WHICH MECHANISM FOR NUCLEAR IMPORT OF PLASMID DNA COMPLEXED WITH POLYETHYLENIMINE DERIVATIVES?

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To investigate the nuclear import mechanism of plasmid/PEI derivative complexes and the putative nuclear targeting of therapeutic genes by the use of oligosaccharides, we have studied the nuclear import of plasmid DNA complexed either with PEI or with lactosylated PEI (LacPEI) in cystic fibrosis human airway epithelial cells (CFTE29o- cells). In synchronized cells that were released from the second thymidine block at the time of LacPEI complex incubation and in which most of the cells have undergone mitosis between the complex incubation and the determination of gene transfer efficiency 24 h later, 56 ± 1% of the cells expressed GFP. In contrast, 26 ± 1% of cells arrested in S phase expressed GFP, showing nevertheless that some complexes were able to cross the nuclear membrane. As for asynchronous cells, 44 ± 1% expressed GFP ($p < 0.05$, Kruskal Wallis-test). Similar results were observed with PEI complexes. The nuclear import of plasmid/LacPEI or unsubstituted PEI complexes was then studied in digitoninpermeabilized cells. Whatever the conditions used, the import buffer alone or supplemented with ATP, cytosolic extract or both, the free biotinylated plasmid DNA or the plasmid DNA complexed with LacPEI or with unsubstituted PEI accumulated at the nuclear periphery and was never observed in the nucleus of digitoninpermeabilized CFTE29o- cells. Our results show that for complexes made with PEI derivatives, the major route for plasmid DNA nuclear entry is a passive nuclear importation during mitosis when the nuclear membrane temporarily breaks down. However, a plasmid DNA importation also occurs in growth-arrested cells; this importation is not related to a classic NLScontaining proteins pathway or to a nuclear targeting mediated by lactosyl residues. We hypothesize that PEI complexes are able to enter the nucleus by alternative routes with novel, unknown mechanisms. By characterizing and understanding them, new gene transfer systems might be developed, aiming to overcome the nuclear barrier and increase transfection efficiency in vivo, when non proliferating cells are the targets.

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