2A) revealed that ΔF508, like wild-type CFTR, showed strong interactions with lumenal calnexin and the cytosolic Hsc-Hsp70/40 and Hsp90 cytosolic components (*see e.g.*, Table 9). These results indicate that ΔF508 interacts with the core machinery directing the folding of wild-type CFTR (*see e.g.*, Fig. 2A).

[0299] Example 4: Hsp90 Co-Chaperone Components in ΔF508 ER Interactome

[0300] The ΔF508 ER interactome was analyzed for the presence of Hsp90 co-chaperone components. Hsp90-dependent folding of a variety of client proteins is transiently regulated by co-chaperones (Picard, 2002, Cell Mol Life Sci 59, 1640-1648; Young et al., 2003, supra; Young et al., 2001, supra). Previous studies have suggested that folding of ΔF508 is kinetically impaired ((Qu et al., 1997, J Bioenerg Biomembr 29, 483-490; Qu et al., 1997, J Biol Chem 272, 15739-15744; Qu and Thomas, 1996, J Biol Chem 271, 7261-7264). Therefore, proteins found in the ΔF508 interactome would be expected to include those associated with folding intermediate(s) sensitive to the Phe 508 deletion that may accumulate in response to a kinetic defect in the folding pathway.

[0301] Results showed that a number of Hsp90 co-chaperone components in the ΔF508 ER interactome were not generally detected in the wild-type proteome (*see e.g.*, Fig. 2A). This finding is consistent with the prediction of accumulated folding intermediate(s) sensitive to the Phe 508 deletion in ΔF508. Hsp90 co-chaperone components in the ΔF508 ER interactome that were not generally detected in the wild-type proteome included the Hsc-Hsp70/Hsp90 organizing protein (HOP), p23, Cdc37, the immunophilin FKBP8 and Aha1. Hsp90 co-chaperones have been studied for their roles as regulators of Hsp90-client interactions to modulate the fold of metastable client proteins including steroid hormone

receptors (SHRs) and signaling kinases (Wegele, et al., 2004, supra). Other chaperone regulators detected included BAG-2/3 that have been studied for their role in regulation of Hsc-Hsp70 function in degradation of CFTR (Arndt et al., 2005, Mol Biol Cell; Dai et al., 2005, J Biol Chem 280, 37634), Hsp105, and the folding chaperonin TCP1.

[0302] The network of the known interactions between Hsc-Hsp70/40, Hsp90 and proteins potentially involved in their regulation illustrates the potential complexity of ΔF508 and wild-type CFTR folding pathways for ER export (*see e.g.*, Fig. 2A).

[0303] Example 5: Effect of Co-Chaperone p23 on ΔF508 Folding in HEK293 cells

[0304] The role of the key co-chaperone regulator p23 (Pratt and Toft, 2003, Exp Biol Med (Maywood) 228, 111-133; Prodromou and Pearl, 2003, Curr Cancer Drug Targets 3, 301-323; Wegele et al., 2004, supra) in HEK293 cells stably expressing ΔF508 was examined at several temperatures. P23, along with HOP and FKBP8, affect ATP-dependent folding steps in the cyclic Hsp90-client interaction pathway.

[0305] To begin to define the role of Hsp90 in folding and export of ΔF508 CFTR, dsRNA and transient transfection was used to control the level of protein expression of selected Hsp90 co-chaperones including p23 (*see* Example 5), HOP (*see* Example 6) and FKBP8 (*see* Example 7) that affect ATP-dependent folding steps in the cyclic Hsp90-client interaction pathway. Following recognition of a client molecule such as CFTR by the Hsc-Hsp70/40 complex, the ubiquitous co-chaperone HOP links the nascent Hsc-Hsp70/40-client complex to Hsp90 (Johnson et al., 1998, J Biol Chem 273, 3679-3686). Subsequently, the co-chaperone regulator p23, in the presence of ATP, displaces Hsc-Hsp70/40 and HOP to form the mature Hsp90-p23-client complex in the ATP-bound state (Wegele et al., 2004, supra). The cycling of Hsp90-client complexes containing p23 are regulated by immunophilins (Johnson and Toft, 1994, J Biol Chem 269, 24989-24993; Wu et al., 2004, Proc Natl Acad Sci U S A 101, 8348-8353). Loss of the immunophilin FKBP52 in the case of the steroid hormone receptor (SHR), the prototypical Hsp90 client (Pratt and Toft, 2003, Exp Biol Med (Maywood) 228, 111-133; Prodromou and Pearl, 2003, supra), destabilizes the intermediate Hs 90-client chaperone complex, preventing hormone loading (Cheung-Flynn et al., 2005, Mol Endocrinol 19, 1654-1666).

[0306] HEK293 cells were maintained in DMEM supplemented with 10% FBS and Pen/Strep as above. HEK293 cells stably expressing ΔF508 CFTR were maintained in the same medium as above plus 150 μg/ml hygromycin B.

[0307] dsRNA and transient transfection were used to control the level of protein

expression of p23. The cDNA clones for p23 was purchased from ATCC (Manassas, VA) and were subcloned into pcDNA expression vector, and the sequence of the coding region was verified by DNA sequencing analysis. dsRNA solutions were prepared by mixing serum and antibiotic free DMEM or MEM-α with the indicated dsRNA at a working concentration of 0.6 μM (human p23, Ambion (Austin, TX) Cat. No. 16704 ID 18391) and 6μl of HiPerFect (Qiagen, Valencia, CA) per well of a 12 well dish. Control dsRNA (Dharmacon Cat. No. CONJB-000015) was added at equal concentration to the dsRNA being tested. The dsRNA mixture (100μl) was added to the cells containing 1.1 ml of the appropriate media at a final concentration of 50 nM and cultured at 37°C/5% CO_2 for 48 h. Upon completion of this incubation, the media was removed and replaced with 1.1 ml of fresh complete medium and 100 μl of freshly prepared dsRNA solution and cultured for an additional 33 h at 37°C/5% CO_2. Where indicated, the cells were subsequently transferred to a 30 °C/5% CO_2 incubator or maintained at 37°C/5% CO_2, for an additional 15 h incubation.

[0308] Over-expression of human p23 was performed by co-transfecting HEK293 with plasmids expressing CFTR ΔF508 and p23 by vaccinia virus infection as previously described (Wang et al., 2004, supra). For samples analyzed at the permissive temperature cells were shifted to 30°C for 15 h prior to harvesting.

[0309] SDS-PAGE and immunoblotting are as described in Example 3.

[0310] Fig. 3 is a series of bar graphs depicting the effect of the Hsp90 co-chaperone p23 on folding and export of ΔF508 from the ER. Fig. 3A is a pair of bar graphs showing percent maximum levels for the steady-state pool of ER glycoform ΔF508 (B), cell surface glycoform ΔF508 (C), and p23 expression. Human dsRNA to p23 (left panel) was used to reduce expression of the indicated protein at 37°C. Scrambled dsRNA was used as a control. Human cDNA to p23 (right panel) were used to overexpress the indicated protein at 37°C. The insets are images of an SDS-PAGE immunoblot for the steady-state pool of ER glycoform (band B) and cell surface glycoform (band C). The steady-state pools of bands B and C were determined using immunoblotting. Fig. 3B is as described for Fig. 3A except that cells were incubated at the permissive temperature (30°) to promote folding and export from the ER. The asterisks (*) indicate statistical significance ($p \leq 0.05$). Experiments were repeated independently in triplicate at least three times with representative results shown. For further methodology information, see Example 5.

[0311] Results showed that dsRNA reduction of p23 levels by ~70% resulted in a comparable (60-70%) reduction in the steady-state pools of both the band B ER glycoform and the small pool of the band C cell surface glycoform when compared to the scrambled mock control (*see e.g.*, Fig. 3A, left panel). Conversely, overexpression (3-5 fold) partially

stabilized band B, but did not result in a significant increase in band C (*see e.g.*, Fig. 3A, right panel). Interestingly, dsRNA reduction of p23 had a similar effect on stability of both band B and C wild-type CFTR in HEK293 (not shown), suggesting that p23 affects the dynamics of normal folding.

[0312] Because ΔF508 CFTR is a temperature sensitive folding mutant (Denning et al., 1992, Nature 358, 761-764), incubation of cells at the permissive temperature (30°) instead of 37°C provides a more energetically favorable folding environment leading to significant levels of cell surface localized ΔF508.

[0313] Results showed that, at steady-state (15 h post temperature-shift from 37°C to 30°C), 40-50% of the total ΔF508 pool in HEK293 cells is typically found in band C (Denning et al., 1992, supra) (*see e.g.*, Fig. 3B, left panel). Notably, even at the permissive folding temperature (30°C), dsRNA reduction of p23 resulted in a significant decrease in the stability of band B and processing to band C (*see e.g.*, Fig. 3B, left panel). At 30°C, overexpression had no effect on band B, but prevented processing to band C (*see e.g.*, Fig. 3B, right panel). A similar dominant negative effect of p23 overexpression has been observed for other Hsp90-dependent signaling pathways reflecting excessive stabilization of the mature client complex (Pratt and Toft, 2003, supra).

[0314] Thus, consistent with the effects on wild-type CFTR, p23 is a modular component of folding that affects the stability of ER ΔF508 at both restrictive and permissive folding conditions. These results emphasize the potential differential role of the local chaperone environment on the kinetically impaired ΔF508 fold.

[0315] Example 6: Effect of Co-Chaperone FKBP8 on ΔF508 Folding in HEK293 cells

[0316] The role of the co-chaperone regulator FKBP8 in HEK293 cells stably expressing ΔF508 was examined. Although unable to identify FKBP52 which is involved in SHR folding (Cheung-Flynn et al., 2005, supra) in the ΔF508 CFTR proteome, the immunophilin family member FKBP8 (Nielsen et al., 2001, Genomics 83, 181-192; Pedersen et al., 1999, Electrophoresis 20, 249-255) was detected. FKBP8 is a membrane-associated immunophilin that has been reported to be localized to both the mitochondria and the ER (Kang et al., 2005, FEBS Lett 579, 1469-1476; Shirane and Nakayama, 2003, Nat Cell Biol 5, 28-37; Weiwad et al., 2005, FEBS Lett 579, 1591-1596).

[0317] dsRNA and transient transfection were used to control the level of protein expression of FKBP8. The cDNA clones for FKBP8 were purchased from ATCC (Manassas, VA) and were subcloned into pcDNA expression vector, and the sequence of the coding

region was verified by DNA sequencing analysis. dsRNA solutions were prepared as in Example 5 but with human FKBP8, Ambion Cat. No. 16704 ID 45182. Over-expression of human FKBP8 was performed by co-transfecting HEK293 with plasmids expressing CFTR ΔF508 and FKBP8 by vaccinia virus infection as previously described (Wang et al., 2004, supra). For samples analyzed at the permissive temperature cells were shifted to 30°C for 15 h prior to harvesting. SDS-PAGE and immunoblotting were as described in Example 3.

[0318] Fig. 4 is a series of bar graphs depicting the effect of the Hsp90 co-chaperone FKBP8 on folding and export of ΔF508 from the ER. Fig. 4A is a pair of bar graphs showing percent maximum levels for the steady-state pool of ER glycoform ΔF508 (B), cell surface glycoform ΔF508 (C), and FKBP8 expression. Human dsRNA to FKBP8 (left panel) was used to reduce expression of the indicated protein at 37°C. Scrambled dsRNA was used as a control. Human cDNA to FKBP8 (right panel) was used to overexpress the indicated protein at 37°C. The insets are images of an SDS-PAGE immunoblot for the steady-state pool of ER glycoform (band B) and cell surface glycoform (band C). Fig. 4B (B) is as described for Fig. 4A except that cells were incubated at the permissive temperature (30°) to promote folding and export from the ER. The asterisks (*) indicate statistical significance ($p \leq 0.05$). Experiments were repeated independently in triplicate at least three times with representative results shown. For further methodology information, see Example 6.

[0319] Results showed that FKBP8 has substantial overlap with the ER marker protein calnexin (not shown), a result consistent with previous reports. Similar to the effect of p23 dsRNA, significant destabilization of ΔF508 in response dsRNA reduction of FKBP8 at 37°C was observed (*see e.g.*, Fig. 4A, left panel). Interestingly, overexpression at 37°C also destabilized CFTR (*see e.g.*, Fig. 4A, right panel) raising the possibility that FKBP8 function is linked to the steady-state concentration of Hsp9O. In contrast, dsRNA reduction of FKBP8 expression reduced (30-40%) the stability of ΔF508 CFTR at 30°C, with a corresponding reduction in the level of band C (-50%) (*see e.g.*, Fig. 4B, left panel), whereas overexpression at 30°C had only a modest effect on stability, yet it interfered with processing to band C (*see e.g.*, Fig. 4B, right panel).

[0320] These results suggest that the Hsp90 co-chaperone FKBP8, like p23, acts as a folding modulator to control ΔF508 stability in the ER. These results emphasize the potential differential role of the local chaperone environment on the kinetically impaired ΔF508 fold.

[0321] Example 7: Effect of Co-Chaperone HOP on ΔF508 Folding in HEK293

cells

[0322] The role of the co-chaperone regulator HOP in HEK293 cells stably expressing ΔF508 was examined. dsRNA and transient transfection were used to control the level of protein expression of HOP. The cDNA clones for HOP were purchased from ATCC (Manassas, VA) and were subcloned into pcDNA expression vector, and the sequence of the coding region was verified by DNA sequencing analysis. dsRNA solutions were prepared as in Example 5 but with human HOP, Ambion Cat. No. 16704 ID 18719. Over-expression of human HOP was performed by co-transfecting HEK293 with plasmids expressing CFTR ΔF508 and HOP by vaccinia virus infection as previously described (Wang et al., 2004, supra). For samples analyzed at the permissive temperature cells were shifted to 30°C for 15 h prior to harvesting. SDS-PAGE and immunoblotting were as described in Example 3.

[0323] Fig. 5 is a series of bar graphs depicting the effect of the Hsp90 co-chaperone HOP on folding and export of ΔF508 from the ER. Fig. 5A is a pair of bar graphs showing percent maximum levels for the steady-state pool of ER glycoform ΔF508 (B), cell surface glycoform ΔF508 (C), and HOP expression. Human dsRNA to HOP (left panel) was used to reduce expression of the indicated protein at 37°C. Scrambled dsRNA was used as a control. Human cDNA to HOP (right panel) was used to overexpress the indicated protein at 37°C. The insets are images of an SDS-PAGE immunoblot for the steady-state pool of ER glycoform (band B) and cell surface glycoform (band C). Fig. 5B is as described for Fig. 5A except that cells were incubated at the permissive temperature (30°) to promote folding and export from the ER. The asterisks (*) indicate statistical significance ($p \leq 0.05$). Experiments were repeated independently in triplicate at least three times with representative results shown. For further methodology information, see Example 7.

[0324] Results showed that, in contrast to the effects of dsRNA reduction of both p23 and FKBP8, the maximal reduction of HOP in response to dsRNA observed in HEK293 cells (40-60%) yielded little change in band B stability or the level of band C (*see e.g.*, Fig. 5A, left panel), whereas overexpression (~4-fold) partially destabilized both B and C (*see e.g.*, Fig. 5A, right panel). Again, dsRNA of HOP did not effect folding or export of ΔF508 at 30°C (*see e.g.*, Fig. 5B, left panel). However, overexpression significantly destabilized the protein suggesting that a prolonged linkage to Hsc-Hsp 70/40 under permissive folding conditions favors targeting for degradation (*see e.g.*, Fig. 5B, right panel).

[0325] These results suggest that that HOP facilitates a link between ΔF508 CFTR and Hsc-Hsp70/40 function in degradation (Arndt et al., 2005, supra; Meacham et al., 1999, surpa; Meacham et al., 2001, supra; Younger et al., 2004, supra). These results emphasize

the potential differential role of the local chaperone environment on the kinetically impaired ΔF508 fold.

[0326] Example 8: Effect of Co-Chaperone Aha1 on ΔF508 Folding in HEK293 cells

[0327] The role of the co-chaperone regulator Aha1 in HEK293 cells stably expressing ΔF508 was examined. The most recently recognized member of the Hsp90 co-chaperone family is Aha1. Aha1 binds the middle domain of Hsp90 and is proposed to function as an ATPase activating protein regulating the ATP cycle of Hsp90 (Harst et al., 2005, Biochem J 387, 789-796; Mayer et al., 2002, Mol Cell 10, 1255-1256; Meyer, 2004, Embo J 23, 1402-1410; Meyer et al., 2003, Mol Cell 11, 647-658; Panaretou et al., 2002, Mol Cell 10, 1307-1318; Siligardi et al., 2004, J Biol Chem 279, 51989-51998).

[0328] dsRNA and transient transfection were used to control the level of protein expression of Aha1. The cDNA clone for Aha1 was amplified by PCR with a *C*-terminal *myc*-tag and cloned into pCDNA3.1+ and the sequence verified by sequencing. dsRNA solutions were prepared as in Example 5 but with 2 μM human Aha1 using 1 μM each of two dsRNAs (Dharmacon, Lafyette, CO) directed to human Aha1 sequences attggtccacggataagct (SEQ ID NO: 9; mRNA transcript SEQ ID NO: 12) and gtgagtaagcttgatggag (SEQ ID NO: 10; mRNA transcript SEQ ID NO: 18), and 6 μl of HiPerFect (Qiagen, Valencia, CA) per well of a 12 well dish. Control dsRNA (Dharmacon Cat. No. CONJB-000015) was added at equal concentration to Aha1 dsRNA. Over-expression of human Aha1 was performed by co-transfecting HEK293 with plasmids expressing CFTR ΔF508 and Aha1 by vaccinia virus infection as previously described (Wang et al., 2004, supra). For samples analyzed at the permissive temperature cells were shifted to 30°C for 15 h prior to harvesting. SDS-PAGE and immunoblotting were as described in Example 3.

[0329] Fig. 6 is a series of bar graphs illustrating that ΔF508 export to the cell surface can be rescued by downregulation of functional Aha1. Fig. 6A is a set of bar graphs showing percent maximum levels for the steady-state pool of ER glycoform ΔF508 (B), cell surface glycoform ΔF508 (C), and Aha1 expression. Human Aha1 dsRNA (left panels) or human Aha1 cDNA (right panels) were used to reduce or overexpress, respectively, Aha1 in HEK293 cells expressing ΔF508 at 37°C (upper panels) or 30°C (lower panels). The insets are images of an SDS-PAGE immunoblot for the steady-state pool of ER glycoform (band B) and cell surface glycoform (band C). Fig. 6B is as described for 5A except that human Aha1 dsRNA was used to reduce Aha1 expression in CFBE41o- cells expressing ΔF508 at 37°C (left panel) or 30°C (right panel). The asterisks (*) indicate statistical significance ($p \leq 0.05$)

using an unpaired, two-tailed t-test (triplicate samples). Representative results shown in triplicate from 4 independent experiments. For further methodology information, see Examples 8-9.

[0330] Results showed that dsRNA reduction of the endogenous level of Aha1 in HEK293 cells by 50-70% effected a marked 3- to 4-fold stabilization of ΔF508 band B (*see e.g.*, Fig. 6A, upper left panel). An even more pronounced stabilization (4- to 5-fold) was observed at 30°C (*see e.g.*, Fig. 6A, lower left panel). Strikingly, at both 37°C and 30°C, stabilization was associated with a corresponding increase in band C reflecting significant cell surface delivery (*see e.g.*, Fig. 6A, left panels), a result not observed with the other co chaperones (*see e.g.*, Fig. 3-5). Because the level of expression of Hsp90 co-chaperones can affect folding and export of ΔF508 at both the permissive (30°C) and restrictive (37°C) folding temperatures, the ΔF508 CFTR may be kinetically trapped in an on-pathway, metastable folded state(s) in response to the endogenous cytosolic pool of Hsp90 co-chaperones that normally facilitate folding of wild-type CFTR. The rescued band C was resistant to processing by endoglycosidase H (not shown), a hallmark of transport through the Golgi complex. In contrast to the effects of dsRNA, overexpression (4-fold) of Aha1 in HEK293 cells expressing ΔF508 significantly destabilized band B at both 37°C (–60%) and 30°C (>90%) with a corresponding loss of processing to band C (*see e.g.*, Fig. 6A, right panels). Under these conditions, change in total pools of Hsc-Hsp70 or BIP was not detected, indicating that it is unlikely that a general ER stress response (Schroder and Kaufman, 2005, Annu Rev Biochem 74, 739-789) was induced by a modest reduction of Aha1 (results not shown).

[0331] By analogy to the dynamic role of Hsp90 in known folding pathways (Wegele et al., 2004, supra; Pratt and Toft, 2003, Exp Biol Med (Maywood) 228, 111-133; Prodromou and Pearl, 2003, supra), the above results suggest that regulation of the CFTR client interaction with Hsp90 through sequential interaction with co-chaperones may temporally coordinate steps in intradomain folding and/or coordinate inter-domain folding to avoid ERAD in the process of achieving the wild-type conformation. The need to coordinate intra- and interdomain folding is consistent with evidence that ΔF508 cannot achieve the proper interdomain interactions of NBD1 with TMD1 to produce a stable fold (Riordan, 2005, supra; Du et al., 2005, Nat Struct Mol Biol 12, 17-25). Moreover, the NBD2 domain, again temporally separated by synthesis of TMD2 (Riordan, 2005, supra), is misfolded in cells expressing ΔF508 CFTR and must dimerize with the NBD1 domain to activate one of the nucleotide binding pockets for channel function (Lewis, 2004, Embo J 23, 282-293). Stalled intermediates in the ΔF508 folding pathway are likely targets for recruitment of components

such as CHIP and their regulatory factors HsBP1 and Bag-1/2 that bind the Hsc-Hsp70/40 complex and target of CFTR to ERAD (Alberti et al., 2004, Mol Biol Cell 15, 4003-4010, ; Meacham et al., 2001, supra). It is now likely the relative abundance and perhaps the balance of specific regulators and co-chaperone components for both Hsc-Hsp70/40 and Hsp90 significantly influence the ability of wildtype and ΔF508 CFTR to fold for export from the ER. This conclusion is consistent with the general observation that ER stability and cell surface availability of ΔF508is highly variable among different cell types.

[0332] Example 9: Effect of Co-Chaperone Aha1 on ΔF508 Folding in CFBE41o- cells

[0333] Because HEK293 cells do not normally express ΔF508 and therefore may represent a special condition that is uniquely sensitive to the level of Aha1 activity (*see* Example 8), the effect of Aha1 dsRNA at 37°C was examined in 1 lung cell line (CFBE41o-) that expresses endogenous levels of ΔF508.

[0334] Human bronchial cell line CFBE41o-derived from a CF patient homozygous for ΔF508 CFTR and the corrected HBE cell line was maintained in MEM supplemented with 10% FBS, Pen/Strep, 2 mM extra glutamine, and 2 μg/ml puromycin. dsRNA preparation and transfection, dsRNA solutions, and over expression of Aha1 were as described in Example 8. SDS-PAGE and immunoblotting were as described in Example 3.

[0335] Similar to the result observed in HEK293 cells, reduction of Aha1 resulted in stabilization of band B (–4-mold) compared to the scrambled control, with a corresponding 4- to 5-fold increase of band C either at 37°C (*see e.g.*, Fig. 6B, left panel) or 30°C (*see e.g.*, Fig. 6B, right panel), a level greater than that observed in the corrected CFBE41o- cell line (HBE) that expresses will-type CFTR (Bruscia et al., 2002, Gene Ther 9, 683-685) (*see* below). Pulse-chase analysis in CFBE41o- expressing ΔF508 revealed a 2-3 fold stabilization of band B in the ER preceding export to the cell surface (not shown). In contrast, no effect of Aha1 dsRNA was observed on stabilization of wild-type CFTR using pulse-chase analysis (not shown) or the steady-state cell surface levels of band C using the HBE cell line, suggesting that it is the ΔF508 mutant that requires an adjustment to the endogenous Aha1 pool to promote more efficient export.

[0336] The stabilization of band B and recovery of ΔF508 band C in response to altered levels of Aha1 suggests that reduced Aha1 activity may regulate Hsp90 chaperone dynamics to promote coupling of ΔF508 to the COPII ER export machinery.

[0337] Example 10: siRNA Design for Silencing Aha1

[0338] Candidate shRNA sequences for targeting the Aha1 gene were obtained from a search tool from Ambion (http://www.ambion.com/techlib/misc/siRNA_finder.html). The cDNA sequence of hAha1 (Ensembl Accession No. ENSG00000100591) was used to generate the exemplary listing. The sequences provide less than 50% GC content and avoid four or more Gs or Cs in a row. The complete listing is provided in Table 1.

[0339] Each shRNA was BLASTed against the human genome to identify possible off-target sites in other genes. Three dsRNAs chosen having a GC content around 40% and few off-target sites in other genes were selected: 99 (SEQ ID NO: 12), 179 (SEQ ID NO: 18) and 256 (SEQ ID NO: 24). Each of these three 19 base pair sequences has no more than 15 consecutive identities with any 5' or 3' untranslated regions, introns or exons of any other genes. The three sequences were cloned into the pSilencer vector (Ambion) to make stable Hela lines with reduced hAha1 expression. dsRNAs corresponding to the 99 and 179 sequences above were created (Dharmacon and Qiagen) for experiments provided herein.

[0340] Example 11: Aha 1 dsRNA Effect on Halide Conductance in CFBE41o- Cells Expressing ΔF508

[0341] While processing to the endo H resistant band C glycoform is a hallmark of transport from the ER to the cis/medial Golgi compartments (*see e.g.*, Examples 8-9), it is possible that the rescued protein was trapped in late trans Golgi or endocytic compartments reflecting unanticipated contribution(s) of the Phe 508 deletion to abnormal sorting in post-ER pathways (*see e.g.*, Fig. 1) (Gentzsch et al., 2004a; Sharma et al., 2004; Swiatecka-Urban et al., 2005). To test for this possibility, CFBE41o- cells expressing ΔF508 were treated with Aha1 dsRNA and surface halide conductance measured using an iodide efflux assay (Loo et al., 2005, supra).

[0342] As a positive control, the halide conductance of the corrected HBE cell line expressing wild-type CFTR was examined. For the Iodide efflux assay, wild type and ΔF508 CFBE41o- cells were *see*ded at a density of 5.0 x 10^5 cells per 60 mm dish and grown under the conditions listed above for 5 days with a change of culture media every 2 days. dsRNA treatment was performed as indicated above with 20 µl of HiPerFect (Qiagen) per 60 mm dish. CFBE41o- cells were shifted to the permissive temperature of 30°C for 15 h prior to iodide efflux analysis (Hughes et al., 2004). Cells were washed 5X with loading buffer (136 mM NaI; 3 mM KNO_3; 2 mM $Ca(NO_3)_2$; 20 mM Hepes and 11 mM glucose) and incubated for 1h at room temperature with 2.5 ml of loading buffer. Cells were subsequently washed 15X with efflux buffer (136 mM $NaNO_3$; 3 mM KNO_3; 2 mM $Ca(NO_3)_2$; 20 mM Hepes and 11 mM glucose) and incubated with 2.5ml of efflux buffer for 1 minute at room temperature and the

media collected for analysis. This incubation was repeated for a total of 4 min. The cells were subsequently incubated with 2.5 ml of stimulation buffer (efflux buffer containing 10 μM forskolin (Sigma) and 50 μM genistein (Sigma)) for 1 min at room temperature and the media collected for analysis. This incubation was repeated for a total of 4 min. The cells were then incubated with 2.5 ml of efflux buffer for 1 min at room temperature and the media collected for analysis. This incubation was repeated for a total of 12 min. The samples were analyzed for iodide content using an iodide selective electrode (Analytical Sensors & Instruments) and a Beckman model 360 pH meter (VWR). The amount of iodide in the collected media was determined by extrapolating from a standard curve of known NaI concentrations. SDS-PAGE and immunoblotting were as described in Example 3.

[0343] Fig. 7 is a line and scatter plot and a bar graph showing the effect of dsRNA Aha1 on iodide efflux by the CFBE41o- cell line. Fig. 7A is a line and scatter plot depicting iodide efflux over time. Iodide efflux was monitored in HBE cells expressing wild-type CFTR (closed boxes) or in ΔF508 expressing CFBE41o- cells that had been incubated at 37°C, or where indicated, at the permissive temperature of 30°C (final 15 h) (closed circles), and transfected with Aha1 (open circles) or scrambled (control) (open boxes) dsRNA. CFTR channels were activated by addition of 10 μM forskolin and 50μM genistein over a 4 min period starting at 1 min and subsequently washed out with efflux buffer. The effect of temperature-shift and dsRNA on CFTR maturation (band B to band C glycoforms) and Aha1 stability is shown in the inset. Fig. 7B is a bar graph depicting the ratio of halide conductance prior to addition of forskolin/genistein (0 min) and at 2 min, the peak period of halide flux. The asterisks (*) indicate statistical significance ($p \leq 0.05$) using the unpaired, two-tailed t-test (triplicate samples) between the temperature-corrected (lane a) and dsRNA-treated (lane c) CFBE41o- cells compared to the scrambled dsRNA-treated control (lane b). There was no statistically significant difference between halide conductance for temperature-corrected (lane a) and dsRNA corrected CFBE41O- cells (lane c) ($p = 0.2$). Experiments were repeated independently at least three times with representative results shown. For further methodology information, see Example 10.

[0344] Results showed that treatment of the CFBE41o- cell line with Aha1 dsRNA resulted in ~70-80% knock-down of endogenous Aha1, leading to stabilization of ΔF508 band B and C at levels ~1.5-fold the 30°C to temperature-corrected control and a 4-fold stabilization of band B over the scrambled dsRNA treated cells (*see e.g.*, Fig. 7A, insert). Whereas HBE cells showed strong halide conductance, no conductance was detected in control CFBE41o- cells that were treated with scrambled dsRNA (*see e.g.*, Fig. 7A). Shift of CFBE41o- to 30°C resulted in recovery of 80-90% of the conductance observed in HBE cells

(*see e.g.*, Fig. 7A). Strikingly, CFBE41o- cells treated with dsRNA, but not scrambled, showed 50-80% recovery of halide conductance compared to that observed in temperature-corrected cells (*see e.g.*, Fig. 7B). These results demonstrate that Aha 1 dsRNA restores halide conductance to CFBE41o- cells expressing ΔF508, thereby achieving functional rescue of CFTR.

[0345] Fig. 8 is a series of cartoons depicting Hsp90 chaperone/co-chaperone interactions directing CFTR folding. Fig. 8A is a cartoon highlighting components involved in wild -type and ΔF508 CFTR folding. They consist of lumenal chaperones (**1**), and a two-state cytosolic system that includes the core components Hsc-Hsp70/40 (**2**) and Hsp90 (**3**) as well as number of Hsc-Hsp70 (**2**) and Hsp90 (**3**) co-chaperone regulator. Additional chaperones such as TCP1 (Spiess et al., 2004, supra) and Hsp105/S100 (**3a**) may also contribute to folding. One or more of these protein interactions are kinetically disrupted by the Phe 508 deletion leading disruption of the Hsp90 ATPase cycle and CF pathophysiology. Fig. 8B is an illustration of the potential role of Hsp90 and the co-chaperones in folding and rescue of ΔF508 CFTR. The ATP/ADP cycle regulating folding for export through ERAF or targeting for ERAD can be dynamically controlled by co-chaperone regulators (X and Y) to adjust the kinetics of the chaperone cycle to the kinetics and energetics of the folding pathway. For example, down-regulation of Aha1 ATPase activity by dsRNA (X) would favor stabilization of ΔF508 for export by reducing Hsp90 ATPase activity, whereas down-regulation of p23 (Y) would favor destabilization leading to ERAD. Fig. 8C is a plot illustrating the relationship between the (co)chaperone concentration in the cytosol (X axis), a hypothetical 'folding stability score' defined by global protein energetics (Sekijima et al., 2005, Cell 121, 73-85) (Z axis), and 'export efficiency' reflecting the level of transport to the cell surface (Y axis). Whereas the more energetically stable wild-type CFTR (dashed curve) responds to the folding activity of the CFTR chaperome response to the normal concentration of Aha1 (box having grid, 'normal chaperome'), the reduced folding energetics of ΔF508 (left solid curve) is unstable in this folding environment and fails to be exported. A change in the set-point of the Hsp90 ATP/ADP cycle afforded by downregulation of Aha1 (grey box, 'rescue chaperome') provides a more productive solvent by adjusting chaperome folding capacity (grid box) to folding of ΔF508 (left curve), while maintaining functionality of the wild-type CFTR fold (right solid curve, grid box). Geldanamycin (GA), a Hsp90 inhibitor, blocks both wild-type and ΔF508 CFTR folding and export by directly binding to Hsp90 and arresting the folding cycle (lower left corner) (Loo et al., 1998, supra).

[0346] In summary, the results above suggest that the intrinsic folding defect in mutant CFTR is kinetically linked to the activity of the Aha1-sensitive Hsp90 ATPase cycle.

The working model developed from results herein emphasizes an environment-sensitive uncoupling from normal cellular folding pathways. In this view, the intrinsic rate of the Hsp90 ATP/ADP cycle controlling Hsp90-client complex interactions is coordinated with the energetics of folding of wild-type CFTR through co-chaperone activity. Whereas the folding energetics driving export of wildtype CFTR is optimized relative to the normal cellular chaperone pool (Fig. 8), a change in the activities of Aha1, and potentially other cochaperones, can alter these (Fig. 8) and the capacity of the chaperone folding/export pathway. In the case of Aha1, reduction of Hsp90 ATPase activity may allow additional time for the kinetically challenged ΔF508mutant (Fig. 8) to engage a 'rescue' chaperone pool (Fig. 8) to favor stability and folding for export. Because partial reduction of the Aha1 pool did not significantly impair the more energetically stable wild-type fold (Fig. 8), these results emphasize that the folding energetics of the Phe 508 deletion may lie outside the normal chaperoned folding boundaries. The ability of a unique local population of chaperones to modulate folding is consistent with recent observations that folding chaperones are now found to regulate specific cellular protein folding pathways (Albanese et al., 2006, Cell 124, 75-88), rather than simply function as inhibitors of protein aggregation (Wickner et al., 1999, Science 286, 1888-1893).

From an evolutionary perspective, genetic modifiers (Qu and Thomas, 1996, supra) are now likely to include folding chaperones that provide a favorable genetic or epigenetic (Cowen and Lindquist, 2005, Science 309, 2185; Queitsch et al., 2002, Nature 417, 618) environment for reduced function of the mutant, yet survival value when challenged with agonists such as cholera toxin where reduced chloride channel function would decrease the possibility of dehydration and death when compared to the wild-type population (Gabriel et al., 1994, Science 266, 107; Thiagarajah and Verkman, 2005, Trends Pharmacol Sci 26, 172). Thus, the activity of chaperone pools may define the difference between a tolerated polymorphism and a deleterious mutation in CF and other protein misfolding diseases.

[0347] Example 12: Hsp90 binding to CFTR is responsive to Aha1 activity

[0348] To determine the effect of Aha1 knock-down on the interaction of ΔF508 with Hsp90, we analyzed the recovery of Hsp90 bound to CFTR following treatment of cells with Aha1 dsRNA. Cells expressing ΔF508 at 37°C were incubated in presence of scrambled or Aha1 dsRNA. Cells were harvested, CFTR immunoprecipitated, and the amount of Hsp90 associated with ΔF508 quantified by immunoblotting. For these experiments, we analyzed the ratio of Hsp90 to CFTR recovered in the immunoprecipitate to determine the relative amount of Hsp90 bound to CFTR under control or knock-down

conditions.

[0349] Fig. 9 illustrates effects of dsRNA Aha1 on Hsp90. HEK293 cells expressing ΔF508 at 37°C were incubated in absence or presence Aha1 dsRNA. Cells were harvested, CFTR immunoprecipitated and the amount of Hsp90 recovered with ΔF508 was quantified by immunoblotting. Left panel: Ratio of Hsp90 to CFTR recovered in the immunoprecipitate. Right panel: fraction of Aha1 remaining in cells following Aha1 dsRNA treatment compared to scrambled control.

[0350] Under conditions in which we observed an ~60% knock-down of Aha1 (Fig. 9, right panel), we observed a 50-60% decrease of bound Hsp90 at reduced levels of Aha1 (Fig. 9, left panel). In contrast, under these conditions we detected no change in the cellular levels of calnexin, BiP, Hsp40, Hsc-Hsp70, Hsp90, HOP FKBP8/38 and p23 compared to the scrambled control, indicating that a reduction in Aha1 can alter the steady-state pool of ΔF508 associated with Hsp90 in the ER. This result is consistent with the observation that Hsp90 and Hsp90 co-chaperone recovery in the CFTR wild-type interactome is reduced relative to the ΔF508 interactome despite comparable levels of band B. The results demonstrate that lowering the level of the Aha1 co-chaperone regulator can modify the kinetic interactions of ΔF508 with Hsp90 to facilitate more efficient progression through the folding pathway, thereby favoring export.

[0351] Example 13: Gene Walking of an Aha gene

[0352] dsRNA design was carried out to identify dsRNAs targeting Aha1. The mRNA sequences of Homo sapiens (NM_012111.1), mus musculus (NM_146036.1) and pan troglodytes (XM_510094.1) Aha 1 were examined by computer analysis to identify homologous sequences of 19 or 21 nucleotides that yield RNAi agents cross-reactive between these three species. Of those identified, 48 such sequences were selected for minimal off-target interactions in rats (at least 3 mismatches to any other gene in the rat genome, or at least two mismatches to any other gene in the rat genome, wherein one of said at least two mismatches is located in a position complementary to position 9 or 10 of the antisense strand of the corresponding RNAi agent, counting 5' to 3') and the corresponding dsRNAs synthesized for screening (AL-DP-7299 - AL-DP-7346, see Table 5. AL-DP-7561, AL-DP-7562, AL-DP-7563 and AL-DP-7564 which are additionally cross-reactive to mus musculus (NM_172391.3) and rattus norvegicus (XM_223680.3) Aha 2, were also synthesized and screened. In addition, a further 40 sequences were selected for minimal predicted off-target interactions in humans (at least 3 mismatches to any other gene in the human genome, or at least two mismatches to any other gene in the human genome,

wherein one of said at least two mismatches is located in a position complementary to position 9 or 10 of the antisense strand of the corresponding RNAi agent, counting 5' to 3') and the corresponding dsRNAs synthesized for screening (AL-DP-9250 - AL-DP-9289, see Table 6). 17 sequences were identified as belonging to both sets (AL-DP-7301, AL-DP-7304, AL-DP-7305, AL-DP-7307, AL-DP-7310, AL-DP-7312, AL-DP-7315, AL-DP-7316, AL-DP-7317, AL-DP-7323, AL-DP-7324, AL-DP-7332, AL-DP-7336, AL-DP-7337, AL-DP-7338, AL-DP-7342, and AL-DP-7344.

[0353] Example 14: dsRNA synthesis

[0354] Source of reagents

[0355] Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

[0356] dsRNA synthesis

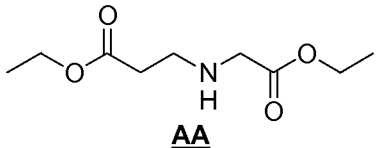
[0357] Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 µmole using an Expedite 8909 synthesizer (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) and controlled pore glass (CPG, 500Å, Proligo Biochemie GmbH, Hamburg, Germany) as solid support. RNA and RNA containing *2'-O*-methyl nucleotides were generated by solid phase synthesis employing the corresponding phosphoramidites and *2'-O*-methyl phosphoramidites, respectively (Proligo Biochemie GmbH, Hamburg, Germany). These building blocks were incorporated at selected sites within the sequence of the oligoribonucleotide chain using standard nucleoside phosphoramidite chemistry such as described in Current protocols in nucleic acid chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Phosphorothioate linkages were introduced by replacement of the iodine oxidizer solution with a solution of the Beaucage reagent (Chruachem Ltd, Glasgow, UK) in acetonitrile (1%). Further ancillary reagents were obtained from Mallinckrodt Baker (Griesheim, Germany).

[0358] Deprotection and purification of the crude oligoribonucleotides by anion exchange HPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman Coulter GmbH, Unterschleißheim, Germany). Double stranded RNA was generated by mixing an equimolar solution of complementary strands in annealing buffer (20 mM sodium phosphate, pH 6.8; 100 mM sodium chloride), heated in a water bath at 85 - 90°C for 3 minutes and cooled to

room temperature over a period of 3 - 4 hours. The annealed RNA solution was stored at –20 °C until use.

[0359] For the synthesis of 3'-cholesterol-conjugated dsRNAs (herein referred to as -Chol-3'), an appropriately modified solid support was used for RNA synthesis. The modified solid support was prepared as follows:

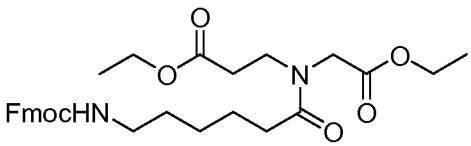
[0360] Diethyl-2-azabutane-1,4-dicarboxylate **AA**



AA

[0361] A 4.7 M aqueous solution of sodium hydroxide (50 mL) was added into a stirred, ice-cooled solution of ethyl glycinate hydrochloride (32.19 g, 0.23 mole) in water (50 mL). Then, ethyl acrylate (23.1 g, 0.23 mole) was added and the mixture was stirred at room temperature until completion of the reaction was ascertained by TLC. After 19 h the solution was partitioned with dichloromethane (3 x 100 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated. The residue was distilled to afford AA (28.8 g, 61%).

[0362] 3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonyl-amino)-hexanoyl]-amino}-propionic acid ethyl ester **AB**

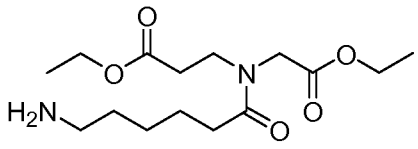


AB

[0363] Fmoc-6-amino-hexanoic acid (9.12 g, 25.83 mmol) was dissolved in dichloromethane (50 mL) and cooled with ice. Diisopropylcarbodiimde (3.25 g, 3.99 mL, 25.83 mmol) was added to the solution at 0°C. It was then followed by the addition of Diethyl-azabutane-1,4-dicarboxylate (5 g, 24.6 mmol) and dimethylamino pyridine (0.305 g, 2.5 mmol). The solution was brought to room temperature and stirred further for 6 h. Completion of the reaction was ascertained by TLC. The reaction mixture was concentrated under vacuum and ethyl acetate was added to precipitate diisopropyl urea. The suspension was filtered. The filtrate was washed with 5% aqueous hydrochloric acid, 5% sodium bicarbonate and water. The combined organic layer was dried over sodium sulfate and concentrated to give the crude product which was purified by column chromatography (50 %

EtOAC/Hexanes) to yield 11.87 g (88%) of AB.

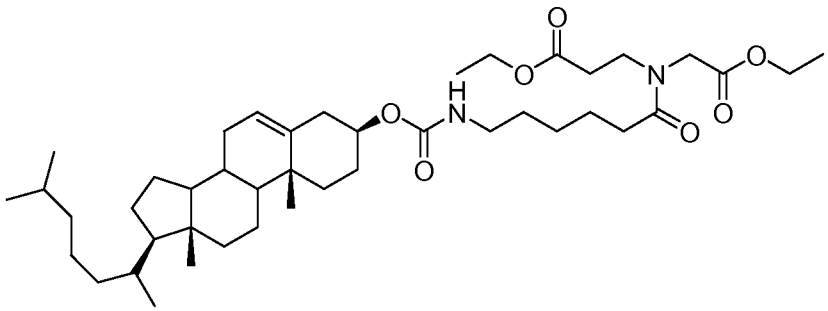
[0364] 3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester **AC**



AC

[0365] 3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoyl]-amino}-propionic acid ethyl ester AB (11.5 g, 21.3 mmol) was dissolved in 20% piperidine in dimethylformamide at 0°C. The solution was continued stirring for 1 h. The reaction mixture was concentrated under vacuum, water was added to the residue, and the product was extracted with ethyl acetate. The crude product was purified by conversion into its hydrochloride salt.

[0366] 3-({6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}ethoxycarbonylmethyl-amino)-propionic acid ethyl ester **AD**

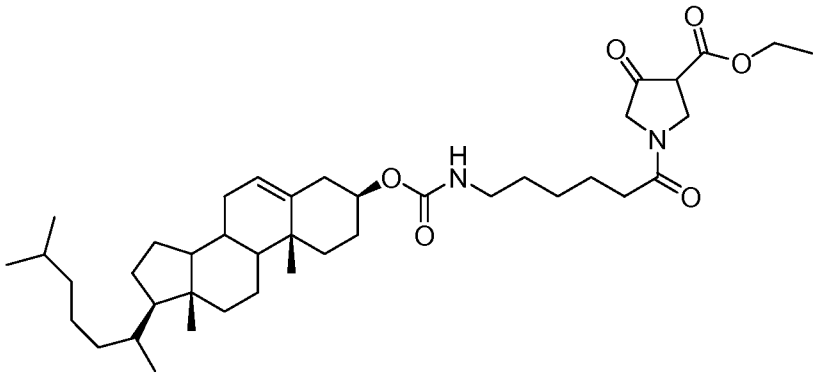


AD

[0367] The hydrochloride salt of 3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester AC (4.7 g, 14.8 mmol) was taken up in dichloromethane. The suspension was cooled to 0°C on ice. To the suspension diisopropylethylamine (3.87 g, 5.2 mL, 30 mmol) was added. To the resulting solution cholesteryl chloroformate (6.675 g, 14.8 mmol) was added. The reaction mixture was stirred overnight. The reaction mixture was diluted with dichloromethane and washed with 10% hydrochloric acid. The product was purified by flash chromatography (10.3 g, 92%).

[0368] 1-{6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-

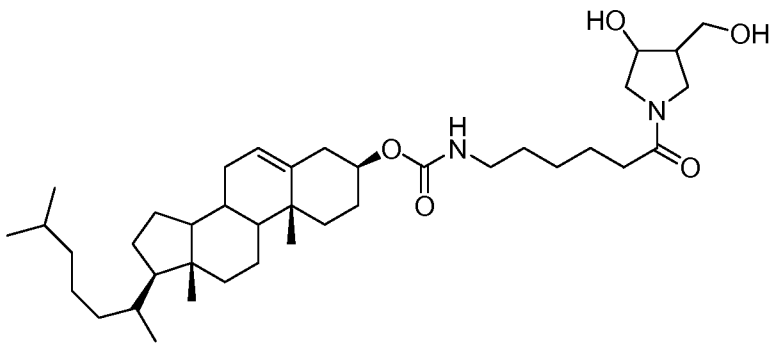
2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-yloxycarbonylamino]-hexanoyl}-4-oxo-pyrrolidine-3-carboxylic acid ethyl ester **AE**



AE

[0369] Potassium t-butoxide (1.1 g, 9.8 mmol) was slurried in 30 mL of dry toluene. The mixture was cooled to 0°C on ice and 5 g (6.6 mmol) of diester AD was added slowly with stirring within 20 mins. The temperature was kept below 5°C during the addition. The stirring was continued for 30 mins at 0°C and 1 mL of glacial acetic acid was added, immediately followed by 4 g of $NaH_2PO_4 \cdot H_2O$ in 40 mL of water The resultant mixture was extracted twice with 100 mL of dichloromethane each and the combined organic extracts were washed twice with 10 mL of phosphate buffer each, dried, and evaporated to dryness. The residue was dissolved in 60 mL of toluene, cooled to 0°C and extracted with three 50 mL portions of cold pH 9.5 carbonate buffer. The aqueous extracts were adjusted to pH 3 with phosphoric acid, and extracted with five 40 mL portions of chloroform which were combined, dried and evaporated to dryness. The residue was purified by column chromatography using 25% ethylacetate/hexane to afford 1.9 g of b-ketoester (39%).

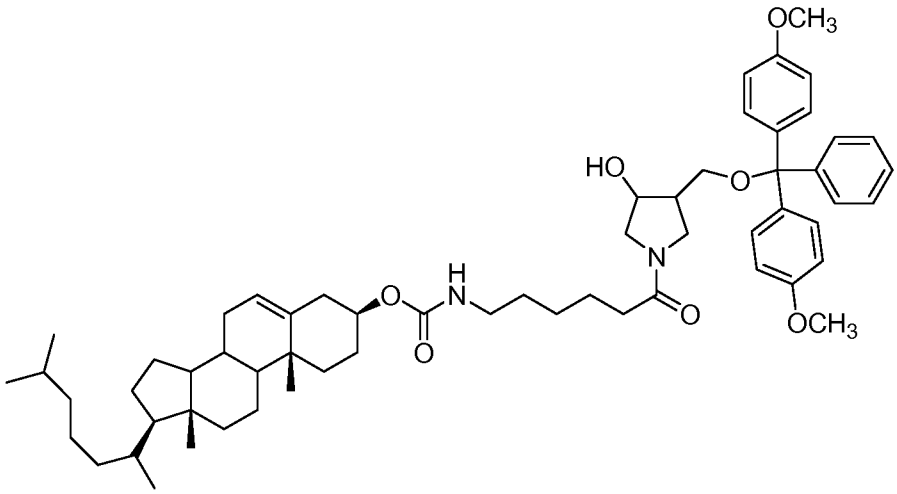
[0370] [6-(3-Hydroxy-4-hydroxymethyl-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AF**



AF

[0371] Methanol (2 mL) was added dropwise over a period of 1 h to a refluxing mixture of b-ketoester AE (1.5 g, 2.2 mmol) and sodium borohydride (0.226 g, 6 mmol) in tetrahydrofuran (10 mL). Stirring was continued at reflux temperature for 1 h. After cooling to room temperature, 1 N HCl (12.5 mL) was added, the mixture was extracted with ethylacetate (3 x 40 mL). The combined ethylacetate layer was dried over anhydrous sodium sulfate and concentrated under vacuum to yield the product which was purified by column chromatography (10% MeOH/$CHCl_3$) (89%).

[0372] (6-{3-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4-hydroxy-pyrrolidin-1-yl}-6-oxo-hexyl)-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AG**

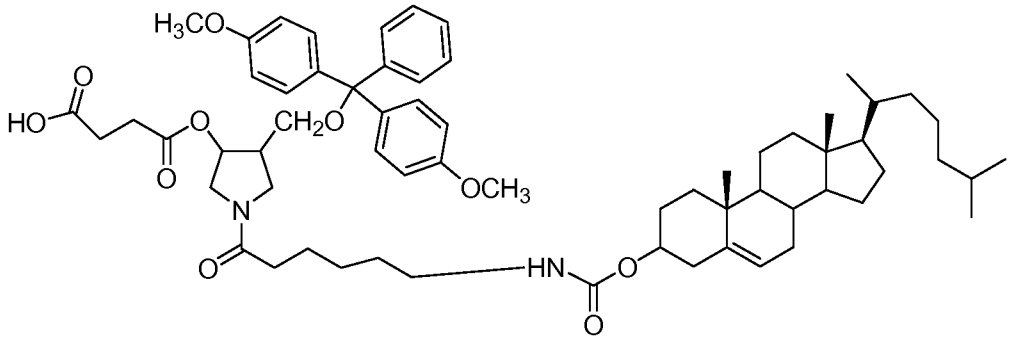


AG

[0373] Diol AF (1.25 gm 1.994 mmol) was dried by evaporating with pyridine (2 x 5 mL) *in vacuo*. Anhydrous pyridine (10 mL) and 4,4'-dimethoxytritylchloride (0.724 g, 2.13 mmol) were added with stirring. The reaction was carried out at room temperature overnight. The reaction was quenched by the addition of methanol. The reaction mixture was concentrated under vacuum and to the residue dichloromethane (50 mL) was added. The organic layer was washed with 1M aqueous sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residual pyridine was removed by evaporating with toluene. The crude product was purified by column chromatography (2% MeOH/Chloroform, Rf = 0.5 in 5% MeOH/$CHCl_3$) (1.75 g, 95%).

[0374] Succinic acid mono-(4-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-1-{6-[17-(1,5-dimethyl-hexyl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-

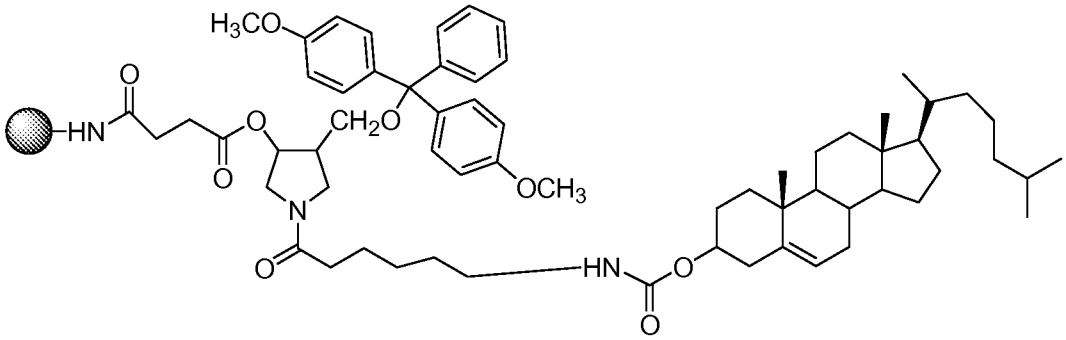
1H cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}-pyrrolidin-3-yl) ester **AH**



AH

[0375] Compound AG (1.0 g, 1.05 mmol) was mixed with succinic anhydride (0.150 g, 1.5 mmol) and DMAP (0.073 g, 0.6 mmol) and dried in a vacuum at 40°C overnight. The mixture was dissolved in anhydrous dichloroethane (3 mL), triethylamine (0.318 g, 0.440 mL, 3.15 mmol) was added and the solution was stirred at room temperature under argon atmosphere for 16 h. It was then diluted with dichloromethane (40 mL) and washed with ice cold aqueous citric acid (5 wt%, 30 mL) and water (2 X 20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was used as such for the next step.

[0376] Cholesterol derivatised CPG **AI**



AI

[0377] Succinate AH (0.254 g, 0.242 mmol) was dissolved in a mixture of dichloromethane/acetonitrile (3:2, 3 mL). To that solution DMAP (0.0296 g, 0.242 mmol) in acetonitrile (1.25 mL), 2,2'-Dithio-bis(5-nitropyridine) (0.075 g, 0.242 mmol) in acetonitrile/dichloroethane (3:1, 1.25 mL) were added successively. To the resulting solution triphenylphosphine (0.064 g, 0.242 mmol) in acetonitrile (0.6 ml) was added. The reaction mixture turned bright orange in color. The solution was agitated briefly using a wrist-action shaker (5 mins). Long chain alkyl amine-CPG (LCAA-CPG) (1.5 g, 61 mM) was added. The suspension was agitated for 2 h. The CPG was filtered through a sintered funnel and

washed with acetonitrile, dichloromethane and ether successively. Unreacted amino groups were masked using acetic anhydride/pyridine. The achieved loading of the CPG was measured by taking UV measurement (37 mM/g).

[0378] The synthesis of dsRNAs bearing a 5'-12-dodecanoic acid bisdecylamide group (herein referred to as "5'-C32-") or a 5'-cholesteryl derivative group (herein referred to as "5'-Chol-") was performed as described in WO 2004/065601, except that, for the cholesteryl derivative, the oxidation step was performed using the Beaucage reagent in order to introduce a phosphorothioate linkage at the 5'-end of the nucleic acid oligomer.

[0379] Nucleic acid sequences are represented below using standard nomenclature, and specifically the abbreviations of Table 12.

Table 12: Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation[a]	Nucleotide(s)
A, a	2'-deoxy-adenosine-5'-phosphate, adenosine-5'-phosphate
C, c	2'-deoxy-cytidine-5'-phosphate, cytidine-5'-phosphate
G, g	2'-deoxy-guanosine-5'-phosphate, guanosine-5'-phosphate
T, t	2'-deoxy-thymidine-5'-phosphate, thymidine-5'-phosphate
U, u	2'-deoxy-uridine-5'-phosphate, uridine-5'-phosphate
N, n	any 2'-deoxy-nucleotide/nucleotide (G, A, C, or T, g, a, c or u)
Am	*2'-O*-methyladenosine-5'-phosphate
Cm	*2'-O*-methylcytidine-5'-phosphate
Gm	*2'-O*-methylguanosine-5'-phosphate
Tm	*2'-O*-methyl-thymidine-5'-phosphate
Um	*2'-O*-methyluridine-5'-phosphate
Af	2'-fluoro-2'-deoxy-adenosine-5'-phosphate
Cf	2'-fluoro-2'-deoxy-cytidine-5'-phosphate
Gf	2'-fluoro-2'-deoxy-guanosine-5'-phosphate
Tf	2'-fluoro-2'-deoxy-thymidine-5'-phosphate
Uf	2'-fluoro-2'-deoxy-uridine-5'-phosphate
A, C, G, T, U, a, c, g, t, u	underlined: nucleoside-5'-phosphorothioate
am, cm, gm, tm, um	underlined: 2-*O*-methyl-nucleoside-5'-phosphorothioate

[a]capital letters represent 2'-deoxyribonucleotides (DNA), lower case letters represent ribonucleotides (RNA)

[0380] Example 15: dsRNA expression vectors

[0381] In another aspect of the invention, Aha1 specific dsRNA molecules that modulate Aha1 gene expression activity are expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Couture, A, et al., *TIG.* (1996), **12**:5-10; Skillern, A., et al., International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, US Pat. No. 6,054,299). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

[0382] The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In a preferred embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

[0383] The recombinant dsRNA expression vectors are generally DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, et al., *Curr. Topics Micro. Immunol.* (1992) 158:97-129)); adenovirus (see, for example, Berkner, et al., BioTechniques (1998) 6:616), Rosenfeld et al. (1991, Science 252:431-434), and Rosenfeld et al. (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, in vitro and/or in vivo (see, e.g., Eglitis, et al., *Science* (1985) 230:1395-1398; Danos and Mulligan, *Proc. Natl. Acad. Sci. USA* (1998) 85:6460-6464; Wilson et al., 1988, Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al., 1990, Proc. Natl. Acad. Sci. USA 87:61416145; Huber et al., 1991, Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al., 1991, Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al., 1991, Science 254:1802-1805; van Beusechem. et al., 1992, Proc. Nad. Acad. Sci. USA 89:7640-19 ; Kay et al., 1992, Human Gene Therapy 3:641-647; Dai et al., 1992, Proc. Natl.Acad. Sci. USA 89:10892-10895; Hwu et al., 1993, J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-

CRIP (Comette et al., 1991, Human Gene Therapy 2:5-10; Cone et al., 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (e.g., rat, hamster, dog, and chimpanzee) (Hsu et al., 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

[0384] The promoter driving dsRNA expression in either a DNA plasmid or viral vector of the invention may be a eukaryotic RNA polymerase I (e.g. ribosomal RNA promoter), RNA polymerase II (e.g. CMV early promoter or actin promoter or U1 snRNA promoter) or generally RNA polymerase III promoter (e.g. U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, e.g. the insulin regulatory sequence for pancreas (Bucchini et al., 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

[0385] In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 -thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

[0386] Generally, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

[0387] dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (e.g. Oligofectamine) or non-cationic lipid-based carriers (e.g. Transit-TKO^{TM}). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single Aha1 gene or multiple Aha1 genes over a period of a

week or more are also contemplated by the invention. Successful introduction of the vectors of the invention into host cells can be monitored using various known methods. For example, transient transfection. can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection. of ex vivo cells can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance.

[0388] The Aha1 specific dsRNA molecules can also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[0389] Example 16: Aha1 dsRNA in vitro screening

[0390] Single Dose Screen in HeLa and MLE 12 cells

[0391] HeLa cells were obtained from American Type Culture Collection (Rockville, MD, cat. No. HB-8065) and cultured in Ham´s F12 (Biochrom AG, Berlin, Germany, cat. No. FG0815) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, Streptomycin 100 µg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

[0392] MLE 12 cells were obtained from American Type Culture Collection (Rockville, MD, cat. No. CRL-2110) and cultured in HITES Medium (1:1 mix Dulbecco's MEM (Biochrom AG, Berlin, Germany, cat. No: F0435) + Ham's F12 (Biochrom AG, Berlin, Germany, cat. No: FG0815)) supplemented to contain 2% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, Streptomycin 100 µg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213), 4 mM L-Glutamin (Biochrom AG, Berlin, Germany, cat. No: K0282), 1 x Insulin/Transferrin/Na-Selenit (Gibco: 51500-056), 10 nM Hydrocortisone (Sigma Munich, Germany, cat.No: H6909), 10 nM ß-Estradiol (Sigma Munich, Germany, cat.No: E2257), and 10 mM HEPES (USB Europe GmBH, Staufen, Germany cat. No.: 16926) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator

(Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

[0393] Transfection and mRNA quantification

[0394] For transfection with dsRNA, HeLa and MLE12 cells were seeded at a density of 2.0 x 10^4 cells/well in 96-well plates and transfected directly. Transfection of dsRNA (30nM) was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer. 24 hours after transfection cells were lysed and Aha1 mRNA levels were quantified with the Quantigene Explore Kit (Genosprectra, Dumbarton Circle Fremont, USA, cat. No. QG-000-02) according to the manufacturer's protocol. Aha1 mRNA levels were normalized to GAPDH mRNA. Readings were obtained in quadruplicates for each dsRNA. dsRNA duplexes unrelated to Aha1 gene were used as control. The activity of a given Aha1 specific dsRNA duplex was expressed as percent Aha1 mRNA concentration in treated cells relative to Aha1 mRNA concentration in cells treated with the control dsRNA duplex.

Table 13: Probe sequences used with Quantigene Explore Kit (Genospectra) in quantification of Homo sapiens (hs) Aha1

FPL Name	Function	Sequence
hsAha1 001	CE	GATGTAAATTCCCATTGCTTCTCTTTTTTCTCTTGGAAAGAAAGT
hsAha1 002	CE	TGAACTCTGTTTTGAGGGTGCTTTTTTCTCTTGGAAAGAAAGT
hsAha1 003	CE	GGGTCTACTGACTCTCCATTCATTGTTTTTCTCTTGGAAAGAAAGT
hsAha1 004	CE	CCTTGCGCTCCTCAGTTTTCTTTTTCTCTTGGAAAGAAAGT
hsAha1 005	CE	GGTTTTTGAAGGAGCAGGCTTAGTTTTTCTCTTGGAAAGAAAGT
hsAha1 006	LE	ACGCTGTTTTCATCAGACAAATTTTTTTAGGCATAGGACCCGTGTCT
hsAha1 007	LE	GCTCACACTAATCTCCACTTCATCCTTTTTAGGCATAGGACCCGTGTCT
hsAha1 008	LE	TCATTAAGGCCACGAGATTTGTTTTTTAGGCATAGGACCCGTGTCT
hsAha1 009	LE	TAGGTAAGATCATGCCCTGGGTTTTTAGGCATAGGACCCGTGTCT
hsAha1 010	LE	ACTCCAACAGGTCTGGCCTGTTTTTAGGCATAGGACCCGTGTCT
hsAha1 011	BL	GTCAGGCTCATCTTTGGCAAG

FPL Name	Function	Sequence
hsAha1 012	BL	TAGAAGTTTCACCCCTTCTTCCT
hsAha1 013	BL	AGTGCTGGCTGCCCCACT

Table 14: Probe sequences used with Quantigene Explore Kit (Genospectra) in quantification of Mus musculus (mm) Aha1

FPL Name	Function	Sequence
mmAhsa 1001	CE	CTCGAACGGCCAGGAACATTTTTCTCTTGGAAAGAAAGT
mmAhsa 1002	CE	GCACTTGCCCTCTTCATTTTCTATTTTTCTCTTGGAAAGAAAGT
mmAhsa 1003	CE	TTGATGGATGCCTCCCCATTTTTTCTCTTGGAAAGAAAGT
mmAhsa 1004	CE	AACTCTGTCTTGAGGGTGCTGATTTTTTCTCTTGGAAAGAAAGT
	CE	TTTGGCCTGGCTTTTTGAATTTTTCTCTTGGAAAGAAAGT
	LE	CAAGCTTGTTCACTTCGGTCACCTCTTTTTAGGCATAGGACCCGTGTCT
	LE	CCTGACTTAGAGGTACCTGTCCAGTTTTTTTAGGCATAGGACCCGTGTCT
	LE	GATTTCCACATGTCCTTTGTACTGCACTTTTTTAGGCATAGGACCCGTGTCT
	LE	ATTTTCATCAGACAAATTGGGTTTTTAGGCATAGGACCCGTGTCT
	LE	TAATCTCCACTTCATCCACGCTTTTTTAGGCATAGGACCCGTGTCT
	LE	TTTCACCCCGTCTTCCTTCATTTTTAGGCATAGGACCCGTGTCT
	LE	ACTGTGGGCAAGATCATGCCCTGAGTATTTTTAGGCATAGGACCCGTGTCT
	BL	AAGATAAGTTTGCCTTTCCTGTTG
	BL	CAGTTTGATGGTCCACTCATAGAAG
	BL	CATCTTTGGCAAGGCTCACAC
	BL	TTAAGGCCACGAGATTTGTGTCAGGCT
	BL	GTAAATTCCCACTGCTTCTCTCAGAAG
	BL	CACTGGATCTACTGACTCTCCATTC
	BL	CTCAGTCTTTAGTGCTGGCTGGCC
	BL	GGAGCAGACTTAGCCTTGCAAGT

[0395] Dose-Response Curves in HeLa cells

[0396] Transfection and mRNA quantification: For transfection with dsRNA, HeLa cells were seeded at a density of 2.0 x 10^4 cells/well in 96-well plates and transfected

directly. Transfection of dsRNA was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer. dsRNAs were concentrated from 30 nM in 3 fold dilutions to 14 pM. 24 hours after transfection Hela cells were lysed and Aha1 mRNA levels were quantified with the Quantigene Explore Kit (Genosprectra, Dumbarton Circle Fremont, USA, cat. No. QG-000-02) according to the protocol. Aha1 mRNA levels were normalized to GAP-DH mRNA. For each dsRNA four individual datapoints were collected. dsRNA duplexes unrelated to Aha1 gene were used as control. The activity of a given Aha1 specific dsRNA duplex was expressed as percent Aha1 mRNA concentration in treated cells relative to Aha1 mRNA concentration in cells treated with the control dsRNA duplex. XL-fit was used to calculate IC_{50} values.

[0397] Table 15 provides values for inhibition of Aha1 expression using various dsRNA molecules of the invention.

Table 15: Residual Aha1 mRNA in % of control in HeLa and MLE12 cells treated with 30 nM solutions of various RNAi agents specific for Aha1, and IC50 for selected RNAi agents determined in HeLa cells

Duplex identifier	HeLa cells, residual mRNA [%]	IC_{50} in HeLa cells [nM]	MLE12 cells, residual mRNA [%]
AL-DP-7299	19 ± 3		105 ± 17
AL-DP-7300	7 ± 2		94 ± 13
AL-DP-7301	3 ± 1	0,035	14 ± 3
AL-DP-7302	17 ± 5		61 ± 16
AL-DP-7303	5 ± 2		23 ± 5
AL-DP-7304	7 ± 3		30 ± 7
AL-DP-7305	6 ± 2		26 ± 6
AL-DP-7306	27 ± 8		45 ± 11
AL-DP-7307	16 ± 6	1,1	27 ± 8
AL-DP-7308	13 ± 5	0,21	20 ± 7
AL-DP-7309	10 ± 3	0,36	22 ± 8
AL-DP-7310	51 ± 13		59 ± 13
AL-DP-7311	4 ± 3	0,07	16 ± 4
AL-DP-7312	14 ± 3		40 ± 8
AL-DP-7313	63 ± 14		80 ± 10
AL-DP-7314	97 ± 21		88 ± 10
AL-DP-7315	76 ± 24		77 ± 10
AL-DP-7316	7 ± 2	0,34	23 ± 6
AL-DP-7317	11 ± 3		44 ± 12
AL-DP-7318	4 ± 2	0,29	16 ± 3
AL-DP-7319	38 ± 7		73 ± 21

Duplex identifier	HeLa cells, residual mRNA [%]	IC_{50} in HeLa cells [nM]	MLE12 cells, residual mRNA [%]
AL-DP-7320	16 ± 4	0,07	15 ± 5
AL-DP-7321	130 ± 36		81 ± 16
AL-DP-7322	4 ± 2	0,045	10 ± 3
AL-DP-7323	24 ± 6		55 ± 11
AL-DP-7324	3 ± 2	0,089	12 ± 4
AL-DP-7325	5 ± 2	0,3	12 ± 4
AL-DP-7326	3 ± 1	0,27	19 ± 7
AL-DP-7327	3 ± 1	0,08	13 ± 7
AL-DP-7328	49 ± 14		67 ± 10
AL-DP-7329	6 ± 2	0,2	18 ± 5
AL-DP-7330	64 ± 19		78 ± 10
AL-DP-7331	5 ± 2	0,55	13 ± 5
AL-DP-7332	95 ± 20		82 ± 15
AL-DP-7333	2 ± 1	0,27	9 ± 3
AL-DP-7334	94 ± 17		83 ± 19
AL-DP-7335	11 ± 5		57 ± 11
AL-DP-7336	22 ± 4		63 ± 12
AL-DP-7337	6 ± 2	0,29	20 ± 5
AL-DP-7338	39 ± 6		56 ± 10
AL-DP-7339	10 ± 1		35 ± 6
AL-DP-7340	8 ± 2	0,61	19 ± 6
AL-DP-7341	17 ± 4		55 ± 16
AL-DP-7342	6 ± 4	0,5	15 ± 3
AL-DP-7343	26 ± 4		103 ± 19
AL-DP-7344	5 ± 2		38 ± 11
AL-DP-7345	53 ± 22		63 ± 15
AL-DP-7346	22 ± 4		44 ± 11
AL-DP-9250	4 ± 1		
AL-DP-9251	51 ± 9		
AL-DP-9252	19 ± 2		
AL-DP-9253	11 ± 1		
AL-DP-9254	7 ± 1		
AL-DP-9255	5 ± 0		
AL-DP-9256	5 ± 0		
AL-DP-9257	7 ± 0		
AL-DP-9258	9 ± 1		
AL-DP-9259	7 ± 1		
AL-DP-9260	15 ± 3		
AL-DP-9261	21 ± 2		
AL-DP-9262	24 ± 4		

Duplex identifier	HeLa cells, residual mRNA [%]	IC_{50} in HeLa cells [nM]	MLE12 cells, residual mRNA [%]
AL-DP-9263	25 ± 6		
AL-DP-9264	7 ± 2		
AL-DP-9265	8 ± 1		
AL-DP-9266	11 ± 2		
AL-DP-9267	45 ± 4		
AL-DP-9268	9 ± 1		
AL-DP-9269	5 ± 1		
AL-DP-9270	6 ± 1		
AL-DP-9271	6 ± 2		
AL-DP-9272	26 ± 8		
AL-DP-9273	11 ± 1		
AL-DP-9274	7 ± 1		
AL-DP-9275	8 ± 1		
AL-DP-9276	4 ± 1		
AL-DP-9277	10 ± 1		
AL-DP-9278	2 ± 0		
AL-DP-9279	3 ± 0		
AL-DP-9280	12 ± 1		
AL-DP-9281	8 ± 2		
AL-DP-9282	3 ± 0		
AL-DP-9283	6 ± 1		
AL-DP-9284	39 ± 2		
AL-DP-9285	4 ± 1		
AL-DP-9286	61 ± 11		
AL-DP-9287	3 ± 1		
AL-DP-9288	27 ± 5		
AL-DP-9289	6 ± 1		

[0398] In summary, AL-DP-7301, AL-DP-7308, AL-DP-7311, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, and AL-DP-7342 inhibited Aha1 expression by at least 80% in both HeLa and MLE12 cells, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337 inhibited Aha1 expression by at least 80% in HeLa cells and by at least 70% in MLE12 cells, AL-DP-7304, AL-DP-7312, AL-DP-7339, and AL-DP-7344 inhibited Aha1 expression by at least 80% in HeLa cells and by at least 60% in MLE12 cells, AL-DP-7306, AL-DP-7317, and AL-DP-7346 inhibited Aha1 expression by at least 70% in HeLa cells and by at least 50% in MLE12 cells, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, and AL-DP-7341 inhibited Aha1 expression by at least 40% in both HeLa and

MLE12 cells, and AL-DP-7302, AL-DP-7315, AL-DP-7319, AL-DP-7328, AL-DP-7330, AL-DP-7336, and AL-DP-7345, inhibited Aha1 expression by at least 20% in both HeLa and MLE12 cells.

[0399] In addition, AL-DP-9250, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9278, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9285, AL-DP-9287, and AL-DP-9289 inhibited Aha1 expression by at least 80% in HeLa cells, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9272, and AL-DP-9288 inhibited Aha1 expression by at least 70% in HeLa cells, AL-DP-9263 inhibited Aha1 expression by at least 60% in HeLa cells, AL-DP-9267 inhibited Aha1 expression by at least 50% in HeLa cells, AL-DP-9251 inhibited Aha1 expression by at least 40% in HeLa cells, and AL-DP-9286 inhibited Aha1 expression by at least 30% in HeLa cells.

[0400] Other Embodiments

[0401] The detailed description set-forth above is provided to aid those skilled in the art in practicing the present invention. However, the invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed because these embodiments are intended as illustration of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description which do not depart from the spirit or scope of the present inventive discovery. Such modifications are also intended to fall within the scope of the appended claims.

[0402] References Cited

[0403] Citation of a reference herein shall not be construed as an admission that such is prior art to the present invention. Specifically intended to be within the scope of the present invention, and incorporated herein by reference in its entirety, is the following publication: Wang, X. et al., Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis, Cell (2006 Nov 17) 127(4):673-5.

CLAIMS

What is claimed is:

1. A dsRNA for inhibiting functional Aha protein expression in a cell, said dsRNA comprising a sense strand and an antisense strand,

wherein said antisense strand comprises a region of complementarity having a sequence substantially complementary to an Aha target sequence, wherein said target sequence is less than 30 nucleotides in length,

wherein said sense strand is substantially complimentary to said antisense strand, and

wherein said dsRNA, upon contact with a cell expressing functional Aha protein, inhibits functional Aha protein expression by at least 20%.

2. A dsRNA according to claim 1, wherein said Aha target sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 12-56.

3. A dsRNA according to claim 1, wherein said dsRNA comprises a sense strand having a sequence selected from the group consisting of SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID NO: 123, SEQ ID NO: 125, SEQ ID NO: 127, SEQ ID NO: 129, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 139, SEQ ID NO: 141, SEQ ID NO: 143, and SEQ ID NO: 145; and

an antisense strand complementary to the sense strand having a sequence selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 140, SEQ ID NO: 142, SEQ ID NO: 144, and SEQ ID NO: 146.

4. A dsRNA according to claim 1, wherein said dsRNA comprises a sense

strand having a sequence of SEQ ID NO: 57 and an antisense strand complementary to the sense strand having a sequence of SEQ ID NO: 58.

5. A dsRNA according to claim 1, wherein said dsRNA comprises a sense strand having a sequence of SEQ ID NO: 69 and an antisense strand complementary to the sense strand having a sequence of SEQ ID NO: 70.

6. A dsRNA according to claim 1, wherein said dsRNA comprises a sense strand having a sequence of SEQ ID NO: 81 and an antisense strand complementary to the sense strand having a sequence of SEQ ID NO: 82.

7. A vector for expressing a shRNA for inhibiting functional Aha1 expression in a cell, said vector comprising a sense strand, a hairpin linker, and an antisense strand,

wherein said sense strand comprising a region of complementarity having a sequence substantially complementary to an Aha target sequence, wherein said target sequence is less than 30 nucleotides in length,

wherein said antisense strand is substantially complimentary to said sense strand, and

wherein said dsRNA, upon contact with a cell expressing functional Aha protein, inhibits functional Aha protein expression by at least 20%.

8. A vector according to claim 7, wherein said Aha target sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 12-56.

9. A vector according to claim 7, wherein said vector comprises a sense strand having a sequence selected from the group consisting of SEQ ID NO: 147, SEQ ID NO: 149, and SEQ ID NO: 151; and

an antisense strand having a sequence selected from the group consisting of SEQ ID NO: 148, SEQ ID NO: 150, and SEQ ID NO: 152.

10. A shRNA for inhibiting functional Aha1 protein expression in a cell, said shRNA comprising a region of complementarity having a sequence substantially complementary to an Aha target sequence, and wherein said target sequence is less than 30 nucleotides in length, and

wherein said shRNA, upon contact with a cell expressing functional Aha protein, inhibits functional Aha protein expression by at least 20%.

11. A shRNA according to claim 10, wherein said Aha target sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 12-56.

12. A shRNA according to claim 10, wherein said shRNA comprises a sequence selected from the group consisting of SEQ ID NO: 153, SEQ ID NO: 154, and SEQ ID NO: 155;

13. A cell or cell population comprising a dsRNA according to claim 1.

14. A cell or cell population comprising a vector according to claim 7.

15. A cell or cell population comprising a shRNA according to claim 10.

16. An isolated antibody that specifically binds functional Aha1, the Hsp90 ATPase binding site for functional Aha1, and/or the functional Aha1-Hsp90 ATPase complex.

17. An agent that decreases intracellular levels of functional Aha1 protein, said agent selected from the group consisting of a small molecule, an antibody, an antisense nucleic acid, an aptamer, a dsRNA, a ribozyme, and any combination thereof.

18. A method of treating a disease associated with misfolding of a protein, the method comprising administering to a subject in need thereof a therapeutically effective amount of at least one agent that decreases intracellular levels of functional Aha1 protein, wherein said agent is selected from the group consisting of a small molecule, an antibody, an antisense nucleic acid, an aptamer, an siRNA, a ribozyme, and combinations thereof.

19. A method according to claim 18, wherein the disease is selected from the group consisting of cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease.

20. A method according to claim 18, wherein the disease is CF.

21. A method according to claim 18, wherein the misfolded protein is a misfolded CFTR.

22. A method according to claim 18, wherein the misfolded protein is a ΔF508 protein.

23. A method of treating a disease associated with misfolding of a protein, the method comprising administering to a subject in need thereof a therapeutically effective amount of at least one dsRNA inhibitor of functional Aha1 expression, said dsRNA comprising a sense strand and an antisense strand,

wherein said antisense strand comprises a region of complementarity having a sequence substantially complementary to an Aha target sequence, wherein said target sequence is less than 30 nucleotides in length,

wherein said sense strand is substantially complimentary to said antisense strand, and

wherein said dsRNA, upon contact with a cell expressing functional Aha protein, inhibits functional Aha protein expression by at least 20%.

24. A method according to claim 23, wherein the disease is selected from the

group consisting of cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease.

25. A method according to claim 23, wherein the disease is CF.

26. A method according to claim 23, wherein the misfolded protein is a misfolded CFTR.

27. A method according to claim 23, wherein the misfolded protein is a ΔF508 protein.

28. A method of treating a disease associated with misfolding of a protein, the method comprising administering to a subject in need thereof a therapeutically effective amount of at least one dsRNA inhibitor of functional Aha1 expression, wherein the dsRNA inhibitor comprises a sequence selected on the basis of

a) the dsRNA comprising a sense strand sequence of about 19 nucleotides to about 25 nucleotides and an antisense strand sequence of about 19 nucleotides to about 25 nucleotides; and

b) the sense strand sequence or antisense strand sequence comprises no more than 15 contiguous nucleotides identical to a contiguous sequence comprised by a 5' untranslated region, a 3' untranslated region, an intron or an exon of any gene or mRNA other than functional Aha1.

29. A method according to claim 28, wherein the disease is selected from the group consisting of cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease.

30. A method according to claim 28, wherein the disease is CF.

31. A method according to claim 28, wherein the misfolded protein is a misfolded CFTR.

32. A method according to claim 28, wherein the misfolded protein is a ΔF508 protein.

33. A method of screening an agent for treating a disease associated with misfolding of a protein, the method comprising:

providing a cell or cell population expressing functional Aha1;

administering a candidate agent to the cell or cell population;

quantifying functional Aha1 activity in the cell or cell population; and

determining whether the candidate agent decreases functional Aha1 activity in the cell or cell population, whereby a decrease in functional Aha1 activity is indicative of

reducing misfolding of the protein.

34. A method according to claim 33, wherein the candidate agent is an dsRNA which inhibits functional Aha1 expression.

35. A method according to claim 34, wherein the dsRNA comprises a) a sequence of from about 19 nucleotides to about 25 nucleotides, and b) the sequence comprises no more than 15 contiguous nucleotides identical to a contiguous sequence comprised by a 5' untranslated region, a 3' untranslated region, an intron or an exon of any gene or mRNA other than an Aha gene or mRNA.

36. A method according to claim 35, wherein the Aha gene or mRNA is a human Aha gene or mRNA.

37. A method according to claim 33, wherein the disease is selected from the group consisting of cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease.

38. A method according to claim 33, wherein the disease is CF.

39. A method according to claim 33, wherein the misfolded protein is selected from the group consisting of a misfolded CFTR, a misfolded fibrillin, a misfolded alpha galactosidase, a misfolded beta glucocerebrosidase, a misfolded rhodopsin, aggregated an amyloid beta and tau, an aggregated amylin, an aggregated alpha synuclein and an aggregated prion.

40. A method according to claim 33, wherein the misfolded protein is a misfolded CFTR.

41. A method according to claim 33, wherein the misfolded protein is a ΔF508 protein.

42. A method of screening for an agent for treating a disease associated with misfolding of a protein, the method comprising:

providing a cell or cell population which expresses functional Aha1;

administering a candidate agent to the cell or cell population;

quantifying Hsp90/ADP complex, Hsp90/ATP complex or a combination thereof in the cell or cell population; and

determining whether the candidate agent decreases the quantity of Hsp90/ADP complex, Hsp90/ATP complex or the combination thereof in the cell or cell population, whereby a decrease in quantity of Hsp90/ADP complex or Hsp90/ATP complex is indicative of decreasing misfolding of the protein.

43. A method according to claim 42, wherein the candidate agent is an dsRNA

which inhibits functional Aha1 expression.

44. A method according to claim 43, wherein the dsRNA comprises a) a sequence of from about 19 nucleotides to about 25 nucleotides, and b) the sequence comprises no more than 15 contiguous nucleotides identical to a contiguous sequence comprised by a 5' untranslated region, a 3' untranslated region, an intron or an exon of any gene or mRNA other than an Aha gene or mRNA.

45. A method according to claim 44, wherein the Aha gene or mRNA is a human Aha gene or mRNA.

46. A method according to claim 42, wherein the disease is selected from the group consisting of cystic fibrosis (CF), Marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease and Creutzfeldt-Jakob disease.

47. A method according to claim 42, wherein the disease is CF.

48. A method according to claim 42, wherein the misfolded protein is selected from the group consisting of a misfolded CFTR, a misfolded fibrillin, a misfolded alpha galactosidase, a misfolded beta glucocerebrosidase, a misfolded rhodopsin, aggregated an amyloid beta and tau, an aggregated amylin, an aggregated alpha synuclein and an aggregated prion.

49. A method according to claim 42, wherein the misfolded protein is a misfolded CFTR.

50. A method according to claim 42, wherein the misfolded protein is a ΔF508 protein.

51. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human Aha gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding an Aha gene, and wherein said region of complementarity is less than 30 nucleotides in length and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO:

214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339.

52. The dsRNA of claim 51, wherein said Aha gene is an Aha1 gene, and preferably a Homo sapiens Aha1 gene.

53. The dsRNA of claim 51, wherein, upon contact with a cell expressing said Aha gene, the dsRNA inhibits expression of said Aha gene in said cell by at least 20%.

54. The dsRNA of claim 53, wherein said at least 20% inhibition of expression of an Aha gene is effected in HeLa and/or MLE12 cells.

55. The dsRNA claim 51, wherein the dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289.

56. The dsRNA of claim 51, wherein said dsRNA comprises at least one modified nucleotide.

57. The dsRNA of claim 56, wherein said modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

58. The dsRNA of claim 56, wherein said modified nucleotide is chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

59. A cell comprising the dsRNA of claim 51.

60. A pharmaceutical composition for inhibiting the expression of an Aha gene in an organism, comprising a dsRNA and a pharmaceutically acceptable carrier, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding an Aha gene, and wherein said region of complementarity is less than 30 nucleotides in length, and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID

NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335,

SEQ ID NO: 337, and SEQ ID NO: 339.

61. The pharmaceutical composition of claim 60, wherein said Aha gene is an Aha1 gene, and preferably a Homo sapiens Aha1 gene.

62. The pharmaceutical composition of claim 60, wherein, upon contact with a cell expressing said Aha gene, the dsRNA inhibits expression of said Aha gene in said cell by at least 20%.

63. The pharmaceutical composition of claim 62, wherein said at least 20% inhibition of expression of an Aha gene is effected in HeLa and/or MLE12 cells.

64. The pharmaceutical composition of claim 60, wherein the dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289.

65. The pharmaceutical composition of 60, wherein said dsRNA comprises at least one modified nucleotide.

66. The pharmaceutical composition of claim 65, wherein said modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

67. The pharmaceutical composition of claim 65, wherein said modified nucleotide is chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

68. A method for inhibiting the expression of an Aha gene in a cell, the method comprising:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the

dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding Aha1, and wherein said region of complementarity is less than 30 nucleotides in length and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263,

SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an Aha gene, thereby inhibiting expression of an Aha gene in the cell.

69. The method of claim 68, wherein the gene is an Aha1 gene, and preferably a homo sapiens Aha1 gene.

70. The method of claim 68, wherein the dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289.

71. The method of claim 68, wherein the method is performed *in vitro*.

72. A method of treating, preventing or managing pathological processes mediated by Aha expression comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a dsRNA, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding Aha1, and wherein said region of complementarity is less than 30 nucleotides in length and wherein the dsRNA effects

cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290, SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313,

SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339.

73. The method of claim 72, wherein the dsRNA is chosen from the group of AL-DP-7301, AL-DP-7308, AL-DP-7318, AL-DP-7320, AL-DP-7322, AL-DP-7324, AL-DP-7325, AL-DP-7326, AL-DP-7327, AL-DP-7329, AL-DP-7331, AL-DP-7333, AL-DP-7340, AL-DP-7342, AL-DP-7303, AL-DP-7305, AL-DP-7307, AL-DP-7309, AL-DP-7316, and AL-DP-7337, AL-DP-7304, AL-DP-7312, AL-DP-7339, AL-DP-7344, AL-DP-7306, AL-DP-7317, AL-DP-7346, AL-DP-7310, AL-DP-7323, AL-DP-7335, AL-DP-7338, AL-DP-7341, AL-DP-7302, AL-DP-7315, AL-DP-7328, AL-DP-7330, AL-DP-7336, AL-DP-7345, AL-DP-9250, AL-DP-9251, AL-DP-9252, AL-DP-9253, AL-DP-9254, AL-DP-9255, AL-DP-9256, AL-DP-9257, AL-DP-9258, AL-DP-9259, AL-DP-9260, AL-DP-9261, AL-DP-9262, AL-DP-9263, AL-DP-9264, AL-DP-9265, AL-DP-9266, AL-DP-9267, AL-DP-9268, AL-DP-9269, AL-DP-9270, AL-DP-9271, AL-DP-9272, AL-DP-9273, AL-DP-9274, AL-DP-9275, AL-DP-9276, AL-DP-9277, AL-DP-9279, AL-DP-9280, AL-DP-9281, AL-DP-9282, AL-DP-9283, AL-DP-9284, AL-DP-9285, AL-DP-9286, AL-DP-9287, AL-DP-9288, and AL-DP-9289.

74. A vector for inhibiting the expression of an Aha gene in a cell, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA, wherein one of the strands of said dsRNA is substantially complementary to at least a part of a mRNA encoding Aha1 and wherein said dsRNA is less than 30 base pairs in length and wherein the dsRNA effects cleavage of an mRNA encoding an Aha gene within the target sequence of a second dsRNA having a sense strand chosen from the group of SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166, SEQ ID NO: 168, SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 194, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 202, SEQ ID NO: 204, SEQ ID NO: 206, SEQ ID NO: 208, SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214, SEQ ID NO: 216, SEQ ID NO: 218, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 228, SEQ ID NO: 230, SEQ ID NO: 232, SEQ ID NO: 234, SEQ ID NO: 236, SEQ ID NO: 238, SEQ ID NO: 240, SEQ ID NO: 242, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 250, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 256, SEQ ID NO: 258, SEQ ID NO: 260, SEQ ID NO: 262, SEQ ID NO: 264, SEQ ID NO: 266, SEQ ID NO: 268, SEQ ID NO: 270, SEQ ID NO: 272, SEQ ID NO: 274, SEQ ID NO: 276, SEQ ID NO: 278, SEQ ID NO: 280, SEQ ID NO: 282, SEQ ID NO: 284, SEQ ID NO: 286, SEQ ID NO: 288, SEQ ID NO: 290,

SEQ ID NO: 292, SEQ ID NO: 294, SEQ ID NO: 296, SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 302, SEQ ID NO: 304, SEQ ID NO: 306, SEQ ID NO: 308, SEQ ID NO: 310, SEQ ID NO: 312, SEQ ID NO: 314, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326, SEQ ID NO: 328, SEQ ID NO: 330, SEQ ID NO: 332, SEQ ID NO: 334, SEQ ID NO: 336, and SEQ ID NO: 338, and an antisense strand complementary to the latter sense strand and chosen from the group of SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167, SEQ ID NO: 169, SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 195, SEQ ID NO: 199, SEQ ID NO: 201, SEQ ID NO: 203, SEQ ID NO: 205, SEQ ID NO: 207, SEQ ID NO: 209, SEQ ID NO: 211, SEQ ID NO: 213, SEQ ID NO: 215, SEQ ID NO: 217, SEQ ID NO: 219, SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 225, SEQ ID NO: 227, SEQ ID NO: 229, SEQ ID NO: 231, SEQ ID NO: 233, SEQ ID NO: 235, SEQ ID NO: 237, SEQ ID NO: 239, SEQ ID NO: 241, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 247, SEQ ID NO: 249, SEQ ID NO: 251, SEQ ID NO: 253, SEQ ID NO: 255, SEQ ID NO: 257, SEQ ID NO: 259, SEQ ID NO: 261, SEQ ID NO: 263, SEQ ID NO: 265, SEQ ID NO: 267, SEQ ID NO: 269, SEQ ID NO: 271, SEQ ID NO: 273, SEQ ID NO: 275, SEQ ID NO: 277, SEQ ID NO: 279, SEQ ID NO: 281, SEQ ID NO: 283, SEQ ID NO: 285, SEQ ID NO: 287, SEQ ID NO: 289, SEQ ID NO: 291, SEQ ID NO: 293, SEQ ID NO: 295, SEQ ID NO: 297, SEQ ID NO: 299, SEQ ID NO: 301, SEQ ID NO: 303, SEQ ID NO: 305, SEQ ID NO: 307, SEQ ID NO: 309, SEQ ID NO: 311, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 319, SEQ ID NO: 321, SEQ ID NO: 323, SEQ ID NO: 325, SEQ ID NO: 327, SEQ ID NO: 329, SEQ ID NO: 331, SEQ ID NO: 333, SEQ ID NO: 335, SEQ ID NO: 337, and SEQ ID NO: 339.

75. A cell comprising the vector of claim 74.

FIG. 1

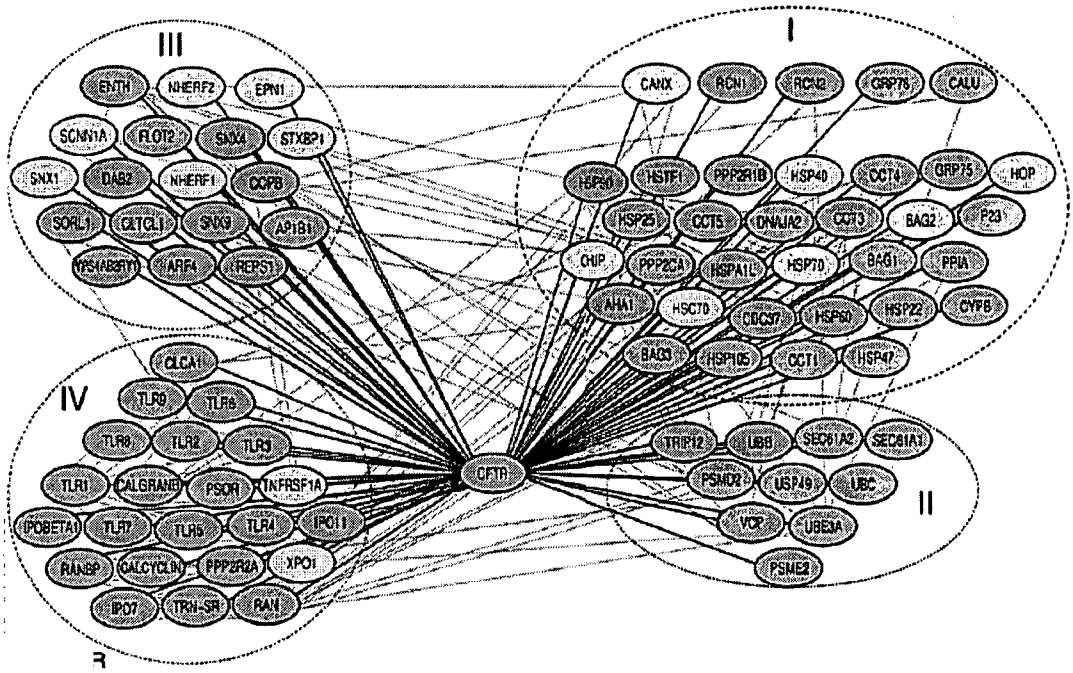

III
I
IV
II
ENTH
NHERF2
EPN1
SCNN1A
FLOT2
SNX4
STXBP1
SNX1
DAB2
NHERF1
CCP8
SORL1
CLTCL1
SNX9
AP1B1
VPS45(ABBP1)
ARF4
REPS1
CALU
HSPA8
HSTF1
PPP2R1B
HSP40
CCT4
GRP75
HOP
HSP25
CCT5
DNAJA2
CCT3
BAG2
P23
CHIP
PPP2CA
HSPA1L
HSP70
BAG1
PPIA
AHA1
HSCT0
CDC37
HSP90
HSP22
CYPB
BAG3
HSP105
CCT1
HSP47
OLCA1
TLR9
TLR6
TLR8
TLR2
TLR3
TLR1
CALGRAN0
PSOR1
TNFRSF1A
IPOBETA1
TLR7
TLR5
TLR4
IPO11
RANBP
CALCYCLIN
FPP2R2A
XPO1
IPO7
TRN-SR
RAN
TRIP12
UBB
SEC61A2
SEC61A1
PSMD2
USP49
UBC
VCP
UBE3A
PSME2
CFTR

FIG. 2A

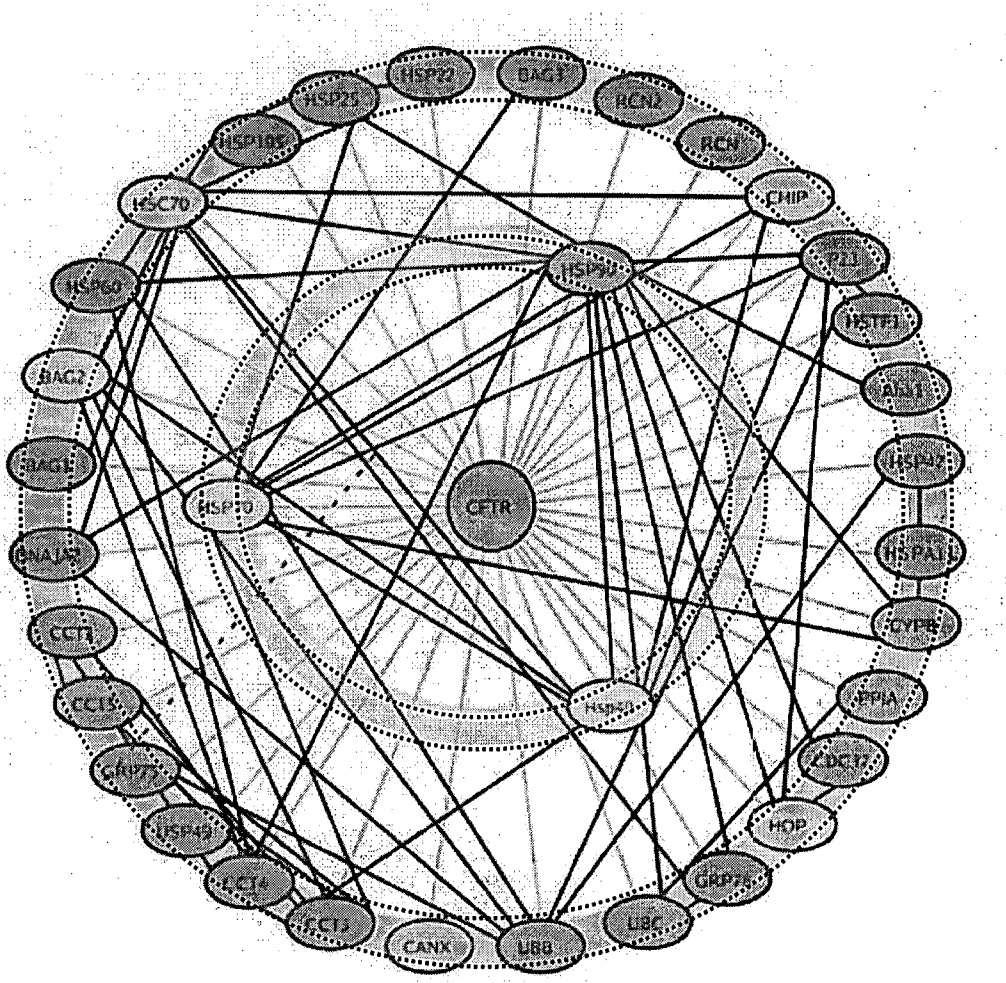

HSP27
BAG1
RCN2
RCN1
HSP105
CHIP
HSC70
P23
HSP90
HSTF1
BAG2
AHA1
BAG3
HSP40
HSP70
CFTR
DNAJB1
HSPA1L
CYPB
CCT1
CCT5
HSP90
PPIA
CAP7
CDC37
HSP40
HOP
CCT4
GRP78
CCT2
UBC
CANX
UBB

FIG. 2B

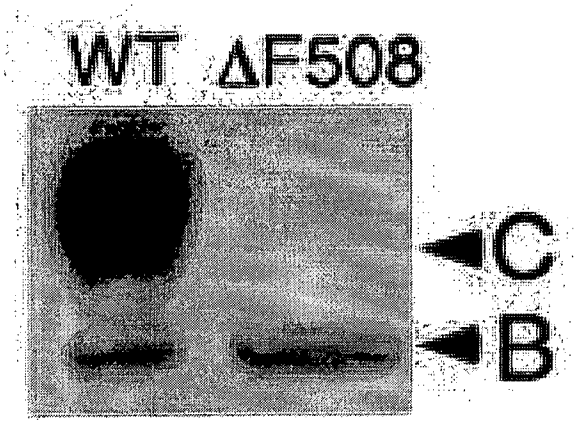


FIG. 3

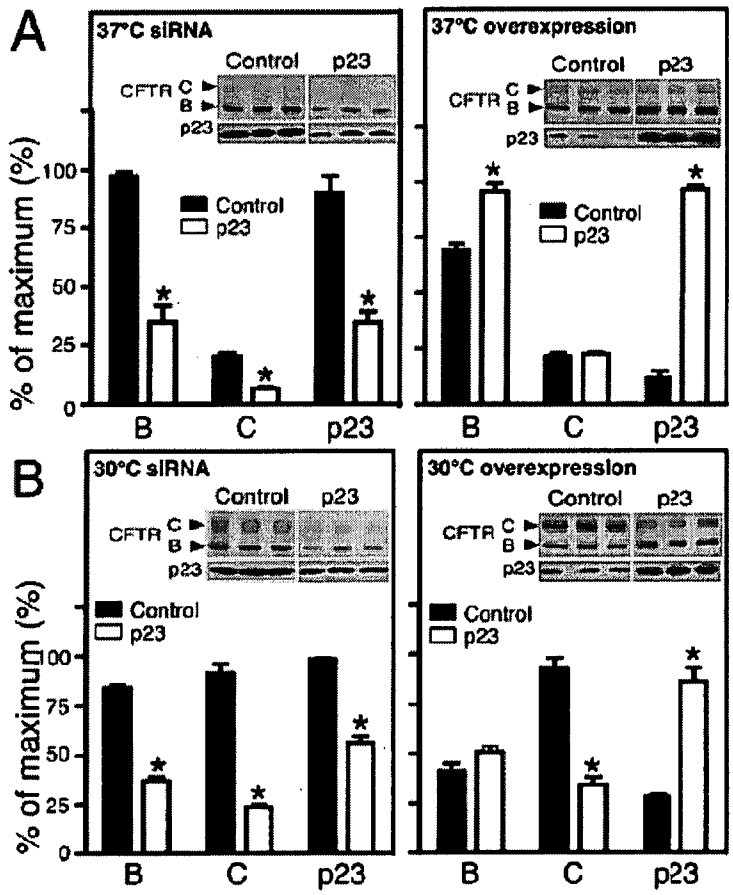

A
37°C siRNA
Control
p23
CFTR C
B
p23
Control
p23
% of maximum (%)
100
75
50
25
0
B
C
p23
37°C overexpression
B
30°C siRNA
30°C overexpression

FIG. 4

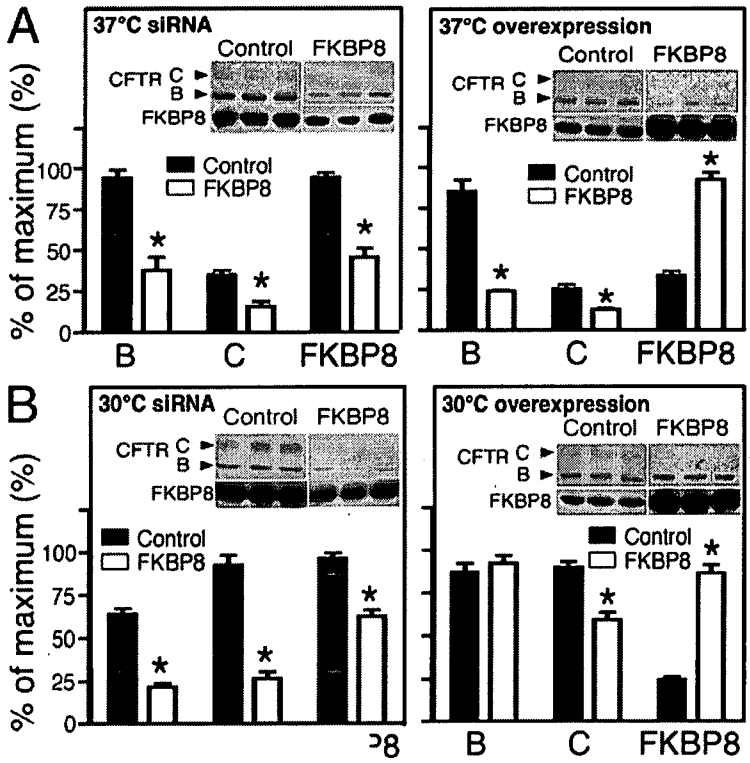

A
37°C siRNA
Control FKBP8
CFTR C
B
FKBP8
Control
FKBP8
% of maximum (%)
100
75
50
25
0
B C FKBP8
37°C overexpression
Control FKBP8
CFTR C
B
FKBP8
Control
FKBP8
B C FKBP8
B
30°C siRNA
Control FKBP8
CFTR C
B
FKBP8
Control
FKBP8
% of maximum (%)
100
75
50
25
0
B C FKBP8
30°C overexpression
Control FKBP8
CFTR C
B
FKBP8
Control
FKBP8
B C FKBP8

FIG. 5

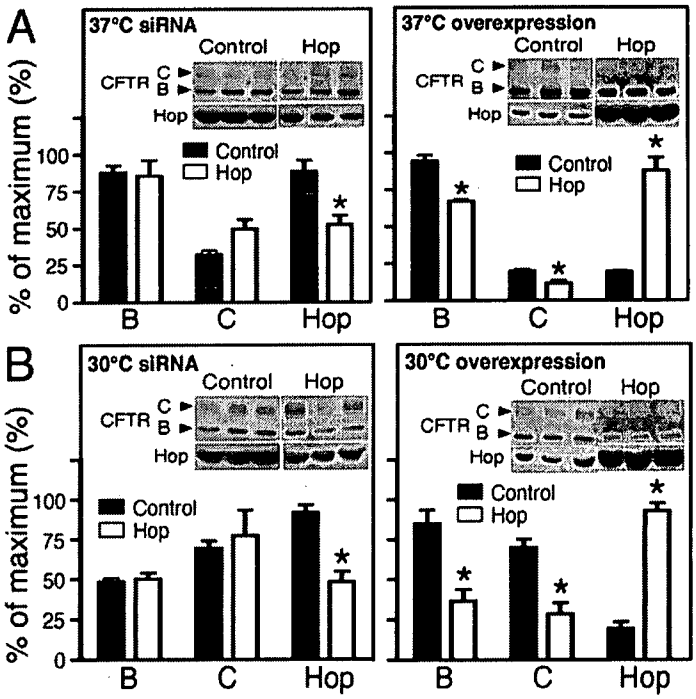

A
37°C siRNA
Control
Hop
CFTR
C
B
Hop
Control
Hop
% of maximum (%)
100
75
50
25
0
B
C
Hop
37°C overexpression
B
30°C siRNA
30°C overexpression

FIG. 6

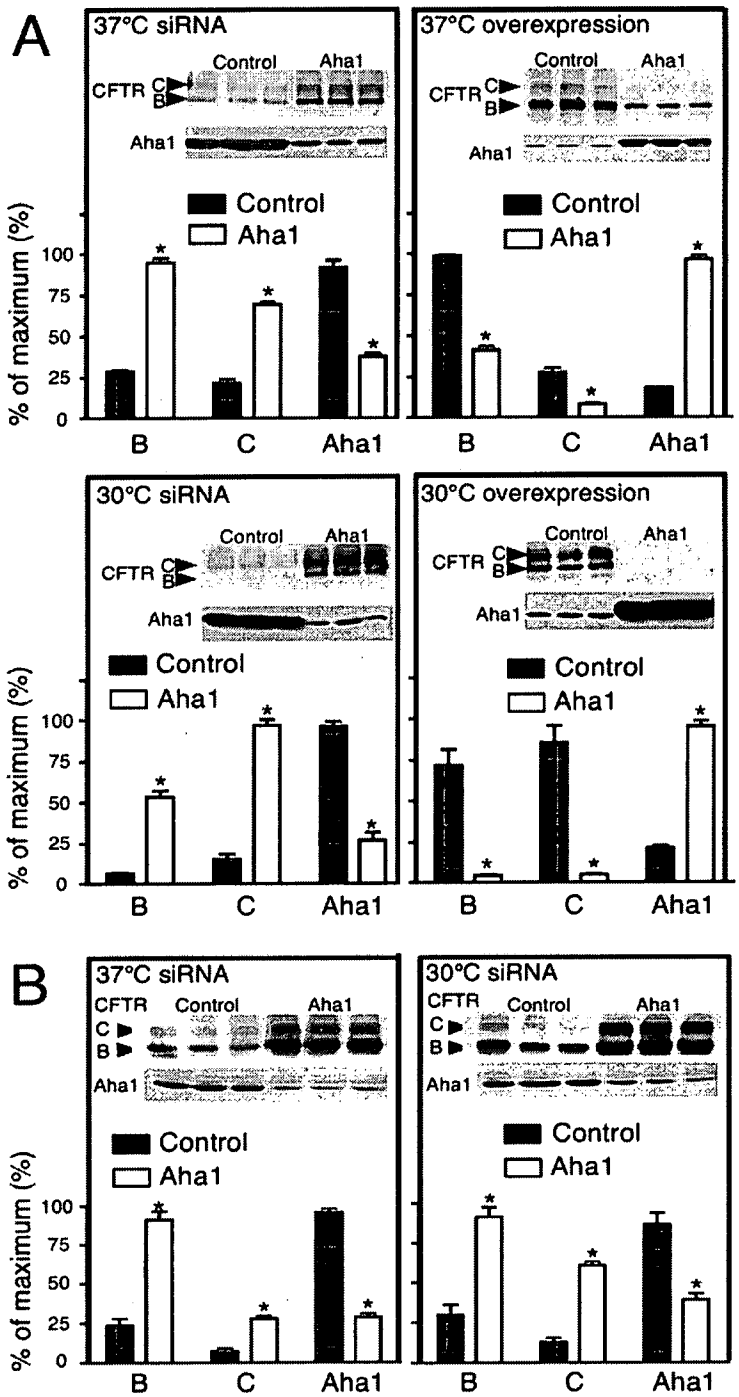

A
37°C siRNA
37°C overexpression
30°C siRNA
30°C overexpression
Control
Aha1
CFTR
C
B
Aha1
Control
Aha1
% of maximum (%)
0
25
50
75
100
B
C
Aha1
B
37°C siRNA
30°C siRNA
CFTR
Control
Aha1
C
B
Aha1
Control
Aha1
% of maximum (%)
0
25
50
75
100
B
C
Aha1

FIG. 7

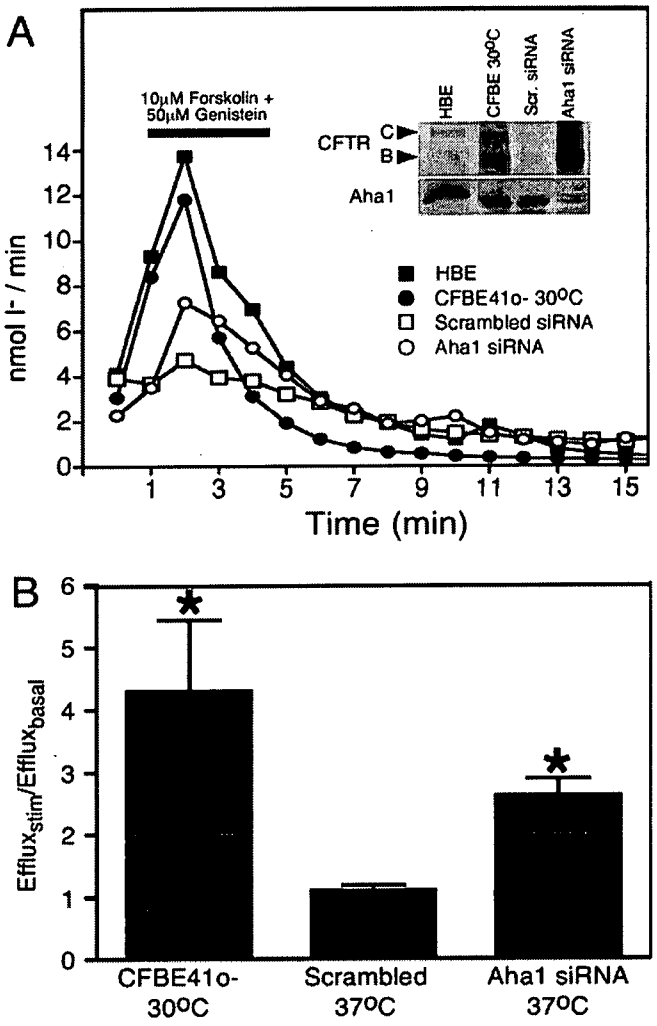

A
10μM Forskolin +
50μM Genistein
HBE
CFBE 30°C
Scr. siRNA
Aha1 siRNA
CFTR C
B
Aha1
HBE
CFBE41o- 30°C
Scrambled siRNA
Aha1 siRNA
nmol I⁻ / min
Time (min)
B
Efflux stim/Efflux basal
CFBE41o-
30°C
Scrambled
37°C
Aha1 siRNA
37°C

FIG. 8A

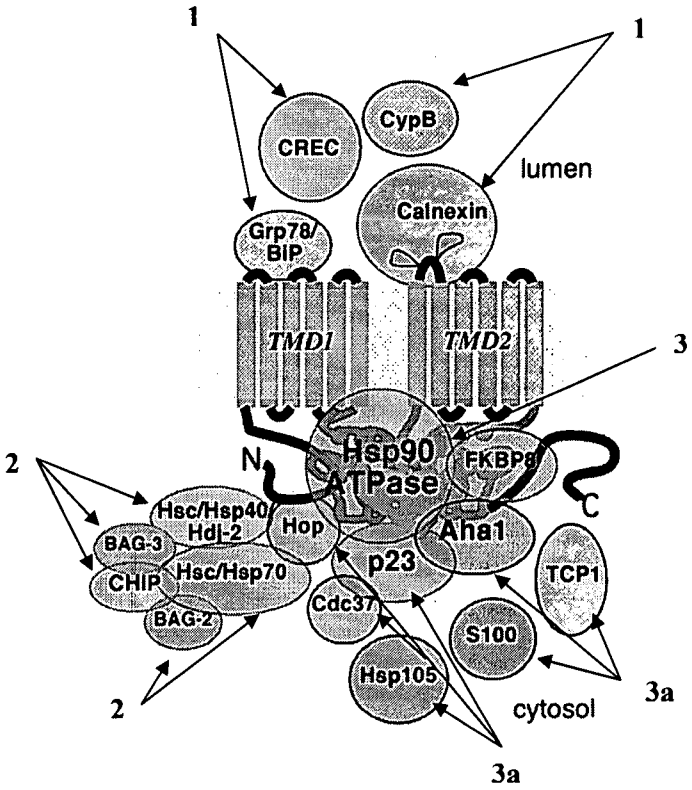

1
1
CypB
CREC
lumen
Calnexin
Grp78/
BIP
TMD1
TMD2
3
2
N
Hsp90
ATPase
FKBP8
C
Hsc/Hsp40
Hdj-2
Hop
Aha1
BAG-3
p23
TCP1
CHIP
Hsc/Hsp70
Cdc37
BAG-2
S100
Hsp105
cytosol
3a
2
3a

FIG. 8B

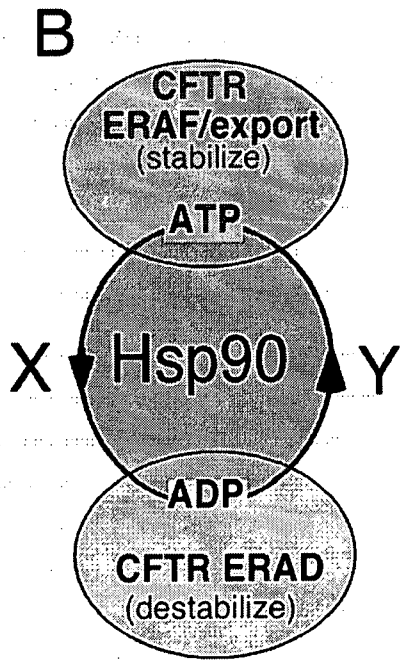

B
CFTR
ERAF/export
(stabilize)
ATP
X
Hsp90
Y
ADP
CFTR ERAD
(destabilize)

FIG. 8C

C

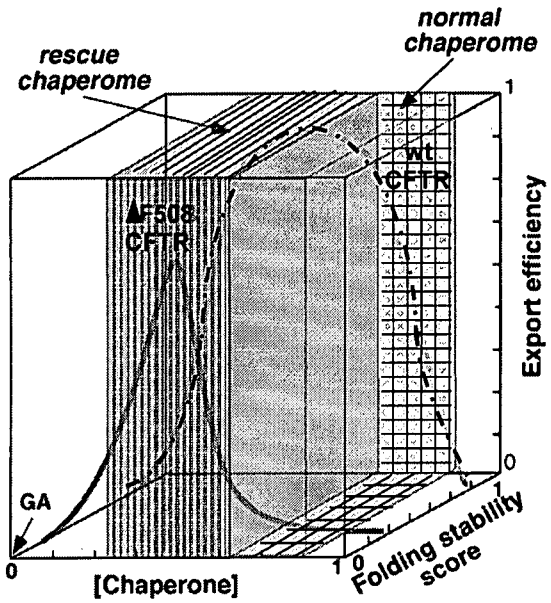
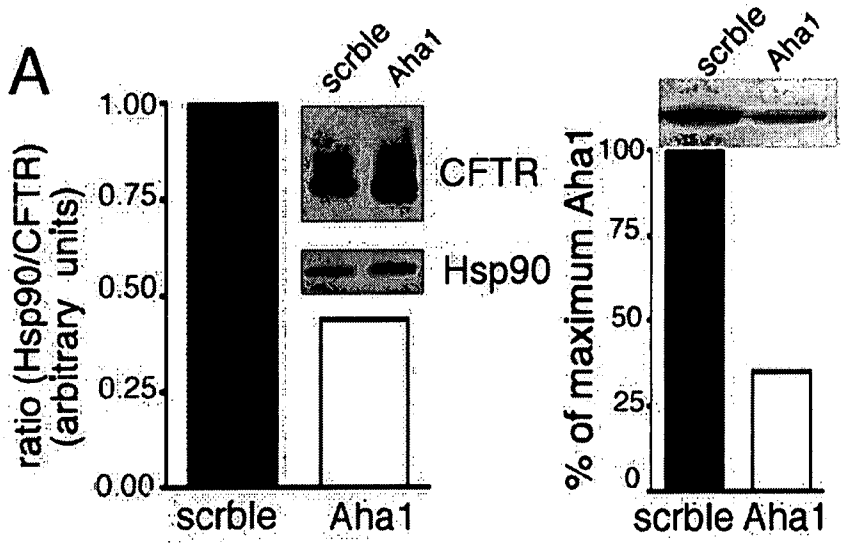

rescue chaperome
normal chaperome
wt CFTR
ΔF508 CFTR
GA
0
[Chaperone]
1
0
Folding stability score
1
0
Export efficiency

FIG. 9


A
scrble
Aha1
CFTR
Hsp90
ratio (Hsp90/CFTR)
(arbitrary units)
1.00
0.75
0.50
0.25
0.00
scrble
Aha1
% of maximum Aha1
100
75
50
25
0
scrble
Aha1