Use of FRAP to measure membrane partition of Cell-penetrating peptides

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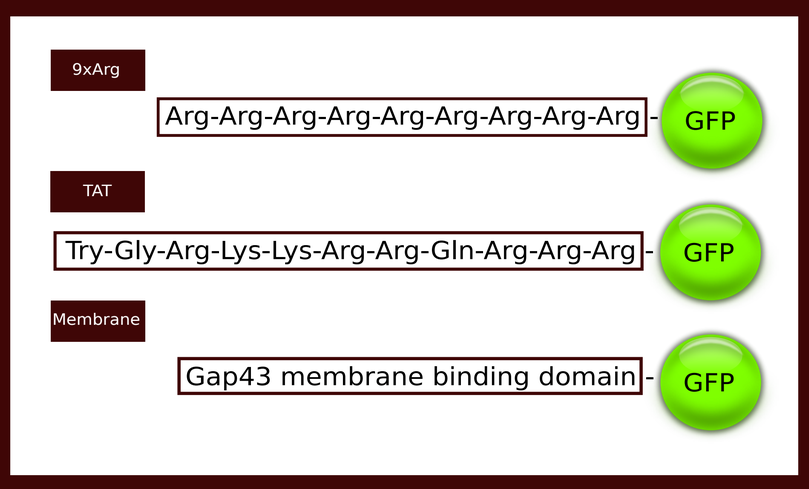
Abstract

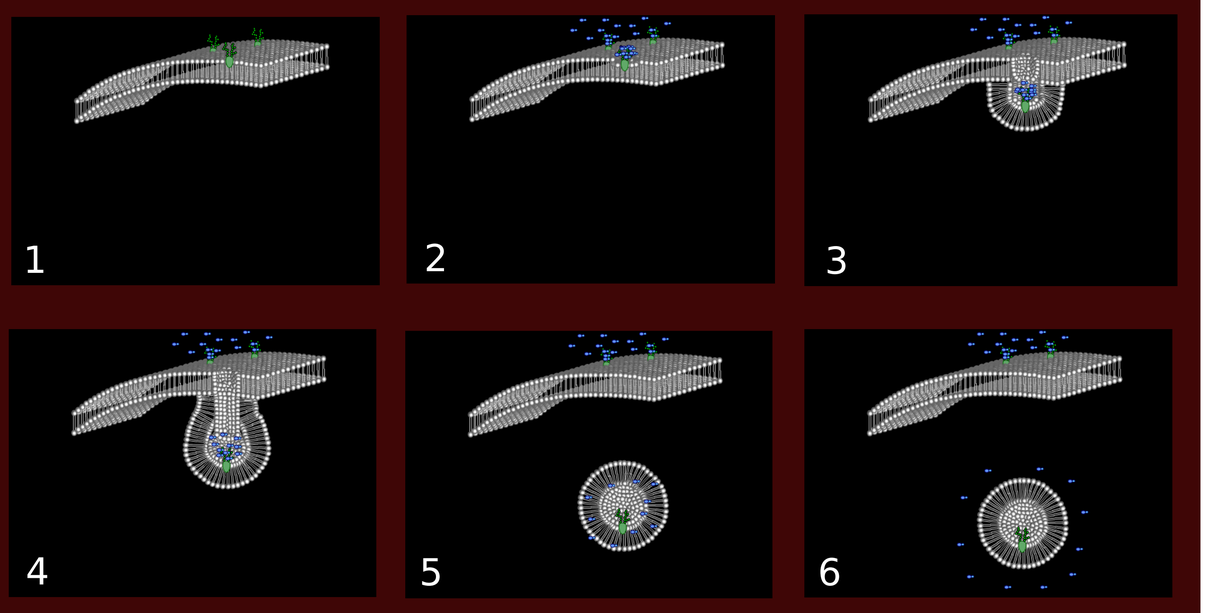
Plasma membrane represents an impermeable barrier for most of proteins. Still some proteins and small peptides enter cells efficiently. The so–called cell penetrating peptides (CPP) or protein transduction domains (PTD's) are defined by their ability to reach the cytoplasmic and/or nuclear compartments in live cells after internalization. CPP are an expanding family, among them one of the best studied is The HIV Tat transactivator sequence and derivates. How do these peptides actually get a across the plasma membrane is still matter of conflict. It has been show that endocytosis contributes to the import of these molecules. Depending of its extracellular concentration, Tat-derived peptides simultaneously use three endocytic pathways: macropinocitosis, clatrin-mediated endocytosis or caveola/lipid-raft-mediated endocytosis. These different mechanisms have in common a transients binding to the membrane. Using the FRAP technique, we have studied the relative membrane affinity of two different Tat-derivates; the so called Tat–peptide, and a 9xArginine peptide. To this end, different cells types (A549, MB-MDA231 and MEFs) were transfected with GFP fusion peptides expression plasmids. Fluorescence recovery kinetics were compared among them. These findings may be relevant at the time to design and explain the biological activity of certain CPP-cargoes.

Protein Transduction Domains (PTD's)



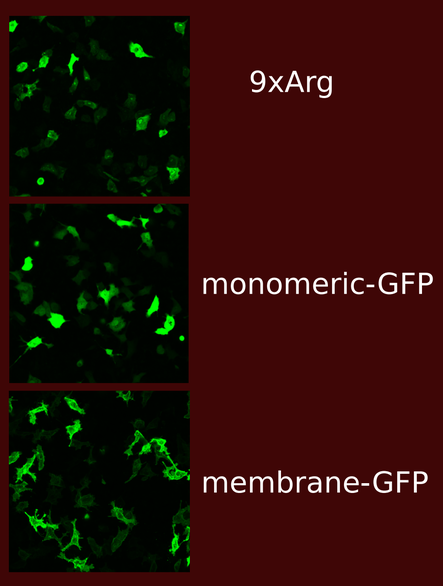
The study consist in the analysis of membrane affinity of two different TAT derivates: TAT and 9xArg. To carry on the experiments, the transduction peptides were fusioned with GFP as it is shown in the figure. TAT is composed of 11aa residues, and the transduction domain 9xArg is formed by 9xArg residues.

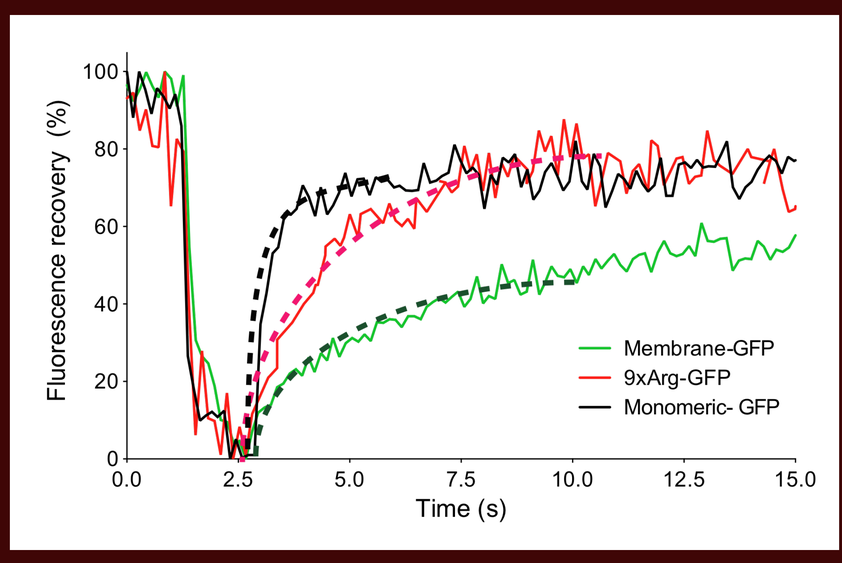
Although penetrating mechanisms are still unclear, the endocytic pathway independent of clathrin is one of the most accepted hypothesis for the transduction process.



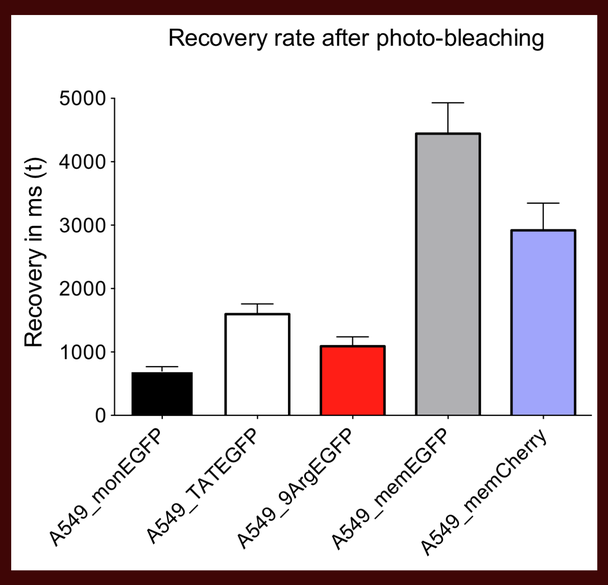
The picture despites the sequential steps of the endocytic transdution process. 2. The transduction domain binds to sulfate heparanes. 3. The binding induces a formation of a small vesicle. 5. The vesicle is endocyted. 6. Small vesicle are leaky and loss part of its contents.
During endocytic process transduction domains binds to lipids and associated proteins.

Frap Analysis



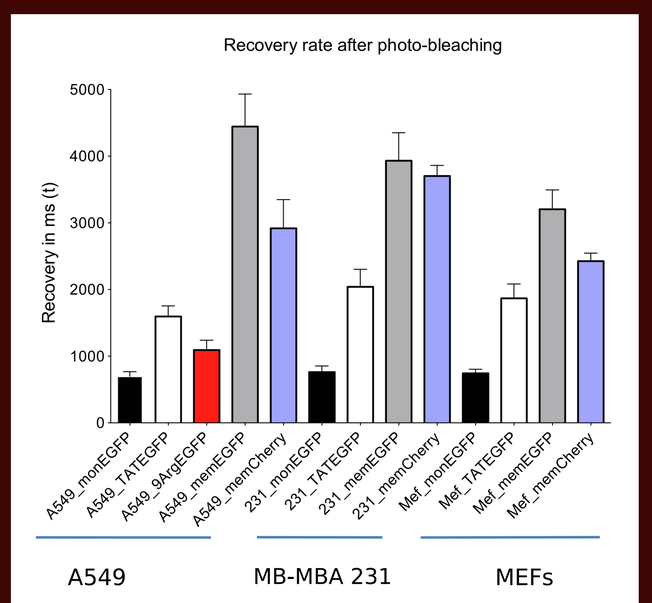


1. Comparative examples of fluorescent recovery after photobleaching from the different fusion proteins. The recovery kinetics were adjusted to a monexponential curve. The faster recovery was obtenined with the monomeric GFP and the slower with the membrane GFP construction. Protein transduction showed a intermediate rate of recovery.



2. Average recovery rate after photobleaching. Two different PTDs of TAT family were tested. A549 cells were transfected with the indicate fusion protein. Monomeric GFP shows the faster recovery, an expected result, because all the protein is cytoplasmatic –simple diffusion- (800ms). Membrane-Cherry or membrane-GFP are predominately associated with membranes, therefore they recover with slow times constant- membrane diffusion- (4500ms and 3200ms), while TAT and 9xArg show an intermediate recovery rate characteristic of partial soluble protein (1800ms and 1200ms) .

3. To ensure that lipid membrane composition or cellular type do not influx recovery rate, frap experiment was repeated in three different cell types (one lung cancer cell, one breast cancer cell and mouse fibroblastes). The results clearly indicate that recovery rate is independent of cellular cell type.



Conclusion

Protein transduction is a useful technique to deliver peptides or proteins to the cellular cytoplasm, even though the molecular mechanism is not well understood. Our results clearly indicate that transduction peptides are associated with membranes, a result expected due to its high density of positive charges in their sequences. Therefore care must be taken at the time to interpret the results obtained using transduction technology.
In a near future, employing a membrane pull down experiment, quantification of total levels of membrane associated protein will be done.

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