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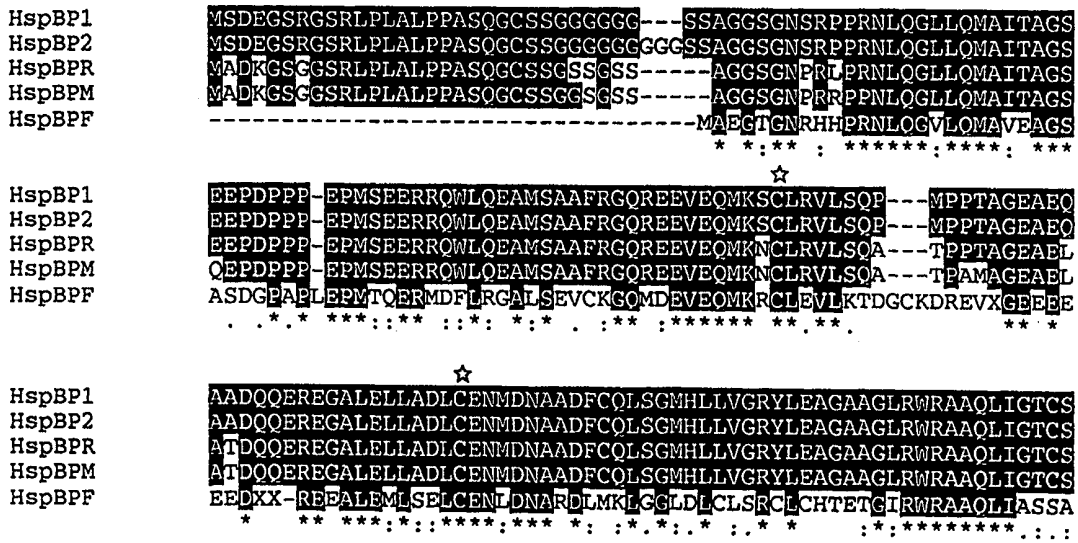
(54) **Title:** DNA ENCODING PROTEINS THAT INHIBIT Hsp70 FUNCTION



(57) **Abstract**

Human heat–shock protein–binding proteins (HspBP–1 and HspBP–2) are disclosed with the polynucleotides which identify and encode them. Genetically engineered expression vectors and host cells comprising the nucleic acid sequences encoding heat–shock protein–binding proteins (HspBP) are also disclosed and a method for producing HspBP polypeptides. Also provided is a process of using HspBP for abrogating heat shock–protein activity in the prevention or treatment of diseases associated with such activity.

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DNA ENCODING PROTEINS THAT INHIBIT Hsp70 FUNCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is based on U.S. Provisional Application 60/109,351, entitled "Inhibition of HSP 70 ATPase Activity and Protein Renaturation By A Novel HSP-Binding Protein," filed by the same inventors on November 20, 1998.

BACKGROUND OF THE INVENTION

Field of Invention

This invention generally relates to the field of molecular medicine and in particular to a novel set of heat-shock protein- binding proteins, and to polynucleotides encoding them, useful in the regulation of physiological events in which one or more 70 kiloDalton heat-shock proteins (Hsp70) are involved, such as normal development, cellular stress responses, heart disease, and cancer.

Description of the Related Art

Practically all organisms respond to heat by inducing the synthesis of a group of proteins called the heat-shock proteins. Although the details of this response vary among organisms, the involvement of Hsp70 and Hsp90 gene families is known to be highly conserved. More recently, it has come to be known that heat shock proteins can be induced by a variety of stress-related stimuli besides heat: anoxia, ethanol and certain heavy metal ions also stimulate increased expression and activity by these proteins. Hence, such proteins commonly are more broadly referred to by those in the art as heat stress or, simply, stress proteins.

Interestingly, stress proteins also are present within

cells under non-stressful conditions (i.e. under normal physiological conditions). Genetic studies in bacteria and lower eukaryotes have demonstrated that Hsp70 is essential for growth at either high or normal temperatures, indicating a crucial role in normal cellular physiology. See generally S. Lindquist and E.A. Craig, *The Heat Shock Proteins*, Annual Revue of Genetics. 22:631-77 (1988).

Particular attention has been focused on Hsp70, a member of a multigene family whose genes are expressed under a wide variety of environmental conditions and are found in all cells. As shown schematically in Fig. 1, Hsp70 and related proteins (such as Hsp72, Hsc70, and Grp78) contain an ATPase domain, a substrate binding domain, and a coupling domain. S. Lindquist and E.A. Craig, Annual Revue of Genetics. 22:631-77 (1988).

In terms of function, studies have shown that Hsp70 plays a role in DNA replication, transport of proteins across membranes, binding of proteins to the endoplasmic reticulum, and uncoating clathrin coated vesicles. S. Lindquist and E.A. Craig, Annual Revue of Genetics. 22:631-77 (1988). Furthermore, Hsp70 is known to associate with nonsterified fatty acids, palmitic acid, stearic acid, and myristic acid and to be involved in signal transduction pathways in the cytoplasm. Hohfeld, Jorg, et al., *Hip, a Novel Cochaperone Involved in the Eukaryotic Hsc70/Hsp40 Reaction Cycle*. Cell vol. 83, 589-598 (November 17, 1995).

Of these functions, perhaps the best studied has been the role of Hsp70 as a "chaperone," a protein that stabilizes other proteins against aggregation and that mediates the folding of newly translated polypeptides in the cytosol and organelles. Proper functioning of Hsp70 as a protein chaperone is dependent on its bound nucleotide state.

Specifically, the ATP form of Hsp70 binds substrate very poorly and therefore must be converted to the ADP form before the misfolded protein can bind. Then, the high affinity of Hsp70 for ATP is utilized to "power" the protein folding and other functions of Hsp70, as much energy is generated by the hydrolysis of bound ATP.

The search for regulators of Hsp70 chaperone function has revealed regulatory factors that form complexes with Hsp70 and assist in determining those substrates with which Hsp70 can associate. For example, the DNAJ-like proteins bind protein substrates exhibiting secondary and tertiary structure but have very low affinity for polypeptides in unfolded conformations. On the other hand, Hsp70 proteins bind unfolded proteins best. Thus, by forming a complex with DNAJ-like protein, Hsp70 proteins can bind with many other proteins of varying conformation. Cyr, D. M. et al., *DnaJ-like Proteins: Molecular Chaperones and Specific Regulators of Hsp70*. TIBS 19 (April, 1994).

Other factors can regulate the substrate binding stability or ATPase activity of Hsp70. Hsp40 stimulates the ATPase of Hsp70 and therefore results in production of the ADP form of Hsp70, which facilitates binding to substrate. Another Hsp70 regulator, the Hip co-chaperone protein, binds to the ATPase domain of Hsp70, thereby promoting the assembly of chaperone complexes and prolonging the time window during which a Hsp70 protein can interact stably with unfolded polypeptides. Hohfeld, Jorg, et al., *Hip, a Novel Cochaperone Involved in the Eukaryotic Hsc70/Hsp40 Reaction Cycle*. Cell vol. 83, 589-598 (November 17, 1995). Similarly, a regulator named Hop modulates the binding of Hsp70 to Hsp90, thereby stimulating Hsp70-mediated refolding of a denatured protein. Johnson, B.D., et al., *Hop Modulates Hsp70/Hsp90 Interactions in Protein Folding*. JBC 273:6, pp. 3679-3686 (February 6, 1998).

A potential regulator of Hsp70 is a 16-kDa protein that is a member of the Nm23/nucleoside diphosphate kinase family. This regulator monomerized Hsc70 (a protein closely related to Hsp70) and assisted in releasing Hsc70 from bound substrate. Leung, S.M. and L.E. Hightower, *A 16-kDa Protein Functions as a New Regulatory Protein for Hsc70 Molecular Chaperone and Is Identified as a Member of the Nm23/Nucleoside Diphosphate Kinase Family*. JBC 272:5, pp. 2607-2614 (January 31, 1997). Also, the cysteine string protein, which is a secretory vesicle protein, and auxilin, a clathrin-associated protein, can both activate Hsc70 ATPase activity. Chamberland, L.H. and R.D. Burgoyne, *Activation of the ATPase activity of heat-shock proteins Hsc70/Hsp70 by cysteine-string protein*. Biochem. J. 322, pp. 853-858 (1997); Braun, J.E.A., et al., *The Cystein String Secretory Vesicle Protein Activates Hsc70 ATPase*. JBC 271:42, pp.25989-25993 (October 18, 1996); Jiang, R.F. et al., *Interaction of Auxilin with the Molecular Chaperone, Hsc70*. JBC 272:10, pp. 6141-6145 (March 7, 1997).

Still other regulators of Hsp70 inhibit Hsp70-mediated refolding. The RAP/HAP46 proteins, which inhibit binding of misfolded proteins to Hsp70, and BAG-1, which causes the release of ADP from Hsp70, both down-regulate Hsp70 activity. Zeiner, M. et al., *Mammalian protein RAP46: an interaction partner and modulator of 70 dDa heat shock proteins*. EMBO J. 16:18, pp. 5483-5490 (1997); Takayama, S. et al., *BAG-1 modulates the chaperone activity of Hsp70/Hsc70*. EMBO J. 16:16, pp. 4887-4896 (1997).

Despite the fact that regulators of Hsp70 protein binding have been discovered and characterized, the functional regulation of Hsp70 is not yet understood. Moreover, the ability to directly abrogate or eliminate Hsp70 ATPase activity through a selectively binding protein has not previously been known. Therefore, the discovery and

isolation of polynucleotides encoding two isoforms of a human heat-shock protein binding protein (HspBP-1 and HspBP-2), is desirable because they provide a means to investigate the effects of heat shock-protein regulation. Such regulation may have consequences in physiological pathways or conditions in which Hsp70 is known to be involved, such as development, apoptosis, cellular stress, heart disease, and cancer.

BRIEF SUMMARY OF THE INVENTION

It is an object of the invention to provide the cloned polynucleotide sequences encoding novel human heat-shock protein-binding proteins.

A second object of the invention is to provide the deduced polypeptide sequences according to the cloned polynucleotide sequences encoding novel human heat-shock protein-binding proteins.

Another object of the invention is to provide rat, mouse, and zebrafish gene homologues of novel human heat-shock protein-binding proteins.

Still another object of the invention is to provide a means of inhibiting the activity of Hsp70 and related proteins using novel heat-shock protein-binding proteins.

Yet another object of the invention is to provide a means of inhibition of the apoptotic activity of Hsp70 and related proteins using novel heat-shock protein-binding proteins.

In accordance with these objectives, the invention features substantially purified human heat-shock protein-

binding proteins (HspBP), designated HspBP-1 and HspBP-2, having the amino acid sequence shown in SEQ ID NO:1 and in SEQ NO:2, respectively. Furthermore, the invention features isolated and substantially purified polynucleotides that encode HspBP-1 or HspBP-2 having the nucleotide sequence shown in SEQ ID NO:3 and SEQ ID NO:4, respectively. Moreover, the invention features nucleic acid sequences encoding polypeptides, oligonucleotides, peptide nucleic acids, fragments, portions or antisense molecules thereof, and expression vectors and host cells comprising polynucleotides that encode human HspBP and its mouse (HspBPM; SEQ ID NO:5), rat (HspBPR; SEQ ID NO:6), and zebrafish (HspBPF; SEQ ID NO:7) homologues. Finally, the invention features pharmaceutical compositions comprising substantially purified HspBP.

Various other purposes and advantages of the invention will become clear from its description in the specification that follows and from the novel features particularly pointed out in the appended claims. Therefore, to the accomplishment of the objectives described above, this invention consists of the features hereinafter illustrated in the drawings, fully described in the detailed description of the preferred embodiments and particularly pointed out in the claims. However, such drawings and description disclose only some of the various ways in which the invention may be practiced.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic view of Hsp70 showing its three functional domains.

Fig. 2A and 2B shows the amino acid sequence alignments among human HspBP-1 (SEQ ID NO:1) and HspBP-2 (SEQ ID NO:2) and the homologous mouse (HspBPM; SEQ ID NO:8),rat

(HspBPR; SEQ ID NO:9), and zebrafish (HspBPF; SEQ ID NO:10) heat-shock protein binding proteins. Amino acid sequence identity among species is highlighted in black. Small stars (*) below residues indicate conservation of any amino acid among all species, while large stars (☆) above residues indicate conservation of the amino acid cysteine (C) among all species. When four of the five amino acids for a particular position are identical, a period (.) or a colon (:) below a residue indicate the degree to which chemical properties, such as size and charge, are shared between the identical and non-identical residues, with a period indicating partial chemical relatedness and a colon indicating high chemical relatedness. The dashes (-) indicate that a protein lacks an amino acid at that particular position of the alignment.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by those of ordinary skill in art of the invention. For example, see the definitions provided by U.S. Patent No 5,955,312 by Hillman and Goli, which is incorporated herein by reference. All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which might be used in connection with the invention.

The invention is based on the discovery of novel polynucleotides encoding two isoforms of human HspBP, and the use of these polynucleotides and proteins in discovering and isolating the homologous polynucleotides and proteins of several different species, including mouse, rat, and zebrafish.

The polynucleotides and proteins of the invention are useful for research on pathways in which active Hsp70 and related proteins participate, such as apoptosis, development, and signal transduction. Furthermore, the invention is useful in the research and in the treatment of maladies involving active Hsp70, such as various types of cancer and heart disease.

For example, it is known that hypoxic stress is a signal that increases the amount of Hsp70 in cardiac tissue, whereupon Hsp70 helps cells survive by binding to partially denatured proteins and assisting in the refolding of these proteins into more stable native structures. Such assistance would be extremely important in proving protection to the heart during periods of hypoxia such as during an infarct or during surgery when blood flow to the heart may be temporarily halted. Thus, discovering, characterizing, and devising ways to down-regulate the expression or activity of Hsp70-inhibiting proteins, such as the HspBP, is clearly useful.

It is also known that harmful conditions, including oxidative stress and UV radiation, can cause programmed cell death (apoptosis). Hsp72, a member of the Hsp70 family, has been shown to inhibit a signal transduction pathway leading to programmed cell death by preventing stress-induced activation of Jun N-terminal kinase, JNK. Gabai, V.L. et al. *Hsp70 Prevents Activation of Stress Kinase*. JBC 272:29, pp. 18033-18037 (July 18, 1997). Moreover, Hsp70 is known to block the apoptotic process by blocking the activation of the caspase protease cascade. Mosser, D.D., et al., *Role of the Human Heat Shock Protein Hsp70 in Protection Against Stress-Induced Apoptosis*. Mol. and Cell. Biol., 17:9, pp. 5317-5327 (Sept., 1997). Thus, HspBP may play a role in promoting apoptosis by halting the inhibitory action of Hsp72 on JNK. By promoting apoptosis, HspBP may be useful in the killing

of, for example, cancer cells.

Although many different methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and material are now described.

The invention encompasses polypeptides comprising the amino acid sequences of SEQ ID NO:1 (HspBP-1; GenBank Acession Number AF093420) and SEQ ID NO:2 (HspBP-2; GenBank Acession Number AF187859). HspBP-1 is 359 amino acids long, while HspBP-2 is 362 amino acids in length. This difference in length is accounted for by the presence of 3 additional glycine residues in HspBP-2 at a glycine-rich region beginning at residue 25.

The invention also encompasses polynucleotides which encode HspBP. Thus, any nucleic acid sequence which encodes an amino acid sequence of a HspBP can be used to produce recombinant molecules which express HspBp. In a particular embodiment, the invention comprises the polynucleotide sequences encoding HspBP-1 (SEQ ID NO:3) and HspBP-2 (SEQ ID NO:4).

The invention further encompasses HspBP variants. A preferred variant is one having at least 90% amino acid sequence similarity to the HspBP amino acid sequences identified by SEQ ID NO:1 and SEQ ID NO:2. Most preferably, however, is a HspBP variant having at least 95% amino acid sequence similarity to SEQ ID NO:1 or SEQ ID NO:2.

As known by those skilled in the art, many commonly available computer programs can be used to search for sequence variants. For example, both the nucleotide and derived amino acid sequences of the human HspBP were used

to search GenBank™, and no matches to known genes or proteins were found. However, when searching the GenBank™ EST data base with the programs BLAST and tBLASTn (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov), a number of significant matches were found in human, mouse, and rat sequences. None of these sequences were for known proteins.

The deduced amino acid sequences of the HspBP of humans (HspBP-1; SEQ ID NO:1 and HspBP-2; SEQ ID NO:2), mice (HspBPM; SEQ ID NO:8), rats (HspBPR; SEQ ID NO:9), and zebrafish (HspBPF; SEQ ID NO:10) are shown in Fig. 2A and 2B in alignment using the CLUSTALW computer program (www.mbshortcuts.com/cgi-bin/mbsalign/alignform.cgi). As indicated by the black shading in Fig. 2A and 2B, the amino acid sequences of HspBP for all species tested are highly conserved.

It will be appreciated by those skilled in the art that, as a result of the degeneracy of the genetic code, a multitude of HspBP-encoding nucleotide sequences, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene, may be produced. The invention contemplates every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence encoding naturally occurring HspBP, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HspBP and their variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring transcription sequences under appropriately selected conditions of stringency, it can be advantageous to

produce nucleotide sequences encoding HspBP or their derivatives possessing a substantially different codon usage. For example, codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HspBP and their derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater stability or half-life, than transcripts produced from the naturally occurring sequence.

As known by one skilled in the art, a DNA sequence, or portions thereof, encoding HspBP and their derivatives may be produced entirely by synthetic chemistry. Subsequently, the synthetic nucleotide sequence may be inserted into any of the many available DNA vectors and cell systems using reagents that are commonly available. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HspBP or any portion thereof.

Also included within the scope of the invention are polynucleotide sequences that are capable of hybridizing to the nucleotide sequences of SEQ ID NO:3 or SEQ ID NO:4 under various conditions of stringency. Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe, as taught in Berger and Kimmel (1987, *Guide to Molecular Cloning Techniques*, Methods in Enzymology, v.152, Academic Press, San Diego, CA).

Natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. One may, for example, screen a peptide

library for inhibitors of HspBP activity by encoding a chimeric HspBP that can be detected by a commercially available antibody. In addition, a fusion protein may be engineered to contain a cleavage site located between the HspBP encoding sequence and the heterologous protein sequence, so that HspBP may be cleaved and purified away from the heterologous moiety.

Methods well known in the art can be used to construct expression vectors containing sequences encoding HspBP and appropriate transcriptional and translational control elements. Methods may include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination in a variety of expression vector/host systems, such as bacteria transformed with recombinant bacteriophage or plasmids or insect cell systems infected with viral expression vectors such as the baculovirus. These methods are described in standard laboratory references, such as Sambrook, J. et al. *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y. (1989).

Altered nucleic acids encoding HspBP which may be used in accordance with the invention include deletions, insertions or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent HspBP. The protein may also show deletions, insertions or substitutions of amino acid residues which produce a silent change and result in functionally equivalent HspBP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of HspBP is retained. For example, negatively charged amino acids aspartic acid and glutamic acid might be substituted for one another.

Also included within the scope of the invention are alleles encoding HspBP. As used herein, an "allele" or "allelic sequence" is an alternative form of the nucleic acid sequence encoding HspBP. Alleles result from a mutation, i.e. a change in the nucleic acid sequence, and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes which give rise to natural deletions, additions or substitutions of amino acids. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

Many ways exist in the art by which HspBP may be used therapeutically. Examples include, but are not limited to, administering HspBP through the introduction of an expression vector into a subject for in vivo therapy, administering a vector expressing antisense of a polynucleotide encoding HspBP, or administering HspBP as part of a pharmaceutical composition. Depending on the route of administration, appropriate agents for use in combination with HspBP for therapy may include any conventional pharmaceutical carrier such as saline or buffered saline (intravenous dosing) and dextrose or water (oral dosing). Further details on techniques for formulation and administration may be found in the latest edition of *Remington's Pharmaceutical Sciences* (Maack Publishing Co., Easton, PA).

In order to further illustrate the invention, the following example is provided. However, this example is not intended in any way to limit the invention.

EXAMPLE

First, screening for Hsp70-interacting proteins using the yeast two-hybrid system was performed according to Bartel,

P.L. et al., "Using the two-hybrid system to detect protein-protein interactions," from *Cellular Interactions in Development: A Practical Approach* (Ed. David Hartley). Oxford University Press (1993). A portion of the cDNA for human Hsp70 (R.Morimoto, Northwestern University) coding for amino acids 1-351 was inserted into the yeast vector pAS2-1 (Clontech, Palo Alto, CA) and used as the "bait plasmid" for the two-hybrid screening procedures. The yeast transfected with this plasmid expressed human Hsp70 as determined by Western blot analysis. These yeasts would not grow on plates lacking histidine and were negative for Beta-galactosidase activity, indicating that Hsp70 alone cannot activate the reporter genes and therefore will not result in false positives.

Then, a human heart cDNA library (Clontech, Palo Alto, CA) containing $3X10^6$ independent clones was screened. This library was made in the pGAD10 cloning vector that had cDNAs fused to the activation domain of the GAL4 transcription activator. Methods for screening of the library were according to the manual provided by Clontech. Clones that lacked the DNA-binding domain/target plasmid but retained the activation domain/library plasmid were isolated using cycloheximide selection (Matchmaker Supplement Kit, Clontech). The candidate Leu+, Trp- clones were then mated to Y187 (MATα) yeast strains carrying different test plasmids. Diploids from the mating were selected (Trp+, Leu+, and His+) and screened for the ability to produce ß-galactosidase.

The cDNA inserts obtained for these ß-galactosidase positive clones can then be sequenced and further characterized by standard molecular biological methods. For example, well-known methods for DNA sequencing may be used which employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase (TM) (U.S. Biochemical Corp., Cleveland OH), Taq polymerase (Perkin Elmer, Norwalk, CN),

or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown Mass.) and the ABI 377 DNA sequencers (Perkin Elmer).

While this example is contemplated to be the preferred mode, it will be understood by those in the art that numerous alternative methodologies may be successfully practiced in lieu of the preferred method described herein.

We claim:

1. An isolated and purified polynucleotide fragment encoding the amino acid sequence of SEQ ID NO:1.

2. A hybridization probe comprising the polynucleotide fragment of Claim 1 and a detectable label.

3. A polynucleotide fragment which is fully complementary to the polynucleotide fragment of Claim 1.

4. A hybridization probe comprising the polynucleotide fragment of Claim 3 and a detectable label.

5. An expression vector containing the polynucleotide fragment of Claim 1.

6. A host cell line containing the expression vector of Claim 5.

7. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 comprising the steps of:

a) culturing a host cell line of Claim 6 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell line culture.

8. An isolated and purified polynucleotide fragment encoding the amino acid sequence of SEQ ID NO:2.

9. A hybridization probe comprising the polynucleotide fragment of Claim 8 and a detectable label.

10. A polynucleotide fragment which is fully complementary to the polynucleotide fragment of Claim 8.

11. A hybridization probe comprising the polynucleotide fragment of Claim 10 and a detectable label.

12. An expression vector containing the polynucleotide fragment of Claim 8.

13. A host cell line containing the expression vector of Claim 12.

14. A method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:2 comprising the steps of:

a) culturing a host cell line of Claim 13 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell line culture.

15. An isolated and purified polynucleotide sequence comprising SEQ ID NO:3.

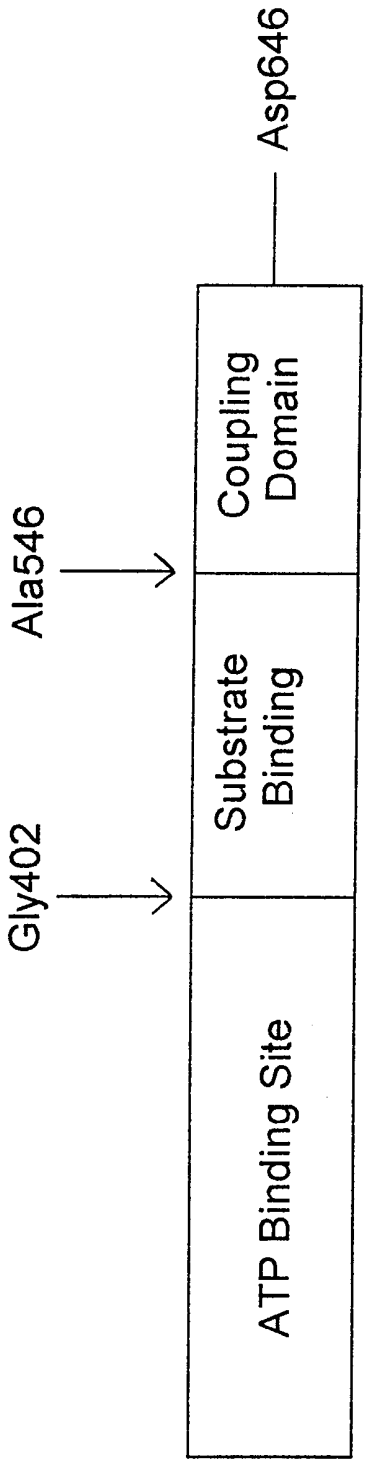
16. An isolated and purified polynucleotide sequence comprising SEQ ID NO:4.

17. A substantially purified human HspBP polypeptide comprising the amino acid sequence of SEQ ID NO:1, or immunologically active fragments thereof.

18. A substantially purified human HspBP polypeptide comprising the amino acid sequence of SEQ ID NO:2, or immunologically active fragments thereof.

19. A pharmaceutical composition comprising a substantially purified human HspBP having the amino acid sequence of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

20. A pharmaceutical composition comprising a substantially purified human HspBP having the amino acid sequence of SEQ ID NO:2 in conjunction with a suitable pharmaceutical carrier.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Substrate Binding
Gly402
ATP Binding Site

FIG. 1

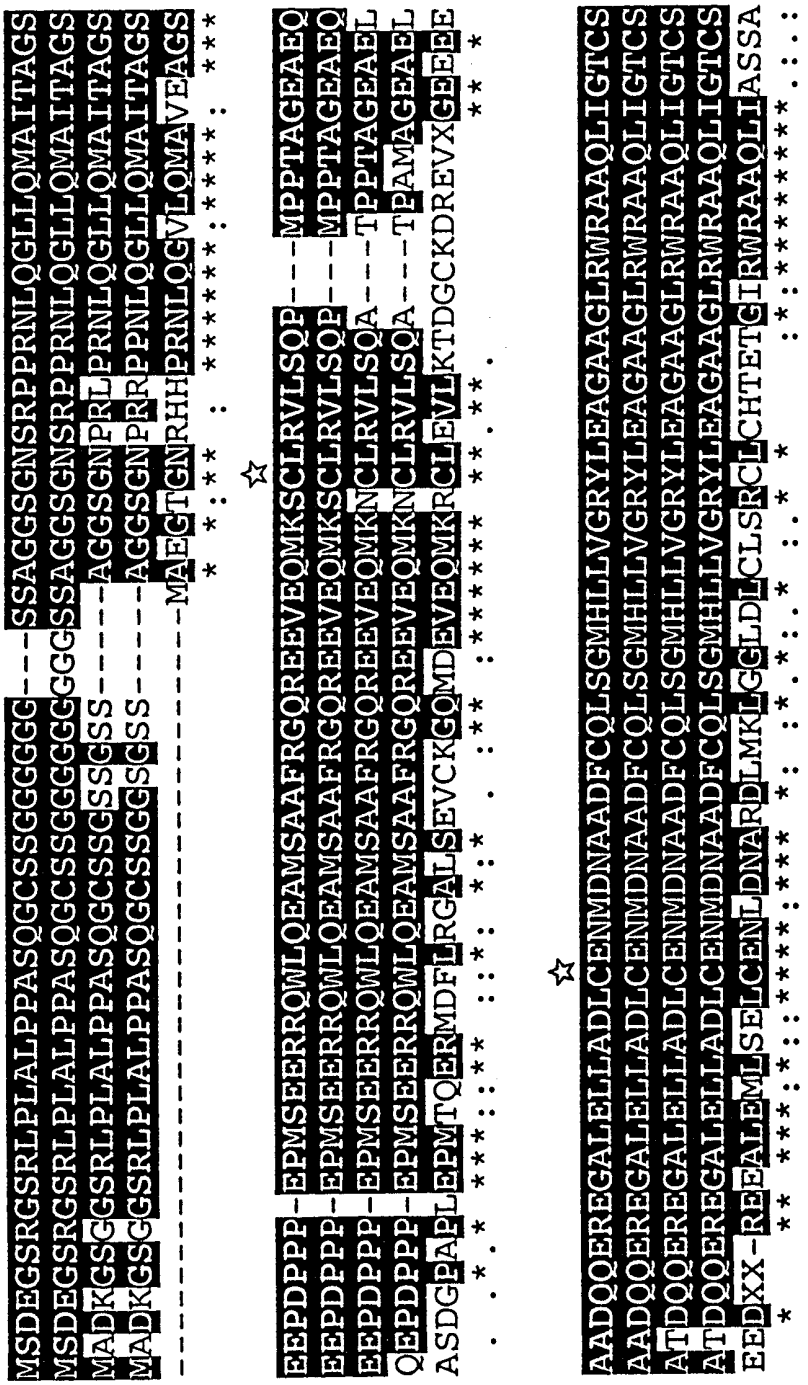
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FIG. 2A

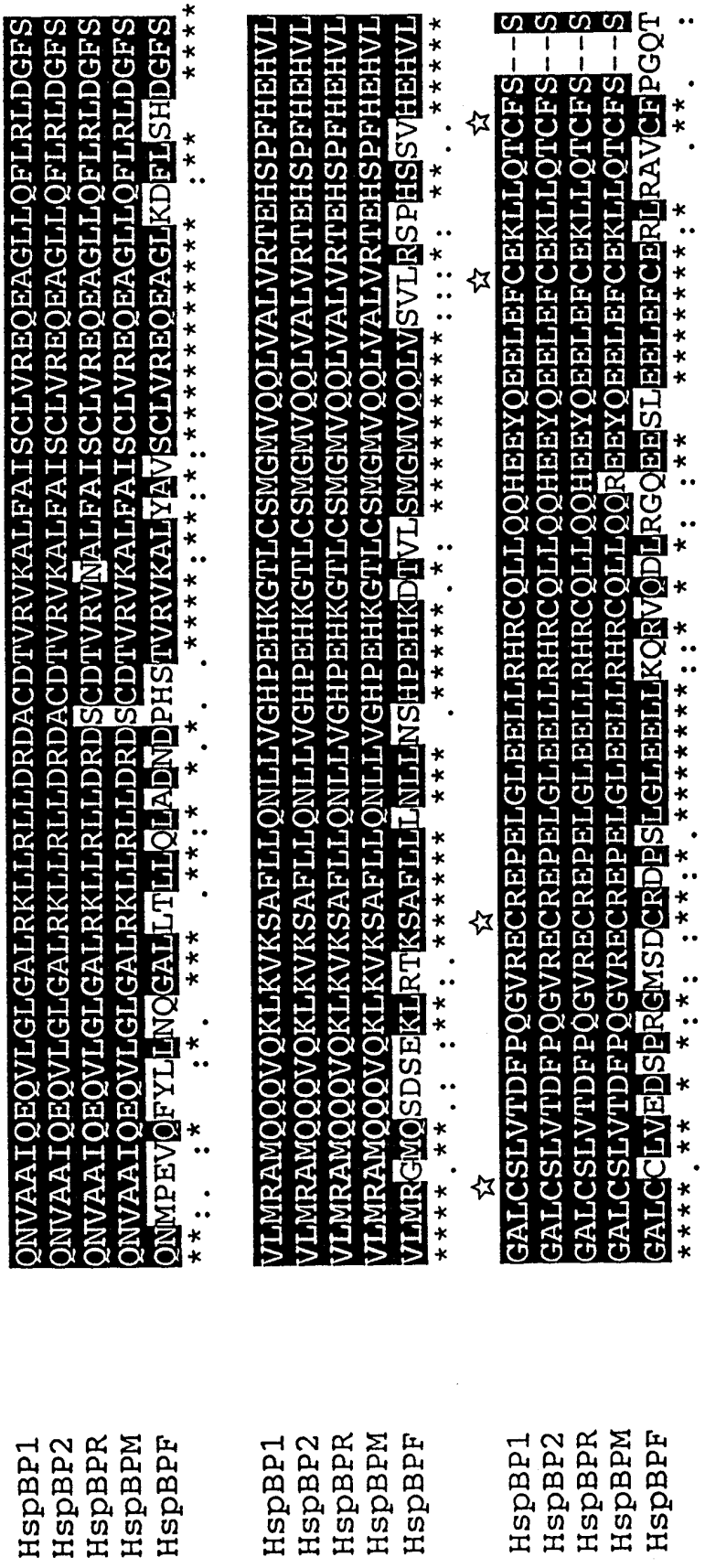
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FIG. 2B

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Arg Lys Leu Leu Arg Leu Leu Asp Arg Asp Ala Cys Asp Thr Val Arg
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Cys Glu Asn Met Asp Asn Ala Ala Asp Phe Cys Gln Leu Ser Gly Met
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His Leu Leu Val Gly Arg Tyr Leu Glu Ala Gly Ala Ala Gly Leu Arg
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Trp Arg Ala Ala Gln Leu Ile Gly Thr Cys Ser Gln Asn Val Ala Ala
165 170 175

Ile Gln Glu Gln Val Leu Gly Leu Gly Ala Leu Arg Lys Leu Leu Arg
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Leu Leu Asp Arg Asp Ser Cys Asp Thr Val Arg Val Asn Ala Leu Phe
195 200 205

Ala Ile Ser Cys Leu Val Arg Glu Gln Glu Ala Gly Leu Leu Gln Phe
210 215 220

Leu Arg Leu Asp Gly Phe Ser Val Leu Met Arg Ala Met Gln Gln Gln
225 230 235 240

Val Gln Lys Leu Lys Val Lys Ser Ala Phe Leu Leu Gln Asn Leu Leu
245 250 255

Val Gly His Pro Glu His Lys Gly Thr Leu Cys Ser Met Gly Met Val
260 265 270

Gln Gln Leu Val Ala Leu Val Arg Thr Glu His Ser Pro Phe His Glu
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His Val Leu Gly Ala Leu Cys Ser Leu Val Thr Asp Phe Pro Gln Gly
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Val Arg Glu Cys Arg Glu Pro Glu Leu Gly Leu Glu Glu Leu Leu Arg
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His Arg Cys Gln Leu Leu Gln Gln His Glu Glu Tyr Gln Glu Glu Leu
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Glu Phe Cys Glu Lys Leu Leu Gln Thr Cys Phe Ser Ser Pro Thr Asp
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Asp Ser Met Asp Arg
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<213> Brachydanio rerio (zebra fish)

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20 25 30

Pro Leu Glu Pro Met Thr Gln Glu Arg Met Asp Phe Leu Arg Gly Ala
35 40 45

Leu Ser Glu Val Cys Lys Gly Gln Met Asp Glu Val Glu Gln Met Lys
50 55 60

Arg Cys Leu Glu Val Leu Lys Thr Asp Gly Cys Lys Asp Arg Glu Val
65 70 75 80

Xaa Gly Glu Glu Glu Glu Glu Glu Asp Xaa Xaa Arg Glu Glu Ala Leu
85 90 95

Glu Met Leu Ser Glu Leu Cys Glu Asn Leu Asp Asn Ala Arg Asp Leu
100 105 110

Met Lys Leu Gly Gly Leu Asp Leu Cys Leu Ser Arg Cys Leu Cys His
115 120 125

Thr Glu Thr Gly Ile Arg Trp Arg Ala Ala Gln Leu Ile Ala Ser Ser
130 135 140

Ala Gln Asn Met Pro Glu Val Gln Phe Tyr Leu Leu Asn Gln Gly Ala
145 150 155 160

Leu Leu Thr Leu Leu Gln Leu Ala Asp Asn Asp Pro His Ser Thr Val
165 170 175

Arg Val Lys Ala Leu Tyr Ala Val Ser Cys Leu Val Arg Glu Gln Glu
180 185 190

Ala Gly Leu Lys Asp Phe Leu Ser His Asp Gly Phe Ser Val Leu Met
195 200 205

Arg Gly Met Gln Ser Asp Ser Glu Lys Leu Arg Thr Lys Ser Ala Phe
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Leu Leu Leu Asn Leu Leu Asn Ser His Pro Glu His Lys Asp Thr Val
225 230 235 240

Leu Ser Met Gly Met Val Gln Gln Leu Val Ser Val Leu Arg Ser Pro
245 250 255

His Ser Ser Val His Glu His Val Leu Gly Ala Leu Cys Cys Leu Val
260 265 270

Glu Asp Ser Pro Arg Gly Met Ser Asp Cys Arg Asp Pro Ser Leu Gly
275 280 285

Leu Glu Glu Leu Leu Lys Gln Arg Val Gln Asp Leu Arg Gly Gln Glu
290 295 300

Glu Ser Leu Glu Glu Leu Glu Phe Cys Glu Arg Leu Arg Ala Val Cys
305 310 315 320

Phe Pro Gly Gln Thr Gln Glu Asp Asn Ala Met Asp Arg
325 330

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27651

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 1/00
US CL : 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 91.1, 320.1; 530/350; 536/23.1, 24.31, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HILLIER et al. Generation and analysis of 280,000 human expressed sequence tags. Genome Research. September 1996, see pages 807-827	1-20
Y	PHAMACIA BIOTECH Overview of molecular biology products. 1996, pages 107, 110-117, 139, 163-165.	2, 4, 5-7, 9, 11, 12-14, 17, 18
Y	STRATAGENE Cloning systems. 1994,see pages 42-43.	5-7,12-14, 17, 18
Y	FIX J.A. Oral conrolled release technology for peptides: status and future prospects. Pharmaceutical Research. 1996, Vol.13,No.12,see pages 1760-1764.	19, 20
Y	TAKAKURA et al. Macromolecular carrier systems for targeted drug delivery: pharmacokinetic considerations on biodistribution. Pharmaceutical Research. 1996. Vol.13,no.6,see pages 820-831.	19,20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
04 February 2000 (04.02.2000)

Date of mailing of the international search report
29 FEB 2000

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/27651

Continuation of B. FIELDS SEARCHED Item 3: WEST, MEDLINE
heat shock protein 70, HSP70, inhibitor, copurifying, coprecipitation, binding protein