Lentiviral transgene vectors

Green light for efficient production of transgenic farm animals

Transgenic animals can be broadly defined as animals that contain recombinant DNA molecules in their genome that have been introduced by human intervention (Wall, 1996). Developmental studies and clinically relevant disease models are based on transgenic laboratory animals, most commonly the mouse. In farm animals, transgenic technology is used to produce large quantities of pharmaceutically relevant proteins and could also provide an unlimited source of genetically modified cells and organs to overcome the growing shortage of suitable human organs for transplantation. Pigs will be the most likely source of these organs as they grow to the appropriate size, have relatively large litters and can be reared in containment to maintain pre-surgical ‘sterility’ (Weiss, 2000). Such xenografts will survive in the recipient only if graft rejection is avoided either by ‘humanizing’ the porcine tissues or by impairing the host’s ability to reject the xenograft by expressing immune-modulatory genes such as human complement regulatory proteins. In addition, disease models using transgenic pigs would be much more clinically relevant than rodent models, owing to the high similarities of human and porcine anatomy and physiology. However, the efficiency with which such transgenic animals can be generated using current approaches is low. This has been vastly improved in a recent study published in EMBO reports, which describes a breakthrough in the genetic engineering of transgenic farm animals through the use of lentiviral gene transfer (Hofmann et al., 2003).

When Rudolf Jaenisch and Beatrice Mintz reported the generation of the first transgenic mammals three decades ago, they used simian virus DNA to transfer genetic material into mouse embryos (Jaenisch & Mintz, 1974). Subsequently, Jaenisch reported the successful integration of mouse retroviral DNA into the mouse genome and the transmission of the integrated viral DNA through the germ line to their offspring (Jaenisch, 1976). The success of Jaenisch’s pioneering work prompted him, as well as several other research groups, to replace viral genes with mammalian genes and express them ectopically in animals (for a review, see Miller, 1997). However, the genes carried by these integrated retroviruses (proviruses) were not expressed in newborn mice. This block in expression puzzled virologists for years; it was not confined to rodents but was also observed in cattle (Chan et al., 1998). This transcriptional repression is thought to be mediated by both cis-acting de novo methylation of the integrated provirus and cell-type-specific trans-acting transcriptional repressors.

The search for alternative methods led to the invention of mechanical procedures to transfer foreign genes into the nucleus. For the past two decades, pronuclear injection has been the most widely used method for generating transgenic mice. Cloned DNA is microinjected directly into the pronucleus of fertilized embryos (Fig 1). An important advantage of pronuclear injection is that it is not limited to certain species; it works well in mice and has also been used to generate transgenic farm animals, including pigs. However, the main drawback of pronuclear injection is its low efficiency. In laboratory animals (mice, rabbits and rats), the transgene is integrated and expressed in not more than 4% of the injected and transferred embryos (Fig 2) (Wall, 1996; Devgan & Seshagiri, 2003). Because the production of rodent embryos is standardized and relatively inexpensive, losses during the injection and culture procedure can be compensated for by high-throughput production and screening. However, for farm animals, in which recovery, culture and transfer of embryos are time-consuming and expensive (a transgenic pig costs about US$30,000 and a transgenic cow about US$300,000), pronuclear injection is even less efficient, and only about 1% of the injected and transferred embryos result in transgenic carriers (Wall, 1996). One factor that contributes to these low efficiencies is technical: whereas the pronucleus of murine zygotes is surrounded by translucent cytoplasm and can therefore be easily targeted with the injection capillary that carries the DNA, the cytoplasm of porcine and bovine zygotes is opaque.

![Fig 1](image-url) | Schematic models showing a comparison between pronuclear DNA microinjection (top) and subzonal lentivirus infection (bottom).
In this latest study, research teams led by Alexander Pfeifer and Eckhard Wolf from the University of Munich have used lentiviral vectors (LVs) to generate transgenic pigs. Although lentiviruses belong to the large family of retroviruses, they differ in several important aspects from the previously used prototypic retroviruses (such as Moloney murine leukemia virus): they carry a larger genome and are able to transduce nondividing cells (reviewed by Pfeifer & Verma, 2001). LVs have been used to transfer genes into delicate cells such as stem cells, neurons and rodent embryos (Miyoshi et al., 1999; Lois et al., 2002; Pfeifer et al., 2002). However, attempts to generate transgenic monkeys with the use of lentiviruses failed (Wolfgang et al., 2001), sparking off a debate about whether LVs can be used for transgenesis in higher mammals.

Hofmann et al. used LVs that carry the gene encoding green fluorescent protein (GFP) to infect zygotes of pigs and cattle. To overcome the protective outer layer of the embryos (the zona pellucida; Fig 1), which is a physical barrier to lentiviral infection, the viral plasmid and the zona pellucida. Subzonal injection has come the way forward for the creation of new animal models for the study of human disease, or as a resource for the production of therapeutic proteins or organs.

Fig 2 | Statistical analysis of transgene expression in pigs generated by lentiviral gene transfer. The efficiencies of DNA microinjection (blue bars) and lentiviral gene transfer (green bars) in pigs are shown. The efficacy of DNA microinjection is reviewed by Wall (1996); the data on lentiviral (LV) transgenesis are from Hofmann et al. (2003).

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The high efficiency of lentiviral transgenesis in the pig is of great interest to academic and industrial researchers alike. This study has paved the way for the creation of the next generation of transgenic farm animals that express 'real' transgenes that confer a specific gene function, or of short interfering RNAs that diminish gene expression. However, such experiments will be limited by the size restriction imposed by the lentiviral genome. Wild-type lentiviruses have a genome of about 8 kb, and the genetic load of LVs (comprising the internal promoter, transgene and enhancer elements) should therefore be less than this. Nevertheless, LVs carrying large and complex complementary DNAs such as coagulation factor VIII together with proper regulatory elements have been engineered and used successfully to express therapeutic levels of the protein in animal models (Fawziul et al., 2001; Kootstra et al., 2003) Even with these limitations in mind, the study by Hofmann et al. shows the way forward for the creation of new animal models for the study of human disease, or as a resource for the production of therapeutic proteins or organs.

REFERENCES


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