

**ROLE OF CLATHRIN- AND CAVEOLAE-MEDIATED ENDOCYTOSIS
IN GENE TRANSFER MEDIATED BY LIPO- AND POLYPLEXES**

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We investigated the internalization and intracellular processing of DOTAP/DNA lipoplexes and PEI/DNA polyplexes by A549 type-II derived pneumocytes. The uptake of FITC-poly-L-lysine labeled complexes was assayed by fluorescence-activated cell sorting and their intracellular fate by fluorescence microscopy. Our data show that polyplexes and lipoplexes are taken up and processed by the cells in different ways. The internalization of DOTAP lipoplexes is diminished by inhibitors of clathrin-mediated endocytosis (chlorpromazine and potassium depletion) but is unaffected by filipin and genistein, which specifically block caveolae-mediated uptake. Contrarily, PEI complexes are taken up by a mechanism involving both caveolae and clathrin coated pits. Transfection mediated by DOTAP lipoplexes is entirely abolished by blocking clathrin-mediated endocytosis, whereas inhibition of the caveolae pathway has no effect on transfection. By contrast, gene delivery and subsequent expression mediated by PEI polyplexes is completely blocked by genistein and filipin and is not affected by inhibitors of clathrin-mediated endocytosis. Our co-localization studies with a lysosomal marker, AlexaFluor-dextran, further revealed that PEI polyplexes taken up by clathrin-mediated endocytosis are targeted to the lysosomal compartment for degradation, while the polyplexes internalized via caveolae escape this compartment, permitting efficient transfection.