Obtaining transgenic animals

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The first studies on the genetic transformation of eukaryotes were initiated by Benoit et al. in 1957 with attempts to transform an entire animal organism by intraperitoneal administration of DNA. However, by the early 1960s, it became clear that it was impossible to transform the organism by parenteral administration of DNA. When injected *in vivo*, labelled, highly polymeric RNA hardly penetrates somatic and germinal cells. The idea of genetically altering animals by introducing genes into fertilised oocytes was put into practice in the 1980s. It became known that foreign genes can stably integrate into the genome of fertilised oocytes and in many cases are then actively functioning, replacing or supplementing the recipient nucleus's own genes. To describe animals whose genotype is altered by the introduction of foreign (exogenous) DNA, the term 'transgenic animal' was introduced (Gordon, Rudl, 1982), the DNA introduced was called a transgene, and the entire process was called a transgenic technology, or transgenesis.

In the first works on transgenesis by Janisch et al. (1974, 1975), the SV40 DNA was injected into the cavity of mouse blastocysts and was exposed in many tissues of the resulting organisms, but was not inherited. In these experiments, the integration of foreign DNA into the genome of recipient animals was not studied in detail. Later, in similar experiments, it was shown that SV40 virus sequences were capable of randomly integrating into the host genome (Kelly and Kondamine, 1982). In 1976, the first transgenic mouse line was obtained by R. Janisch as a result of infection of mouse embryos with Moloney's leukemia retrovirus (M-MuLV). In a series of experiments it was proved that the transformation of mouse embryos with the M-MuLV provirus was accompanied by the incorporation of the latter into the host genome. The M-MuLV provirus entered germ cells with high frequency and was transmitted to the offspring as a Mendelian trait. A number of mouse lines containing exogenous Moloney's provirus sequences in multiple chromosomal sites have been

obtained. Since early 1980s hundreds of genes have been introduced into various mouse lines.

These studies have greatly contributed to the identification of mechanisms of gene regulation and tumor development, the nature of immunological specificity, the molecular genetics of growth and development, and other functional biological processes. Transgenic mice have played a role in the study of the possibility of large-scale synthesis of drugs, as well as in the creation of transgenic lines to model various human genetic diseases.

Three main methodological approaches are used to obtain transgenic mice: infecting embryos at early stages of development with retroviral vectors; microinjecting DNA into the male pronucleus of a fertilised egg; introducing genetically modified embryonic stem cells into a preimplanted embryo at an early stage of development.

Retroviruses have several features that allow their use for gene transfer: the incorporation of retroviruses into the genome is effective; retroviruses can infect embryonic cells in the early stages of development, both *in vivo* and *in vitro*; the provirus is stably incorporated into the chromosome and embryos infected with the virus in the early stages of development can transmit the virus to offspring.

However, the use of retroviruses to insert genes into mammalian embryonic cells poses several problems. R. Janisch et al. in 1981 showed that wild-type retroviruses infect germ cells, but their expression in these cells is inhibited.

The use of retroviral vectors has significant drawbacks. Retroviral-derived animals are usually mosaic: they have some body cells that contain the transgene and others that do not. Although these vectors are created so that they are defective in replication, the genome of the retrovirus strain (helper virus), which is needed to produce large amounts of vector DNA, can enter the same nucleus as the transgene. Despite all measures taken, helper retroviruses can replicate in a transgenic animal, which is unacceptable if these animals are to be used as food. Since alternative methods of transgenesis exist, retroviral vectors are rarely used to create transgenic animals of commercial value.