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Article Addendum

Cell-penetrating peptides

From mammalian to plant cells

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Abbreviations: CPP, cell-penetrating peptide; *p*VEC, peptide vascular endothelial-cadherin

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Internalization of cell-penetrating peptides, well described in mammalian cell system, has recently been reported in a range of plant cells by three independent groups. Despite fundamental differences between animal cell and plant cell composition, the CPP uptake pattern between the mammalian system and the plant system is very similar. Tat, Tat-2 *p*VEC and transportan internalisation is concentration dependent and non saturable, enhanced at low temperature (4°C), and receptor independent. The use of CPPs as nanocarrier for macromolecular delivery in plant cells is now achievable and the advances made in mammalian cells greatly enhance our understanding of cell-membrane and CPP-macromolecule complex interaction in plant. The cross membrane nanocarrier ability of CPPs promises new avenues in the field of plant biotechnology.

Cell-penetrating peptides (CPPs) are a class of short peptides with a property to translocate across cell membranes.[1] The distinct ability of CPPs to deliver macromolecules that are otherwise restricted to cross the membrane has lead to development of novel peptide-mediated gene and protein delivery methods in human cells for therapeutic purposes.[2] Most of the investigations pertaining to CPPs, such as structural and functional studies, have been carried out in the animal system. Plant systems largely remained unexplored in this context till the emergence of recent reports focusing on translocation of arginine rich CPPs in plants cells. Cellular internalization of cell penetrating peptides such as *p*VEC, transportan, monomer and dimer of HIV-1 Tat basic domain, and penetratin has been reported in tobacco protoplasts derived from suspension cell culture or triticale mesophyll protoplasts.[3-6] In triticale, leaf bases that are highly meristematic in nature and the root tips known for high mitotic activity showed significant internalization of both *p*VEC and transportan. Tat_{49-57} RKKRRQRRR basic domain, one of the shortest known cell penetrating peptide, can independently translocate across the plasma membrane with predominant accumulation in the plant cell nucleus.[4,7] Tat and its oligomers can traverse plasma membrane with the cargo complex carrying macromolecules of much higher mass and size than their own. Cellular uptake of arginine rich intercellular domain (AID) fusion proteins has been shown in onion epidermal and root tip cells and tomato root cells.[6] Synthetic cationic homoarginine (R12) oligopeptide has also been shown to deliver dsRNA into tobacco cells to induce post transcriptional gene silencing in plants.[8]It is interesting to note that similar to cell-penetrating peptides, virE2 protein of *Agrobacterium* (natural genetic engineer of plants) has nuclear localization sequence (bipartite) that can mediate nuclear uptake of ssDNA in plant cells. Are there more plant specific sequences that can be employed as CPPs for delivering macromolecules that are otherwise rendered impermeant by the cell membrane? Such questions can form the basis of future investigations on role of CPPs in plants.

Also, the process of cellular internalization of CPPs still remains unresolved and complex. However, as observed in mammalian cell lines, our studies in plant protoplasts indicated that the uptake of Tat, *p*VEC and transportan was concentration dependent, non-saturable and does not involve endocytosis. It is notable that the uptake of negative controls, mutated Tat and scrambled *p*VEC neither increased at the highest concentration provided nor influenced by the various physical chemical factors investigated, confirming that peptide sequence plays an important role in plant cell permeation properties of CPPs. Our studies also indicate that the extent of internalization of CPPs not only varies with the mammalian cell lines and different tissues but also with the organism investigated.[1,3,4,7,9] It is interesting as well as intriguing that Tat, *p*VEC and transportan have very different amino acid compositions, still these peptides show significant similarity in their uptake pattern both in plant as well as animal cells. However, noncovalent protein transduction of fluorescent proteins using arginine rich CPPs in plant root tip cells is inhibited in the presence of same macropinocytic inhibitors.[7,10] It is possible that the permeation properties of CPP change upon complexing with macromolecules and according to recipient cell, as a result, cargo is internalised by an altogether different mechanism(s)

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where as CPPs alone are transduced directly into the cells in an energy, temperature and receptor independent manner.

Macromolecular components (proteins, drugs or nucleic acid) of the CPP-driven cargo complex cannot surpass the entry barrier posed by cell membrane via conventional transport mechanisms. Dual ability of cell penetrating peptides to translocate cargo complexes of much bigger size than their own across the plasma membrane and also act as nuclear localization signal have demonstrated potential to revolutionize the field of bio-nanomedicine research.[11,12] The delivered proteins maintain their functionality and are biologically active in the cells. One major distinction between plant and the animal cells is that plant cells have a cell wall surrounding the cell membrane. Therefore, somatic plants cells can pose challenges in the uptake of cell-penetrating peptides. Being single celled and without cell wall, protoplasts offer a comparable system to mammalian cell lines although the cell membrane composition of the two cells differs significantly. They can also be easily isolated and purified in abundance. Other single cell and micro-calli culture systems are amenable to green plants regeneration. Most commercial plants are challenging targets for genetic manipulations through conventional methods of transformation. At the cross road of plant cell culture and nanocarrier technology, we see tremendous potential of CPPs such as Tat basic domain as vectors for alternate, simple and cost-effective strategies for gene transfer in cells of these commercially important plant species. Synthetic or in vivo produced nucleic acid, proteins and CPPs are blocks that conjugate to form nano-complexes in a relatively predictable manner. The growing interest in CPP-mediated molecule delivery in plants can give rise to an entirely new field of 'phyto-nanobiotechnology'!

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