Title: PEPTIDES FOR USE AS TRANSLOCATION FACTORS

Abstract: Proteins that contain the amino acid sequence motif X'X'XX'X', where X' = R or K and X'' and X''' = any amino acid have been found to translocate and can therefore be used in the manufacture of compositions for therapeutic applications. The proteins may also be used as translocation factors to deliver proteins or nucleic acids into a cell.
PEPTIDES FOR USE AS TRANSLOCATION FACTORS

Field of the Invention

The present invention relates to the identification of an amino acid motif that aids translocation of proteins, the preparation of proteins as translocation agents and therapeutic agent conjugates.

Background to the Invention

Gene therapy provides the potential to cure selected genetic diseases. However, a major obstacle is the effective delivery of the gene or protein of interest to the target site. A variety of viral and non-viral vectors have been developed to deliver genes or gene products in to various cells, tissues and organs by ex vivo or in vivo strategies. Among viral-based vectors, retroviruses, adenoviruses, adeno-associated viruses and herpes viruses have been most extensively studied. Among non-viral-based vectors, liposomes and cationic lipid-mediated systems have been used to introduce plasmid DNA directly into animals. However, one of the main challenges of gene therapy remains the design of effective delivery systems.

Histones have also been proposed for use as a vehicle for gene delivery via transfection. Histones are the proteins responsible for the nucleosomal organisation of chromosomes in eukaryotes. The core histones H2A, H2B, H3 and H4 form the core structure of the nucleosome, and the linker histone H1 seals two rounds of DNA at the nucleosomal core.

Zaitsev et al, Gene Therapy, 1997; 4: 586-592 discloses certain nuclear proteins, including histone, which can be prepared to act as DNA carriers via transfection.

Translocation refers to the delivery of proteins across a cell membrane and is therefore different from transfection which relates only to DNA.

Various methods for the delivery of therapeutic proteins across a cell membrane have been proposed. For example, Prochiantz, Current Opinion in Neurobiology, 1996; 6: 629-634, relates to the use of antennapedia to transport proteins across a cell membrane. Other transporting proteins have also been identified including VP22 and tat.
Although these systems are generally useful, it is desirable to identify other suitable transport proteins, particularly those derived from a human source, which would be less likely to be immunogenic.

**Summary of the Invention**

The present invention is based on the surprising finding that proteins and peptides comprising a specific amino acid motif can translocate across a cell membrane.

According to a first aspect of the invention, a protein, or a fragment thereof that retains the biological activity of the protein, comprising an amino acid sequence $X^1X^2X^3X^1$, where $X^1 = R$ or $K$ and $X^2 = X^3 = \text{any amino acid}$, is used in the manufacture of a composition for the treatment of a disease characterised by a deficiency in the production or function of the endogenous protein, or to regulate a biochemical pathway.

In this aspect of the invention, the protein containing the motif can be used in therapy, for example, to replace or supplement a patient's endogenous protein that may be produced inefficiently in the cell. As the protein contains the motif, it will be able to enter the cell by translocation, thereby being delivered to its site of activity. Alternatively, the protein is administered to control a particular biochemical pathway, e.g. a signalling pathway.

According to a second aspect of the invention, a therapeutic agent that has its site of activity within a cell, is used in the manufacture of a composition to treat or diagnose a disease, wherein the agent is conjugated to a peptide that comprises the amino acid sequence defined above. The agent is therefore able to gain entry into the cell using the translocating properties of the peptide.

According to a further aspect of the present invention, there is a conjugate of a peptide capable of translocating across a cell membrane, and a therapeutic, diagnostic or cosmetic agent, wherein the peptide comprises the amino acid sequence motif defined above.

Surprisingly, it has been found that peptides and proteins that comprise the defined amino acid sequence can act via translocation, to deliver a covalently bound therapeutic, diagnostic or cosmetic agent intracellularly.
In contrast to conventional translocating agents such as tat, VP22 or antennapedia, the present invention permits human-derived peptides to be used as the translocation factor, thereby reducing the risk of adverse immunological reactions that may result from the use of non-human peptides.

According to a fourth aspect of the invention, an antibody has affinity for a protein comprising an amino acid sequence as defined above.

According to a fifth aspect of the invention, an expression vector is prepared that expresses a conjugate of the invention in the form of a fusion protein.

Description of the Invention

The present invention is based on the surprising finding that peptides comprising a particular amino acid motif are capable of undergoing translocation across a cell membrane. The critical sequence is:

\[ X^1 X^2 X^3 X^1 \]

where \( X^1 \) is Arginine or Lysine and \( X^2 \) and \( X^3 \) are any amino acid. In the preferred embodiment, \( X^2 \) is Alanine or Valine and \( X^3 \) is Arginine or Lysine.

Identifying this sequence enables many different translocating peptides to be produced.

In the context of the present invention, the term "translocation" refers to the ability of an agent, protein or conjugate, to cross a cellular membrane, i.e. to enter a cell.

The different motif sequences that may be used in the invention include those shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>KKAKK</td>
<td>KRARK</td>
</tr>
<tr>
<td>KKAKR</td>
<td>KRAKK</td>
</tr>
<tr>
<td>KKARK</td>
<td>KRAKR</td>
</tr>
<tr>
<td>KKARR</td>
<td>KRARR</td>
</tr>
<tr>
<td>KKVKK</td>
<td>KRVKK</td>
</tr>
<tr>
<td>KKVKR</td>
<td>KRVKR</td>
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<td>KKVRK</td>
<td>KRVRK</td>
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<td>KKVR</td>
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<tr>
<td>KKVRR</td>
<td>RKVRK</td>
</tr>
<tr>
<td></td>
<td>RRVRR</td>
</tr>
</tbody>
</table>
The translocating peptide may be in a truncated form or in a synthetic form, which can be produced readily, without the need to undergo time-consuming and expensive purification steps. Truncated forms are often produced more readily in recombinant expression systems, i.e. in a recombinant mammalian or bacterial expression system. In addition, truncated forms may be less immunogenic and therefore more suitable for administration of the therapeutic agent.

In a preferred embodiment, the translocating peptide fragment comprises no more than 50, preferably no more than 40, and most preferably no more than 30 amino acid residues. The defined sequence motif may be present more than once in the peptide. The peptide is preferably derived from or based on a mammalian protein, preferably a human protein. This reduces the risk of promoting an immunogenic response.

According to the invention, the identification of the defined amino acid motif allows the native protein to be used in therapy. The realisation that a particular endogenous protein has the ability to translocate, enables new therapies to be developed. In this aspect, the natural protein may be administered to a patient, in any pharmaceutically acceptable form, to replace or supplement the endogenous protein, which is either not being produced (or produced in an insufficient amount), or which is produced in an aberrant form. The natural protein is administered knowing that the protein can translocate across the cell membrane, and localise in the defective cells. Accordingly, no other translocation factor is required to facilitate the delivery of the protein.

This therapy is therefore a viable alternative to gene therapy. Rather than attempting to introduce a gene that encodes a product, the product itself can be administered and will translocate across a cellular membrane.

Proteins that comprise the motif may therefore be used in the manufacture of a composition for the treatment of a disease characterised by a deficiency in production of the endogenous protein. Suitable proteins are identified in Tables 2 - 5, with those proteins shown in Table 2 being of particular interest.

The proteins may not only be administered as a replacement therapy, but may be used in any therapeutic context. For example, the proteins may be
required to influence a particular regulatory pathway or to upregulate or
downregulate control systems within a cell. Suitable therapies will be apparent
to the skilled person.

The proteins to be administered may be produced using techniques
known to those skilled in the art. For example, recombinant DNA technology
permits the large-scale production of proteins in cell culture, and this can be
adapted in the present invention.

Functional variants of the translocating peptides/proteins may also be
used. For example, proteins with high levels (greater than 70%, preferably
greater than 90% and more preferably greater than 95%) of sequence similarity
to the endogenous protein are within the scope of the present invention. The
term "similarity" is known in the art. The term refers to a comparison between
amino acid sequences, and takes into account not only identical amino acids in
corresponding positions, but also functionally similar amino acids in
corresponding positions. Thus similarity between polypeptide sequences
indicates functional similarity, in addition to sequence similarity.

Levels of similarity between amino acid sequences can be calculated
using known methods. In relation to the present invention, publicly available
computer based methods for determining similarity include the BLASTP,
BLASTN and FASTA programmes (Atschul et al., J. Molec. Biol., 1990; 215:403-
410), the BLASTX program available from NCBI, and the Gap program from
Genetics Computer Group, Madison WI. The levels of similarity referred to
herein, are determined using the Gap program, with a Gap penalty of 12 and a
Gap length penalty of 4.

The variants may be produced using standard recombinant DNA
techniques such as site-directed mutagenesis. The variants may also have
conserved amino acid substitutions (although not in the critical amino acid
sequence), e.g. replacement of a hydrophobic residue for a different hydrophobic
residue. All this will be apparent to the skilled person, based on conventional
protein technology. The variants must retain the functional ability to translocate
across a cellular membrane.
If used as a replacement therapy, to correct a deficiency of the endogenous protein, the variants should also retain the biological activity of the endogenous protein, i.e. that activity that is deficient in the patient.

Compositions containing the protein to be administered may comprise any suitable excipient, diluent or buffer. No other translocating factor is required.

In a preferred embodiment of the invention, the protein to be administered is IRAK1 and/or MYD88. Both of these proteins are involved in the signalling pathway that suppresses production of IL-1 and TNF and these proteins can be translocated into cells to suppress the inflammatory signalling pathway through a decrease in the production of IL-1.

In a further preferred embodiment, the transcription factor E2A is used in therapy. E2A is a molecule known to be involved in controlling proliferation of stem cells, and a mutated form has been identified in childhood leukemia patients. Administering the non-mutated form allows patients to be given a normal source of the protein, helping to ameliorate the condition.

In a further preferred embodiment, Fanconi anaemia can be treated by administering the product of the Fanconi A gene.

In a still further embodiment, Bruton Globulinaemia is treated by administering cytoplasmic tyrosine kinase (NCBI Accession No. Q06187), a mutation in which has been implicated as a causative factor in this disease.

The identification of the motif on particular proteins also enables the identification of suitable therapeutic targets. For example, the realisation that a particular protein has the capability to translocate across a cell membrane, and that in doing so, a disease may be caused or spread, enables the production of suitable therapies designed to prevent this. This is particularly suitable when the protein is a product of an oncogene or a tumourigenic cell. Realising that the protein has the motif and may therefore be implicated in the spread of the disease to neighbouring cells, allows, for example, antibodies to be designed to target the proteins when released from the tumour cell, thereby preventing the proteins from entering other cells.

An example of this is the finding that the protein product of the human APC gene (NCBI Accession No. M74088) contains the motif and that the
oncogenic form may be spread by translocation, thereby spreading the disease. The oncogenic product is therefore a viable target for antibody therapy.

Accordingly, the present invention includes antibodies that bind with high affinity to a protein having the defined amino acid motif, and to the use of the antibodies in therapy to treat a disease caused by or promoted by the protein. In particular, the present invention includes antibodies that bind to any of the proteins identified in Tables 2 or 3.

The present invention also includes the inhibition of any of the proteins with the defined motif which are oncogenic, in particular any oncogenic protein of those shown in Tables 2 or 3.

The protein to be administered to a subject may also be used as a cosmetic. For example, DNA-repair proteins that help protect against the damaging effects of UV light or X-ray damage are known. The protein xeroderma pigmentosa, contains the amino acid motif responsible for translocation, and is therefore a suitable product for inclusion in a topical composition for cosmetic use. Used in this way, the protein will translocate across the cell membrane to exert its effect within the target cells. The proteins may therefore be prepared for topical administration, for example in creams or ointments, as will be appreciated by the skilled person.

The present invention also enables peptides comprising the motif to be used as the translocating vehicle for the delivery of other therapeutic, diagnostic or cosmetic agents across a cell membrane to effect entry of the agent into the cell or across an intracellular compartment.

Peptides having the motif may therefore be used to prepare conjugates having the translocating region and a heterologous therapeutic agent.

The term "conjugate" refers to a chimeric molecule formed from a translocating peptide and a therapeutic, diagnostic or cosmetic agent.

The conjugates are therefore hybrid molecules not found together in their natural form. The peptide and agent are covalently linked. The covalent linkage may be in the form of a chemical linker molecule, or the product may be in the form of a fusion protein.
The term "peptide" used herein, is intended to refer to oligo and poly-
peptides and proteins.

The translocating peptides may comprise the defined sequence motif
more than once, for example two or three motifs may be present.

The translocating peptides may also comprise a high percentage of Lys
and Arg residues, typically greater than 5%, preferably more than 10%. The
folding of the proteins/peptides and the ionic charge may also be factors that
influence translocation.

In addition to the translocating peptides identified herein, the conjugates
comprise a discrete, i.e. heterologous, therapeutic, diagnostic or cosmetic agent.
In the context of the present invention, a reference to "therapy" or "therapeutic
agent" also includes prophylactic treatments, e.g. vaccination. Examples of
suitable therapeutic and diagnostic agents include polynucleotides, proteins,
peptides, antibodies, enzymes, antigens, growth factors, hormones, non-protein
therapeutic or diagnostic agent, enzyme inhibitors, cytotoxic agents and contrast
agents.

A protein therapeutic agent is preferably at least 100 amino acids in size.
The present invention is particularly useful for longer sequences, e.g. at least
150, 200, 300, 400 or 1000 amino acids in size. For the avoidance of doubt, the
term "protein" as used herein also encompasses polypeptides of the required
length; although the term "polypeptide" generally means sequences of from 2 to
100 amino acids in length, usually 2 up to 60.

The therapeutic agent may comprise nucleic acid, e.g. a reporter gene.
The nucleic acid may be DNA or RNA, in either single stranded or double
stranded form.

The nucleic acid may encode a therapeutic agent, e.g. an enzyme, toxin,
immunogen, etc. or may itself be the therapeutic agent. For example, anti-sense
RNA or DNA may be used to target and disrupt expression of a gene. All this will
be apparent to the skilled person.

The therapeutic agent may also be a chemical compound, i.e. an organic
or inorganic molecule. Any suitable pharmacological agent is within the scope
of the present invention. Preferred chemical molecules include cytotoxic agents and growth factors.

It is preferable that the agent to be delivered has its site of activity within a cell. For example, if the agent is a therapeutic protein, the natural site of activity of that protein should be within a cell.

It will be evident to the skilled person which therapeutic agents/proteins have their site of activity within a cell. The agent may be a ligand for a molecule within the cell or may be a target of ligands within the cell. Agents that act outside of the cell are not intended to be within the scope of the invention. For example, agents that act on extracellular receptors, e.g. insulin, do not need to be translocated to exert a therapeutic effect, and are therefore not intended to be a part of the conjugates.

In one embodiment, the conjugates will not contain the sequence KKAKK or KKARK.

The conjugates of the invention may be produced via techniques known to those skilled in the art. The peptide and agent are linked via a covalent attachment. In one embodiment the agent is a peptide (or protein) and the conjugate is a fusion protein. The production of fusion proteins is known to those skilled in the art and comprises the production of a recombinant polynucleotide that encodes, in frame, both the peptide and the agent.

For example, nucleic acid encoding a suitable conjugate may be incorporated into a suitable expression vector or plasmid for further manipulation. As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof. Selection and use of such vehicles are well known to the skilled person. Many vectors are available, and selection of appropriate vector will depend on the intended use of the vector, e.g. whether it is to be used for DNA amplification or for DNA expression, the size of the DNA to be inserted into the vector, and the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the
following: an origin of replication, one or more marker genes, an enhancer element, a promoter, a transcription termination sequence and a signal sequence.

The conjugates may also be produced by the use of bifunctional reagents which are capable of reacting with the peptide and agent. For example, conjugation of the peptide and agent may be achieved by reagents such as N-succinimidyl 3-(2-pyridyl-dithio)propionate (SPDP) which form a disulphide bridge. Alternative conjugation reagents include: glutaraldehyde, cystamine and EDAC.

Preferably, the agent is linked by a cleavable linker region to the peptide region. Preferably, the cleavable linker region is a protease-cleavable linker, although other linkers, cleavable for example by small molecules, may be used. These include Met-X sites, cleavable by cyanogen bromide, Asn-Gly, cleavable by hydroxylamine, Asp-Pro, cleavable by weak acid, and Trp-X, cleavable by, \textit{inter alia}, NBS-skatole. Protease cleavage sites are preferred due to the milder cleavage conditions necessary and are targeted by, for example, factor Xa, thrombin and collagenase. Any of these may be used. The precise sequences are available in the art and the skilled person will have no difficulty in selecting a suitable cleavage site. By way of example, the protease cleavage region targeted by Factor Xa is I E G R. The protease cleavage region targeted by Enterokinase is D D D D K. The protease cleavage region targeted by Thrombin is L V P R G. Preferably, the cleavable linker region is one which is targeted by endocellular proteases.

Additional cell transportation signals may be present. For example, nuclear localisation signals may be an additional component of the constructs. This may aid the transport of the therapeutic component to the correct intracellular location. Suitable signals are known and identified in the prior art.

The proteins, whether to be administered as a replacement therapy, or for use as a translocating peptide, may also be modified to include additional substituents that help to target the protein/peptide to a particular cell. For example, the proteins may be glycosylated. Alternatively, the proteins/conjugates may be delivered to a particular target tissue/organ by
known targeting techniques, for example those based on antibody targeting using liposomes to carry the proteins/conjugates.

It is apparent that the compositions and constructs of the invention are intended for therapeutic use, although diagnostic use is also envisaged. In the context of therapy, it will also be apparent that veterinary use is to be included.

Applications for the conjugates of the present invention include:

1. Antigen delivery system.

An antigen is any agent that when introduced into an immunocompetent animal stimulates the production of a specific antibody or antibodies that can combine with the antigen. However, the antigen may need to be combined with a carrier to be able to stimulate antibody production or specific T cells (helper or cytotoxic). This is where the present invention may be useful as a carrier for transporting the antigen from one side of the cell membrane to the other such that it can stimulate antibody production. By way of example, bacterial and viral antigens translocated by the conjugates in the cell cytoplasm may be processed and associated with MHC class 1 molecules. This antigen processing and presenting pathway is known to activate specific CD8 cytotoxic lymphocytes.

2. Gene therapy.

Gene therapy may include any one or more of: the addition, the replacement, the deletion, the supplementation, the manipulation etc. of one or more nucleotide sequences in, for example, one or more targeted sites - such as targeted cells. If the targeted sites are targeted cells, then the cells may be part of a tissue or an organ. General teachings on gene therapy may be found in Molecular Biology, Ed Robert Meyers, Pub VCH, such as pages 556-558.

By way of further example, gene therapy can also provide a means by which any one or more of: a nucleotide sequence, such as a gene, can be applied to replace or supplement a defective gene; a pathogenic nucleotide sequence, such as a gene, or expression product thereof can be eliminated; a nucleotide sequence, such as a gene, or expression product thereof, can be added or introduced in order, for example, to create a more favourable phenotype; a nucleotide sequence, such as a gene, or expression product thereof can be added or introduced, for example, for selection purposes (i.e. to
select transformed cells and the like over non-transformed cells); cells can be
manipulated at the molecular level to treat, cure or prevent disease conditions
such as cancer (Schmidt-Wolf and Schmidt-Wolf, 1994, Annals of Hematology
69; 273-279) or other disease conditions, such as immune, cardiovascular,
neurological, inflammatory or infectious disorders; antigens can be manipulated
and/or introduced to elicit an immune response, such as genetic vaccination. In
a particularly preferred embodiment, the compositions may be used to introduce
functional proteins in the cytoplasm of genetically deficient cell types.

3. Cancer therapy.

The conjugates may be used to transport into cancer cells molecules that
are transcription factors and are able to restore cell cycle control or induce
derdifferentiation. For example, it is understood that many cancer cells would
undergo apoptosis if a functional P-53 molecule is introduced into their
cytoplasm. The present invention may be used to deliver such gene products.

Further, cytotoxic agents may be delivered to the cancer cell to destroy the cell.

4. Antibacterial and antiviral therapy.

For example, the compositions may be used to transport in the cytoplasm
of infected cells recombinant antibodies or additional DNA-binding molecules
which interfere with a crucial step of bacterial and viral replication.

5. Use in expression systems for the production of protein.

For example, it is desirable to express exogenous proteins in eukaryotic
cells in culture so that they get processed correctly. However, many exogenous
proteins are toxic to eukaryotic cells. In manufacturing exogenous proteins it is
therefore desirable to achieve temporal expression of the exogenous protein.

The system may therefore be used in connection with an inducible promoter for
this or any other application involving such a system.

6. Contrast imaging

A suitable contrast agent may be part of the conjugate to allow imaging
to be carried out.
7. Cosmetic methods

The conjugates may also be used to administer cosmetic agents within a cell. For example, the cosmetic agent may be a protein (or nucleic acid encoding a protein) that helps protect against the effects of U.V. damage.

Compositions of the invention may optionally comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may further comprise any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s), and other carrier agents that may aid or increase entry into the target site.

The compositions may be adapted for any route of administration, including intramuscular, intravenous, topical, intradermal or subcutaneous.

The delivery of one or more therapeutic genes or proteins according to the invention may be carried out alone or in combination with other treatments or components of the treatment. Diseases which may be treated include, but are not limited to: cancer, neurological diseases where the agent is required intracellularly, inherited diseases, heart disease, stroke, arthritis, viral infections and diseases of the immune system. Suitable therapeutic genes include those coding for tumour-suppressor proteins, enzymes, pro-drug activating enzymes, immunomodulatory molecules, antibodies, engineered immunoglobulin-like molecules, conjugates, hormones, membrane proteins, vasoactive proteins or peptides, cytokines, chemokines, anti-viral proteins, antisense RNA or DNA and ribozymes.

The amount to be administered to a patient will depend on the usual factors: age of the patient, weight, severity of the condition, route of administration, activity of the therapeutic etc. All this can be determined by conventional methods known to the skilled person.

Proteins that contain the motif include:

1) transcription factors;
2) DNA repair enzymes;
3) proteins involved in the growth and modelling of tissue and bones;
4) oncogenes involved in the genesis; of lung tumour and colon carcinoma; and
5) a series of proteins which have genes that are mutated in a number of hereditary diseases.

Table 2 lists of proteins (or genes for the proteins) that comprise the motif and therefore have the capability to translocate. The proteins may therefore be useful in various aspects of the invention either as a target, e.g. for antibody therapy, for replacement therapy, or as a therapeutic agent for delivery across a cell membrane.

<table>
<thead>
<tr>
<th>Protein/Gene</th>
<th>NCBI Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral infectivity factor vif</td>
<td>S43001</td>
</tr>
<tr>
<td>Xeroderma pigmentosum group c</td>
<td>AAA82720</td>
</tr>
<tr>
<td>Melastatin 1</td>
<td>NP_002411</td>
</tr>
<tr>
<td>Histone deacetylase 1</td>
<td>NP_004955</td>
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<tr>
<td>Spi-1 proto-oncogene</td>
<td>X52056</td>
</tr>
<tr>
<td>Human APC gene</td>
<td>M74088</td>
</tr>
<tr>
<td>Homosapiens coiin (P80)</td>
<td>NM_004645</td>
</tr>
<tr>
<td>Bone morphogenetic protein-4</td>
<td>P12644</td>
</tr>
<tr>
<td>Thyrotroph embryonic factor</td>
<td>HSU06935</td>
</tr>
<tr>
<td>Bone morphogenetic protein-3</td>
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<tr>
<td>dek gene</td>
<td>X64229</td>
</tr>
<tr>
<td>Interleukin-1 receptor-associated kinase (IRAK1)</td>
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<tr>
<td>MYD-88</td>
<td>AAB49967.1</td>
</tr>
<tr>
<td>Fanconi A gene product</td>
<td>015360</td>
</tr>
<tr>
<td>Transcription factor E2A</td>
<td>AAH01728.1</td>
</tr>
</tbody>
</table>

Further suitable proteins are listed in Table 3-5, with the NCBI accession numbers identified.
### Table 3

Search carried out using the ExPASy software with the motif [R/K]-[R/K]-x-[R/K]-[R/K], where X = any amino acid.

1. Neuroendocrine protein 7B2 precursor (Secretory granule endocrine protein I; Secretogranin V). Acc. No. P05408
5. AF-6 protein. Acc. No. P55196
7. Amphiregulin precursor (Colorectum cell-derived growth factor) (CRDGF). Acc. No. P15514
16. Bone morphogenetic protein 3b precursor (Growth/differentiation factor 10) (GDF-10) (Bone inducing protein). Acc. No. P55107
17. Bone morphogenetic protein 2 precursor. Acc. No. P12643
20. BN51 protein. Acc. No. P05423
22. Tyrosine-protein kinase BTK (Bruton's tyrosine kinase) (Agammaglobulinaemia tyrosine kinase) (B cell progenitor kinase). Acc. No. Q06187
23. Brain-cadherin precursor (BR-cadherin) (Cadherin-12) (N-cadherin 2) (Cadherin, neural type, 2). Acc. No. P55289
26. Protocadherin alpha 3 precursor (PCDH-alpha3). Acc. No. Q9Y5H8
27. Cell division cycle 7-related protein kinase (CDC7-related kinase). Acc. No. O00311
28. Protein kinase CLK2. Acc No. P49760
31. Cylicin I (Multiple-band polypeptide I). Acc. No. P35663
32. Cylicin II (Multiple-band polypeptide II). Acc. No. Q14093
33. Dystroglycan precursor (Dystrophin-associated glycoprotein 1) [Contains: Alpha-dystroglycan; Beta-dystroglycan]. Acc. No. Q14118
34. Serine/threonine-protein kinase DCAMKL1 (Doublecortin-like and CAM kinase-like 1). Acc. No. O15075
35. Doublecortin (Lissencephalin-X) (Doublin). Acc. No. O43602
36. DEK protein. Acc. No. P35659
38. Dystrophin. Acc. No. P11532
40. Steroid hormone receptor ERR1 (Estrogen-related receptor, alpha) (ERR-
alpha) (Estrogen receptor-like 1). Acc. No. P11474
42. Estrogen-related receptor gamma (ERR gamma-2). Acc. No. O75454
43. Estrogen receptor (Estradiol receptor) (ER-alpha). Acc. No. P03372
44. Endothelin-1 precursor. Acc. No. P05305
45. Endothelin-2 precursor. Acc. No. P20800
46. Ellis-van Creveld syndrome protein (DWF-1). Acc. No. P57679
47. 51 kDa FK506-binding protein (FKBP51) (Peptidyl-prolyl cis-trans isomerase) (PPIase) (Rotamase) (54 kDa progesterone receptor-associated immunophilin) (FKBP54) (P54) (FF1 antigen) (HSP90-binding immunophilin). Acc. No. Q13451
48. FOSB protein (G0/G1 switch regulatory protein 3). Acc. No. P53539
49. P55-C-FOS proto-oncogene protein (Cellular oncogene C-FOS) (G0S7 protein). Acc. No. P01100
50. FOS-related antigen 1. Acc. No. P15407
51. FOS-related antigen 2. Acc. No. P15408
52. Fragile X mental retardation syndrome related protein 2. Acc. No. P51116
54. Glucocorticoid receptor. Acc. No. P04150
55. Growth/differentiation factor 5 precursor (Cartilage-derived morphogenetic protein 1). Acc. No. P43026
58. Granzyme B precursor (T-cell serine protease 1-3E) (Cytotoxic T-lymphocyte proteinase 2) (Lymphocyte protease) (SECT) (Granzyme 2) (Cathepsin G-like 1) (Fragmentin 2) (Human lymphocyte protein) (HLP) (C11). Acc. No. P10144
59. Histone H1′ (H1.0) (H1(0)). Acc. No. P07305
60. Histone H1A (H1.1). Acc. No. P16401
61. Histone H1B (H1.4). Acc. No. P10412
62. Histone H1C (H1.3). Acc. No. P16402
63. Histone H1D (H1.2). Acc. No. P16403
64. Histone H1T. Acc. No. P22492
65. Histone H1X. Acc. No. Q92522
67. Core histone macro-H2A.2 (Histone macroH2A2) (mH2A2). Acc. No. Q9P0M6
69. Histone H2B.a/g/k (H2B.1 A) (H2B/a) (H2B/g) (H2B/k). Acc. No. P02278
70. Histone H2B.c (H2B/c). Acc. No. Q99880
73. Histone H2B.f (H2B/f) (H2B.1). Acc. No. P33778
74. Histone H2B.h (H2B/h). Acc. No. Q93078
75. Histone H2B.j (H2B/j). Acc. No. Q93079
77. Histone H2B.n (H2B/n) (H2B.2). Acc. No. P23527
80. Histone H2B.s (H2B/s). Acc. No. P57053
84. Histone deacetylase 1. Acc. No. Q13547
85. Antimicrobial peptide hepcidin precursor (Liver-expressed antimicrobial peptide) (LEAP-1) [Contains: Hepcidin 25; Hepcidin 20]. Acc. No. P81172
86. Hepatocellular carcinoma protein HHCM. Acc. No. Q05877
89. Heat shock cognate 71 kDa protein. Acc. No. P11142
90. Heat shock 70 kDa protein 1-HOM. Acc. No. P34931
91. Human T-cell leukemia virus enhancer factor. Acc. No. P32314
92. Calpain inhibitor (Calpastatin) (Sperm BS-17 component). Acc. No. P20810
93. Caspase-1 inhibitor Iceberg. Acc. No. P57730
94. Importin alpha-1 subunit (Karyopherin alpha-1 subunit) (SRP1-beta) (RAG cohort protein 2) (Nucleoprotein interactor 1) (NPI-1). Acc. No. P52294
95. Interleukin-1 receptor-associated kinase 1 (IRAK-1). Acc. No. P51617
96. Interferon regulatory factor 2 (IRF-2). Acc. No. P14316
98. Nuclear factor NF-kappa-B p100 subunit (H2TF1) (Oncogene LYT-10) (LYT10). Acc. No. Q00653
101. Leukemia associated protein 2. Acc. No. Q043262
102. Lysosomal trafficking regulator (Beige homolog). Acc. No. Q96998
105. Thimet oligopeptidase (Endopeptidase 24.15). Acc. No. P52888
112. Myb-related protein B (B-Myb). Acc. No. P10244
113. Myogenic factor MYF-5. Acc. No. P13349
114. Myogenic factor MYF-6. Acc. No. P23409
115. Myogenin (Myogenic factor MYF-4). Acc. No. P15173
119. Proprotein convertase subtilisin/kexin type 7 precursor (EC 3.4.21.-) (Proprotein convertase PC7) (Subtilisin/kexin-like protease PC7) (Prohormone convertase PC7) (Lymphoma proprotein convertase). Acc. No. Q16549
120. Polycystin precursor (Autosomal dominant polycystic kidney disease protein 1). Acc. No. P98161
122. Polymyositis/scleroderma autoantigen 1 (Autoantigen PM/Scl 1) (Polymyositis/scleroderma autoantigen 75 kDa) (PM/Scl-75) (P75 polymyositis-scleroderma overlap syndrome associated autoantigen). Acc. No. Q06265
123. Prostatic acid phosphatase precursor. Acc. No. P15309
126. 31 kDa transforming protein (Transcription factor PU.1). Acc. No. P17947
129. Retinoblastoma binding protein 2 (RBBP-2). Acc. No. P29375
130. Retinoblastoma-like protein 1 (107 kDa retinoblastoma-associated
131. Retinoblastoma-like protein 2 (130 kDa retinoblastoma-associated
protein) (PRB2) (P130) (RBR-2). Acc. No. Q08999
133. X-linked retinitis pigmentosa GTPase regulator. Acc. No. Q92834
134. Semaphorin 3A precursor (Semaphorin III) (Sema III). Acc. No. Q14563
135. Semaphorin 3B precursor (Semaphorin V) (Sema V). Acc. No. Q13214
136. Semaphorin 3C precursor (Semaphorin E) (Sema E). Acc. No. Q99985
137. Semaphorin 3D precursor. Acc. No. Q95025
138. Mothers against decapentaplegic homolog 1 (SMAD 1) (Mad-related
protein 1) (Transforming growth factor-beta signaling protein-1) (BSP-1)
(hSMAD1) (JV4-1). Acc. No. Q15797
No. P08294
140. Striatin. Acc. No. O43815
141. Treacle protein (Treacher collins syndrome protein). Acc. No. Q13428
142. Thyrotroph embryonic factor. Acc. No. Q10587
143. T-lymphoma invasion and metastasis inducing protein 1 (TIAM1 protein).
Acc. No. Q13009
144. TNG1 protein. Acc. No. P56846
Q03169
146. Tuberin (Tuberous sclerosis 2 protein). Acc. No. P49815
P53804
148. Utrophin (Dystrophin-related protein 1) (DRP1) (DRP). Acc. No. P46939
149. Wiskott-Aldrich syndrome protein interacting protein (WASP interacting
150. Wiskott-Aldrich syndrome protein family member 1 (WASP-family protein
member 1) (Verprolin homology domain-containing protein 1). Acc. No. Q92558
151. Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein
member 2) (Verprolin homology domain-containing protein 2). Acc. No.
152. Wiskott-Aldrich syndrome protein family member 3 (WASP-family protein member 3) (Verprolin homology domain-containing protein 3). Acc. No. Q9UPY6
154. DNA-repair protein complementing XP-B cells (Xeroderma pigmentosum group B complementing protein) (DNA excision repair protein ERCC-3) (Basal transcription factor 2 89 kDa subunit) (BTF2-P89) (TFIIH 89 kDa subunit). Acc. No. P19447
155. DNA-repair protein complementing XP-C cells (Xeroderma pigmentosum group C complementing protein) (P125). Acc. No. Q01831
156. DNA-repair protein complementing XP-F cell (Xeroderma pigmentosum group F complementing protein) (DNA excision repair protein ERCC-4). Acc. No. Q92889

Table 4
Search carried out using the ExPASy software with the motif [R/K]-x-[R/K]-[R/K]-[R/K], wherein X = any amino acid.

3. Apoptosis related protein APR-2. Acc. No. Q9Y5M1
4. Beta-secretase precursor (Beta-site APP cleaving enzyme) (Beta-site amyloid precursor protein cleaving enzyme) (Aspartyl protease 2) (Asp 2) (ASP2) (Membrane-associated aspartic protease 2) (Memapsin-2). Acc. No. P56817
6. Fetal alzheimer antigen (Fetal Alz-50-reactive clone 1). Acc. No. Q12830
7. Caspase-10 precursor (ICE-like apoptotic protease 4) (Apoptotic protease Mch-4) (FAS-associated death domain protein interleukin-1B-converting enzyme 2) (FLICE2). Acc. No. Q92851
17. Neurogenic differentiation factor 3 (Neurogenic basic-helix-loop-helix protein) (Neurogenin 1). Acc. No. Q92886
18. Parathyroid hormone precursor (Parathyrin) (PTH) (Parathormone). Acc. No. P01270
20. Ski oncogene (C-ski). Acc. No. P12755
21. Spastin. Acc. No. Q9UBP0
23. T-cell acute lymphocytic leukemia-1 protein (TAL-1 protein) (STEM cell
24. Tumor necrosis factor ligand superfamily member 13 (A proliferation-
inducing ligand) (APRIL) (TNF-and APOL-related leukocyte expressed ligand 2)
(TALL-2) (TNF-related death ligand-1) (TRDL-1). Acc. No. O75888
25. Tumor necrosis factor receptor superfamily member 18 precursor
(Glucocorticoid-induced TNFR-related protein) (Activation-inducible TNFR
family receptor). Acc. No. Q9Y5U5
27. Vascular endothelial growth factor B precursor (VEGF-B) (VEGF related
factor). Acc. No. P49765
28. Vascular endothelial growth factor precursor (VEGF) (Vascular
permeability factor) (VPF). Acc. No. P15692

Table 5
Search carried out using the ExPASy software with the motif [R/K]-[R/K]-[R/K]-x-
[R/K], wherein X = any amino acid.
1. Transcription factor AP-1 (Proto-oncogene C-JUN) (P39) (G0S7). Acc.
   No. P05412
2. T-cell surface glycoprotein CD3 zeta chain precursor (T-cell receptor T3
   zeta chain). Acc. No. P20963
3. T lymphocyte activation antigen CD86 precursor (Activation B7-2 antigen)
   (CTLA-4 counter-receptor B7.2) (FUN-1). Acc. No. P42081
4. T-cell surface glycoprotein CD8 beta chain precursor (Antigen CD8B).
   Acc. No. P10966
5. Cell division cycle 2-like protein kinase 5 (Cholinesterase-related cell
division controller) (CDC2-related protein kinase 5). Acc. No. Q14004
   interval MAGE superfamily 6) (DAM6). Acc. No. O15479
7. Parathyroid hormone-related protein precursor (PTH-rP) (PTHrP). Acc.
   No. P12272
   Acc. No. P04156
9. Transforming growth factor beta 2 precursor (TGF-beta 2)
(Glioblastoma-derived T-cell suppressor factor) (G-TSF) (BSC-1 cell growth inhibitor) (Polyergin) (Cetermin). Acc. No. P08112


11. DNA-repair protein complementing XP-B cells (Xeroderma pigmentosum group B complementing protein) (DNA excision repair protein ERCC-3) (Basal transcription factor 2 89 kDa subunit) (BTF2-P89) (TFIIH 89 kDa subunit). Acc. No. P19447

12. DNA-repair protein complementing XP-C cells (Xeroderma pigmentosum group C complementing protein) (P125). Acc. No. Q01831

13. Hypothetical 28.3 kDa protein (TCF3 (E2A) fusion partner in childhood leukemia). Acc. No. Q9UPA6

The following Examples illustrate the invention.

Example 1

This experiment was carried out to compare the translocation efficiency of different protein delivery systems: lipofectin reagent, antennapedia and histone H1.4B. The histone H1.4B protein contained the defined motif.

The quantitative measurement of translocation was carried out by reading the activity of a β-Galactosidase enzyme that was included as part of a fusion protein with the translocation agents. When provided with the appropriate galactoside substrate, the enzyme deglycosylates the substrate leading to the accumulation of dioxetane. During a following incubation in a different buffer, the dioxetane becomes deprotonated and decomposes with emission of light (425 nm) that can be read using a luminometer. This system, therefore, provided a sensitive means of detecting the amount of the β-Galactosidase present in the samples, and ultimately, the amount of fusion protein delivered into the cells.

The experiments were carried out using 8-well chamber slides. 3x10^4 Hela cells were seeded into each well, supplemented with 400 µl RPMI + 10% FCS medium and incubated overnight at 37°C and 5% CO_2. The following day, the appropriate dilutions of protein were made into RPMI medium and added to the wells. Both lipofectin delivery and translocation experiments were carried out for 4 hours.

The experiments were carried out on two different days: the first day a
negative control β-Galactosidase and the H1.4B-Bgal were tested, and the second day the antennapedia fusion (Antp-βgal) was tested. A lipofectin delivery of β-Galactosidase, provided by Roche, was performed on both days as positive control for delivery into the cells.

The amount of each protein construct was determined after previous tests that showed these were the best values to obtain a linear reading using a luminometer. It was decided to start with the amount of each that gave a value close to $6 \times 10^7$ rlu, as this is the limit of the equipment’s capacity. From that value, three consecutive 1/5 fold dilutions were made in each case.

These tests also showed that the different constructs containing the β-Galactosidase had different activities. Therefore, the original activity of the different proteins was also measured in parallel to the activity recovered after translocation, in order to make sure that all the proteins were standardised to an original maximum activity of around $6 \times 10^7$ rlu.

The experimental protocol was as follows:

1. Wash cells (split previous night into chamber slides: 2-3 $\times 10^4$ cells in 0.4ml complete RPMI/well) 2 times with incomplete RPMI.
2. Add 0.5 ml complete RPMI/well.
3. Add peptide (from 200 ng/well diluted in steps of 1:2 to blank).
4. Leave for 4 hours in incubator.

Fixing of chamber slides and development with Avidin-Fluorescein

1. Remove medium from chamber slides.
2. Wash slide 3 times with 1 x PBS.
3. Eliminate most liquid, pull off chamber and let the slide dry.
4. Freeze at -20°C for at least 20min.
5. Fix in 3% formaldehyde in 1 x PBS for 15 – 20 min.
6. Rinse in 0.5% BSA in PBS.
7. Block with 5% BSA in 1 x PBS for 20min.
8. Rinse 3 times with 0.5 M NaCl in PBS.
9. Add Avidin-fluorescein solution (1:4000 dilution in 0.1 M NaHCO$_3$, 0.5 M NaCl, pH 8.5) 50 µl/ well and incubate for 30 min in dark humid chamber.
10. Wash 10 times with 0.5 M NaCl in PBS.
11. Remove plastic sections around wells.
12. Add 2-3 drops of 4,6-diamidino-2-phenylindole (DAPI) or fluorescent stain and seal slide with nail varnish.
13. Check in microscope and store in dark humid chamber.

The results after translocation were obtained by reading the β-Galactosidase recovered from the cell extracts and are shown in Tables 6 and 7. In all cases the values obtained follow a linear pattern which indicates that the system is efficient. The results for the β-Galactosidase negative controls shows an increasing tendancy that does not correlate with original expectations, as this molecule does not translocate and no difference of activity should be observed when the amount of protein is increased. However, this artefact may be due to the washes or to molecules of protein that did not get into the cells but attached to the cellular membrane when they were added to the wells. In any case, the values for the biggest amounts of β-Galactosidase protein are clearly lower than those obtained from the delivery systems.

As for the comparison between lipofectin delivery, Antp-β-Gal and H1.4B-β-Gal, the histone H1.4B translocates at a rate five times higher than that of lipofectin and antennapedia, as the β-Galactosidase activity recovered after translocation is five times higher.
<table>
<thead>
<tr>
<th>Amount of protein</th>
<th>Protein activity (rlu)</th>
<th>Activity after transfection (rlu)</th>
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</thead>
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<tr>
<td>Blank</td>
<td>8.578</td>
<td>27.347</td>
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<tr>
<td>Blank</td>
<td>10.204</td>
<td>26.662</td>
</tr>
<tr>
<td>1.6 ng</td>
<td>22.026.968</td>
<td>50.353</td>
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<td>47.212</td>
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<tr>
<td>8 ng</td>
<td>43.535.464</td>
<td>140.342</td>
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<td>8 ng</td>
<td>43.392.116</td>
<td>162.319</td>
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<td>40 ng</td>
<td>62.436.592</td>
<td>635.228</td>
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<td>40 ng</td>
<td>61.919.408</td>
<td>675.640</td>
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<th>Amount of protein</th>
<th>Protein activity (rlu)</th>
<th>Activity after translocation (rlu)</th>
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</thead>
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<td>640 pg</td>
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<td>3.2 ng</td>
<td>51.004.204</td>
<td>29.527</td>
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<tr>
<td>3.2 ng</td>
<td>50.660.716</td>
<td>35.227</td>
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<tr>
<td>15 ng</td>
<td>24.657.924</td>
<td>56.941</td>
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<tr>
<td>16 ng</td>
<td>24.504.798</td>
<td>55.164</td>
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<td>80 ng</td>
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<td>242.200</td>
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<tr>
<td>80 ng</td>
<td>overload</td>
<td>298.589</td>
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<th>Amount of protein</th>
<th>Protein activity (rlu)</th>
<th>Activity after translocation (rlu)</th>
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<tbody>
<tr>
<td>1.6 ng</td>
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<td>49.243</td>
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<td>1.6 ng</td>
<td>3.694.802</td>
<td>35.667</td>
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<td>8 ng</td>
<td>11.583.624</td>
<td>70.352</td>
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<td>8 ng</td>
<td>12.377.050</td>
<td>60.663</td>
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<tr>
<td>40 ng</td>
<td>33.230.640</td>
<td>355.641</td>
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<td>40 ng</td>
<td>33.458.668</td>
<td>333.496</td>
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<tr>
<td>200 ng</td>
<td>58.670.112</td>
<td>2.687.583</td>
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<td>200 ng</td>
<td>56.713.392</td>
<td>2.989.107</td>
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### Table 7

#### β-Gal positive control (Roche) transfection

<table>
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<th>Activity after transfection (rlu)</th>
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<tr>
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<td>Blank</td>
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<td>1.6 ng</td>
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<td>40.121</td>
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<td>1.6 ng</td>
<td>10,740.756</td>
<td>33.578</td>
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<tr>
<td>8 ng</td>
<td>33,610.784</td>
<td>164.745</td>
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<tr>
<td>8 ng</td>
<td>34,080.292</td>
<td>157.913</td>
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<tr>
<td>40 ng</td>
<td>57,920.416</td>
<td>715.773</td>
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<tr>
<td>40 ng</td>
<td>57,083.376</td>
<td>744.405</td>
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#### Antp-β-Gal translocation

<table>
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<th>Activity after tranlocation (rlu)</th>
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<td>2 ng</td>
<td>628.733</td>
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<td>10 ng</td>
<td>2,638.336</td>
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<td>10 ng</td>
<td>2,660.380</td>
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</tr>
<tr>
<td>50 ng</td>
<td>10,787.237</td>
<td>39.725</td>
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<tr>
<td>50 ng</td>
<td>11,323.412</td>
<td>25.066</td>
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<tr>
<td>250 ng</td>
<td>36,136.704</td>
<td>549.104</td>
</tr>
<tr>
<td>250 ng</td>
<td>37,321.488</td>
<td>682.181</td>
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#### Tat-β-Gal translocation

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<th>Amount of protein</th>
<th>Protein activity (rlu)</th>
<th>Activity after tranlocation (rlu)</th>
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<td>0.8 ng</td>
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<td>4.317</td>
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<td>0.8 ng</td>
<td>1,620.746</td>
<td>5.587</td>
</tr>
<tr>
<td>4 ng</td>
<td>6,443.625</td>
<td>6.318</td>
</tr>
<tr>
<td>4 ng</td>
<td>5,424.017</td>
<td>5.454</td>
</tr>
<tr>
<td>20 ng</td>
<td>20,843.930</td>
<td>30.984</td>
</tr>
<tr>
<td>20 ng</td>
<td>22,358.902</td>
<td>25.516</td>
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<tr>
<td>100 ng</td>
<td>53,224.476</td>
<td>220.384</td>
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<tr>
<td>100 ng</td>
<td>49,537.884</td>
<td>128.548</td>
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</table>
Example 2

Various peptides were prepared synthetically and conjugated to a biotin molecule. The conjugates were then tested for translocation using the experimental protocol described in Example 1.

The conjugates are identified in Table 8, together with the identification of the proteins from which they are derived.

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Genbank Nb</th>
<th>Peptide amino acid sequence</th>
<th>SEQ ID NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEF</td>
<td>gi:4507431</td>
<td>Biotin-MIKKAKVVVPDEQKDEK-Amide</td>
<td>1</td>
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<tr>
<td>BMP-3</td>
<td>gi:115072</td>
<td>Biotin-TLKKARRKQWIEPRNCARR-Amide</td>
<td>2</td>
</tr>
<tr>
<td>Spi-1</td>
<td>gi:4507175</td>
<td>Biotin-GEVKVKKLYQFSGEVL-Amide</td>
<td>3</td>
</tr>
<tr>
<td>APC</td>
<td>gi:182397</td>
<td>Biotin-SSRKAKKPAQTASKLPPPVAR-Amide</td>
<td>4</td>
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<tr>
<td>P80-coilin</td>
<td>gi:4758024</td>
<td>Biotin-NLSLRKAKKRAFAQLEG-Amide</td>
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</tr>
<tr>
<td>BMP-4</td>
<td>gi:115073</td>
<td>Biotin-HALTRRRRAKRSPKHSHQ-Amide</td>
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</tr>
<tr>
<td>IRAK</td>
<td>gi:8928535</td>
<td>Biotin-CLHRRAKRPMTQVYER-Amide</td>
<td>7</td>
</tr>
<tr>
<td>Melastatin1 (p2)</td>
<td>gi:3243075</td>
<td>Biotin-TKGGRGKGKGGKGGKVK-Amide</td>
<td>8</td>
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<tr>
<td>HDAC1</td>
<td>gi:13128860</td>
<td>Biotin-SNFKKAKRVKTEDEKEKDP-Amide</td>
<td>9</td>
</tr>
<tr>
<td>Caspase-1 inhibitor Iceberg</td>
<td>gi:10954343</td>
<td>Biotin-QLLRKRRIFIHSVGAGT-Amide</td>
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<tr>
<td>Dyskerin p1</td>
<td>gi:4503337</td>
<td>Biotin-IKKEKKSKDKKKAKAGLES-Amide</td>
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<tr>
<td>Dyskerin p2</td>
<td>gi:4503337</td>
<td>Biotin-LPKKHKKKKERRKLSPED-Amide</td>
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<tr>
<td>E2A leukemia p1</td>
<td>gi:12804613</td>
<td>Biotin-AARGRRRQRELNRKYQA-Amide</td>
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<tr>
<td>E2A leukemia p2</td>
<td>gi:12804613</td>
<td>Biotin-GPSGRKRRVPRDGRAGNA-Amide</td>
<td>14</td>
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<tr>
<td>Major prion protein precursor</td>
<td>gi:2944217</td>
<td>Biotin-GLCKKRKPFGWNTGGSR-Amide</td>
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<tr>
<td>Bruton’s tyrosine kinase</td>
<td>gi:2117817</td>
<td>Biotin-HRTKKPLPPTEEDQILKKP-Amide</td>
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<tr>
<td>Sex-determining region Y protein</td>
<td>gi:226981</td>
<td>Biotin-PNYKYPRRRACKMLPKNCS-Amide</td>
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<tr>
<td>Baculoviral IAP repeat-Containing protein 5</td>
<td>gi:4502145</td>
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<tr>
<td>Apoptosis Inhibitor Survivin</td>
<td>gi:2315863</td>
<td>Biotin-NKIAKETNKKKEFEETAKKVRRA-Amide</td>
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Granzyme B
  Precursor  gi:4758494  Biotin-QLERKAKRTRAVQPLRLPS-Amide
Parathyroid hormone  gi:4506267  Biotin-SQRPRKKEDNVLSHEKSLGE-Amide
Toll-interleukin 1
    receptor
    domain-containing
    adapter protein  gi:20140483  Biotin-GKMAFRQTLKKPKKRPNSPEST-Amide

In each case, translocation was observed.
CLAIMS

1. Use of a protein, or a fragment thereof that retains the biological activity of the protein, comprising an amino acid sequence $X^1X^1X^2X^3X^4$, where $X^1 = R$ or $K$ and $X^2$ and $X^3$ = any amino acid, in the manufacture of a composition for the treatment of a disease characterised by a deficiency in the production or function of the endogenous protein, or to regulate a biochemical pathway.

2. Use according to claim 1, wherein $X^2 = A$ or $V$.

3. Use according to claim 1 or claim 2, wherein $X^3 = R$ or $K$.

4. Use according to any preceding claim, wherein the amino acid sequence is KKAKK or KKVKK.

5. A protein according to any preceding claim, which is a human-derived protein.

6. Use according to claim 1, wherein the protein is any of those identified in Table 2.

7. Use according to claim 1, wherein the protein is selected from the group consisting of: IRAK1, MYD88, transcription factor E2A, Fanconi A gene product and cytoplasmic tyrosine kinase.

8. A method for the treatment of disease, the disease being characterised by a deficiency in the production or function of an endogenous protein comprising the amino acid sequence defined in any of claims 1 to 4, comprising administering to a patient a composition comprising the functional protein, or a functional fragment thereof comprising the defined amino acid sequence.

9. An antibody having affinity for a protein comprising an amino acid sequence as defined in any of claim 1 to 4.

10. An antibody according to claim 9, wherein the protein is any of those identified in Table 2, or a mutant thereof.

11. An antibody according to claim 9 or claim 10, wherein the protein is a product of an oncogene.

12. An antibody according to any of claims 8 to 10, which has affinity for the protein product of the human APC gene.
13. A conjugate of a peptide capable of translocation across a cell membrane, and a therapeutic or diagnostic agent, wherein the peptide comprises the amino acid sequence \( X^1 X^2 X^3 X^4 \), where \( X^1 \) is R or K and \( X^2 \) and \( X^3 \) = any amino acid.

14. A conjugate according to claim 13, wherein \( X^2 = A \) or \( V \).

15. A conjugate according to claim 13 or claim 14, wherein \( X^3 = R \) or K.

16. A conjugate according to any of claims 13 to 15, with the proviso that the peptide does not contain the sequence KKAKK or KKARK.

17. A conjugate according to any of claims 12 to 16, wherein the peptide comprises the amino acid sequence KKVKK.

18. A conjugate according to any of claims 13 to 17, wherein the agent is a therapeutic protein.

19. A conjugate according to any of claims 12 to 18, wherein the agent is an antibody.

20. A conjugate according to claim 19, wherein the antibody has affinity for a protein comprising the sequence defined in any of claims 1 to 4.

21. A conjugate according to any of claims 13 to 20, wherein the agent has its site of activity within a cell.

22. A conjugate according to any of claims 13 to 17, wherein the agent is a polynucleotide molecule.

23. A conjugate according to any of claims 13 to 17, wherein the agent is a contrast agent.

24. A conjugate according to any of claims 13 to 20, which is a fusion protein.

25. A conjugate according to any of claims 13 to 23, wherein the agent is conjugated to the peptide via a chemical linker molecule.

26. A conjugate according to any preceding claim, wherein the peptide comprises at least 20 amino acids.

27. A conjugate according to any preceding claim, for use in therapy or diagnosis.

28. An expression vector that encodes a fusion protein according to claim 24.

29. A recombinant cell line comprising an expression vector according to claim 28.
30. A process for the production of a therapeutic or diagnostic agent capable of being delivered across a cell membrane, comprising covalently attaching the agent to a peptide comprising the amino acid sequence as defined in any of claims 1 to 4.

31. Use of a therapeutic agent that exerts its therapeutic effect within a cell, in the manufacture of a composition to treat a disease, wherein the agent is conjugated to a peptide that comprises the amino acid sequence defined in any of claims 1 to 4.

32. Use of a conjugate of a peptide capable of translocation across a cell membrane and a therapeutic or diagnostic agent, in the manufacture of a composition to treat or diagnose a disease, wherein the peptide comprises the amino acid sequence defined in any of claims 1 to 4.

33. Use of an antibody in the manufacture of a medicament for the treatment of a tumour, wherein the antibody has affinity for a protein product of an oncogene, and comprises an amino acid sequence as defined in any of claims 1 to 4.

34. Use according to any of claims 31 to 33, wherein the conjugate is as defined in any of claims 13 to 26.

35. A composition, for therapeutic use, consisting of a protein as defined in any of claims 1 to 6, and a pharmaceutically acceptable carrier.
SEQUENCE LISTING

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Artificial Sequence

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Description of Artificial Sequence: Synthetic peptide conjugated with biotin

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