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Importance of Gene Manipulation Techniques

Shahid Raza, Sahrish Faooqi and Hira Mubeen
University of South Asia, Lahore, Pakistan.

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Corresponding Author

Shahid Raza

Abstract

Genetic modification allows changes in genes to transfer genetic material in different organisms. It is possible to introduce foreign genes into crop plants and express these in specific tissues. Transfer of genes can help to improve crop protection. Genetic modification can be done with various viral and non viral methods. Direct manipulation of DNA sequences can lead to different expression levels. Choosing the best method for gene delivery can be a difficult task, particularly to transfer genetic material in a proper way. This review highlights, basic mechanism and importance and differnet methods used for efficient gene delivery. Hence, understanding of intracellular machinery plays a crucial role for designing a more effective gene delivery environment.

INTRODUCTION

Genetic engineering is used for direct genetic modification of organisms to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms (Izquierdo, 2001; Karp, 2002). Most techniques are related to the direct manipulation of DNA oriented to the expression of particular genes. The most accepted purpose of genetic engineering is focused on the direct manipulation of DNA sequences. These techniques involve the capacity to isolate, cut and transfer specific DNA pieces, corresponding to specific genes (Lewin, 1999; Klug and Cummings, 2002). Genetic modification of organisms using molecular genetics and recombinant DNA technology is more difficult and costly than in simpler organisms. In mammals, techniques for reproductive manipulation of gametes and embryos such as cloning and procedures for artificial reproduction such as in vitro fertilization, embryo transfer and artificial insemination, are frequently an important part of these processes (Murray et al. 1999; Izquierdo, 2001).

Current research in genetic manipulation of organisms is oriented toward a variety of different possible medical, pharmaceutical and agricultural applications. The research in genetic engineering of mammalian cells is based on using gene therapy technique to cure genetic diseases such as cystic fibrosis by replacing the damaged copies of the gene by normal ones in foetuses or infants (Izquierdo, 2001; NHGRI, 2001; Coutelle and Rodeck, 2002). Genetically engineered animals such as the ‘knockout mouse' in which one specific gene is ‘turned off' are used to model genetic diseases in humans and to discover the function of specific sites of the genome (Majzoub and Muglia, 1996). Production of specific therapeutic human proteins such as insulin in the mammary gland of genetically modified milking animals like goats is another useful application of genetic manipulation (Murray et al. 1999; Wall, 1999). Removal or alteration of an existing gene via homologous recombination required the use of ES cells and was limited to the mouse until the advent of nuclear transfer cloning procedures (Houdebine, 1998; Murray et al. 1999; Rao, 2000).

TRANSGENE TECHNOLOGY

Transgenic technology is another important way to introduce a gene from one individual in the genome of another. Transgenic animals have any of these genetic modifications with potential use in studying mechanisms of gene function, changing attributes of the animal in order to synthesize proteins of high value, create models for human disease or to improve productivity or disease resistance in animals (Chien, 1996; Majzoub and Muglia, 1996; Houdebine, 1998; Houdebine, 2002; Murray et al. 1999; Rao, 2000; Felmer, 2004). In the early 80´s, several research groups reported success in gene transfer and the development of transgenic mice (Gordon et al. 1980; Palmiter et al. 1982; Murray et al. 1999).

Gene Delivery Methods

Gene delivery is a method used to introduce foreign DNA into host cells for genetic modification of crops. Different methods of gene delivery are now being used for a various types of cells and tissues, from bacterial to mammalian. Generally, the methods can be categoriezed as non-viral and viral.Viral-mediated gene delivery systems consist of viruses that are modified to bereplication-deficient, but which can deliver DNA for expression. Adenoviruses, retroviruses, and lentiviruses are used as viral gene-delivery vectors (Escors and Breckpot, 2010). Non-viral methods include physical methods such as electroporation, microinjection and gene gun, continuous infusion, and sonication and chemical, such as lipofection. Gene delivery systems use various methods to allow uptake of the gene that has been selected to target the cell (Conwell and Huang, 2005). Understanding intercellular traffic and targeting mechanism is the most important factor in designing a more effective gene delivery system. Cell targeting refers to delivery of the therapeutic agent to a specific compartment or organelle of the cell. It is the most commonly used mechanism in endocytosis gene therapy, particularly in cellular uptake of non-viral gene delivery systems (Prokop and Davidson, 2007). After the cellular uptake of the delivery system by endocytosis, cellular release takes place to initiate DNA transcription and translation and to produce the related protein. A successful gene delivery procedure involves minimizing potential inhibitory inflammatory response while also overcoming certain barriers at each step of the gene delivery procedure, in order to optimize gene activity (Conwell and Huang, 2005).

Transgenic methods

Microinjection of DNA and now nuclear transfer, are two commonly used methods for production of transgenic livestock successfully. Once a specific fusion gene containing a promoter and the gene to be expressed has been cloned and characterized, sufficient quantities are isolated, purified and tested in cell culture if possible and prepared for preliminary mammalian gene transfer experiments. In contrast with nuclear transfer studies, DNA microinjection experiments were first performed in themouse (Izquierdo, 2001). Frequently the used promoters have not allowed an efficient control of the expression of the transgene. It was assessed that it is necessary to develop more complex constructions that activate or repress the expression of the transgene more precisely. In livestock, knowledge of effects of specific genes and gene combinations on important traits could lead to theirenhanced control to create new more useful populations. The use of specific gene information can help to increase rates of genetic improvement and open opportunities for using additive and non-additive genetic effects of domestic species.

Physical Gene Delivery Methods

A variety of different physical or mechanical means for translocating nucleic acids into cells have been developed.They use a better approach for gene delivery.

Microinjection is the simplest gene delivery method, but one of the most difficult to apply. It entails the direct injection of DNA into the nuclei of target cells using fine glass needles under microscopy. This method of gene transfer is the nearly 100% efficient but time-consuming, typically allowing only a few hundred cells to be transfected per experiment.

Electroporation is a method of introducing nucleic acids into cells by exposing the cells to a rapid pulse of high voltage current causing pores in the cell membraneto open temporarily. This allows exogenous DNA to pass through the pores and into thecytoplasm of the cells. Typically, the gene transfer efficiency is relatively low andelectroporation frequently results in a high incidence of cell death. Nevertheless, withelectroporation there is practically no limit to the size of DNA that can be delivered. In addition, there are few restrictions on the cell types to which this technique can be applied.

Gene Gun is a method used for the direct introduction of DNA into various tissues. In this method, plasmid DNA is coated onto metal microparticles and then blasted into cells using either electrostatic force or gas pressure. Some of the DNA becomes trapped by a few cells and may then be expressed to sufficient levels. This technique is fast, simple and safe and it can transfer genes to a wide variety of tissues.

Naked DNA involves direct injection of DNA leading to gene expression in vivo and can be used to treat certain genetic diseases. As a result, the use of naked DNA seems to belimited to only a few applications involving easily accessible tissues such as skin, muscles and hard tumors.

Gene Transfer Procedures
Gene transfer procedures are frequently categorized by whether the delivered gene remains separate from the host cell chromosome or integrated into the host cell chromosome.

Transient Gene Delivery
Expression of the transgene typically dissipates after a given period of time usually within several days because the expression vector is either degraded or expelled from the host cell.

Stable Gene Delivery
Expression of the transferred gene is prolonged, or stable, because the vector is integrated into the host cell chromosome. Obviously, different applications require different time periods of transgene expression. Hence, a careful comparison between different gene delivery methodologies will allow a suitable choice for generating the desired transient or stable expression.

Discussion

Gene delivery is important for introducing foreign DNA into host cells for genetic modification of various crops. There are many viral and non viral methods of gene delivery developed for a various types of cells and tissues from bacterial to mammalian. The gene delivery system generally includes polymers, liposomes, dendrimers, and cationic lipids. Non-viral vectors have many advantages, such as easy of synthesis, effective targeting of cell/tissue, low immune response, and potential to use plasmid at desired molecular weight. The biggest disadvantage of non-viral vectors in clinical use is low transfection efficiency. A large number of viral and non-viral gene delivery systems have been developed.

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