Chapter 2

Penetratin Story: An Overview

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Abstract

Cell-penetrating peptides are short, often hydrophilic peptides that get access to the intracellular milieu. They have aroused great interest both in academic and applied research. First, cellular internalization of CPPs often involves the crossing of a biological membrane (plasma or vesicular), thus challenging the view of the non-permeability of these structures to large hydrophilic molecules. Secondly, CPPs can drive the internalization of hydrophilic cargoes into cells, a rate-limiting step in the development of many therapeutic substances. Interestingly, the two most used CPPs, TAT and penetratin peptides, are derived from natural proteins, HIV Tat and Antennapedia homeoprotein, respectively. The identification of the penetratin peptide, summarized in this review, is intimately linked to the study of its parental natural protein.

Key words Penetratin, Cell-penetrating-peptide, Homeodomain, Homeoprotein

1 Introduction

It is extremely striking that the transgression of the dogma of membrane impermeability to hydrophilic molecules at the origin, 20 years ago, of the cell-permeable peptide field stems from the study of two unrelated transcriptional regulators, HIV Tat protein and Antennapedia homeoprotein. In both cases, the necessity to verify their purely intracellular activity had motivated the addition of these proteins in the extracellular medium, with unexpected results that suggested internalization by cultured cells. These results have led to the development of the first cell-permeable peptides, to expression strategies based on direct protein delivery—instead of classical nucleic acid transfection—and to the search for the underlying biological function of protein transduction.

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2 The Origin of an Unexpected Observation

In 1988, the capture of HIV-TAT by cells and its transport to the nucleus were described [1, 2]. At the same time, our laboratory was trying to correlate neuronal shape and position and in this position/shape context had started to investigate the function of homeoprotein transcription factors.

In the mid-1980s, we observed that brain neurons in culture adopt different polarity patterns depending on the origin of the astrocytes on which they were plated [3, 4]. It was particularly striking that dendrites would only develop when neurons and astrocytes were derived from the same structure. This allowed us to establish a theoretical link between developmental morphogenetic programs and positional information. At the time, the homeoprotein family of transcription factors that link organ shape to their positional information was discovered in Drosophila. We asked whether morphogenetic programs acting at the multicellular levels might also act at the single-cell level, on neuronal shape.

Homeoproteins are defined by nature of their DNA-binding domain, the homeodomain. This domain is highly conserved across homeoproteins and species, and is composed of three α-helices, the third helix being more particularly dedicated to the recognition of the DNA target site [5]. We wanted to test our hypothesis by injecting a homeodomain within live neurons. The logic was that the injected homeodomain would gain access to the nucleus and displace endogenous homeoproteins away from their cognate sites, thus revealing their morphological function at the single-cell level. We used the homeodomain of Antennapedia for practical reasons and on the basis of the strong sequence conservation between homeodomains. To analyze the role of homeoproteins in neuronal morphogenesis we developed a protocol aimed at antagonizing transcriptional activity of endogenous homeoproteins. This was achieved through the mechanical internalization of FITC-labeled homeodomains into live post-mitotic neurons [6–8]. The addition of exogenous Drosophila Antennapedia homeodomain (AntpHD) induced strong neurite outgrowth as expected that was attributed to a competition between the homeodomain and endogenous homeoproteins for their binding sites [8]. But the surprise was total when adding the homeodomain into the culture medium, for a control, we observed the same phenotype. This suggested either that the effect of the injected homeodomain was due to its leakage outside of the cells—an artifact—or that the homeodomain was internalized. We verified the latter possibility and observed, much to our surprise, that the 60-amino acid-long polypeptide was captured by the cells and addressed to their nuclei [8].

3 Homeodomain Translocation

In an attempt to analyze the neurite-promoting function of the homeodomain and its mechanism of action, two different point mutations affecting the specificity of protein/DNA interactions (AntpHD 50A) or the structure of the homeodomain (AntpHD 48S) were introduced [9–11]. The DNA-binding capacity of the three mutants is either decreased (AntpHD 50A) or completely abolished (AntpHD 48S) and the biological activity (neurite outgrowth stimulation) is lost in all cases [9–11]. Most importantly, translocation into live cells is lost only in the AntpHD 48S mutant, into which a single-serine residue replaces three amino acids (tryptophan 48, phenylalanine 49, and glutamine 50). Tryptophan 48 (Trp 48) and phenylalanine 49 (Phe 49) are conserved in all homeodomains, and important for the homeodomain structure [10].

This observation was so unexpected and disturbing that we decided to identify the mechanism involved in homeodomain capture. Interestingly, the intracellular distribution showing uniform cytoplasmic staining and nuclear accumulation was at odd with endocytosis. Indeed, uptake was observed at 4 °C, with the same uniform cytoplasmic staining. To preclude that this diffusion was due to AntpHD redistribution following fixation, the same experiments done with an FITC-tagged homeodomain, on live cells, and with the help of confocal microscopy gave identical results [8]. Finally, it was verified that the AntpHD was retrieved, intact, from the cells at both temperature, demonstrating very limited degradation [12].

4 The Penetratin Peptide

The results with AntpHD 48S suggested the presence of a cell translocation sequence in the third helix. The 16 amino acids of the helix (amino acids 43–58 of the homeodomain) were synthesized and internalization into live cells was followed thanks to an N-terminal biotin [13]. Shorter versions of the same peptide, with N-ter or C-ter deletions, are not internalized suggesting that this sequence, thereafter penetratin, is necessary and sufficient for internalization.

Similarly to AntpHD, penetratin can be internalized by an energy-independent mechanism at both 4 and 37 °C and has access to the cytoplasm and nucleus from which it is retrieved without apparent degradation [13]. Penetratin's high content in basic amino acids is reminding of TAT and oligoarginine peptides. In contrast, a unique feature of penetratin is the presence of hydrophobic residues, in particular tryptophans, which are critical to the translocation process [13]. Indeed, penetratin and other basic

PTDs differ in their cellular behaviors, even in the same experimental setup [14–17]. More strikingly, significant differences have been reported between penetratin-like sequence issues from different homeoproteins, despite extensive conservation of this motif among homeoproteins [18, 19]. Although biophysical and biological studies have greatly helped to our comprehension of penetratin behavior, a full understanding of its mechanism of translocation is still in wait.

4.1 Charge and Hydrophobicity: A Dual Mode of Interaction

Because penetratin composed of D-amino acids (D-penetratin) and an *inverso* form of the peptide are internalized as efficiently as penetratin [20], it was concluded that a chiral membrane receptor (usually a protein) is not required for cellular translocation. On the other hand, the specific ability of penetratin to form multimers in the presence of ionic detergents has led to a close examination of penetratin/lipid interactions [13]. Biophysical studies have established that penetratin preferentially interacts with anionic phospholipids mainly through electrostatic interactions, followed by limited peptide insertion into the bilayer [21–23]. Although the first studies strongly suggested that penetratin binds to the lipid headgroups, a situation not in favor of direct translocation across pure lipid bilayers, a more recent diversification of the experimental models and techniques has revealed a different picture [24, 25]. Penetratin actually crosses pure lipid bilayers, either in the presence of an applied transmembrane pH gradient [26, 27] or in response to a self-generated potential resulting from asymmetric peptide aggregation at one side of the bilayer (electroporation-like mechanism) [21]. The spontaneous insertion of non-aggregated penetratin in the inner leaflet of lipid bilayers was also reported using a novel solid-state NMR technique [28]. In these experimental setups, the behavior of penetratin greatly depends on the lipid composition of the vesicles. The absence of penetratin translocation reported by other groups could reflect an unfavorable lipid composition [29–31].

It must be kept in mind that penetratin/lipid interaction is a reciprocal process affecting both partners. Penetratin adopts a random coil structure in an aqueous environment but becomes structured in the presence of anionic phospholipids. At a low peptide/lipid ratio (1/325), the peptide adopts an α-helical conformation [13, 32–35]. At a high peptide/lipid ratio (1/10), the peptide forms antiparallel β-sheets [23, 35, 36]. Conversely, penetratin alters the organization of lipid bilayers and the orientation of lipid acyl chains is modified upon the deep insertion of penetratin into membrane bicelles [37]. When applied on a brain lipid mixture preparation, penetratin induces the formation of hexagonal phases [33]. We have proposed that this transient remodeling of lipid organization induced by penetratin places the peptide in a pseudo-hydrophilic environment and allows its transfer from the extracellular

medium to the cytoplasm of the cell. Other perturbations of the lipid bilayer upon penetratin addition have been reported, which can account for the translocation process. Penetratin induces a negative curvature of the lipid bilayer of giant unilamellar vesicles that contains liquid disordered domains [38] and has been shown to stimulate a membrane repair response, following the transient perturbation of plasma membrane integrity [39].

4.2 Influence of Structural Parameters on Peptide Translocation

Mutation analysis has confirmed the contribution of both hydrophobic and electrostatic properties to penetratin translocation. Mutation of basic residues favors peptide insertion in the acyl chains but destabilizes the bilayer [40]. A similar situation is observed upon addition of fluorescent probes to penetratin, which increases its hydrophobicity [41], and induces a transient destabilization of the plasma membrane in live cells demonstrated by the uptake of a cell-impermeant DNA dye and the appearance of phosphatidylserine at the cell surface [42]. Taken together many arguments suggest that a subtle balance between hydrophobic and electrostatic properties of penetratin is required for its translocation. In fact, even minimal modifications, such as substitution of the two Trp residues by two Phe residues, modify peptide/lipid interactions and impair translocation in live cells [13, 37, 41, 43]. By contrast neither peptide helicity nor amphipathicity seems to be required for peptide internalization [20]. Indeed, increasing the amphipathicity of penetratin by mutations increases the toxicity of the peptide rather than its translocation efficiency [44].

A common feature of basic CPPs, including penetratin, is their strong electrostatic interaction with the complex carbohydrates that decorate the cell surface, likely preceding the interaction with the lipid bilayer. It was shown that the presence of Trp residues also modulates this very early step along the internalization process and consequently impacts on the internalization pathway used [45].

4.3 One or More Mechanisms of Penetratin Internalization

Recent studies on the mechanism of internalization of penetratin in live cells have revealed a more complex picture than previously thought, and concluded to a predominant endocytic uptake and vesicular localization of this peptide [14, 15]. This proposal is at odd with a direct translocation process demonstrated by several internalization protocols, in particular at 4 °C, and biophysical studies. In fact there is no reason to exclude that penetratin can be captured by endocytosis depending on cell type and tagging procedure. It remains that, in contrast with Tat, endocytosis is not a prerequisite for penetratin transfer into the cytoplasm and nucleus. Among modifiers of penetratin uptake are the highly negatively charged carbohydrates that surround most cells, in particular glycosaminoglycans (GAGs) [46]. The complex sugars could restrict penetratin access to the membrane, promote penetratin

aggregation [47], and induce endocytosis, with the possibility (or not) that the peptide crosses the bilayer later, once within endosomes. Indeed, penetratin escape from artificial vesicles driven by a proton gradient has been reported, supporting this mechanism [48]. Moreover, the non-endocytic component of penetratin internalization (i.e., observed at 4 °C) is poorly affected by the absence of GAGs, while the endocytic component dramatically decreases [46].

The diversity of mechanisms has also been illustrated by several studies [40, 49, 50]. For example, the intracellular distribution of internalized penetratin greatly differs between Hela and MC57 cell lines, or the macropinocytosis inhibitor ethylisopropylamiloride (EIPA) decreases penetratin uptake added at high (50 μM) but not low (10 μM) concentration [17, 51].

5 The First Applications

Soon after the observation of homeodomain translocation, we have demonstrated the use of this process for the efficient cell delivery and biological activity of hydrophilic molecules was published. Both antisense oligonucleotides (against the β-amyloid precursor protein) and protein domains (C-terminus domain of rab3a) were efficiently internalized by cells in culture upon fusion to AntpHD [52, 53]. The first in vivo application of AntpHD-mediated vectorization was the induction of T-cell responses by a peptide derived from the HLA-cw3 cytotoxic T cell epitope [54]. It appeared very quickly that the 16-amino acid-long peptide penetratin could substitute advantageously for AntpHD, both for oligopeptide and oligonucleotide delivery [55, 56]. Since this time, this vectorization strategy has expanded dramatically [57], and proven to be highly versatile toward the nature of the transported cargo (from small drugs to nanoparticles) and the biological context (both ex vivo and in vivo). Most importantly, a large panel of peptides has been characterized on the basis of their cell-penetrating behavior although only some of them have been validated with a biological cargo.

In physiological situations, many of the successful applications of penetratin-driven delivery rely on the topical or targeted delivery of the compound. Improvement of the bio-distribution and bioavailability will consist in one of the major challenges for the therapeutical development of this promising strategy.

6 Conclusion

More than 10 years after the initial reports, one can ask whether CPP-based cellular delivery has reached maturity. The naïve view of a universal magic CPP bullet that delivers any hydrophilic

molecule into the cell has been replaced by a more complex picture, where for instance the nature of the transported cargo, its mode of linkage to the CPP, or the targeted intracellular compartment have to be considered. Our knowledge in this field still remains largely empirical, rather than predictive, and often relies on the setting up of dedicated experimental protocols, such as those described in this book.

References

1. Frankel AD, Pabo CO (1988) Cellular uptake of the tat protein from human immunodeficiency virus. Cell 55:1189–1193
2. Green M, Loewenstein PM (1988) Autonomous functional domains of chemically synthesized human immunodeficiency virus tat trans-activator protein. Cell 55:1179–1188
3. Denis-Donini S, Glowinski J, Prochiantz A (1984) Glial heterogeneity may define the three-dimensional shape of mouse mesencephalic dopaminergic neurones. Nature 307: 641–643
4. Chamak B, Fellous A, Glowinski J, Prochiantz A (1987) MAP2 expression and neuritic outgrowth and branching are coregulated through region-specific neuro-astroglial interactions. J Neurosci 7:3163–3170
5. Gehring WJ, Qian YQ, Billeter M, Furukubo-Tokunaga K, Schier AF, Resendez-Perez D, Affolter M, Otting G, Wüthrich K (1994) Homeodomain-DNA recognition. Cell 78: 211–223
6. Ayala J, Touchot N, Zahraoui A, Tavitian A, Prochiantz A (1990) The product of rab2, a small GTP binding protein, increases neuronal adhesion, and neurite growth in vitro. Neuron 4:797–805
7. Borasio GD, John J, Wittinghofer A, Barde YA, Sendtner M, Heumann R (1989) ras p21 protein promotes survival and fiber outgrowth of cultured embryonic neurons. Neuron 2: 1087–1096
8. Joliot A, Pernelle C, Deagostini-Bazin H, Prochiantz A (1991) Antennapedia homeobox peptide regulates neural morphogenesis. Proc Natl Acad Sci U S A 88:1864–1868
9. Bloch-Gallego E, Le Roux I, Joliot A, Volovitch M, Henderson CE, Prochiantz A (1993) Antennapedia homeobox peptide enhances growth and branching of embryonic chicken motoneurons in vitro. J Cell Biol 120: 485–492
10. Le Roux I, Joliot AH, Bloch-Gallego E, Prochiantz A, Volovitch M (1993) Neurotrophic activity of the Antennapedia homeodomain depends on its specific DNA-binding properties. Proc Natl Acad Sci U S A 90:9120–9124
11. Le Roux I, Duharcourt S, Volovitch M, Prochiantz A, Ronchi E (1995) Promoter-specific regulation of gene expression by an exogenously added homeodomain that promotes neurite growth. FEBS Lett 368: 311–314
12. Joliot A, Triller A, Volovitch M, Pernelle C, Prochiantz A (1991) alpha-2,8-Polysialic acid is the neuronal surface receptor of antennapedia homeobox peptide. New Biol 3: 1121–1134
13. Derossi D, Joliot A, Chassaing G, Prochiantz A (1994) The third helix of the Antennapedia homeodomain translocates through biological membranes. J Biol Chem 269:10444–10450
14. Duchardt F, Fotin-Mleczek M, Schwarz H, Fischer R, Brock R (2007) A comprehensive model for the cellular uptake of cationic cell-penetrating peptides. Traffic 8:848–866
15. Maiolo JR, Ferrer M, Ottinger EA (2005) Effects of cargo molecules on the cellular uptake of arginine-rich cell-penetrating peptides. Biochim Biophys Acta 1712:161–172
16. Manceur A, Wu A, Audet J (2007) Flow cytometric screening of cell-penetrating peptides for their uptake into embryonic and adult stem cells. Anal Biochem 364:51–59
17. Nakase I, Niwa M, Takeuchi T, Sonomura K, Kawabata N, Koike Y, Takehashi M, Tanaka S, Ueda K, Simpson JC et al (2004) Cellular uptake of arginine-rich peptides: roles for macropinocytosis and actin rearrangement. Mol Ther 10:1011–1022
18. Balayssac S, Burlina F, Convert O, Bolbach G, Chassaing G, Lequin O (2006) Comparison of penetratin and other homeodomain-derived cell-penetrating peptides: interaction in a membrane-mimicking environment and cellular uptake efficiency. Biochemistry 45: 1408–1420
19. Harreither E, Rydberg HA, Amand HL, Jadhav V, Fliedl L, Benda C, Esteban MA,

Pei D, Borth N, Grillari-Voglauer R et al (2014) Characterization of a novel cell penetrating peptide derived from human Oct4. Cell Regen (Lond) 3:2

20. Derossi D, Calvet S, Trembleau A, Brunissen A, Chassaing G, Prochiantz A (1996) Cell internalization of the third helix of the Antennapedia homeodomain is receptor-independent. J Biol Chem 271:18188–18193
21. Binder H, Lindblom G (2003) Charge-dependent translocation of the Trojan peptide penetratin across lipid membranes. Biophys J 85:982–995
22. Christiaens B, Symoens S, Verheyden S, Engelborghs Y, Joliot A, Prochiantz A, Vandekerckhove J, Rosseneu M, Vanloo B, Vanderheyden S (2002) Tryptophan fluorescence study of the interaction of penetratin peptides with model membranes. Eur J Biochem 269:2918–2926
23. Persson D, Thorén PE, Nordén B (2001) Penetratin-induced aggregation and subsequent dissociation of negatively charged phospholipid vesicles. FEBS Lett 505:307–312
24. Fragneto G, Bellet-Amalric E, Charitat T, Dubos P, Graner F, Perino-Galice L (2000) Neutron and X-ray reflectivity studies at solid-liquid interfaces: the interaction of a peptide with model membranes. Phys Rev B Condens Matter 276–278:501–502
25. Fragneto G, Graner F, Charitat T, Dubos P, Bellet-Amalric E (2000) Interaction of the third helix of antennapedia homeodomain with a deposited phospholipid bilayer: a neutron reflectivity structural study. Langmuir 16: 4581–4588
26. Björklund J, Biverståhl H, Gräslund A, Mäler L, Brzezinski P (2006) Real-time transmembrane translocation of penetratin driven by light-generated proton pumping. Biophys J 91:L29–L31
27. Magzoub M, Pramanik A, Gräslund A (2005) Modeling the endosomal escape of cell-penetrating peptides: transmembrane pH gradient driven translocation across phospholipid bilayers. Biochemistry 44:14890–14897
28. Su Y, Mani R, Hong M (2008) Asymmetric insertion of membrane proteins in lipid bilayers by solid-state NMR paramagnetic relaxation enhancement: a cell-penetrating Peptide example. J Am Chem Soc 130:8856–8864
29. Bárány-Wallje E, Keller S, Serowy S, Geibel S, Pohl P, Bienert M, Dathe M (2005) A critical reassessment of penetratin translocation across lipid membranes. Biophys J 89:2513–2521
30. Persson D, Thorén PEG, Esbjörner EK, Goksör M, Lincoln P, Nordén B (2004) Vesicle size-dependent translocation of penetratin analogs across lipid membranes. Biochim Biophys Acta 1665:142–155
31. Terrone D, Sang SLW, Roudaia L, Silvius JR (2003) Penetratin and related cell-penetrating cationic peptides can translocate across lipid bilayers in the presence of a transbilayer potential. Biochemistry 42:13787–13799
32. Drin G, Mazel M, Clair P, Mathieu D, Kaczorek M, Temsamani J (2001) Physico-chemical requirements for cellular uptake of pAntp peptide. Role of lipid-binding affinity. Eur J Biochem 268:1304–1314
33. Berlose JP, Convert O, Derossi D, Brunissen A, Chassaing G (1996) Conformational and associative behaviours of the third helix of antennapedia homeodomain in membrane-mimetic environments. Eur J Biochem 242: 372–386
34. Lindberg M, Gräslund A (2001) The position of the cell penetrating peptide penetratin in SDS micelles determined by NMR. FEBS Lett 497:39–44
35. Magzoub M, Kilk K, Eriksson LE, Langel U, Gräslund A (2001) Interaction and structure induction of cell-penetrating peptides in the presence of phospholipid vesicles. Biochim Biophys Acta 1512:77–89
36. Bellet-Amalric E, Blaudez D, Desbat B, Graner F, Gauthier F, Renault A (2000) Interaction of the third helix of Antennapedia homeodomain and a phospholipid monolayer, studied by ellipsometry and PM-IRRAS at the air-water interface. Biochim Biophys Acta 1467: 131–143
37. Zhang W, Smith SO (2005) Mechanism of penetration of Antp(43-58) into membrane bilayers. Biochemistry 44:10110–10118
38. Lamazière A, Chassaing G, Trugnan G, Ayala-Sanmartin J (2009) Tubular structures in heterogeneous membranes induced by the cell penetrating peptide penetratin. Commun Integr Biol 2:223–224
39. Palm-Apergi C, Lorents A, Padari K, Pooga M, Hällbrink M (2009) The membrane repair response masks membrane disturbances caused by cell-penetrating peptide uptake. FASEB J 23:214–223
40. Christiaens B, Grooten J, Reusens M, Joliot A, Goethals M, Vandekerckhove J, Prochiantz A, Rosseneu M (2004) Membrane interaction and cellular internalization of penetratin peptides. Eur J Biochem 271:1187–1197
41. Esbjörner EK, Lincoln P, Nordén B (2007) Counterion-mediated membrane penetration: cationic cell-penetrating peptides overcome Born energy barrier by ion-pairing with

phospholipids. Biochim Biophys Acta 1768:1550–1558

42. Dupont E, Prochiantz A, Joliot A (2007) Identification of a signal peptide for unconventional secretion. J Biol Chem 282:8994–9000
43. Magzoub M, Eriksson LEG, Gräslund A (2003) Comparison of the interaction, positioning, structure induction and membrane perturbation of cell-penetrating peptides and non-translocating variants with phospholipid vesicles. Biophys Chem 103: 271–288
44. Drin G, Déméné H, Temsamani J (2001) Translocation of the pAntp peptide and its amphipathic analogue AP-2AL. Biochemistry 40:1824–1834
45. Bechara C, Pallerla M, Zaltsman Y, Burlina F, Alves ID, Lequin O, Sagan S (2013) Tryptophan within basic peptide sequences triggers glycosaminoglycan-dependent endocytosis. FASEB J 27:738–749
46. Jiao CY, Delaroche D, Burlina F, Alves ID, Chassaing G, Sagan S (2009) Translocation and endocytosis for cell-penetrating peptide internalization. J Biol Chem 284: 33957–33965
47. Ghibaudi E, Boscolo B, Inserra G, Laurenti E, Traversa S, Barbero L, Ferrari RP (2005) The interaction of the cell-penetrating peptide penetratin with heparin, heparansulfates and phospholipid vesicles investigated by ESR spectroscopy. J Pept Sci 11:401–409
48. Madani F, Perálvarez-Marín A, Gräslund A (2011) Liposome model systems to study the endosomal escape of cell-penetrating peptides: transport across phospholipid membranes induced by a proton gradient. J Drug Deliv 2011:897592
49. Letoha T, Gaal S, Somlai C, Czajlik A, Perczel A, Penke B (2003) Membrane translocation of penetratin and its derivatives in different cell lines. J Mol Recognit 16:272–279
50. Letoha T, Gaál S, Somlai C, Venkei Z, Glavinas H, Kusz E, Duda E, Czajlik A, Peták F, Penke B (2005) Investigation of penetratin peptides. Part 2. In vitro uptake of penetratin and two of its derivatives. J Pept Sci 11:805–811
51. Fischer R, Waizenegger T, Köhler K, Brock R (2002) A quantitative validation of fluorophore-labelled cell-permeable peptide conjugates: fluorophore and cargo dependence of import. Biochim Biophys Acta 1564:365–374
52. Allinquant B, Hantraye P, Mailleux P, Moya K, Bouillot C, Prochiantz A (1995) Downregulation of Amyloid Precursor Protein inhibits neurite outgrowth in Vitro. J Cell Biol 128:919–927
53. Perez F, Lledo PM, Karagogeos D, Vincent JD, Prochiantz A, Ayala J (1994) Rab3A and Rab3B carboxy-terminal peptides are both potent and specific inhibitors of prolactin release by rat cultured anterior pituitary cells. Mol Endocrinol 8:1278–1287
54. Schutze-Redelmeier MP, Gournier H, Garcia-Pons F, Moussa M, Joliot A, Volovitch M, Prochiantz A, Lemonnier FA (1996) Introduction of exogenous antigens into the MHC class I processing and presentation pathway by Drosophila antennapedia homeodomain primes cytotoxic T cells in vivo. J Immunol 157:650–655
55. Théodore L, Derossi D, Chassaing G, Llirbat B, Kubes M, Jordan P, Chneiweiss H, Godement P, Prochiantz A (1995) Intraneuronal delivery of protein kinase C pseudosubstrate leads to growth cone collapse. J Neurosci 15:7158–7167
56. Troy CM, Derossi D, Prochiantz A, Greene LA, Shelanski ML (1996) Downregulation of Cu/Zn superoxide dismutase leads to cell death via the nitric oxide-peroxynitrite pathway. J Neurosci 16:253–261
57. Dupont E, Prochiantz A, Joliot A (2006) Penetratins. CRC, Boca Raton, FL