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REVIEW

Cell-Penetrating Peptides: a Useful Tool for the Delivery of Various Cargoes Into Cells

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Summary

Cell-penetrating compounds are substances that enhance the cellular uptake of various molecular cargoes that do not easily cross the cellular membrane. The majority of cell-penetrating compounds described in the literature are cell-penetrating peptides (CPPs). This review summarizes the various structural types of cell-penetrating compounds, with the main focus on CPPs. The authors present a brief overview of the history of CPPs, discuss the various types of conjugation of CPPs to biologically active cargoes intended for cell internalization, examine the cell-entry mechanisms of CPPs, and report on the applications of CPPs in research and in preclinical and clinical studies.

Key words

Cell-penetrating peptides • Cell penetration • Peptides • Drug delivery

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Introduction

Drugs that are to be transported from the bloodstream to the target cells must overcome cell membranes, which are formed by an amphiphilic phospholipid bilayer. Small hydrophobic compounds cross the cell membrane relatively easily, by diffusion. However, hydrophilic substances dissolved in the blood or bound to plasma proteins are not able to penetrate the membrane without energy-dependent processes. Similarly, macromolecular substances that can serve as carriers of hydrophobic drugs do not cross the cell membrane directly but need to be transported to the cytoplasm, usually *via* energy-dependent mechanisms (Hampl *et al.* 2015). Cell-penetrating compounds are substances that can penetrate the cells *via* both energy-dependent and energy-independent processes, and can be used to deliver various "cargoes" into cells. The cargo can consist of either a low-molecular-weight drug or a macromolecular delivery system that also includes other components such as targeting ligands (Bohmova and Pola 2016) and diagnostic labels. Cell-penetrating peptides (CPPs), on which we mainly focus in this review, represent the largest and most thoroughly studied class of cell-penetrating compounds.

CPPs are usually short oligopeptides consisting of 5-30 amino acid residues. Both their chemical structure and conformation are highly variable; their only common feature is the ability to penetrate the cell membrane without the involvement of energy-dependent processes. CPPs' major disadvantages include the lack of cell-type selectivity, the impossibility of oral administration, and low plasma half-life due to enzymatic degradation and renal filtration (Mie and Mørck 2015).

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History of CPPs

In 1988 and 1989, CPPs were described independently by two scientific research groups, both of whom reported on peptides derived from the transactivator of transcription (HIV-1 TAT) protein of the human immunodeficiency virus. The primary role of this protein is to enhance the efficiency of viral transcription and replication. Both publications described the synthesis of shorter oligopeptides derived from the HIV-1 TAT protein and the ability of these oligopeptides to penetrate cell membranes (Green and Loewenstein 1988, Viscidi *et al.* 1989). The resulting peptide sequence, GRKKRRQRRRC, which showed the highest penetration efficiency, was termed TAT (Vivès *et al.* 1997).

In 1994, penetratin (RQIKIWFQNRRMKW-KK), a CPP consisting of 16 amino acids, was identified. This peptide is derived from the third α-helix of the Antennapedia-based homeoprotein, which was first discovered in *Drosophila* (Derossi *et al.* 1994).

Lin *et al.* (1995) described the peptide AAVLLPVLL-AAP, which was derived from the structure of the hydrophobic region of Kaposi's fibroblast growth factor (K-FGF). It was shown that the peptide was able to efficiently penetrate the cell nucleus.

One year later, two other CPPs were reported: peptide VTVLALGALAGVGVG, containing residues 747-762 of the C-terminal segment of human integrin β_3, and peptide YKSAVTTVVNPKYEGK, representing the homologous portion of the integrin β_1 cytoplasmic tail (residues 788-803) (Liu *et al.* 1996).

The connection of the neuropeptide galanin and the peptide toxin mastoparan, which is present in wasp venom, led to the discovery of a new CPP. This consisted of the 27-amino-acid sequence GWTLNSAGYLLGKIN-LKALAALAKKIL, referred to as transportan (Pooga *et al.* 1998).

Many other CPPs were reported during the following years: for example, peptide pVEC (LLIILRRRIRKQAHAHSK) (Elmquist *et al.* 2001), which originated from the structure of vascular endothelial cadherin (CD144); or Pep-1 (KETWWETWWTEWSQPKKKRKV), consisting of three domains. The first segment, KETWWETWWTEW, enables efficient cell membrane targeting thanks to the hydrophobic interactions of tryptophan with proteins of the cell membrane. The second domain is the lysine-rich hydrophilic part, KKKRKV, which is derived from the nucleus localization sequence (NLS) of Simian virus 40 (SV-40). The remaining third part containing proline serves as a flexible linker between the two other domains.

The peptide sequence CSIPPEVKFNKPFVYLI (C105Y) and its shorter version, PFVYLI, were derived from α1-antitrypsin, and were reported in 2006 (Rhee and Davis 2006).

The pH low-insertion peptide (pHLIP), a 38-peptide derived from the bacteriorhodopsin C helix, has the fascinating ability to switch from a random coil conformation to an α-helix that penetrates the cell membrane within a few seconds, when the pH falls below 7.0 (Reshetnyak *et al.* 2006). Therefore, this peptide and its applications would merit a separate review.

Since the discovery of CPPs more than 25 years ago, the number of scientific publications demonstrating the use of CPPs for delivery of various cargoes is still growing.

Structural classification of CPPs

CPPs can be classified according to various criteria such as their chemical structure, the natural protein from which they are derived, or the mechanism of cell entry. In this review, we classify CPPs based on their chemical structures. The various resulting structures also affect the ability of peptides to penetrate cell membranes.

Cationic CPPs

Positively charged peptides contain multiple lysine and arginine residues. Polyarginines are a very well-studied group of CPPs. For instance, Tünnemann *et al.* (2007) demonstrated that a minimum of eight arginine residues are required for enhanced cell membrane penetration; a higher number further increases the penetration efficiency.

In another work, the preparation of oligopeptides with 4, 6, 8, 10, 12, and 16 arginine residues was described (Futaki *et al.* 2001). It was shown that while R4 had extremely low transfection activity, R6 and R8 exhibited maximal internalization into the cells and accumulation in the nucleus. Surprisingly, a higher number of arginines led to a lower level of internalization. A complex of peptide R16 with a model protein did not show any significant internalization.

Among the CPPs containing multiple Lys or Arg residues, TAT peptide (GRKKRRQRRR) and penetratin (RQIKIWFQNRRMKWKK) are the most studied. They also act as nuclear localization sequences (NLSs), which will be described later. Both peptides penetrate all types

of mammalian cells except two types of epithelial cells: CaCO-2 (colonic carcinoma cells) and MDCK (Madin-Darby canine kidney cells) (Violini *et al.* 2002).

NLSs are special category of CPPs: these peptides are able to deliver various cargoes into the cell nucleus *via* nuclear pores that the peptides recognize using specific nuclear transportation processes (Lange *et al.* 2007). These lysine- or arginine-rich peptides usually originate from DNA and RNA polymerases. A typical example of an NLS is PKKKRKV, derived from SV-40 antigen (Kalderon *et al.* 1984).

Other examples of positively charged CPPs are summarized in Table 1.

Table 1. Positively charged CPPs.

Name of CPP	AA Structure	Reference
AIP6	RLRWR	Wang *et al.* (2011)
DPV6	GRPRESGKKRKRKRLKP	de Coupade *et al.* (2005)
HIV-1 TAT (48-60)	GRKKRRQRRRPPQ	Green *et al.* (1988), Viscidi *et al.* (1989)
IRS-tag	RYIRS	Dong *et al.* (2003)
Mini-penetratin	RRMKWKK	(Moede *et al.* (1999)
Penetratin	RQIKIWFQNRRMKWKK	Derossi *et al.* (1994)
Polyarginines	R8, R9, R10, R12	Tünnemann *et al.* (2007)
R9F2C	RRRRRRRRRFFC	Moulton *et al.* (2004)

Amphipathic CPPs

Amphipathic (or amphiphilic) CPPs have alternating regions of polar (hydrophilic) amino acids and non-polar (hydrophobic) amino acids in their structure. The resulting charge can be positive, neutral, or negative. Some examples of amphipathic CPPs classified according to their overall charge are shown in Table 2. Among the amphipathic CPPs, proline-rich peptides represent a specific category. A proline residue lacking a hydrogen bond to the nitrogen atom of the pyrrolidine ring contributes to the disruption of the secondary structure of the peptide chain.

Hydrophobic CPPs

Hydrophobic CPPs have a high content of hydrophobic amino acid residues such as alanine, leucine, isoleucine, phenylalanine, tryptophan, methionine, and tyrosine; these are listed in Table 3.

Other cell-penetrating compounds

Compounds other than peptides that can also penetrate cell membranes without the need for an energy supply are much less frequently described in the literature. Similarly to peptides, amphiphilic surfactants also contain both hydrophilic and hydrophobic parts. Examples of such compounds are natural saponins, which are used in molecular biology or microbiology as membrane-permeating substances (Wojciechowski *et al.* 2014).

Another example of cell-penetrating compounds is the group of so-called peptide-like molecules (PLM) with structures resembling a peptide chain. They consist of unnatural α, β, or γ-amino acid residues, instead of proteinogenic amino acids or isosteric peptides (Wipf *et al.* 2009). However, their behavior is similar to that of native peptides. A representative of PLM, the anti-HIV drug ritonavir has also been recently described as an efficient P-glycoprotein inhibitor that significantly enhances cell penetration of polymer conjugates with covalently bound ritonavir, when compared to the polymer alone (Koziolová *et al.* 2016).

Types of CPP attachment to the cargo

The attachment of CPP to the cargo that is intended to be delivered into the intracellular compartments can be either covalent (cleavable or non-cleavable) or can be based on non-covalent interactions. Both approaches have their advantages and disadvantages; the choice of the type of bond usually depends on the particular structures of both the CPP and the cargo. The simplest method is based on direct mixing of the two components (the CPP and the cargo) (Kamei *et al.* 2016).

Table 2. Amphipathic CPPs.

Name of CPP	AA Structure	Reference
Amphipathic cationic		
CADY	GLWRALWRLLRSLWRLLWRA	Crombez *et al.* (2009)
EB-1	LIRLWSHLIHIWFQNRRLKWKKK	Lundberg *et al.* (2007)
hCT (9-32)	LGTYTQDFNKFHTFPQTAIGVGAP	Tréhin *et al.* (2004)
PTD4	YARAAARQARA	Ho *et al.* (2001)
MAP	KLALKALKALKAALKLA	Poon and Gariepy (2007)
Pep-1	KETWWETWWTEWSQPKKRKV	Morris *et al.* (2001)
pVEC	LLIILRRRIRKQAHAHSK	Elmquist *et al.* (2001)
SynB1	RGGRLSYSRRRFSTSTGR	Rousselle *et al.* (2000)
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL	Pooga *et al.* (1998)
Vp1	APKRKSGVSK	Saphire *et al.* (2000)
Amphipathic neutral		
MAP17	QLALQLALQALQAALQLA	Scheller *et al.* (1999)
PreS2	PLSSIFSRIGDP	Oess and Hildt (2000)
Amphipathic anionic		
GALA	WEAALAEALAEALAEHLAEALAEALEALAA	Li *et al.* (2004)
MAP12	LKTLTETLKELTKTLTEL	Oehlke *et al.* (1999)
Proline-rich		
$(PPR)_n$	(PPR)3, (PPR)4, (PPR)5, (PPR)6	Daniels and Schepartz (2007)
$(PRR)_n$	(PRR)3, (PRR)4, (PRR)5, (PRR)6	Daniels and Schepartz (2007)
Bac-7	RRIRPRPPRLPRPRPRPLPFPRPG	Sadler *et al.* (2002)
SAP	VRLPPPVRLPPPVRLPPP	Martin *et al.* (2011)

Table 3. Hydrophobic CPPs.

Name of CPP	AA Structure	Reference
BIP	VPMLK(E)	Sawada *et al.* (2003)
C105Y	(CSIPPEVKFNK)PFVYLI	Rhee and Davis (2006)
β3-integrin	VTVLALGALAGVGVG	Liu *et al.* (1996)
K-FGF	AAVLLPVLLAAP	Lin *et al.* (1995)
NF-κB	VQRKRQKLMP	Lin *et al.* (1995)
Pep-7	SDLWEMMMVSLACQY	Gao *et al.* (2002)
β1-tail	YKSAVTTVVNPKYEGK	Liu *et al.* (1996)

Covalent attachment

A covalent attachment between the CPP and the cargo molecule is the most commonly used bond. There can be either a direct covalent bond between the two components (Goswami *et al.* 2015), or they can be linked together *via* transporting systems such as polymer carriers (Golan *et al.* 2016), metal nanoparticles (de la Fuente and Berry 2005), or liposomes (Ding *et al.* 2015). The most common types of covalent bond are amide, disulfide (Herce *et al.* 2013) or triazole-based originating from a "click" reaction (Shabanpoor *et al.* 2015). Spacers are often used to adjust the optimal distance between the CPP and the cargo. These spacers can be attached to the side-chain functional groups of the CPP, such as the lysine amino group or cysteine thiol group, or even the carboxylic group or amino group at the C or N-terminus

of the peptide, respectively. An advanced method of attaching the CPP to another protein (or peptide) molecule is the preparation of a fusion protein using recombinant DNA technology (Mie *et al.* 2003). However, this method is applicable only when the cargo to be internalized is a protein or another peptide.

Non-covalent approach

The main advantage of the non-covalent approach is that the complex between the CPP and the cargo is formed upon the mere mixing of the two components. The application of the well-known strong interaction between avidin and biotin (Wierzbicki *et al.* 2014), or the use of electrostatic interactions between a positively charged CPP and a negatively charged polyanionic transporting system (Xu *et al.* 2010) or cargo, e.g. siRNA (Peng *et al.* 2017), are examples of non-covalent methods. The disadvantage of non-covalent attachment is the lower stability of the complex in the body environment.

Cellular uptake mechanisms

The cellular uptake mechanism of CPPs depends on a variety of factors, such as the type of CPPs, concentration, incubation time, temperature, membrane structure, type of cell, primary and secondary structure of CPP, and cargo type. Direct penetration of CPPs *via* both energy-independent pathways and energy-dependent pathways (usually related to endocytosis) have been discussed recently (Copolovici *et al.* 2014, Reissmann 2014). It was found that direct penetration occurs only at high concentration of CPPs, while endocytosis is present in almost all cases (Guidotti *et al.* 2017).

Direct penetration

Direct penetration occurs even at a low temperature (4 °C) or in the presence of inhibitors of endocytosis. It is an energy-independent process based on the interaction of positively charged CPPs with negatively charged components of the cell membrane, such as heparan sulfate and a phospholipid barrier. Direct penetration into the cytosol (Murray *et al.* 2016) involves various mechanisms of CPP entrance, such as pore formation and destabilization of the cell membrane. Pore formation includes the barrel stave model and the toroidal pore model (Madani *et al.* 2011), while the carpet-like model and inverted micelle formation induce membrane destabilization and direct internalization of CPPs.

The **barrel stave model** (Fig. 1) involves the helix conformation of the CPP. The hydrophobic residues of the helix structure face toward the hydrophobic tails of the lipid bilayer, and the hydrophilic residues of the CPP form the internal environment of the pore. This model is unique for alamethicin, which induces the formation of transmembrane pores containing a 3-11 parallel helical structure (Brogden 2005).

In addition to the barrel stave model, the **toroidal pore model** (Fig. 1) is an approach that depends on the helix conformation of the CPP with differences in the mechanism of pore formation. The pores are formed by peptides that are associated with the polar head groups of lipids inside the cell membrane. The hydrophilic core of the toroidal pore is lined with both the inserted peptides and hydrophilic head groups of the phospholipid cell membrane (Matsuzaki *et al.* 1996, Yang *et al.* 2001). Magainins, melittin, and protegrins are alpha-helix peptides that induce toroidal pore formation; these toroidal pores are larger and more variable than the barrel stave type (Brogden 2005).

The **carpet-like model** (Fig. 1) describes the destabilization of the cell membrane (Madani *et al.* 2011). In this model, peptides are in parallel orientation to the membrane surface. Essential interactions in this model are electrostatic interactions between anionic phospholipid head groups and positively charged peptides. The CPP concentration must be above a threshold concentration and high enough to form the carpet-like membrane coating; consequently, membrane permeation occurs after a sufficient amount of the membrane is covered with CPPs. In the carpet-like model, in contrast with the barrel stave model, the peptides are not internalized into the hydrophobic core. After the hydrophilic groups of the peptides bind to the phospholipid head groups, rotation of the peptide leads to destabilization and reorganization of the cell membrane (Shai 1999). The lipid bilayer transforms into a micelle, forming a transient hole (Khandia *et al.* 2017).

An **inverted micelle** (Fig. 1) is formed between two cell membrane bilayers, as a hexagonal structure in which the CPP is surrounded by the hydrophobic part of the membrane (Khandia *et al.* 2017). In addition to the interaction between the hydrophobic residues of CPPs and the hydrophobic part of the membrane, the interaction between positively charged CPPs and the negatively charged part of the cell membrane has also been shown to be involved (Guo *et al.* 2016, Islam *et al.* 2018). The HIV-1 TAT peptide and octaarginine are effectively internalized through inverted micelle formation (Khandia *et al.* 2017).

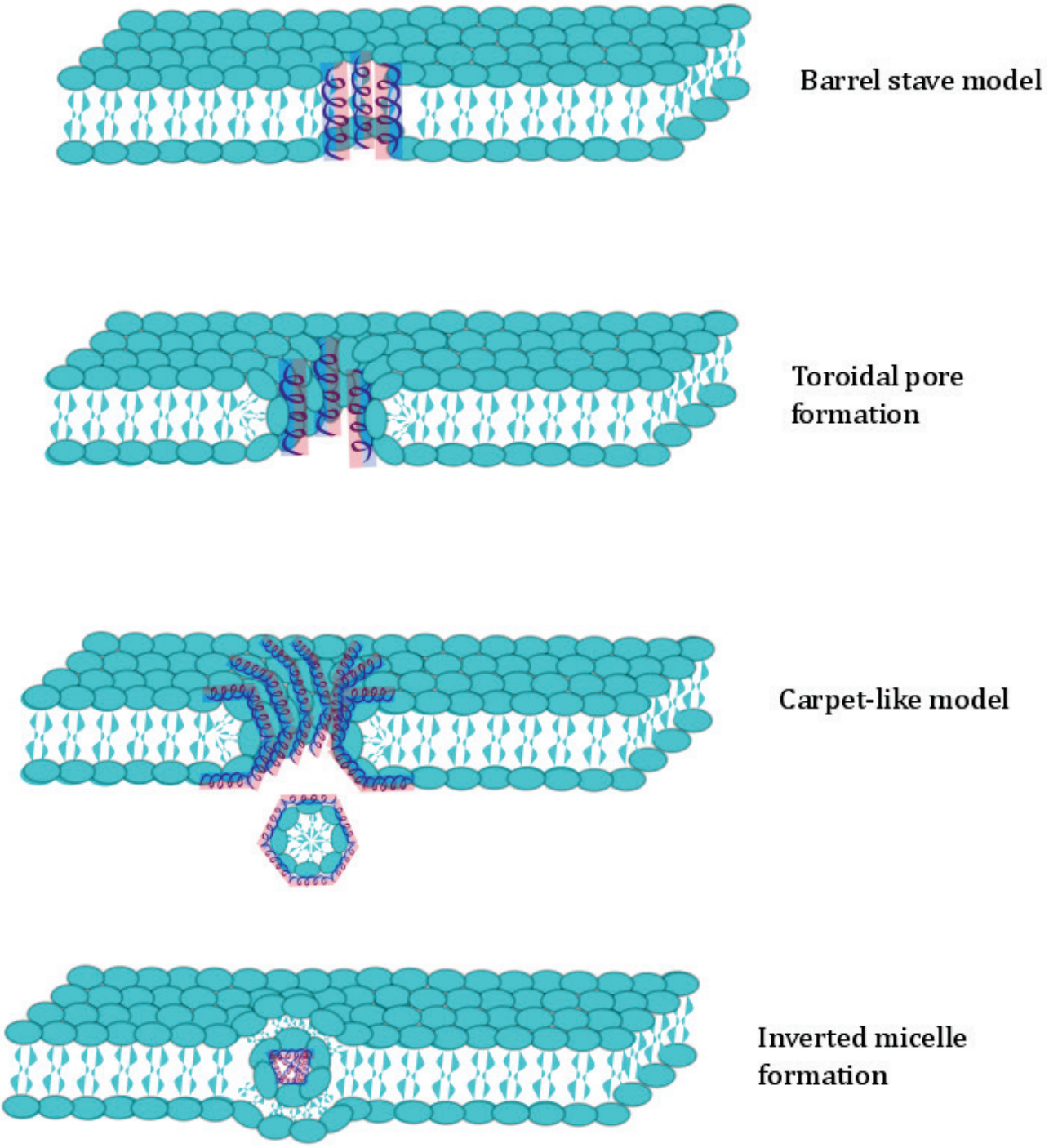

Fig. 1. Schematically depicted models of direct penetration of CPPs *via* cell membrane. The hydrophilic parts of the peptides are colored red and the hydrophobic parts of the peptides are colored blue.

Endocytosis

The entrance mechanism of CPPs was first described as a receptor-independent and non-endocytic uptake. This presumption was based on observations of fluorescently labeled CPPs in fixed cells: it was found that the fixation of cells could lead to the artificial redistribution of CPPs inside cells. Later, a number of studies showed the contribution of endocytosis to the different CPP internalization mechanisms and their cargo molecules (Richard *et al.* 2003). Endocytosis is an energy-dependent mechanism and consists of several pathways, including phagocytosis for the uptake of large particles, and pinocytosis for the internalization of the fluid surrounding the cell. Pinocytosis includes

micropinocytosis, clathrin- and/or caveolin-dependent endocytosis, clathrin- and/or caveolin-independent endocytosis, and dynamin-dependent and/or dynamin-independent endocytosis. CPPs can utilize more endocytic pathways during internalization, and thus increase their uptake into the cells (Heitz *et al.* 2009). Macropinocytosis results in the formation of vesicles called macropinosomes, which are formed during inward folding of the plasma membrane. Clathrin and caveolin are proteins that are present in the intracellular part of the cell membrane during endocytosis; they are required for the invagination of the membrane and formation of vesicles that are coated with these proteins. The clathrin-coated vesicles are a few hundred nanometers in diameter, while the caveolin-coated vesicles have a diameter below one hundred nanometers. Dynamin is a protein involved in the invagination of the cell membrane and is necessary for the formation of these vesicles (Jones 2007).

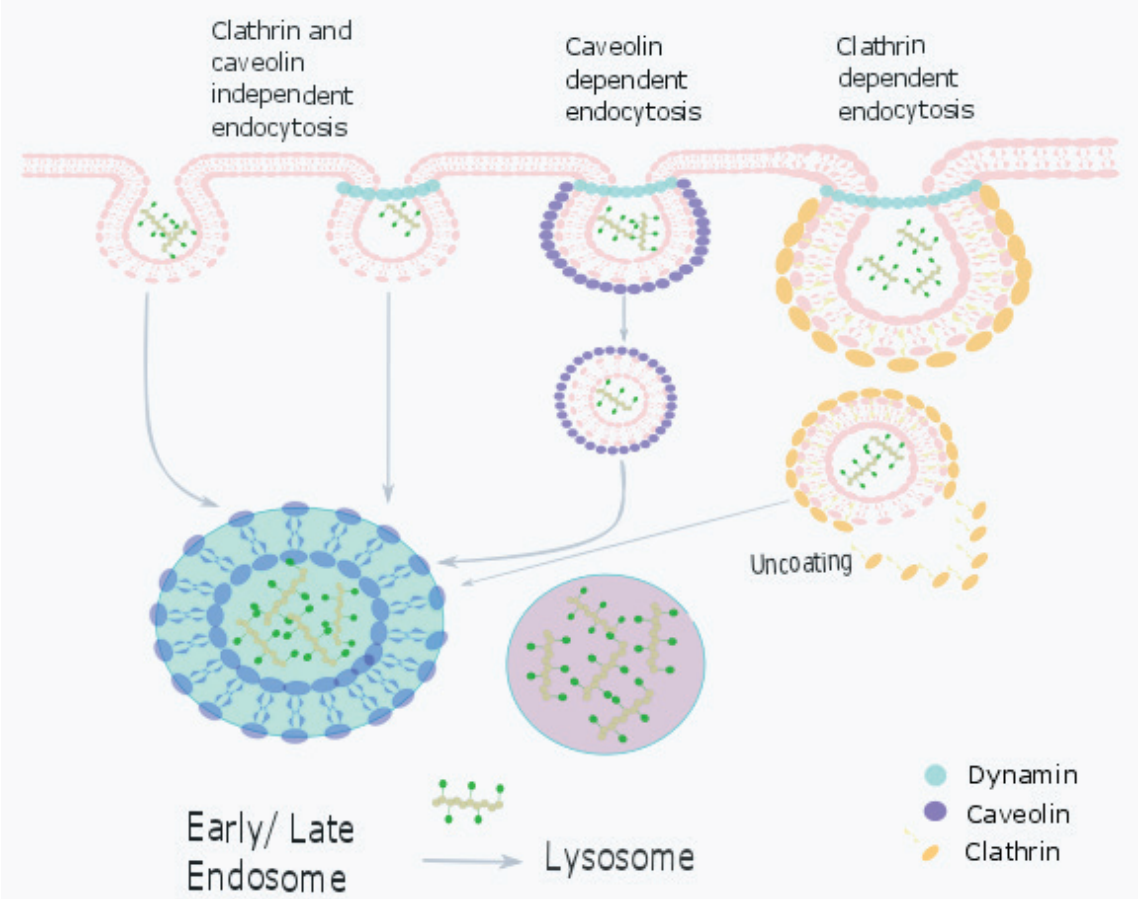

Fig. 2. Cellular uptake of CPPs by endocytic pathways.

Endosomal escape of CPPs

The route of cellular uptake of the CPP-cargo complex has been discussed in detail above. The limitations of delivery *via* endocytosis include cargo entrapment inside the endosome and subsequent possible destruction in the acidic lyso-endosome, whereas direct penetration transports the cargo directly to the cell cytoplasm. There is contradictory evidence regarding endosomal accumulation, endosomal escape, and lysosomal degradation. The CPP-cargo complex interacts with phospholipid bis(monoacylglycero)phosphate (BMP), which is a part of the endosomal membrane. The fusion of membranes of the lysosome and the CPP-cargo-containing endosome is crucial for cargo escape to the cytosol. For example, the TAT peptide induces lipid mixing and subsequent leaky fusion of membranes. Similarly, polyarginines are also able to escape from the late endosome (Appelbaum *et al.* 2012, Yang *et al.* 2010).

The changes in pH values during transitions from early to late endosomes are essential for transition from an inactive to an active membrane-disruptive form of pH-dependent membrane-active peptides (PMAPs),

e.g. the HA2 peptide (which corresponds to the 23 N-terminal residues of the hemagglutinin HA2 subunit of the influenza virus X31 strain) (Wharton *et al.* 1988). When inserted into the membrane, PMAPs induce membrane fusion, leakage and lysis (Esbjörner *et al.* 2007, Turk *et al.* 2002).

Multivalent CPPs increase endosomolytic activity through stronger interaction with BMPs in membranes, and they escape from endosomes more efficiently than monomeric CPPs. Multivalency can be achieved by attaching a protein oligomerization domain to the CPP, chemical conjugation of CPPs to dendrimers, or by attaching CPPs to branched oligopeptides, such as the fork-like structure of lysine or glutamic acid. However, this approach also presents the following limitations: chemical synthesis of multivalent CPPs is more difficult; there is a higher risk of immunogenic properties; and it is necessary to balance the number of branches of CPP, to elicit a strong enough but not too extensive reaction.

Folded proteins containing a pentaarginine motif were able to escape from endosomes to the cytoplasm (Appelbaum *et al.* 2012). Indeed, cyclization of arginine-rich CPPs led to a more efficient cellular uptake and delivery to the cytoplasm and nucleus. For release from endosomes and effective cytosolic delivery, the presence of D- and L-arginine residues is probably necessary, as it was reported that peptides containing only L-arginine (polypeptide R8) were eventually incorporated within endosomes (Ma *et al.* 2012). Thus, an endosomolytic agent called dfTAT (disulfide-linked TAT) was developed to destabilize endosomes (Najjar *et al.* 2015) and to enable cargo escape to the cytoplasm.

Another strategy uses CPP-bound calmodulin and a cargo with a calmodulin binding site. After cellular uptake, the level of calcium (which is needed for stable CPP-cargo bonds) decreases, and the cargo leaves the endosome before its transformation to the acidic late endosome (Salerno *et al.* 2016).

Targeted subcellular localization

An important advantage of some CPPs is direct transport to the cell organelles through the recognition of specific localization sequences inside the cells.

In cancer treatment, the most promising approach is targeting the nucleus with DNA-damaging agents or nucleic acids for gene therapy, as mentioned above (Cartier and Reszka 2002). Another important target is mitochondria, which are targeted by short artificially designed peptides such as Szeto-Schiller (SS) peptides, rather than by natural sequences (Mahon *et al.* 2007, Szeto 2006). Moreover, Cerrato *et al.* developed several CPPs designed especially for mitochondria targeting (Cerrato *et al.* 2015).

Lysosomes are organelles that are involved in macromolecular turnover, and they provide nutrition by autolyzing dysfunctional organelles during starvation periods. The dysfunction of lysosomal enzymes can lead to the accumulation of substrates that compromise cellular function; this condition is referred to as the lysosomal storage condition (Lübke *et al.* 2009). Receptors on the cell surface (folate, transferrin, vascular endothelial growth factor) are used for the endosome/lysosome targeting of degradative enzymes (Ni *et al.* 2006).

Due to the addition of a targeting sequence to the CPP-cargo complex, the cargo was successfully delivered to the nucleus, nucleolus, lysosome, peroxisome, mitochondria, and endoplasmatic reticulum.

Preclinical and clinical use of CPPs

Several preclinical studies have been performed on experimental animals in the search for an effective model for various therapeutic uses: these include cerebral ischemia, ALS, myocardial injury, cancer, muscular dystrophy, cardiology, anti-prion treatment, and both viral and bacterial infections (Copolovici *et al.* 2014). Some of these studies reported promising results.

For example, the RI-TAT-p53C' protein was developed to restore the pro-apoptic activity of the p53 protein that is responsible for cell cycle arrest and apoptosis following oncogenic stress (Vousden and Lu 2002, Snyder *et al.* 2004). Some of the chemotherapeutics are delivered as prodrugs and metabolized into effective forms that are often insoluble (Meyer-Losic *et al.* 2008).

CPPs can be used for targeted gene delivery in gene therapy: compared to other procedures, they provide less toxic and significantly more efficient transfection methods. For instance, CPP-DNA complexes are better protected in an extracellular space than naked DNA. In pulmonary treatment, TAT-PEG-poly(ethylene imine) polymers carrying plasmid DNA demonstrated approximately 600 % higher transfection efficiency *in vivo* than plasmid DNA alone.

Furthermore, CPP-bound siRNAs show higher stability and delivery efficiency *in vivo*. For instance,

MPG-8 targets cyclin B1, thus preventing tumor growth in animal models. In the mouse model, the TAT-conjugated system can deliver siRNA of epidermal growth factor receptor (EGFR) and AKT serine/threonine kinase 2 (Akt2) in glioblastoma (Crombez *et al.* 2009, Michiue *et al.* 2009).

Currently, over 25 clinical trials involving CPPs are in progress, including some in phase III (LeCher *et al.* 2017). No immunogenicity and good toleration by patients was shown using the p28 peptide derived from bacterial azurin: this is because p28 enters the nucleus, then binds to p53 and prevents its degradation, which leads to cancer cell apoptosis (Warso *et al.* 2013). Moreover, the same protein has been tested in progressive tumors of the central nervous system.

Conclusions

In this review, we have attempted to summarize the most important cell-penetrating peptides, although there has been insufficient scope to cover all the systems described to date.

Since their discovery, for the last three decades cell-penetrating compounds have been attracting the attention of researchers in various fields. The ability of CPPs to penetrate cell membranes and to deliver various biologically active cargoes into cells is especially relevant to biomedical applications such as drug delivery and diagnostics. With respect to the tremendous progress in solid-phase peptide synthesis, and consequently, also the improved affordability and availability of practically any synthetic peptide, the scientific attractiveness of CPPs is still growing.

Conflict of Interest

There is no conflict of interest.

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