Multi-author Review

Transgenic vertebrates

The Editors wish to thank Dr. D. F. Went and Prof. G. Stranzinger for coordinating this multi-author review.

Transgenic vertebrates. Introduction

D. F. Went and G. Stranzinger

Department of Animal Science, ETH-Zentrum, CH-8092 Zürich (Switzerland)

It was only about 15 years ago that the first transgenic animals carrying foreign DNA in soma and germ line cells were generated by exposing mouse embryos to infectious retrovirus[1]. About 4 years later the technique of microinjecting recombinant DNA into one of the pronuclei of a fertilized egg to generate transgenic animals was established (see Rusconi, this review). This technique has its drawbacks, but for the moment it remains the method of choice in most experiments where foreign DNA is being introduced into the soma and germ line cells of mammals. In these past 15 years a vast and evergrowing number of investigations using transgenic animals has been accomplished. Most of these experiments were carried out with laboratory animals, mainly mice. The mouse model is very suitable for several reasons: adequate techniques for embryo culture and handling have been developed, inbred strains with specific genetic and phenotypic characteristics are available and, last but not least, mouse experimenting is relatively economical.

The possibility of altering the genome of animals by introducing new gene variants did not only stimulate work in basic research areas such as regulation of genes or development and response of the immune system, but also promoted applied research engaged in the genetic engineering of farm animals. Farm animals contribute to the well-being of mankind in several ways and they have already been subject to genetic modification for many years through natural and artificial selection. Because of a long generation interval and the negative correlation between some major traits it would be desirable for animal breeders to realize effective and fast-working techniques for genetic improvement of animals. Scientists, trying to apply the methods developed for mice to farm animals, met with several technical difficulties, e.g. the impaired visibility of the pronuclei. These difficulties have now largely been overcome, but insufficient access to gene constructs important for animal production and the high cost of research on transgenic farm animals remain major obstacles.

Today, it does not seem practical to collect all published data on transgenic animals in a single review, nor would any prominent author accept such an undertaking. Therefore, a multi-author review on this topic was conceived and even in this case we – the review coordinators – had to limit this review to certain topics. Being engaged ourselves in animal science and in animal breeding in particular, we are familiar with the needs and shortcomings in this field and therefore we have given somewhat more attention to applied aspects. However, it becomes more and more apparent that to be successful in genetic engineering of domestic animals, apart from identifying the relevant genes, a better understanding of the regulation of transgenes in transgenic animals is needed. For example, the attempts to use farm animals for the concept of molecular farming (i.e. for the production of valuable – human – proteins) clearly have shown the need for a better knowledge of basic mechanisms of gene regulation. Likewise, if improvement of disease resistance of farm animals by the introduction of transgenes is attempted, this must be preceded by extensive and meticulous tests on the regulation and effectiveness of such transgenes in laboratory animals. Nevertheless, extrapolations from laboratory animals to farm animals concerning the effects of transgenes and their products on developmental and physiological processes can be erroneous. Great expectations based on the observation of 'dramatic growth' of mice possessing integrated metallothionein-growth hormone fusion genes could not be substantiated for farm animals as yet (see Ward and Nancarrow, this review). This means that in the future if the use of transgenic farm animals in food, textile fiber or pharmaceutical production is envisaged, research on the larger domestic animals must continue and cannot be substituted for by investigations on the much cheaper mouse model.

We have chosen to start this review with a chapter by Rusconi on transgene regulation in laboratory animals; this information on regulation of genes forms a basis for subsequent chapters. The following two chapters by Iglesias and Bluethmann, respectively, deal with the immune system and describe the contributions to an understanding of B cell and T cell development made by studies using transgenic mice. The topics of transgenesis in commercially important vertebrates other than mammals (fish and birds) are then covered by Houdebine/Chourrout and Shuman, respectively. The differences in gametic and embryonic development as well as the increasing importance of these animal groups for human nutrition

led us to include these chapters in this review. The three final chapters outline studies on transgenic farm animals in particular with regard to utilization for production of pharmaceutical proteins (Wilmut et al.), modification of important production traits (Ward and Nancarrow) and attempts to improve disease resistance (Müller and Brem).

In this review we have not addressed the topic of oncogenes and transgenic mice, which undoubtedly is of increasing importance for cancer research, nor the use of transgenic mice as disease models. Also, studies on mammalian development with the help of transgenic animals were not included. Results of such studies may well affect applications and strategies in animal breeding some time in the future.

1 Jaenisch, R., Germ line integration and Mendelian transmission of the exogenous Moloney leukemia virus. Proc. natl Acad. Sci. USA *73* (1976) 1260–1264.

0014-4754/91/090865-02$1.50 + 0.20/0

Transgenic regulation in laboratory animals

S. Rusconi

Institut für Molekularbiologie II der Universität Zürich, Irchel, Winterthurerstrasse 190, CH-8057 Zürich (Switzerland)

Abstract. This chapter is an attempt to summarize some commonly accepted and some more subjective opinions about the regulation of transgene expression in laboratory animals. After a short historical introduction, I present some general notions regarding gene structure/function. The spotlight shifts then to the description of the most popular techniques for gene transfer, including the targeted gene replacement. The different approaches are briefly discussed in terms of intrinsic advantages and limitations regarding gene expression patterns. Furthermore, the role of enhancers, promoters and other *cis*-acting elements such as silencers and dominant control regions as well as their involvement in the chromatin on-off state are discussed on the basis of a specific example studied in our laboratory. The review concludes by presenting recent results and the new perspectives opening in the field of 'surrogate' (also called 'reversed') genetics. Some problems which remain to be solved both at the technical as well as at the social-ethical level are also briefly presented.

Key words. Transgenic mice; microinjection; recombinant DNA; gene expression; transcription factors; chromatin; homologous recombination; episomal maintenance; embryonic stem cells; germ line; position-effect; mosaicism; globin genes.

Why and how did it all start?

It is a common belief that direct genetic transformation of increasingly complex organisms by the genetic carrier material has received more serious attention only since the classical experiment of Avery and colleagues[2]. I would like to go beyond this rather scholastic belief by daring to say that this has been the pervasive dream that has accompanied all studies in the field of modern genetics. Direct genetic transformation relies on the possibility of physically breaking the barrier of the compartment in which the genetic material is normally stored (i.e. the cell or the nucleus) with chemical, physical or biological tricks and depositing new, and possibly defined, genetic material into the host genome. In this first phase one is mainly confronted with the technical issue of efficiency of the process of gene transfer. Down the road, other important problems such as the stability and the faithful expression of the newly introduced genetic information arise and, as we shall see, several related aspects have yet to be completely elucidated. It is only after solving the majority of these problems that we can exploit the technique of direct genetic transformation in its full potential, that is to obtain useful information about specific gene expression patterns and their complicated network of primary and secondary metabolic effects in the context of the entire organism. Beside the mere academic interest, the application of direct gene transfer for the 'improvement' of the genetic repertoire of economically important animal and plant species also seems to receive increasing attention (see chapters by other contributors to this multi-author review).

In the last few decades, the booming of techniques allowing rapid and easy cloning of genes from various sources, along with a formidable progress in basic embryo culture procedures and manipulation (refs 12 and 50, and references therein) have provided the tools for the modern transgene techniques. In fact, several very early attempts to transform entire organisms had failed, probably due to the rather primitive stage in the techni-