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CHEMBIOCHEM Highlights

Dodging Endosomes: Effective Cytosolic Antibody Delivery

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With the pool of new small-molecule drugs drying up over the last decades, great promise has been found in the exploitation of the much larger protein-derived molecules, so-called "biologicals". In particular, antibodies have attracted a lot of attention because of their high selectivity and affinity for their targets. Despite enormous progress in the development of antibodies as drugs, their full potential cannot be reached as many are ineffective owing to their inability to reach their intracellular target.[1] Hence, there is a great need for strategies that facilitate the delivery and cellular uptake of these drugs by cells.[2] In this light, cell-penetrating peptides (CPPs) are very interesting as they have been shown to be able to enter cells rather easily and to take a variety of cargoes across the otherwise mostly impermeable plasma membrane without inducing cytotoxicity.[3–6] Therefore, CPPs have been included as components in drug-delivery methods as a possible solution to this permeation problem.[7] However, even though some CPP conjugates have entered clinical trials,[8] a major issue is that with large cargoes such as antibodies, CPPs very often lead to localization in endosomes, which still could in essence be considered to be outside the cell, and mostly result in complete degradation of the proteins.[9–10] To overcome this limitation, a variety of approaches have been investigated.

One recent elegant approach was reported by the Futaki group.[11] They reconsidered using pH-sensitive peptides, of which the GALA peptide is an archetypal example, to achieve endosomal escape.[12] These peptides only perturb membranes after they get activated by a drop in pH, which is often found in late endosomes. It was postulated that because of the strong negative charge on this type of peptide, optimal effectivity cannot be achieved. Therefore, Futaki designed new endosomolytic peptides based on the cationic spider venom M-lycotoxin (Figure 1). This was combined with the idea of incorporating negative charge in the peptide through glutamic acids in order to modulate its membrane activity. Protonation at lower pH should restore its membrane-lytic activity. A variety of M-lycotoxin derivatives were examined, and it was found that the incorporation of a single Glu led to optimal cytosolic delivery of a model macromolecule, the 10-kDa dextran. Next, it was shown that bioactive proteins—such as Saporin, a ribosome-inactivating protein, and a recombinase called Cre, which catalyses site-specific recombination of DNA—could also be released into the cytosol. Subsequently, antibodies were shown not only to be delivered to the cytosol but also to be able to find and bind to an intracellularly expressed target protein. Finally, even exosomes—cell-derived vesicular structures of 30–100 nm—could be stimulated to release their contents into cells. When the authors investigated the uptake-and-release mechanism of the peptide in more detail, to their surprise, they hardly found any endosomolytic activity. Moreover, the expected conformational change upon acidification was absent. It was hypothesised that endosomal membrane perturbation was mainly achieved by electrostatic interactions. The incorporation of Glu modulates this activity. As the initial uptake of the peptide is endosomal and nonspecific, an even better result might be expected if it could be combined with a component that induces this pathway.

With the similar goal of cytosolic delivery in mind, the Cardoso and Hackenberger groups[13] took up a very different approach. Earlier they had reported that cyclic-arginine-rich CPPs are able to transduce large cargo directly into the cytosol and set out to show that these could also be applied to single-domain antibodies called nanobodies for intracellular targeting.[14] Making use of expressed protein ligation, they were able to produce green-fluorescent-protein-binding nanobodies (GBPs) tailored with a cyclic deca-arginine (cR_{10}). It was shown that these CPP–nanobody conjugates could enter fibroblast cells and bind to intracellularly expressed GFP (Figure 2, left). Furthermore, they demonstrated that GFP fusion proteins can also be targeted without much interference from subsequent protein–protein interaction of these fused proteins. In addition, they established that not only could the cR_{10}–nanobody constructs enter cells by themselves, but that also interacting proteins could be taken up as well, for example GFP-fused P53 (Figure 2, right). As all these cR_{10} constructs mainly ended up in nucleoli due to the preferential localization of the cyclic CPP itself, cleavable variants of the cR_{10}–nanobody chimeras were prepared. These conjugates entered cells as efficiently as their noncleavable counterparts and were shown now to be able to bind to their target GFP–PCNA fusion protein and localize in the nucleus outside of the nucleoli.

These two examples of effective intracellular delivery, that is, beyond endosomes, could be the next step in the development of efficient drug-delivery vehicles for large cargoes such as antibodies. They will open up a whole new pool of targets that have been inaccessible to date. Of course, in vivo experiments need to be performed to verify whether these approaches can be applied in situations beyond the test tube. Moreover, although very elegant methodologies, they do not solve another problem associated with CPPs, namely that they suffer from poor cellular specificity. Nevertheless, new steps have been taken to generally applicable approaches to bring

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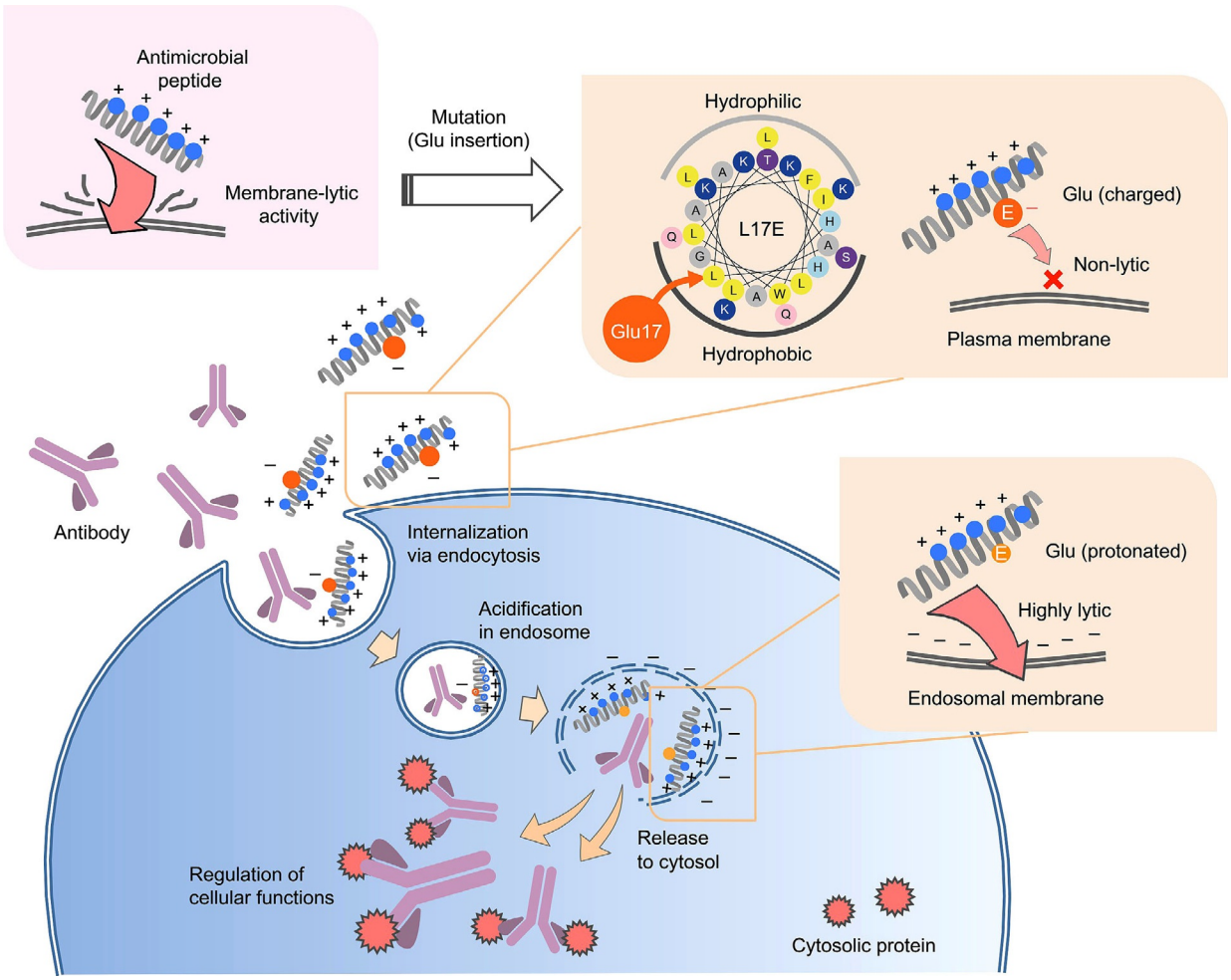

Figure 1. Design concept of endosomolytic peptides. Reprinted with permission from ref. [11]. Copyright: Nature Publishing Group, 2017.

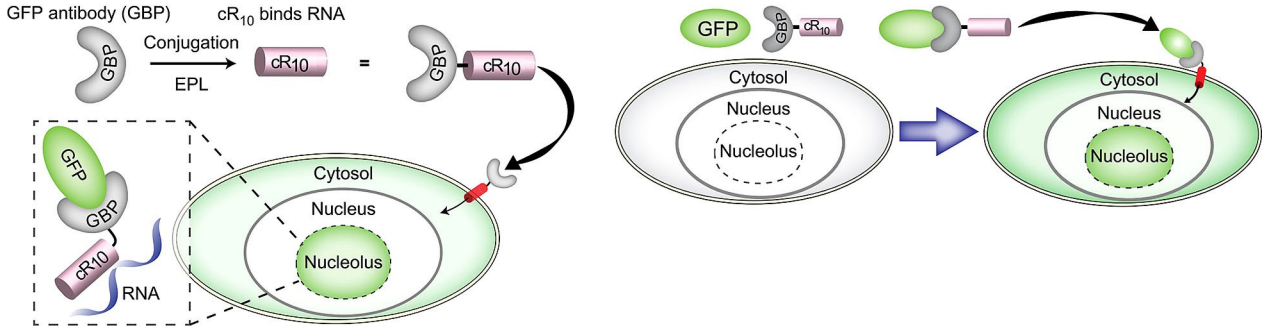

Figure 2. A schematic of cR_{10}-mediated cellular uptake of a nanobody that binds intracellularly expressed GFP (left) and a GFP nanobody that binds extracellularl eGFP and subsequently enters into living cells (right). Reprinted with permission from ref. [13]. Copyright: Nature Publishing Group, 2017.

CPPs from the laboratory to the clinic, a journey that has already lasted for over 20 years.

Conflict of Interest

The authors declare no conflict of interest.

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