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Abstract

The direct intracellular delivery of proteins has, until recently, been difficult to achieve, due primarily to the bioavailability barrier of the plasma membrane. During the past 15 years, a variety of peptides called protein transduction domains (PTDs) or cell penetrating peptides (CPPs), have been characterized for their ability to translocate into live cells. The most commonly studied are homeodomain transcription factors such as Antennapedia, the herpes simplex virus (HSV) type 1 protein VP22, and the human immunodeficiency virus (HIV-1) transactivator TAT protein. Recently, polyarginine exhibits even greater efficiency in terms of delivery of several peptides and proteins. Numerous examples of biologically active full-length proteins and peptides have been delivered to cells and tissues, both in vitro and in vivo. These studies offer new avenues for treatment of several diseases. The main mechanism of protein transduction is an electrostatic interaction with the plasma membrane, penetration into cells by macropinocytosis, and a release to cytoplasm and nuclei by retrograde transport. Moreover, the intercellular transfer of endogenous transcription factors, such as TAT and homeoproteins, seems to point to an original and important mode of signal transduction. The protein transduction systems have opened up several possibilities, not only for the development of new peptide/protein drugs but also for consideration of their physiological and developmental implications.

KEYWORDS: protein transduction, protein transduction domain, cell penetrating peptide, macropinocytosis, intercellular transfer

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Review

Protein Transduction Technology: A Novel Therapeutic Perspective

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The direct intracellular delivery of proteins has, until recently, been difficult to achieve, due primarily to the bioavailability barrier of the plasma membrane. During the past 15 years, a variety of peptides called protein transduction domains (PTDs) or cell penetrating peptides (CPPs), have been characterized for their ability to translocate into live cells. The most commonly studied are homeodomain transcription factors such as Antennapedia, the herpes simplex virus (HSV) type 1 protein VP22, and the human immunodeficiency virus (HIV-1) transactivator TAT protein. Recently, polyarginine exhibits even greater efficiency in terms of delivery of several peptides and proteins. Numerous examples of biologically active full-length proteins and peptides have been delivered to cells and tissues, both *in vitro* and *in vivo*. These studies offer new avenues for treatment of several diseases. The main mechanism of protein transduction is an electrostatic interaction with the plasma membrane, penetration into cells by macropinocytosis, and a release to cytoplasm and nuclei by retrograde transport. Moreover, the intercellular transfer of endogenous transcription factors, such as TAT and homeoproteins, seems to point to an original and important mode of signal transduction. The protein transduction systems have opened up several possibilities, not only for the development of new peptide/protein drugs but also for consideration of their physiological and developmental implications.

Key words: protein transduction, protein transduction domain, cell penetrating peptide, macropinocytosis, intercellular transfer

The plasma membrane consists of a lipid bilayer into which proteins and glycoproteins are inserted. In general, the hydrophobic nature of these lipids makes it impossible for the vast majority of peptides and proteins to cross the membrane. A significant exception to this rule have recently been pointed out, with the identification of several protein transduction domains (PTDs), also known as cell-penetrating peptides (CPPs), that are capable of transducing cargo across the membrane, allowing proteins to accumulate within the cell. The most commonly studied are homeodomain transcription factors such as Antennapedia (Antp) [1–6], the herpes simplex virus (HSV) type 1 protein VP22 [7], and the human immunodeficiency virus (HIV-1) transactivator TAT protein [8–13]. Recently, poly-arginine (polyR) and

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poly-lysine have been shown to exhibit even greater efficiency in terms of delivery of several peptides and proteins [14–18]. Significantly, when synthesized as recombinant fusion proteins or covalently cross-linked to full-length proteins, these PTDs are capable of delivering biologically active proteins. These PTD fusion proteins are found in both the cytoplasm and the nucleus. Studies by our group, along with those by others, have demonstrated how these protein transduction systems have opened up several possibilities for the development of new peptide/protein drugs [1, 12, 14, 15, 19, 20]. Here, we review some of the most recent advances in this rapidly expanding area of research.

Intracellular Delivery of PTD-Conjugated Macromolecules

The most impressive aspect of PTD-mediated delivery and its therapeutic potential is its size independence. The ability of PTDs to deliver cargo into cells is not limited only to proteins or peptides. Several PTDs have been shown to mediate the efficient intracellular accumulation of non-organic molecules. The broad utility of protein transduction technology is illustrated schematically in Fig. 1.

Protein and peptide delivery. Proteins have been evolutionarily selected to perform specific functions. Thus, the ability to deliver a wide variety of full-length, functional proteins has tremendous potential as a biological tool for studying cellular processes as well as for developing novel and potentially very specific therapeutic agents. To date, a growing number of transducible proteins covering a wide range of sizes and functional classes have been successfully used to study intracellular mechanisms. These include PDX-1 [1–3], BETA2/NeuroD [19], Ngn3 [20], enhanced green fluorescent protein (EGFP) [1, 19], Cre recombinase [9, 10], and p53 [21]. PTDs have been shown to deliver proteins in excess of 100 KDa into cultured cells and most, if not all, cells in mammalian model systems [8].

Peptides can be used in a vast range of applications in pharmaceutical research. By linking peptide sequences to PTDs, previously non-cell-penetrating peptides have been introduced into cells, thereby altering existing protein-protein or protein-oligonucleotide interactions. Several peptides have been fused to PTDs, thus enabling their entry into the cytoplasm. The introduced peptides inhibit protein binding or protein complex formation [11, 12, 14–16]. Subsequently, several reports have described the delivery of peptides and proteins *in vivo* [12, 14]. Moreover, Barka T *et al.* recently showed the advantages and versatility of protein transduction over viral transgene delivery [22, 23]. Their studies using retroviral vectors expressing β-galactosidase [22] were compared with results from the injection of TAT-β-galactosidase fusion protein *in vivo* [23]. Injection of TAT-β-galactosidase by retrograde duct injection resulted in transduction into 100 % of the rat salivary gland cells in a concentration-dependent manner, whereas viral delivery could only achieve 30–50% efficiency.

Delivery of antisense oligonucleotides. The use of antisense techniques as a pharmaceutical tool is interesting because this technology potentially has the ability to downregulate the expression of virtually any desired gene. Antisense techniques are based on sequence-specific oligonucleotide analogs that, after introduction to the cytosol, can hybridize with complementary mRNA strands. This hybridization causes translational arrest or recruitment of RNaseH, thereby altering the gene expression in the cell [24]. The development of antisense therapy has focused mainly on improving methods for oligonucleotide delivery into cells. Although the cellular uptake of naked oligonucleotides is poor, it is significantly increased by coupling the oligonucleotide to a transporter peptide, leading to decreased expression of the gene product of the targeted gene. Morris *et al.* describe a potent new strategy for oligonucleotide delivery based on the use of a short peptide vector, termed MPG, which contains a hydrophobic domain, derived from the fusion sequence of HIV gp41, and a hydrophilic domain, derived from the nuclear localization sequence of SV40 T-antigen [25]. Tung *et al.* reviews the preparation and applications of PTD-oligonucleotide conjugates [26]. These strategy of oligonucleotide delivery into cultured cells based on a peptide vector offers several advantages including efficiency, stability and absence of cytotoxicity, when compared to other commonly used approaches of delivery. The interaction with PTD strongly increases both the stability of the oligonucleotide to the nuclease and the crossing of the plasma mem-

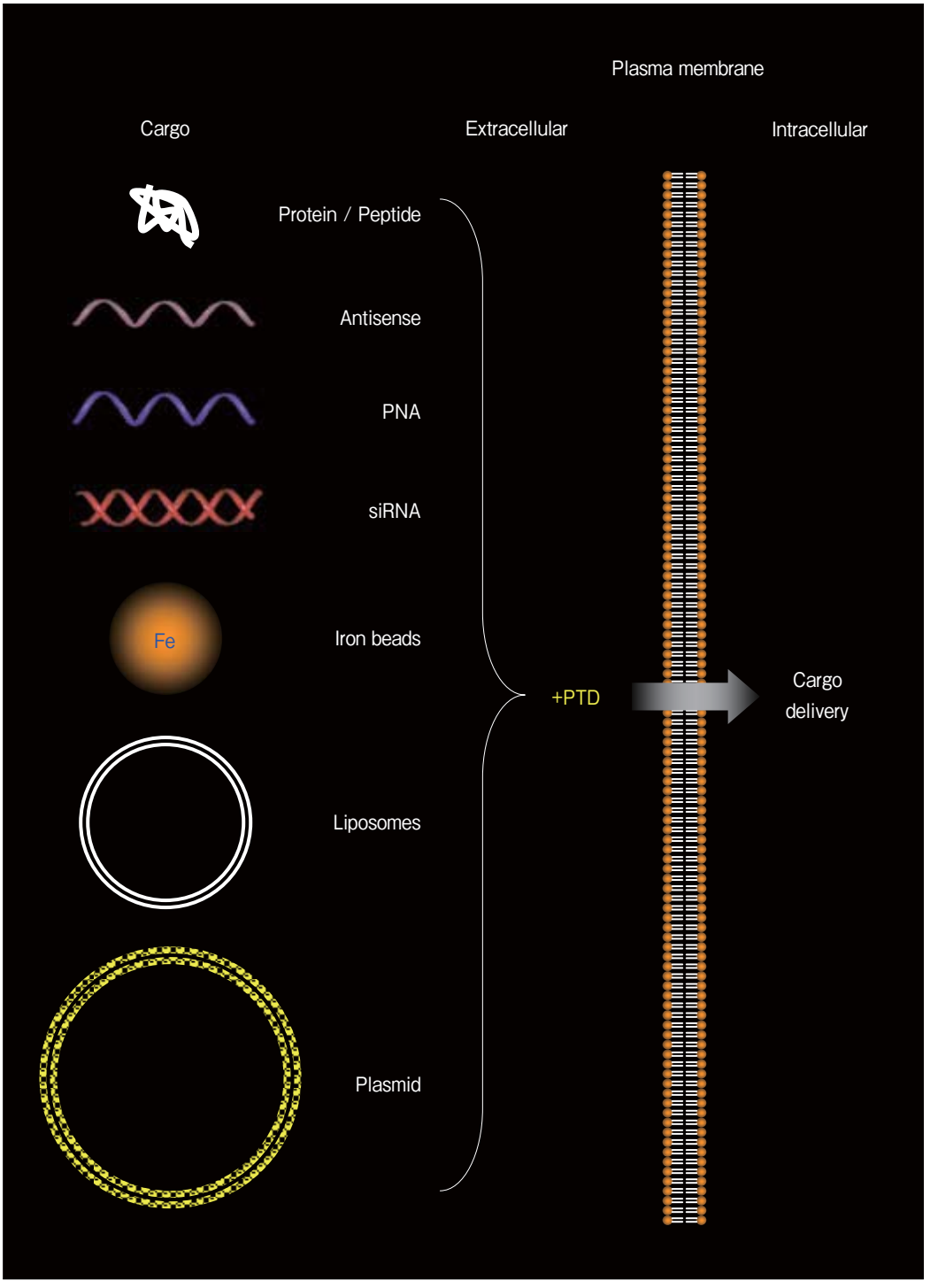

Fig. 1 Utility of protein transduction technology. A wide variety of cargo has been covalently linked to PTDs. Currently, most molecules can be transduced into cells when linked to a PTD.

brane.

The rapid degradation of natural oligonucleotides inside the cell prohibits their use in antisense technology; however, many oligonucleotide analogs have been used in antisense techniques, with varying results. Astriab-Fisher *et al.* showed that PTD-phosphorothionate oligonucleotide conjugate was internalized into cells under serum-replete conditions and inhibited expression of the target molecule [27]. PTD-PNA could also inhibit the expression (see '*Delivery of peptide nucleic acids (PNA)*' section). The development of modified oligonucleotides is constantly progressing [24]. Increased stability, enhanced RNA binding affinity and lower toxicity are just some of the aspects to be considered when choosing a suitable oligonucleotide.

Delivery of peptide nucleic acids (PNAs). PNAs form stable complexes with DNA and RNA, have low toxicity and, unlike naturally occurring oligonucleotides, are not sensitive to nucleases. These features make them an ideal tool for antisense therapy. As is the case for the majority of large molecules, the cellular uptake of these oligonucleotides is poor, but coupling to PTDs increases their uptake and thus their applicability as tools for the highly specific downregulation of desired gene products. In many cases the PTD-PNA construct is not synthesized as a continuous chain because synthesis of a continuous chain could interfere with the internalization of the construct and the PNA-mRNA interaction. Therefore, the method most frequently employed is coupling of the PNA to the peptide via a disulfide bond [28, 29]. The disulfide bond has no significant effect on internalization and, once inside the cell, the conditions of the intracellular environment cause the reduction of the disulfide bond, thereby releasing PNAs, which are then free to interact with the desired target [30]. Moreover, PTD-PNA has been shown to enhance the transfection efficacy of plasmids [31]. In combination with the transfection agent polyethyleneimine (PEI), the efficacy of plasmid transfection was improved up to eightfold (see '*Plasmid delivery*' section).

siRNA delivery. The recent discovery of the RNA interference pathway in a wide variety of eukaryotic organisms has provided a novel means of characterizing gene function in mammalian cells. Short, interfering RNAs (siRNAs) have considerable potential as a powerful tool in molecular biology research and in the future as pharmaceutical drugs. However, the major drawback with the use of siRNA, as with most oligonucleotide-based drugs, is the low yield of cellular uptake. By contrast, the use of a mixture of PTD and siRNA directed towards glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA increased the cellular uptake of the siRNA several fold compared with naked siRNA, and the targeted mRNA was downregulated. PTD and siRNA are not covalently linked, but form a complex through electrostatic interactions [32]. The peptide carrier constitutes an excellent tool for the delivery of siRNA into cells.

Delivery of iron nanoparticles. Weissleder's group [33–35] recently delivered into cells 41-nm biocompatible, dextran-coated superparamagnetic iron oxide nanoparticles conjugated to TAT-PTD. Derivatized particles were internalized into lymphocytes over 100-fold more efficiently than non-modified particles. These particles were also internalized into both hematopoietic and neural progenitor cells at levels up to 10–30 pg per cell. Moreover, the iron incorporation did not affect cell viability, differentiation, or proliferation of $CD34^+$ human stem cells. Following intravenous injection into immunodeficient mice, 4% of magnetically $CD34^+$ cells homed to bone marrow per gram of tissue, and single cells could be detected by magnetic resonance (MR) imaging in tissue samples. The method has potential applications for *in vivo* tracking of magnetically labeled cells by MR imaging and for recovering intracellularly labeled cells from organs.

Liposome delivery. The therapeutic delivery of polar compounds is often inefficient owing to the difficulty of crossing the lipid membrane of cells. As a consequence, delivery of these compounds within liposomal carriers has been the focus of increasing attention, but has been hampered by inefficient cellular uptake and consequent degradation through the endocytic pathway. Recently, Torchilin *et al.* [36] demonstrated that relatively large drug carriers, such as 200-nm liposomes, can be delivered into cells by a TAT peptide attached to the liposome surface. Incubation of TAT liposomes with several cells results in intracellular localization of certain liposomes. Confocal analysis showed that the liposomes remained intracellularly intact 1 h after trans-

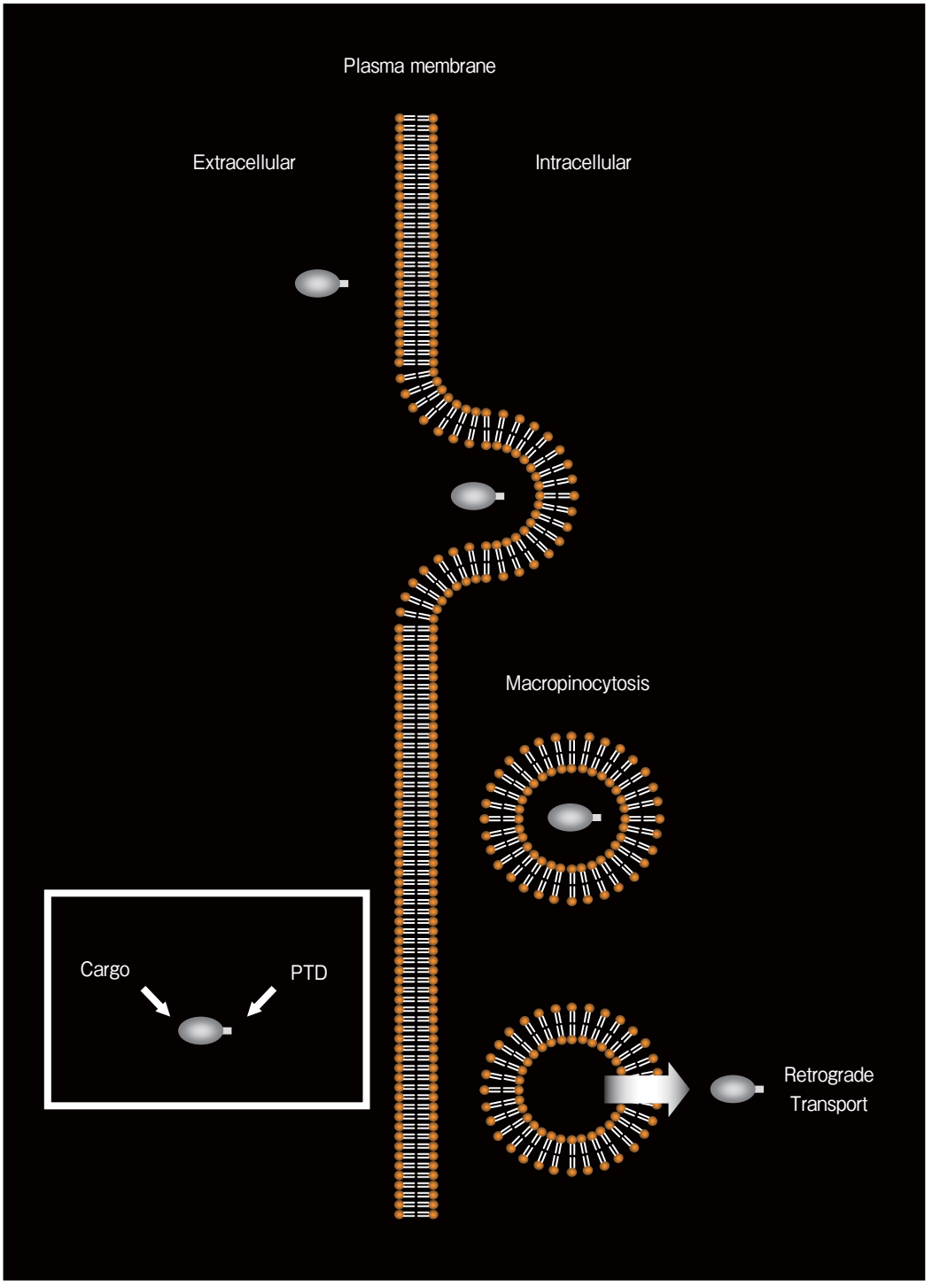

Fig. 2 Mechanism of protein transduction. The primary mechanism of protein transduction is an electrostatic interaction with the plasma membrane, penetration into cells by macropinocytosis, and release to the cytoplasm and nuclei by retrograde transport.

duction. The approach may have important implications for drug delivery directly into the cell cytoplasm.

Plasmid delivery. Although viral methods have several advantages, they also have many drawbacks such as the immunological response provoked by exposure to viral infection. Other currently employed standard methods, including the application of polycationic agents, electroporation and microinjection, are inefficient for use *in vivo*. Polycationic agents have toxicity and targeting problems, electroporation causes high cell mortality and microinjection can only be applied to one cell at a time. However, the use of non-viral synthetic vectors minimizes the risk of triggering an immune response in the treated individual because these vectors lack viral components. PTDs can also deliver plasmids. Sakaguchi *et al.* [37] designed a new system for transporting oligonucleotides into cell nuclei. The vehicle is composed of glutathione-S-transferase, 7 arginine residues, the DNA-binding domain of GAL4 and a nuclear localization signal, which are linked with flexible glycine stretches. The p53-responsive element linked to the GAL4 upstream activating sequence was efficiently transferred by the vehicle protein into the nuclei of primary cultures of neuronal cells, embryonic stem cells, and various human normal cells. The peptide sequence PKKKRKV has mostly been used as a nuclear localization signal (NLS), but it has been suggested that this peptide also has cell-penetrating features, and it has been shown to translocate proteins larger than 970 kDa into the nucleus [38]. The linking of the SV40 NLS peptide sequence via PNA to a reporter gene carrying the vector, transfecting cells and PEI, resulted in an up to eightfold increase in the nuclear uptake of EGFP or *lacZ*-carrying plasmids, compared with only PEI acting as the transfecting agent [31].

Transfection by PTD vectors might use the counter charge between negatively charged plasmid DNA and the positively charged peptide. Hyndman *et al.* [39] demonstrated that incorporation without covalent linkage of a TAT PTD peptide into gene delivery lipoplexes improved gene transfer. Furthermore, sterilized R8 (arginine-8) peptides have been shown to transfect luciferase-containing plasmids into the nucleus with an efficiency that is comparable to Lipofectamine™ [40]. To avoid loss of translocation as a result of the PTD interacting with DNA, the PTD can be designed as a branched complex to interact with DNA and mediate membrane translocation. Eight-branched TAT has been used to transfect cells, with results equivalent to standard Lipofectamine™ strategies [41]. These systems can be a convenient and powerful tool for specifically disrupting the function of DNA-binding proteins.

Delivery of genome of bacterial phage and virus. An extreme case is the intracellular addressing of bacterial phages and viruses [5, 42, 43]. Eguchi *et al.* constructed recombinant lambda phage particles displaying TAT-PTD on their surfaces and carrying mammalian marker genes as part of their genomes [42]. When animal cells are briefly exposed to the TAT-phage, significant expression of phage marker genes is induced with no harmful effects to the cells. Given the size of the phage genome, the technology could therefore be used to introduce up to 20,000 bp of foreign coding DNA into cells. Similarly, incubating a combination of viral vectors and PTDs before viral infection can improve viral gene delivery. The use of adenovirus and Antp-PTD simultaneously significantly improved the efficiency of the gene delivery of green fluorescent protein (GFP) and β-Gal by adenovirus *in vivo* and *in vitro* [43]. Antp-PTD can promote viral uptake without genetic coupling, perhaps through the interaction of Antp-PTD with coat proteins. A possible mechanism is that the amphipathic peptide binds the virus or phage through its hydrophobic side and the lipids through its charged side.

These data may have significant implications for the future of other molecule delivery systems *in vitro* and *in vivo*.

Mechanisms of Protein Transduction

One structural cellular membrane component is required for PTD internalization. It is shown that increasing concentrations of heparin could compete with TAT-PTD internalization [44]. Not surprisingly given their highly basic charge, TAT peptides bind to heparin [45–47]. This work led to the discovery that cell-surface heparin sulfate proteoglycans appear to be key mediators of peptide internalization [48]. Consistent with this observation, cells genetically impaired in the biosynthesis of fully sulfonated

heparin sulfate proteoglycans were selectively impaired for transduction by a TAT fusion protein [48], poly-arginine, and BETA2/NeuroD PTD (Noguchi *et al.* unpublished data). In addition, PTD uptake could be competitively inhibited by the selective degradation of the heparin sulfate sidechains using glycosaminoglycan lyases (Noguchi *et al.* unpublished data). The ubiquitous presence of heparin sulfate might explain the observation that PTDs and conjugated proteins are able to enter a wide variety of cells. This interaction at the cell surface is likely to mediate the uptake of other arginine-rich PTDs. For example, Antp-PTD has also been found to interact strongly with cellular membranes [49].

Early studies showed that the internalization of TAT-PTD, Antp-PTD, or polyR-PTD was not significantly inhibited by incubation at 4 °C, by depletion of the cellular ATP pool, or by inhibitors of endocytosis; additionally, internalization did not depend on a specific primary sequence, implying that receptor recognition was not necessary [18, 50, 51]. Therefore, it had been commonly accepted that the internalization of PTD-containing proteins involves neither endocytosis nor specific protein transporters. However, some mechanistic studies suggest that both the import and nuclear localization of PTD-containing proteins occur only during fixation and not in living cells [52, 53]. Lundberg and Johansson showed that fixation with methanol induces an artificial influx of both VP22 protein from the *Herpes Simplex* virus and the positively charged histone H1 [52]. Additionally, both TAT-PTD and VP22-PTD were shown to mediate cell binding but not translocation [53]. These data suggest that the protein transduction does not occur in living cells. However, a large number of studies have recently reported PTDs in living cells using both reporter proteins and/or functional peptides/proteins. Indeed, protein transduction MUST work in living cells because many published studies have demonstrated PTD-mediated delivery of BIOLOGICALLY ACTIVE proteins/peptides. The following three models have emerged to explain PTD uptake in recent reports.

One model is the perpendicular insertion of amphipathic peptides into the membrane, with hydrophobic residues juxtaposing the lipids and the assembled hydrophilic faces forming a channel through oligomerization. This would allow efficient passage of hydrophilic cargoes, but would also be a threat to cell survival. Several peptides can form pores at the cell surface and, indeed, induce cell death. One example of this is provided by defensins [54], a class of small peptides that form holes in microbial membranes and are produced by the invertebrate immune system and by some vertebrate cells.

A second model proposes that peptides bind to the polar heads of lipids in the membrane through electrostatic interaction, resulting in transient formation of inverted micelles that engulf the peptide and its cargo, even at 4 °C. The re-opening of the micelle inside the cell would then result in release of the PTD − cargo construct into the cytoplasm. Some experiments suggest that Antp-PTD might use this pathway. The addition of Antp-PTD to a film of brain lipids induces the formation of inverted micelles. [55] A peptide of the same length, charge and hydrophobicity in which a tryptophan has been changed into a phenylanine (W6F variant) still binds the lipids [56, 57] but is not internalized [58] and does not induce inverted micelle formation. However, inverted micelle formation would limit cargo size and require the presence of hydrophobic amino acids, which are absent in TAT-PTD and poly-arginine-PTDs; thus, such a mechanism is unlikely to be a common PTD delivery mechanism.

A third model is endocytosis followed by passage from the vesicle into the cytoplasm; although this implies vesicle disruption, the plasma membrane and cell viability are not affected. Some studies have shown that TAT-PTD, polyR-PTD, Antp-PTD, and arginine-rich peptide SynB enter cells by endocytosis rather than by temperature- or energy-independent translocation [59, 60]. We observed, for the first time, protein transduction in real time. TAT-PTD, 11R-PTD, and BETA2/NeuroD transcription factor, which has an arginine- and lysine-rich PTD, penetrates into cells by macropinocytosis, a type of endocytosis, and is released from the endosome homogeneously into the cytoplasm and nuclei by retrograde transport [2, 19]. Antp-PTD and PDX-1 protein, which has Antp-like PTD, is also internalized into cells by the same mechanism [2, 3]. Recently, the role of endosomal acidification and retrograde transport for the uptake of Antp-PTD, TAT-PTD, and 9R-PTD was reported [61]. A number of well-characterized toxins reach the cytosol of

eukaryotic cells after binding to the cell surface, undergoing endocytosis and retrograde transport to the Golgi apparatus and endoplasmic reticulum [62, 63, 64]. The arginine-rich motif of 8–10 amino acids in the A subunits of these toxins, reported to be transported by means of retrograde transport, is similar to those of TAT-PTD, Antp-PTD [61] and PDX-1-PTD. PTDs, even Antp-PTD, transport by a similar mechanism to that of these protein toxins.

In conclusion, the primary mechanism of protein transduction is an electrostatic interaction with the plasma membrane, penetration into cells by macropinocytosis, and release to the cytoplasm and nuclei by retrograde transport. However, it is not possible to completely eliminate the possibility of partial uptake across the plasma membrane via other mechanisms.

Future Objectives

Protein transduction technology has some disadvantages. One is that transduced proteins only have a limited active half-life, so that their effects are only transient. Michiue *et al.* showed that mutant proteins of p53 in which multiple lysine residues in the C-terminal were replaced by arginines were effectively delivered into glioma cells and were resistant to Mdm2-mediated ubiquitination [65]. The mutant proteins displayed higher transcription regulatory activity and induced powerful inhibition of glioma cell proliferation. These findings suggest that ubiquitination-resistant proteins may become a more effective strategy for protein transduction technology.

Another disadvantage is accumulation of PTDs or PTD-fused proteins in the endocytic compartment. The main mechanism of protein transduction is penetration into cells by macropinocytosis and release to the cytoplasm and nuclei by retrograde transport. Although PTDs or PTD-fused proteins can be transduced into cells by macropinocytosis, such proteins are often entrapped inside macropinosomes in the cytoplasm. Therefore, high dose concentrations of PTDs or PTD-fused proteins are needed for the technology to function effectively. To overcome this problem, the proteins were linked with the NH_2-terminal domain of influenza virus hemagglutinin-2 subunit (HA2), which is a pH-dependent fusogenic peptide that induces the lysis of membranes at low pH levels [21, 66]. The pH-sensitive HA2 peptide markedly and specifically enhanced macropinosome escape [66]. HA2- and polyarginine-fused p53 induced p21(WAF1) transcriptional activity and inhibited the growth of cancer cells more effectively than a polyarginine-fused p53 protein [21]. Moreover, we developed methods of enhancing endosome escape using photo-sensitive PTD [6]. These techniques can enhance the efficiency of the protein transduction system.

In some protein transduction experiments, the activity of internalized proteins was not retained following insertion into the cell. To resolve this issue, proteins and compounds could be coupled to PTDs via a linker designed to release the active proteins and drugs within cells. Organ-specific delivery involving PTDs also poses a complex challenge with respect to the utility of this technology as a clinical tool. The technology cannot be applied to insoluble proteins due to difficulties associated with purification of these molecules. Overcoming these obstacles will require advances involving refined PTD sequences and improved application.

Physiological and Developmental Implications

PTDs are defined by their ability to reach the cytoplasmic and/or nuclear compartments in live cells after internalization. The initial discovery of PTDs originated from the unexpected observation that certain full-length proteins or protein domains can translocate across the plasma membrane. This was first shown for the HIV TAT transactivator [67, 68] and for the homeodomain of the *Drosophila melanogaster* transcription factor Antennapedia [69], and has since expanded to include 'non-natural' peptides that share this property. In the case of homeodomain- and TAT-derived peptides, and in contrast with many other PTDs, uptake seems to reflect an *in vivo* biological process. In support of this, the translocating activity of these peptides correlates with that of their parental full-length proteins [70], strongly suggesting that, in some circumstances, these proteins may have paracrine activities as part of a physiological process, in that they are released by one kind of cell and then internalized by other cells. Accordingly, the full-length proteins are also secreted, probably by unconventional secretion pathways that do not involve a secretion signal sequence

[71, 72]. Recent reports of homeoproteins show that HOXB4 protein stimulates the non-oncogenic proliferation of CD34 haematopoietic stem cells [73], and that PDX-1 protein can transform duct cells into insulin producing cells [1–3]. Moreover, we reported that BETA2/NeuroD protein, one of the bHLH transcription factors, can also be transduced into cells due to its own arginine- and lysine-rich sequence [19]. BETA2/NeuroD and PDX-1 proteins are important in pancreatic development [74, 75]. The *pdx-1* gene has A-boxes, and the PDX-1 protein can positively autoregulate its expression [76]; the *BETA2/NeuroD* gene also has E-boxes, and the BETA2/NeuroD protein can stimulate its own transcription [77]. The positive autoregulation of these proteins leads us to consider a potential paracrine role in neogenesis. Once BETA2/NeuroD and PDX-1 proteins are transduced into progenitor cells, endogenous *BETA2/NeuroD* and *pdx-1* gene transcription are amplified by these proteins, stimulate insulin transcription, and may facilitate their differentiation to insulin-producing cells.

Although the exact function of this intercellular transfer is not yet clear, recent data on transcription factors, in particular, homeoproteins, suggest that, after transfer, the transcription factor can regulate transcription and translation in the recipient cell, thus acting as a 'messenger protein' [4, 78]. As homeoprotein expression is regionally specified, it has been speculated that this corresponds to the passage of positional information between cells [4, 78].

Conclusions

The present protein transduction system has low toxicity and a high yield of delivery. The use of this system opens up interesting therapeutic perspectives. The interest in protein translocation across membranes is not limited to PTDs and their use as a tool with which to investigate cell biology, or conversely to their biotechnological applications. The intercellular transfer of endogenous transcription factors, such as TAT and homeoproteins, seems to point to an original and important mode of signal transduction. The physiological significance of these pathways requires further exploration.

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