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Cover story

Unraveling the penetration: Model giant plasma membrane vesicles for study of cell-penetrating peptides

Cell-penetrating peptides (CPPs) have been studied as possible alternative transfection reagents for almost twenty years. While several promising applications of CPPs as carriers for bioactive cargoes have been announced, their mechanisms of cellular internalization are still not clearly understood. A large number of studies have demonstrated that CPPs and CPP–cargo complexes at moderate peptide concentrations are taken up mainly by endosomal pathways. Those “Trojan horse” peptides, however, are shown to possess diverse properties depending on the material of the horse (physicochemical properties and concentration of CPP), the number of soldiers inside the horse (amount of cargo of the CPP–cargo complex), and nature of the wall of the city of Troy (contents of plasma membrane). It has been convincingly shown that in certain conditions rapid non-endosomal uptake of CPPs also takes place leading to a diffuse staining of the cytoplasm, as if “flooding the city with horses”. The detailed mechanism of this phenomenon is still incompletely understood, but molecular rearrangements in the plasma membrane must occur to enable the process. The possibility that the CPP penetration is preceded by some signaling cascades resulting in the membrane rearrangements for the peptide influx has been actively investigated.

The study by Säälik et al. in this issue exploited a cell-free system to study the interactions between the CPPs and plasma membrane components exploiting the giant plasma membrane vesicles (also called membrane blebs) [1]. Membrane of the vesicles retains its molecular richness but lacks the processes fuelled by cellular energy. Säälik et al. made two surprising observations when fluorescent CPPs were added to the giant plasma membrane vesicles. The CPPs preferred loosely packed membrane areas (i.e., liquid-disordered lipid phase) as the interaction site. This contradicts the earlier data that the cellular uptake of CPPs and CPP–cargo complexes is highly dependent on the presence of membranous cholesterol- and lipid-rafts, which are known to constitute the tightly packed membrane areas (i.e., liquid-ordered lipid domains). Another very intriguing observation is that CPPs crossed the membrane and accumulated within the giant plasma membrane vesicles, corroborating the direct penetration of peptides across the bilayer.

When CPPs are interacting with living cells, however, they first have to face the active membrane barrier supported by the actin cortex and

wide arsenal of energy-driven mechanisms which are designed to retain the membrane homeostasis and potential. This would most likely lead to endocytosis as the dominating CPP/CPP–cargo uptake mechanisms in cells. The carrier and cargo, then, have to find a way from entrapping vesicles to the cytoplasm. The likelihood of such endosomal escape increases as the endosomes mature and CPPs accumulate. This leads to destabilization of membrane of some vesicles and subsequent release of its contents to the cytosol. Such a scenario has been proposed in majority of cases including the study on PF6 (a stearylated derivative of Transportan10, an amphipathic CPP) complexed with antisense oligonucleotides by Hassane et al., which is also published in this issue [2]. The exact mechanisms of the CPP uptake by cells require further studies, and the giant plasma membrane vesicles provide an additional means of studying such mechanisms. The ability of direct penetration across the bilayer by CPPs suggests that they also have a great potential for penetration across the membrane of endosomes. Thus, in addition to the study on cellular uptake, the giant plasma membrane vesicles, with further modifications, can be used as a tool to study the endosomal escape of CPP–cargo complexes and other drug delivery systems.

References

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