INTRACELLULAR TRAFFICKING OF DNA COMPLEXED TO LACTOSYLATED POLYLYSINE IN CYSTIC FIBROSIS AIRWAY SURFACE EPITHELIAL CELLS

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Among various glycosylated polylysines, lactosylated polylysine has been shown to be the most efficient vector for gene transfer into immortalized cystic fibrosis airway surface epithelial cells, the ΣCFTE cells (Fajac et al., Hum Gene Ther 1999; 10 : 395-406). However, 24 h after the transfection step, less than 10 % of the cells were transfected. In order to better understand the intracellular barriers to an efficient gene expression, we have investigated the intracellular trafficking of the plasmid DNA complexed to lactosylated polylysine. We used a biotinylated DNA and complexed it to a fluoresceinylated and lactosylated polylysine. The ΣCFTE cells were incubated in the presence of the complexes for 1 hour at 4°C in order to allow the binding of the complexes to the cell membrane without enabling their uptake by the cells. Then, the complexes were withdrawn and the cells incubated at 37°C. At various times from 10 min to 48 hours, the biotinylated DNA was evidenced with rhodamine-labeled streptavidin, the nucleus with DAPI and the intracellular trafficking of the complexes studied by confocal microscopy. As expected, after one hour at 4°C, the complexes were seen at the cell membrane. After 10 to 15 min of incubation at 37°C, the complexes were seen inside the cells. After 1 hour, some of them began to reach the nuclear membrane and they could be detected inside the nucleus after 2 hours. An important localization inside the nucleus was seen between 4 and 8 hours. After 24 hours, complexes were not seen anymore in the nucleus and they were seen as clusters in one area of the cell. After 48 hours, nearly no more complexes could be detected inside the cells. Our results which show the presence of the complexes inside the nucleus in more than 10 % of the cells suggest that the presence of the plasmid DNA inside the nucleus is not sufficient to allow an efficient gene expression. Other factors such as the dissociation of the plasmid from the lactosylated polylysine that we have never been able to detect, are likely to be necessary.

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