972. Transcription and Expression of Plasmid DNA: Modulation by Polyethylenimine/Plasmid DNA Complex Formation

Stephanie Grosse,[1] Isabelle Honore,[1] Florence Favatier,[1] Natacha Frison,[2] Michel Monsigny,[2] Isabelle Fajac.[1]

[1]*Faculte Cochin, Laboratoire Physiologie Respiratoire, Paris, France;* [2]*Glycobiologie-VTI, CBM-CNRS, Orleans, France.*

Polyethylenimine (PEI) is an efficient vector for gene transfer into various mammalian cells, including airway epithelial cells. In order to obtain an enhanced-cell specific transfection, we have developed a lactosylated PEI (Lac-PEI) and shown that Lac-PEI was more efficient than unsubstituted PEI for gene transfer into immortalized cystic fibrosis airway epithelial ΣCFTE29o- cells. When the intracellular trafficking of Lac-PEI or PEI/plasmid complexes was studied in ΣCFTE29o- cells, some whole Lac-PEI or PEI/plasmid complexes were found in the cell nucleus. Our aim was to evaluate if the transcription and the expression of a plasmid complexed with either Lac-PEI or PEI were impaired.

Various amounts of PEI (average Mr: 25 000, branched form) or of Lac-PEI were complexed with plasmids in order to reach defined polymer nitrogen/DNA phosphate (N/P) ratios (from 2.5 to 20). The plasmids used encoded genes for the firefly luciferase (pCMV-*Luc*, 5 kb), the green fluorescent protein (pCMV-*GFP*, 5 kb) or the cystic fibrosis transmembrane conductance regulator protein (pCMV-*CFTR*, 10 kb). Knowing that both the size and the zeta potential which indicates the surface charge of complexes are N/P ratio-dependent, they were both measured by using a Zetasizer. The transfection efficiency was measured 24 h after one h incubation of ΣCFTE29o- cells in the presence of complexes. The initiation of transgene transcription was analyzed in an acellular nuclease S1 transcription assay. The nuclear expression of transgene was assessed in living cells upon an intranuclear microinjection of complexes.

The size of complexes made with Lac-PEI or PEI at any N/P ≥ 10 was around 200 nm. At N/P ≤ 5, the size could not be determined either because the size of particles was too disperse or much too large. For complexes made with Lac-PEI or PEI at a N/P of 10, the zeta potential was strongly positive and reached a plateau (+30 mV and +40 mV, respectively). The transfection efficiency varied with the N/P ratio: the highest luciferase activity was achieved with a N/P of 10 for Lac-PEI (3.2±0.8 10^8 RLU/mg of protein) and 5 for PEI (2.4±0.4 10^8 RLU/mg of protein), whereas it was lower when N/P < 5 or > 10 were used. When plasmids of different size (5 or 10 kb) were complexed with Lac-PEI or PEI, transgene transcription was the most efficient at a N/P of 10 but was lower than that observed with the naked plasmid. When transgene expression was assessed in living cells upon injection of complexes into the nucleus, the expression was also dependent of the N/P ratio: the highest expression was observed when the N/P was 10 with both vectors (90±4% of GFP-positive cells), slightly lower than that observed with the naked plasmid (98±4% of GFP-positive cells).

The presence of PEI in a plasmid/PEI complex appears to be a limiting step of the intranuclear transgene expression. Modifications of PEI leading to a more efficient dissociation of the plasmid from PEI could lead to an improvement of transgene processing in the nucleus.

Association Vaincre la Mucoviscidose and INSERM