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Testes Mediated Gene Transfer – An approach to create Transgenic Animal

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Introduction

A genetically modified organism (GMO) or engineered organism (GEO) is an organism whose genetic material has been altered using genetic engineering techniques. Transgenic organisms, a subset of GMOs, are organisms that have inserted DNA from a different species. Technique used to create GMOs or transgenic organisms, is called recombinant DNA technology. This technology uses the DNA molecular from different sources and combine them to create one single molecule, having new set of genes. This is called recombinant DNA, which is then transferred to another organism by variety of methods to give that organism a new set of gene or property. Paul Berg, in 1972, created first recombinant DNA molecules by combining DNA from the monkey virus SV40 with that of the lambda virus. A year later, Herbert Boyer and Stanley Cohen created the first transgenic organism by inserting antibiotic resistance genes into the plasmid of an E. coli bacterium. Rudolf Jaenisch created the first transgenic mouse by introducing foreign DNA into its embryo. The first transgenic livestock was produced almost 20 years ago by microinjection of foreign deoxyribonucleic acid (DNA) into the pronuclei of zygotes. DNA microinjection has been mostly used forthe production of transgenic animals, however, as microinjection has several significant shortcomings – including low efficiency, random integration and variable expression patterns related to the site of integration – research has focused on alternative methodologies for improving efficiency of generating transgenic livestock. The use of spermatozoa has been studied in recent years for gene transfer in transgenic animal technology and several distinct approaches have been used. The first report that exogenous DNA could be introduced into sperm was made by Brackett et al. (1971). After it, several studies in distinct species have reported the generation of transgenic animals using spermatozoa as vectors to carrier foreign DNA to the ova (Campos et

al., 2011). One such approach utilizing sperm as a carrier for transgeneis the direct introduction of foreign DNA into testes, calledas testis-mediated gene transfer (TMGT), whichallows for naturalmating and mass transfer of transgene. This technique exempts the useof other procedures such as in-vitro fertilization (IVF) and embryotransfer (ET). Sato et al. (2002) demonstrated this method byway of direct, but surgical injection of DNA solution into testes with subsequently "in□vivo" electroporation to improve theuptake of foreign DNA by epididymal epithelial cells. Shen et al. (2006) demonstrated efficient generation of rabbits and micethrough TMGT using surgical injection in testes with a DMSO/DNA complex to improve uptake of foreign DNA by sperm cells. It has been shown that sperm cells incubated with exogenous DNA can actually carry the DNA into eggs, and the gene thus transferred is transcriptionally active, in addition, it is also reported that DNAs injected into the testis of either rats or mice can be transferred into eggs via sperm at fertilization (testis-mediated gene transfer). The transgenes could be expressed and transmitted in the descendants, suggesting that injected DNAs were stably integrated in the genome of offspring. This suggests that the foreign DNA can spontaneously bind to a certain portion of living spermatozoa and be introduced into the oocyte, which results in generating transgenic animals at least in some instances through a process yet to be defined (Chang et al., 1999). Method The basic idea behind production of transgenic organism by testes mediated gene transfer is to insert the transgene construct into the testes of the male recipient animal, so that the transgene is "taken up" by the spermatozoa and when fertilization occurs it is transferred to the progeny creating a transgenic offspring. There are several methods to transfect the spermatogonial cells with the transgene. A simplified procedure for testes mediated gene transfer is shown in fig 1. There can be two different strategies that can be used either the testes cells are first harvested and then incubated with the transgene so that they can take up the transgene and then selected for positive cells &injected into the testes of target animal (As shown from A to C in Fig.1) or alternatively the transgene could be directly injected into the testes (As shown from H to C in Fig.1). After the injection sufficient time is given for the integration of transgene with spermatozoa (D in Fig. 1), then the animal carrying transgene in spermatozoa is mated with a normal female to produce the progeny of transgenic animals (E – F in Fig.1). There are various ways and routes to transfer the transgene into the testes of animal, either via direct injection to seminiferous tubules or interstitial space or to vas deferens. One of the simplestmethods to produce transgenic animalisthe gene transfer into male stem cells such as spermatogonia by direct introduction of foreign DNA into seminiferous tubules.Yamazaki et al. (1998, 2000) first demonstrated the use of direct introduction of plasmid DNA in seminiferous tubules and subsequent in vivo electroporation (EP), they showed that almost all spermatogenic cells within seminiferous tubules could be transfected by DNA, but the males undergoing DNA introduction failed to produce transgenic mice despite mating with normal females. Huang et al.(2000) employed gene delivery to seminiferous tubules and ICSI of DNA□transfected sperm cells. They first infused seminiferous tubules of young mice aged 2 weeks old with transgene tagged with yellow fluorescent protein and then used electroporation to encouragetransfection. They were able to produce transgenic pups having successfully integrated transgene. This was the first report of use of TMGT-ICSI in successful production of transgenic offsprings.Attempts to infect germ-line stem cells with viral vectors or transfect them by other techniques such as introduction of plasmid DNA and subsequent whole-testis electroporation have met with little success (Sato, 2005). Nagano et al. (2000) used in-vitro

retroviral-mediated gene delivery into spermatogonial stem cells of both adult and immature mice. They reported the stable integration and expression of a transgene in 2-20% of stem cells and after transplantation of the transduced stem cells into the testes of infertile recipient mice, approximately 4.5% of progeny from these males were transgenic, and the transgene was transmitted to and expressed in subsequent generations. They further demonstrated that introduction of retroviral vector into seminiferous tubules of mice resulted in production of transgenic mice with an efficiency of 35%. The introduced DNA was integrated into the host chromosome and the gene transmission pattern from one generation to the next was Mendelian (Nagano et al., 2000). This alternative route for transgenesis is now applied to species includingpigs and goats (Honaramooz et al., 2003). Testis-mediated gene transfer (TMGT) based upon direct introduction of DNA into the interstitial space of mammalian testis Injecting the plasmid vector with cationic liposome directly into the interstitial space of mammalian testis is another effective method to create transgenic organism. Ogawa et al.(1995) demonstrated that injection of linearized lacZ expression plasmid mixed with cationic liposome (Lipofectin™) into testes of adult mouse via the scrotum resulted in transmission of the foreign DNA sequences to the progeny (blastocysts) through fertilization. They found that 80.0% of the blastocysts, derived from mating with males receiving the transgenic DNA, were stained for X□Gal. These findings demonstrated that DNA exogenously introduced into the mouse testis is transferred to eggs via spermatozoa with relatively high efficiency. Gene transfer to vas deferens Another way to transfect sperm cells with exogenous DNA is direct introduction of DNA into spermatozoa in the vas deferens. Huguet and Esponda (1998, 2000) injected a plasmid DNA encoding the GFP into mouse vas deferens. The night after injection, males were mated with normal estrus females. When the resulting offspring were analyzed, four of 53 newborn were found to be positive for presence of the GFP gene by PCR. In the positive animals, some tissues exhibited GFP expression. They suggested the possibility of use of this method as a simple alternative to thecreation of transgenic animals. These studies also demonstrate that epididymal and vas deferens secretions do not block binding of DNA to spermatozoa. Further improvement in TMGT is still required for use of it as an alternative method for transgenic animal production, as the results are highly variable among different research groups. Sato (2005) has suggested the introduction of DNA into the testis of a very young male, probably around 5 to 10 days of age, because young testes are smaller than adult ones and have actively proliferating spermatogonial cells. Therefore, it appears that sperm cells in a testis would be easily accessible for exogenous DNA contact. Similarly repeated injections of DNA can also be investigated. This strategy is aimed at increasing the frequency of DNA contact with testicular spermatozoa. Sato and Nakamura (2004) utilized the repeated injection of DNA regimen, however, repeated injections (up to 6 times) of plasmid/liposome complex failed to improve the TMGT system. If efficient methods for TMGT are developed, initially in lab animals, then this technology will provide a powerful tool for production of domestic transgenic animals such as cows, pigs and horses, which is considered difficult with presently available microinjection techniques (Krimpenfort et al., 1991). Mechanism DNA association with sperm in vitro has been quantitatively examined in several species of animals and it has been shown that relatively small portion of spermatozoa are capable of binding DNA and DNA binding is confined exclusively to the posterior region of the sperm head. In addition, it has been also reported that electroporation or liposome is needed to facilitate the association;without these treatments, DNAs are easily

removed by washing with the medium containing DNase.These agents (lipsomes, electroporation, calcium phosphate, gene gun etc.) increase the efficiency of uptake of DNA by spermatozoa cells. One of the cheapest agents to increase the efficiency is the use of calcium phosphate, originally discovered by F. L. Graham and A. J. Vander Eb in 1973. When used with HEPES buffered saline, a fine precipitate of the positively charged calcium and the negatively charged phosphate is formed, binding the DNA to be transfected on its surface. The suspension of the precipitate is then injectedinto the testes cells to be transfected. By a process not entirely understood, the cells take up some of the precipitate, and with it, the DNA. Other methods use liposomes to bind the DNA and get it into the cell. A liposome is an artificially-prepared vesicle composed of a lipid bilayer. It is very efficient method to introduce the foreign DNA with liposomes i.e. small, membrane-bounded bodies that are in someways similar to the structure of a cell and can actually fuse with the cell membrane, releasing the DNA into the cell. For eukaryotic cells, transfection is better achieved using cationic liposomes (or mixtures), because the cells are more sensitive. The negatively charged DNA binds to the polycation and the complex is taken up by the cell via endocytosis. Electroporation is another popular method, although requiring an instrument and affecting the viability of many cell types, it creates micro-sized holes transiently in the plasma membrane of cells under an electric discharge. It is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA. Therefore, there are many types of agents available for efficient introduction of foreign DNA into the cells. The exact mechanism of how the sperm cells take up the foreign DNA is still not clearly known. Chang et al., 1999 worked out the possible mechanism for uptake of DNA by sperm during testes mediated gene transfer. They observed that injected DNA is bound to almost all of the epididymal spermatozoa suggesting that intratesticular environment might be more favourable for sperm-DNA association than in vitro incubation. The longer period of contact of spermatozoa with DNA in vivo also contribute to the increased efficiency of the binding. When spermatozoa were incubated with DNA in vitro in the presence of liposome, the treatment with DNase removed only partially the DNA, suggesting that at least some DNA molecules are internalized in sperm heads (Bachiller et al., 1991). Although this process is not fully elucidated, it does not appear to be a simple passive process as thought earlier. The weak association of spermatozoa with exogenous DNA in-vivo may be at least partially explained by the blocking factor(s) for sperm-DNA association present in the seminal plasma. However, Chang et al. (1999) was able to show that the exogenous DNA injected with liposomes is not integrated into genome of the sperm but get adsorbed on its surface and carried by the spermatozoa and the integration only occurs after fertilization. Conclusion The testes mediated gene transfer technology opens up new important perspectives in the field of animal transgenesis. The conventional approach using pronuclear microinjection is still effective as a means of producing transgenic animals. However, it is both costly and labor-intensive and requires long periods of time to perform it. The use of TMGT would be cheaper and more rapid, with quick and effective delivery of genes to target tissues. The exogenous DNA injected into thetestis can be transferred into eggs by sperm cells and integrated in the genome of offspring under

a certain experimental condition, hence the testis-mediated gene transfer method will be a useful

tool as an alternative to produce transgenic animals by the conventional methods and the efficiency could be improved if the precise mechanisms underlying it can be revealed by future studies.

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