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Review Article

TRANSGENIC CHICKENS-AN OVERVIEW

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INTRODUCTION

Transgenic chickens hold great promise in basic biological research and in industrial applications. A number of different strategies to allow the manipulation of the avian genome have been investigated with varying degrees of success. Infection of chick embryos with retroviral vectors has successfully produced transgenic chickens by exploiting the natural abilities of retroviruses to enter cells and integrate into the host chromosomes.

ACCESSING THE AVIAN GENOME

As originally defined by Gordon and Ruddle (1981), transgenic animal can be described as an organism carrying recombinant DNA molecules that were intentionally introduced by human intervention.

RETROVIRUS-MEDIATED GENE TRANSFER

Retroviruses are composed of a diploid singly-standard RNA genome contained within a core of viral structural proteins which is in turn surrounded by a membrane envelope studded with viral glycoproteins (Varmus, 1988). Infection of susceptible cells takes place by receptor-mediated endocytosis following binding of viral envelope proteins to cellular receptors, resulting in the eventual release of the viral core into the cytoplasm of the host cell. Within the cell, retrovirally encoded reverse transcriptase and intergrase enzymes catalyze reverse transcription of the single-stranded RNA genome into double-stranded proviral DNA in the cytoplasm and integration of the proviral DNA into random sites in the host chromosomes. Viral promoters drive transcription of viral RNA genomes and viral proteins are translated from full-length and spliced viral transcripts. Full-length RNA gnomes are particles bud from infected cells enclosed in an envelope derived from the host cell membrane.

In general, retrovirus-mediated gene transfer can successfully introduce transgenes into precursors to both somatic and germline cell lineages, resulting in the production of

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transgenic birds. Advantages of this method include efficient and accurate integration of a single vector copy into chromosomal DNA of most infected cells (or occasionally a few copies) and high levels of transgene expression (Vile *et al*., 1996).

MICROINJECTION OF DNA

Microinjection of DNA directly into the fertilized ovum is now routinely used for the production of transgenic mice, rabbits, sheep, pigs, and cattle (Eyestone, 1997). In the case of transgenic mammals, the injected embryos are transferred into recipient females for the remainder of embryonic development. Aprroximately 2.5 hours after lay of the preceding egg, avian ova obtained from the oviduct of laying hens are fertilized, but the pronuclei located in the germinal disc have not yet fused (Perry, 1987). At this point the egg consists of only the yolk surrounded by a capable of dense albumen the final components of the albumen have not been secreted, and the shell membranes and shell have not yet formed. Attempts to transfer the oviductal eggs back into a recipient oviduct in order to complete egg formation were extremely difficult in practice and the rate of success was very low (Olson and Neher, 1948). Hence, initial work towards the genetic manipulation of chickens by microinjection of DNA focused on the development of an in vitro culture system that would enable normal development of the fertilized ovum until hatch.

Ono and Wakasugi (1984) were able to culture 2.5-day old quail embryos to hatch in surrogate chicken egg shells. However, the three-chicks which hatched were unable to stand and died within two days. No chicks hatched when shell-less culture methods were used (Ono and Wakasugi, 1984). Rowlett and Simkiss (1987) were successful in culturing 3-day old chick embryos to hatch using surrogate turkey and chicken egg shells. The hatched birds were slightly smaller than controls, but were otherwise normal and were reared through to sexual maturity. In contrast, to shell less culture methods, the use of surrogate shell cultures allows the normal development of embryos to hatch, partly because the egg shell provides the embryo with approximately 80 % of the calcium and 20 % of the magnesium it requires (Ono and Wakasugi, 1984). In addition, the physical shape provided by the recipient egg shell is thought to better preserve the normal spatial relationships of the embryo, yolk sac, and albumen, as well as permitting development of extra embryonic membranes (Rowlett and Simkiss, 1987).

IN OVO TRANSFECTION OF EMBRYOS

Nonviral methods of gene transfer have also been attempted at later and more accessible stages of development, including the Stage X blastoderm (Maruyama *et al*., 1988)

and embryonic stages after 24-48 hours incubation (Muramatsu *et al*., 1997). The transfection protocols were carried out on embryos in intact eggs (in ovo) access to the embryos was achieved by drilling small windows into the eggshell, which were then released for incubation. Liposome-DNA complexes have been injected into the subgerminal cavity of stage X blastoderms, with expression of reporter genes being detected in extra and intra embryonic tissues, including PGCs, after 48-96 hours of incubation (Maruyama *et al*., 1998). Embryos 24-48 hours old have been transfected in intact eggs by injection of liposome DNA complexes, microparticle bombardment, and in ovo electroporation following injection of plasmid DNA into the embryo (Muramastu *et al*., 1997). Varying degrees of gene expression in extra-and intra embryonic tissues were detected after another 48 hours of incubation and in ovo electroporation was particularly effective (Muramatsu *et al*., 1997).

SPERM-MEDIATED GENE TRANSFER

In the process of fertilization, spermatozoa naturally transfer "foreign" DNA into the ovum. Viewed in this light, sperm are ideal vehicles for the transfer of exogenous DNA sequences into the egg. Brackett and Colleagues (1971) were the first to demonstrate that heterologous DNA incorporated in sperm could be transferred into the egg during fertilization when they observed the production of infectious SV40 virus from rabbit ova fertilized with spermatozoa incubated in the presence of SV40 DNA. Autoradiography studies showed that the DNA bound to the post-acrosomal area of the spermatozoa (Brackett *et al*., 1971). Because artificial insemination is routinely used with poultry, in principle sperm-mediated methods offer a rapid, simple and attractive route to transgenesis in chickens.

Various attempts at genetic manipulation of the chicken have been made using irradiated sperm (Shoffner *et al*., 1990). In this technique, donor male gametes are irradiated and insemination into recipient hens, followed by a second insemination with normal, unirradiated semen. The hypothesis behind this procedure is that chromatin fragments from a supermerary sperm pronucleus of irradiated origin are incorporated into the zygotic nucleus where they are subsequently integrated into the genome (Shoffner *et al*., 1990). Using this technique, genes controlling egg and feather colour (Swinbanks, 1986) and major histocompatibility haplotypes (Burmstead *et al*., 1987) have apparently been successfully transferred to 0.5-3.5 % of progeny. The genetically transferred traits were also observed in the offspring of these birds.

In order to use this approach to produce transgenic chickens, a number of prerequisites must be fulfilled. A source of donor cells capable of contributing to the

formation of germline chimeras must be identified. To enable genetic modification the cells must survive in vitro manipulations in tissue culture while still maintaining their ability to be incorporated within a recipient embryo so that the genetic modification can be passed on in vivo. Two sources of donor cells have been identified in chickens; the PGCs and the stage X blastoderm.

Primordial germ cell chimeras

As the PGCs are the precursors of the cells which compose the germline, they are excellent candidates for genetic modification. Chick PGCs can be isolated from embryos at three different stages of development: from the germinal crescent from the blood of stage 13-15 (Hamburger and Hamilton, 1951) chick embryos (Vick *et al*., 1993) and from the gonads of stage 27-30 embryos (Allioli *et al*., 1997). Each source has advantages and disadvantages (Wentworth *et al*., 1989). It can be difficult to prepare viable singly-cell suspensions from the germinal crescent without reaggregation of the cells or contamination with other cell types. While PGCs from the blood are easy to isolate, they are present in very low concentrations. Gonadal PGCs are present in greater numbers and proportion than in the germinal crescent or the blood and they actively proliferate in vivo once they have reached the gonads, thus, they may be easier to manipulate in culture if similar in vitro rates of proliferation can be maintained (Allioli *et al*., 1997).

Two different approaches have been taken to increase the ratio of donor to receipient PGCs. The first involves the separation or enrichment of PGCs in the donor cell preparations by density gradient centrifugation (Naito *et al*., 1994), elutriation, or fluorescent activated cell sorting (FACS) (Wentworth *et al*., 1989).

CONCLUSION

Although a number of routes to transgenesis in chickens have been investigated, it is not possible to routinely produce transgenic chickens with the same ease as transgenic mice. Some methods, such as retrovirus mediated gene transfer and microinjection of DNA into the fertilized ovum, have successfully produced transgenic birds, however more research is required to overcome technical limitations and low efficiencies of gene transfer. Other methods which are appealing due to their rapid and simple nature, such as sperm mediated gene transfer and the direct transfection of embryos, are currently being investigated but have yet to produce a bird with a stable integrated transgene. Overall, much research is still required for the development of practical and economical techniques for accessing the avian genome.

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