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Transgenic vertebrates. Conclusions and outlook

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Transgenic vertebrates in basic and applied research

Generation of transgenic animals is a fascinating way to examine the effect of genes and their products on developmental, morphogenetic and physiological processes in the live animal. This kind of research is in part dependent on the feasibility to control the level of expression of the transgene. As summarized by Rusconi in this review, much has been learned in the past few years about regulation of transgenes. New control regions have been found and several (to some extent) inducible transcription systems have been developed. Nevertheless, gene expression of most gene constructs used at present in transgenics cannot be controlled at will. This circumstance hampers research at the basic level and more seriously at the applied level. For example, in studying B cell development in transgenic mice it would be helpful if the transcriptional onset of the transgenes (such as H chain genes) could be predicted or, still better, be induced at a specific developmental stage. On the other hand, studies in which the effects of (over)expression of transgenes (such as interleukin genes) are examined in vivo may still yield useful information in spite of varying expression levels of the transgenes (see Iglesias, this issue). The same is true of studies on allelic exclusion which denotes the phenomenon of only the maternal or paternal allele of a gene being expressed in any given cell. This is the case for genes that encode T cell receptor (TCR) proteins. Transgenic mice having integrated a productively rearranged TCRβ transgene show different levels of expression of the transgene. This variation helped to interpret the process of allelic exclusion. When transgene expression was high, the endogenous TCRβ genes were suppressed. When it was low, both transgenic and endogenous TCRβ chains could be detected on the surface of the same cell, indicating that allelic exclusion is a regulated event and not a matter of probability (see Bluethmann, this issue).

An important new method in the field of gene transfer is the possibility of gene targeting by homologous recombination. This demanding technique (emphasized in the chapters by Rusconi, Bluethmann and Wilmut et al.) may solve many of the problems of varying expression of transgenes and also become the method of choice for gene disruption, alteration or replacement. It is hoped that in the future the efficiency of this method can be improved and that it can be applied to animal species other than mice as well.

A considerable number of investigations on transgenesis in vertebrates deals with fish (cf. Houdebine and Chourrout, this issue). Generation of transgenic fish seems not to be a major problem in spite of the invisibility of the pronuclei in most (yolk-rich) fish eggs. However, expression of the transgenes is usually poor, probably due to the fact that most of the transgenes used so far were constructed for work with mammals and contain mam-

malian genes. Many of the fish studies have been done for applied purposes. Some reports claim to have produced transgenic fish growing faster than controls due to the effect of the integrated transgene. Transgenic fish may become the first vertebrates serving as food for humans in the not too distant future.

The specific features of avian development have necessitated the development of suitable gene transfer methods other than DNA-microinjection into pronuclei. Retroviral infection of multi-celled bird embryos is technically relatively simple and the retroviruses can be used as replication-competent or replication-defective vectors for gene transfer (see Shuman, this review). This technique is now used in most experiments attempting to generate transgenic birds (chicken and quail). The easy accessibility of embryonic stages from the blastoderm stage onwards should make the transgenic bird embryo a favorable model for studies of expression of transgenes during embryonic development. For applied purposes limitations of the retrovirus vector techniques are still severe. One of the problems is the general occurrence of germ line mosaicism resulting in a low number of germ line transgenics; this calls for extensive screening tests. A substantial increase in the (as yet small) number of research groups at present engaged in the generation of transgenic birds is also highly desirable.

In the field of 'molecular farming' a breakthrough has happened recently. Although in most cases it is still unclear why one gene construct is effective while another is hardly expressed, in some cases a high level of secretion of a valuable gene product in the milk of transgenic mice has been obtained (see Wilmut et al., this issue). As soon as this can be repeated in a farm animal, tests for commercial applications will begin. However, it is likely that still more problems will turn up. For example, Wall et al. (ref. 47 in Wilmut et al., this issue) report that 2 out of 3 transgenic pig lines displaying a high level of secretion of a heterologous milk protein in their milk stopped lactation after a few days. Thus, a high activity of a transgene in the mammary gland may cause physiological problems to the expression system.

Physiological problems for transgenic farm animals may also be expected when modification of their metabolism or introduction of new biochemical pathways for improving important production traits is envisaged (see Ward and Nancarrow, this issue). As pointed out above, transgenic laboratory animals are mainly used to study, elucidate and eventually understand biological processes of live animals. In such cases the effect (or absence) of a given (trans)gene and its product on development, physiology and metabolism of the transgenic animal frequently results in deviations from the normal biological performance. From pathological symptoms a possible function of the gene product can be inferred. For breeders of farm animals however, the foremost goal is to breed healthy, sturdy animals with good reproductive performance who transmit excellent production traits to their offspring. Thus, uncontrolled disturbance of the delicate physiological balance by the introduction of transgenes is highly undesired. The outcome of experiments in which the endocrine system of transgenic domestic animals was modified does underline these concerns and again demonstrates the need for efficient control of transgene expression. To what extent the insertion of new biochemical pathways by gene transfer will be tolerated by the animal species in question, is still a matter of speculation. Some exciting new ideas and concepts to improve quantity and/or quality of sheep wool are tested at present or will be tested in the near future and the first results are encouraging (see Ward and Nancarrow, this issue). It is conceivable that genetic modification of structural proteins contained in wool or milk may be less hazardous to the health status of animals than genetically altering the level of metabolically important proteins such as proteohormones.

The overview of Müller and Brem (this issue) on disease resistance in farm animals outlines the enormous complexity of the biological processes involved in the defence mechanisms against pathogenic agents. Aiming at the improvement of disease resistance of livestock by gene transfer this complexity may represent a disadvantage; however, it may also be advantageous. A disadvantage obviously is the frequent polygenic origin of susceptibility to infections. On the other hand, the various levels of defence mechanisms and the large number of molecules acting as modulators of immune reactions represent a potential for a variety of opportunities to support resistance defence mechanisms by the transfer of an appropriate (effective) transgene. Of course, this ambitious goal is dependent on a thorough knowledge of defence mechanisms in disease resistance in general and on a precise notion of the participating elements involved in the attack and suppression of a specific pathogenic agent in the animal species in question. At the moment, we are far from having achieved this stage.

Only in a few instances has it been possible to link disease resistance to a single genetic locus. One of these loci, the mouse Mx system, confers resistance to influenza virus infections and may be of prospective interest to animal breeders (see, e.g., ref. 15 in Shuman, this issue). Müller and Brem (this issue) have summarized their studies trying to generate transgenic pigs expressing the Mx gene. Problems arising in these studies again center on low expression levels, difficulties with inducibility and also on rearrangements of the gene constructs. These attempts may exemplify some of the pitfalls to be encountered on the way to the goal of using transgenic farm animals for economic reasons.

Transgenic vertebrates and public opinion

The importance of transgenic mice for basic research cannot be overstated. This is indirectly shown by the strong increase in the number of papers dealing with

transgenic mice (see Rusconi, this issue). In Switzerland, the number of projects using transgenic animals increased almost twelvefold from 1986 to 1990 (1986: 4 projects; 1990: 47 projects; data according to SKBS). More directly, the scientific impact of transgenic mice research is shown in some of the chapters of this review. This significance, which may seem obvious to many biologists and medical scientists, is stressed here because, in the last few years, several industrialized countries have encountered opposition from animal protection groups against the use of animals in research and of the production of transgenics in particular. One of the arguments is that this kind of research can be carried out using cell, tissue or organ cultures. While some studies can be and indeed are being done under in vitro conditions, the effects of (mutant) genes and gene products linked to the immune system ultimately must be studied in the complex and complete organism (see chapters by Iglesias and Bluethmann, this issue). Resistance from the public to the generation of larger transgenic mammals such as transgenic livestock is even more pronounced. In addition to animal welfare aspects, concerns center on safety aspects in case of controlled release of transgenic organisms and on ethical questions (cf. Ward and Nancarrow, this issue). The concerns of these groups (animal rights activists, ecologists, scientists in ethics) should be taken seriously and, clearly, every measure to improve the welfare of the animals must be taken into consideration. However, it is also clear that even in the case of installation and observance of optimal conditions for maintenance, handling and care of test animals extreme animal protection groups will not relinquish in their journalistic and political activities against all animal research. This is going to be a serious problem for research in biology and biomedicine and, at least in Switzerland, a real menace to future work on transgenic animals. The review coordinators have had experience in this area by suffering from a totally unjustified attack from animal rights activists. They know how frustrating, time- and money-consuming, and work-blocking such involvements can be. The only effective way to cut the ground from under such extremists in the future may be to openly and understandably explain to the public how and why the animals are kept and what the research aims are. It should also be pointed out that animal research occasionally may lead to applications which are beneficial to *all* living organisms. With regard to transgenic farm animals, it should be emphasized by scientists involved in such studies that even though their studies began with some prospective application in mind, their results usually provide a multitude of new and important data on the biology, physiology and genetics of the animals in question.

A highly controversial topic is that of patenting transgenic animals. In this context we cannot discuss the problem adequately, however, we want to express some personal opinions. The advantages to the economy and technical progress of our society, offered by the opportunity of patenting inventions and innovations, indeed are obvious. The search for new and creative solutions to produce goods and commodities, to satisfy the necessities of life and to solve environmental problems of our societies is strongly stimulated by the prospect of profit. It is also reasonable to assume that private companies are more prepared to invest money in projects with transgenic animals if such animals can be patented. On the other hand, most scientists in the field of biology and medicine are motivated by the thirst for knowledge, the prospects of scientific recognition and career promotion, and/or the finding of new treatments for diseases rather than by the prospect of monetary reward. Therefore, we do not believe that a political decision rejecting animal patenting will affect biomedical research significantly. Indeed, the opposite may be true. In the case of licensing animal patents, the linkage between animal research and profit-thinking may well have an adverse effect on public acceptance and recognition of animal experimentation as a need and benefit for the advancement of biomedical science as well as of human and animal health care in the years to come.

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