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CYTOSKELETAL INVOLVEMENT IN THE CELLULAR TRAFFICKING OF PLASMID/PEI DERIVATIVE COMPLEXES

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Polyethyleneimine (PEI) is a synthetic cationic polymer with endosome buffering capacity that has been shown to mediate efficient in vitro and in vivo gene transfer into various mammalian cells. We have developed lactosylated PEI and showed that it is more efficient than unsubstituted PEI for in vitro gene transfer into airway epithelial cells. Our aim was to study the cytoskeletal involvement in the cellular trafficking of complexes made with plasmid/PEI derivatives. Lactosylated PEI (25 kDa, branched form) contained about 5% of its amino groups substituted with a lactosylthiocarbamoyl unit. Lactosylated or sugar-free PEI (75 nmol) was complexed to a plasmid (2.5 µg) encoding either green fluorescent protein (GFP) or luciferase (N/P ratio of 10). Immortalized cystic fibrosis airway epithelial cells (ΣCFTE29o- cells) were incubated in the presence of complexes and cytoskeletal inhibitors, and the number of transfected cells was determined by flow cytometry 24 h later. Complexes were also generated with fluorescein-labeled PEI derivatives and the cell fluorescence intensity was determined by flow cytometry. In the presence of nocodazole (25 µM) to disrupt microtubules or of cytochalasin D (25 µM) to depolymerize actin filaments, the gene transfer efficiency with lactosylated PEI or sugar-free PEI was dramatically decreased, showing the involvement of both microfilaments and microtubules in gene transfer efficiency: for instance with complexes made of lactosylated PEI : 29±3% of the cells were transfected in control experiments, while 3±1% of the cells (more than 90% inhibition) were transfected in the presence of nocodazole or of cytochalasin D; $p<0.05$). The uptake of fluoresceinylated complexes studied by flow cytometry was decreased by 50 % in the presence of cytochalasin D for both types of complexes ($p<0.005$) and unchanged in the presence of nocodazole, suggesting a predominant role for actin microfilaments in complex uptake. When the inhibitors were added to the cell culture after the complex uptake had occurred, gene transfer efficiency was similar in the presence of cytochalasin D, as compared with control conditions. However, it was decreased by 75% in the presence of nocodazole, suggesting the involvement of microtubules in the intracellular trafficking of complexes. The involvement of actin microfilaments and of microtubules during the key steps of gene transfer was verified by confocal microscopy after labeling of complexes, microfilaments and microtubules. Our results show a major cytoskeletal involvement in the cellular trafficking of complexes made of PEI derivatives : actin microfilaments in the early steps of complex uptake and microtubules in their trafficking towards the nucleus, probably through guided transport of complex-containing endosomal vesicles.

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