Transfection Encyclopedia Article

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Contents

Transfection

Transfection is the process of delivering foreign molecules such as **DNA** into eucaryotic cells. After **cloning** a **gene**, scientists analyze its characteristics by reintroducing it into various **cell** types. In order to study the regulation of **gene expression**, the relevant DNA sequence can be mutated and transfected into cells, and its activities can be studied under different physiological conditions. Cell lines that express proteins can be established. The protein can be purified for further biomedical research. Large-scale production of a protein can be used as a drug.

There are two types of transfections that are routinely done in mammalian systems--transient and stable, or permanent transfections. In transient transfection, the **plasmids** get into the cell **nucleus**, but are not integrated into the chromosomes. There can be several copies of plasmids in the cells. As a result, the expression level is high. The transfected gene can be analyzed between one to four days after induction of the DNA, depending on the vectors. Supercoiled plasmid DNA is used in transient transfection to achieve high efficiency. In stable transfection, the transfected DNA integrates into the chromosomes or exists as an episome. The cells that have integrated, or episomal foreign DNA, can be distinguished by selectable markers located on the plasmids. The commonly used markers include genes encoding aminoglycoside phosphotransferase and hygromycin B-phosphotransferase, among others. Linear DNA is normally used in stable transfections to facilitate the optimal integration of the foreign DNA into the host **genome**.

Over the years, many methods have been developed to introduce DNA into mammalian cells. The four basic methods include: calcium phosphate transfection, DEAE-dextran transfection, electroporation, and liposome-mediated transfection. In the first two methods, the treatment of cells resulted in DNA attaching to the cell surface. The DNA is then endocytosed by uncharacterized pathways. Electroporation uses electric field to produce holes on cell membrane. The DNA enters the cells through these holes. This method is versatile in transfecting various cell types. The mechanisms of the liposome-mediated transfection is not well understood. Presumably, negatively charged phosphate groups on DNA bind to the positively charged surface of the liposome, and the residual positive charge then mediates binding to negatively charged sialic acid residues on the cell surface.