

## Visions & Reflections

# Internalization of cationic peptides: the road less (or more?) traveled

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**Abstract.** Facilitating the entry of molecules into mammalian cells is of great interest to fields as diverse as cell biology and drug delivery. The discovery of natural protein transduction domains and the development of artificial ones, including polyarginine, provides a means

to achieve this goal. Here, we comment on key chemical and biological aspects of cationic peptide internalization, including the physiological relevance of this process.

**Keywords.** Endocytosis, heparan sulfate proteoglycan, HIV-TAT, polyarginine, protein transduction domain.

### Introduction

The ability of polycations to enhance the cellular uptake of macromolecules has been known for nearly 50 years [1]. Interest in this phenomenon reemerged in 1988 with the finding that exogenous TAT protein from the human immunodeficiency virus (HIV-TAT) could enter mammalian cells [2, 3]. Many natural and synthetic cationic peptides likewise have this ability [4]. These peptides have been classified as ‘protein transduction domains’ (PTDs) or ‘cell-penetrating peptides’ (CPPs).

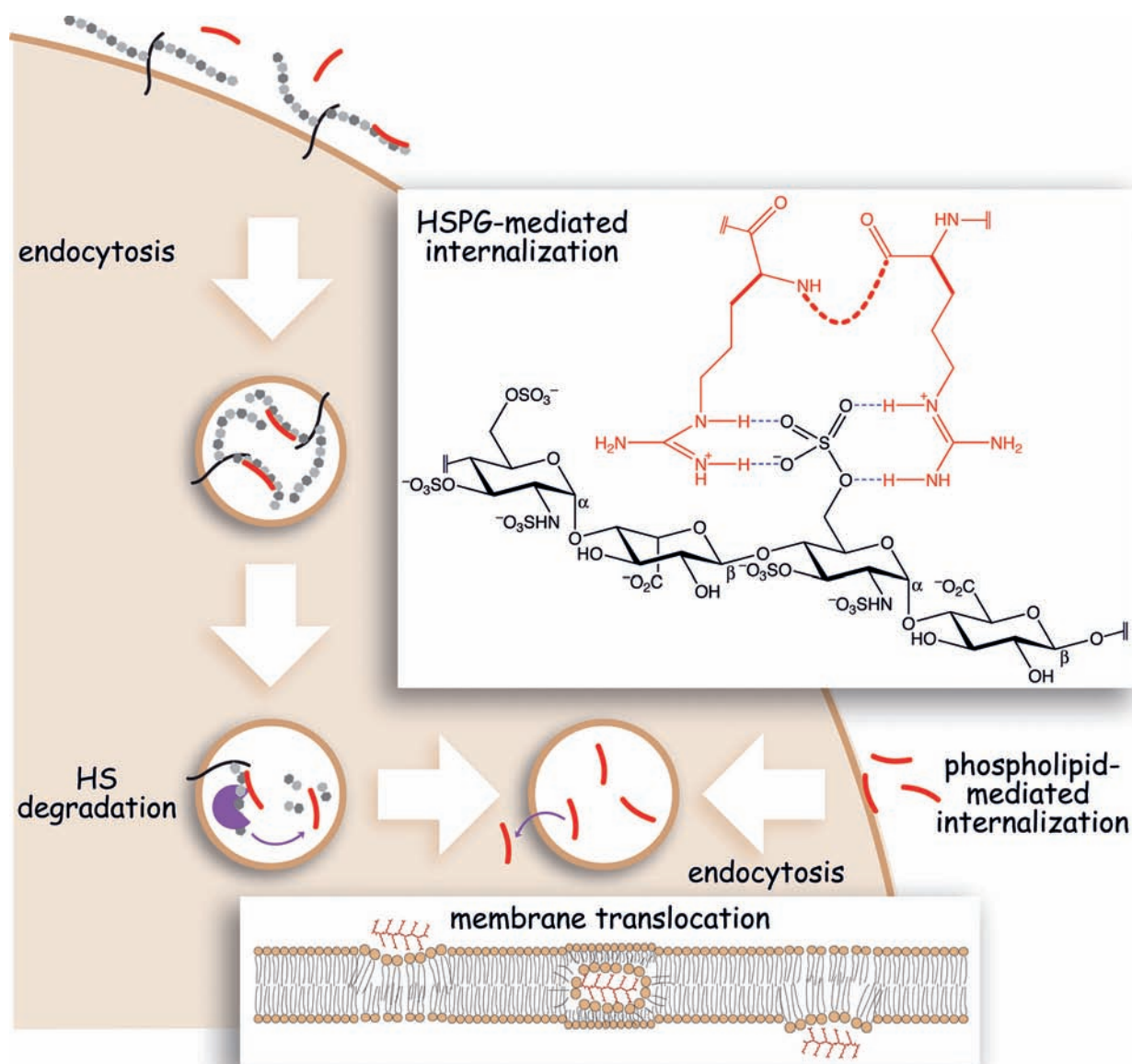
Rather than discuss the detailed mechanism of PTD internalization or potential applications for these peptides, which have been well covered in the literature [5–7], we wish to take a step back and discuss more general issues about the cellular internalization of cationic molecules. We focus on one PTD – polyarginine – as the basis for this discussion, and speculate on the role of its guanidinium groups in cellular uptake. Further, we discuss whether the pathway of PTD internalization is unique to cationic peptides, or whether these peptides hijack path-

ways that have evolved for the internalization of endogenous cationic macromolecules. It is important to note that we shall limit our discussion to experiments performed with live cells, as fixation has been shown to alter the internalization of PTDs [8].

### Role of charge in PTD internalization

HIV-TAT is perhaps the best-studied PTD. Structure-function studies defined an 11-residue, basic sequence corresponding to a portion of the RNA-binding domain of HIV-TAT as being necessary for cellular uptake [9]. This region contained six arginine residues that were especially critical. This finding led several groups to examine the uptake of simple polybasic peptides, such as polyarginine and polylysine. Polyarginine was found to be superior to other homopolymeric amino acids [10], and the optimal length of polyarginine necessary for transport was found to be between 5 and 11 residues, with octa- and nonaarginine being transported most efficiently [10, 11]. Other PTDs are of similar size and likewise contain a high percentage of basic residues [12–14].

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**Figure 1.** Scheme of putative routes for polyarginine internalization. Polyarginine (red) interacts with either HSPGs (black/gray) or lipids (brown) at the cell surface and undergoes endocytosis. Polyarginine is released from HSPGs by glycosidase activity within endocytic compartments. Free polyarginine crosses the endocytic membrane into the cytosol. (Upper inset) Possible hydrogen bonds between two guanidinium groups of polyarginine (red) and a sulfuryl group of heparan sulfate (black). (Lower inset) Possible mechanism for the membrane translocation of polyarginine in which cationic guanidinium groups interact with anionic lipid head groups to form a neutral complex that traverses the bilayer.

### Why is polyarginine internalized by cells?

What physicochemical properties of polyarginine allow for translocation into cells? Is polyarginine internalization purely a biophysical phenomenon, or have cells evolved a mechanism(s) for the uptake of cationic peptides and proteins? If so, why?

Polyarginine is a natural polymer of guanidinium groups. Like its enantiomer, poly(D-arginine) enters cells efficiently, indicating that the internalization process is not stereospecific [10]. In addition, guanidinium groups dis-

played from numerous non-proteinogenic scaffolds can facilitate internalization [15–17]. These results indicate that a peptide scaffold is not essential for PTD uptake.

Why is the guanidinium group important for uptake? Primary guanidinium groups bear a positive charge under physiological conditions and have the potential to donate up to five hydrogen bonds to electron-rich functionalities [18], such as the carboxyl, phosphoryl, and sulfuryl groups of cell-surface carbohydrates and phospholipids (Fig. 1). The favorable Coulombic component makes such hydrogen bonds especially strong [19]. Using *N*-methylated ar-

ginine residues, Rothbard and co-workers have elegantly demonstrated that the ability of the guanidinium group to form hydrogen bonds is indeed essential for efficient cellular uptake [20, 21].

The role of the guanidinium group in PTD internalization is not understood completely. Our group has shown that polyarginine forms a tight interaction with heparin, a mimic for the heparan sulfate proteoglycans (HSPGs) on the surface of mammalian cells (Fig. 1) [22, 23]. HIV-TAT peptide and protein have also been shown to interact with heparin [24–27]. Many groups have shown that in glycosaminoglycan (GAG)-deficient cell lines, internalization of PTDs is decreased [4, 22, 28, 29]. These results suggest that the role of the guanidinium functionality in PTD uptake could be to facilitate binding to the cell surface through interactions with cell-surface HSPGs.

An alternative role for the guanidinium functionality in uptake could be to facilitate binding to cell-surface lipids (Fig. 1). Rothbard and co-workers have shown that PTDs, such as polyarginine, can form neutral complexes with phospholipids within endocytic vesicles, which in turn can cross cellular membranes in the presence of a chemical gradient [21]. Likewise, Matile and co-workers have shown that cationic peptides and hydrophobic anions can form neutral complexes that translocate across bilayers [30, 31]. Merkle and co-workers have reported that the cationic peptide pVEC (and its derivatives) can induce phospholipid phase transitions, suggesting another mechanism by which PTDs could reach the cytosol [32].

### Do cells have a mechanism for internalizing cationic molecules?

The transport of molecules into and out of cells is a highly regulated process. Nonetheless, many cationic proteins freely enter cells. Basic fibroblast growth factor (bFGF) does so via caveolae-mediated endocytosis [33]. Bovine pancreatic ribonuclease (RNase A) and its homologs interact strongly with HSPGs [34] and are internalized by dynamin-independent endocytosis [35]. Cellular entry of the cationic growth factor, midkine (MK), is mediated by the lipoprotein receptor-related protein (LRP) [36]. (Interestingly, LRP has also been implicated as a possible mediator of HIV-TAT internalization [37].) One common link between many of these internalized cationic growth factors is that they form biologically relevant interactions with HSPGs on the cell surface. Indeed, HSPGs play an important role in the internalization of a diverse group of macromolecules, including growth factors [38], polyamines [39] and viruses [40].

There is evidence that, in addition to being found at the cell surface, HSPGs are transported to both the cytosol and the nucleus [41]. The importance of this trafficking is not understood, but suggests that some fraction of

cell-surface HSPGs must be able to escape degradation in the lysosome and traffic to other regions of the cell. It is therefore conceivable, perhaps likely, that cationic proteins or peptides that associate with HSPGs could traffic with these carbohydrates.

The interaction between cationic proteins and anionic carbohydrates on the cell surface could be important for either signaling (for growth factors) or patterned gene expression (for cationic transcription factors). Belting and co-workers have proposed that extracellular transcription factors form gradients similar to those observed for morphogens, and that their biological effects are regulated by HSPG-mediated internalization [42]. PTDs could exploit this very same pathway to enter cells.

### Conclusions

Much is known about the internalization of PTDs and the role of HSPGs in complex biological processes. The interaction between PTDs and HSPGs is also fairly well established. HSPGs play an important role in the internalization and trafficking of other cationic molecules. Still, the role HSPGs play in the internalization process has not been studied extensively. Although it remains possible that PTD internalization is dictated through direct interactions with the plasma membrane, we must not discount the possibility that PTDs follow a pathway that evolved for another purpose – the uptake of cationic growth factors and other signaling molecules.

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