POTOCYTOSIS AND CELLULAR EXIT OF COMPLEXES MAY LIMIT THE EFFICIENCY OF GENE TRANSFER BY POLYCATIONS

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Although polycations are among the most efficient nonviral vectors for gene transfer, their gene expression is still too low for efficient in vivo applications. In order to better understand the intracellular barriers impeding an efficient gene transfer, the trafficking of plasmid DNA complexed with various polycations was studied by electron microscopy in human airway epithelial cells.

Gold labeled plasmid DNA (5 µg) was complexed with polycations using a charge ratio PEI nitrogen/DNA phosphorous of 10 : unsubstituted PEI (Mr ~ 25 000) and lactosylated PEI, lactosylated (15 µg) and mannosylated (10 µg) polylysine (polymerisation degree: 190). Complexes were incubated for 1 h in the presence of immortalized cystic fibrosis airway epithelial cells (ΔCTTE29o- cells) or primary human bronchial epithelial cells. At various times, from 1 h up to 24 h, cells were fixed, stained with 2% osmic acid and 2.5% aqueous uranyl acetate, dehydrated and embedded in araldite/epon resin. Ultrathin sections were then examined by transmission electron microscopy.

The cellular process of complexes was found to vary with their size and with the polycation derivative used. Large complexes (diameter > 200 nm) entered the cells though two routes : macropinocytosis as well as clathrin-coated pits for complexes made with a glycosylated polycation. Complexes with a diameter of 100 to 200 nm made with a glycosylated polycation entered the cells through a single route: clathrin-coated pits. Both types of complexes were then found in endosomal vesicles. They accumulated in lysosomes or in vesicles near the nucleus and the nuclear entry was quite limited. In contrast, small complexes with a diameter lower than 100 nm were taken up through caveolae and followed a traffic pattern of potocytosis leading to the endoplasmic reticulum. Finally, some complexes were seen to exit the cells after 24 h either by regurgitation for complexes made with PEI derivatives or through an exosome like pathway for complexes made with polylysine derivatives.

Our electron microscopic study confirms previous results, obtained by ... and others, showing that gene transfer in eukaryotic cells based on the use of polycations is a multi-step process in which the entry into the nucleus is one of the main limiting steps. Moreover, this study suggests previously unsuspected barriers to an efficient gene transfer by using polycation derivatives as vectors; indeed, small complexes, that are thought to be the best approach to achieve an efficient in vivo gene transfer, were taken up through caveolae and followed a traffic pattern of potocytosis leading to the endoplasmic reticulum, a dead end. Finally, complexes were seen to exit the cells after 24 h and this exit of complexes may account for the well established short time efficiency of gene transfer based on synthetic vectors.

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