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Transgenic mice: A powerful tool for basic research

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In December 1982 an issue of Nature flashed a giant mouse accompanied by a normal litter mate as its cover picture. The king sized mouse owed its impressive appearance to the gene for rat growth hormone (*Palmiter et al.* 1982). The gene had been planted among the mouse's own genes and had become part of the animal's genetic constitution. During the couple of years preceding this experiment a few, less photogenic, experiments of the same type had been performed. Since then very many other such »transgenic mice«, i.e. mice with foreign genes inserted among their own genes, have been created and have given rise to lines of mice with new heritable characters. We want to describe here how and why such experiments are performed and to give a few examples of experiments that have been done. Several reviews that cover the field can be recommended: for biological aspects in general (*Brinster* and *Palmiter* 1986), for aspects of molecular biology (*Palmiter* and *Brinster* 1986), for oncogenesis (*Hanahan* 1986), and for technical aspects (*Hogan et al.* 1986). There are several textbooks that cover general aspects of gene transfer; recent examples are (*Kingsman and Kingsman* 1988) and (*Winnacker* 1987).

Let us state at the outset that to us the techniques are valuable not as tools to create bigger and better creatures but as an avenue of basic research investigating how cells and organisms are organized. We shall describe some examples of the usefulness of work with transgenic mice in this area.

HOW IT IS DONE

Under the microscope, a solution of DNA containing a gene to be studied is injected directly into the male pronucleus of a fertilized egg cell. The eggs are then implanted into the oviduct of a foster mother, who gives birth to progeny, some of which have incorporated the foreign DNA into one of their chromosomes. Variations of the technique exist, e.g. a virus can be used as carrier of the gene to be introduced; we shall not discuss such variations here.

In principle the technique is simple. In practice work with transgenic mice is time-consuming and demanding on both manual skill and expensive equipment. The limiting time factor lies in the generation time of the mouse. Figure 1 shows a flow scheme for the creation of a transgenic mouse line.

Injecting DNA into one-cell zygotes

The details are as follows. Four categories of mice enter each experiment: Eggs are obtained from donor females (D), in whom ovulation has been induced by hormone treatment and who has been mated with normal males (C). DNA is injected into the eggs, which are then implanted into »pseudopregnant« females (B), made physiologically prepared to serve as foster mothers to the treated eggs by mating with sterile (vasectomized) males (A). Most often all four categories of mice are of the same genetic type – they are all first-generation hybrids of two inbred strains.

Each experiment is begun three days before the microinjection with administration of follicle-stimulating hormone to 6-8 weeks old egg donors (D), followed 48 hours later by human chorionic gonadotropin. At the same time each egg donor is placed in a mating cage with a male. This »superovulation« procedure allows isolation of 20-30 eggs per mouse compared to 6-10 from mice in normal estrus. (What we call »eggs« for short is actually fertilized »zygotes« at the one-cell stage). To assure that the eggs are at the same developmental stage the mice are

kept on a controlled light cycle, so that copulation takes place around midnight. The zygotes will then be at a stage suitable for DNA injection in the afternoon of the following day. In a typical experiment 6-10 out of 10 hormone-treated mice will show visual signs of fertilization, i.e. copulation plugs blocking the vagina. From these mice between 100 and 200 fertilized zygotes can be collected. Approximately 200-400 molecules of DNA in 1-2 picoliter are injected into each zygote, preferably into the male pronucleus, which is larger than the female pronucleus and further away from the polar body (Fig. 1). 25-30 zygotes of those that appear unharmed by the injection are then implanted into the oviduct of each foster mother.

Testing the offspring

From 2-3 weeks of age the progeny can be tested for the presence of foreign DNA in their tissues by isolating DNA from 1 cm of their tails. The tail DNA is subjected to a »hybridization test«: The DNA samples are dried down on a membrane filter and are tested for binding to a probe of radioactive DNA prepared from the same DNA as was injected. DNA molecules bound to the filter will bind specifically to the radioactive DNA probe if they contain sequences of the same origin. The positive samples are indentified after locating the radioactivity on the membrane using an X-ray film, on which areas exposed to radioactive DNA are identified as dark spots after development. More sophisticated tests of a similar nature, so called Southern blots, are then undertaken to show whether the DNA has been inserted in a simple way without rearrangement (Fig. 1). The identified transgenic mice are called »the founder generation«. The efficiency of generating transgenic mice varies, but under optimal conditions between 20 and 30% of mice born after re-implantation will be positive for transgene sequences. As soon as they reach maturity, they are back-crossed to normal mice of the desired genetic background. Approximately 30-40% of the founders are mosaics, i.e. not all somatic and germ cells contain the new DNA. Therefore many of their progeny must be tested to find transgenic offspring. Such low-frequency inheritance indicates that the integration of the injected DNA took place *after* the first mitotic division of the zygote. (Conversely, in some founders the new genes may be found at more than one locus.) In later generations, however, the new genes normally segregate in a classical mendelian way. The analysis of a »transgenic line« can meaningfully take place in the first and second generation following the founder.

WHY IT IS DONE

The techniques leading to »transgenic mice« have primarily been applied for specific purposes within the study of gene regulation. The final object of such research is to illuminate on different levels how genes can instruct a fertilized egg cell to give rise to such an enormously complex structure as a living animal. These experiments build on knowledge derived from simpler systems. We shall start by outlining some of this background.

The molecular biologist's view of living things.

Virtually every cell of a man or a mouse carries a complete description of all the proteins that are to be synthesized in the organism, a description encoded in the DNA in the form of some 3×10^9 base pairs. For virtually every biochemical function one or several proteins are required, and for the synthesis of each protein a gene is required. There are somewhere between 10,000 and 100,000 protein genes in the mammalian cell. Some of these are active in almost every cell, others are active in only a subset of cell types, and others again are active in only one cell type. Miraculously the built-in master plan of the zygote allows it to differentiate into many cell types, each using the regulatory program differently.

Molecular biology tries to understand how the genetic information in the DNA controls synthesis of specific proteins in time and space. When a protein is studied from this view point, the gene for the protein is first identified. The search then turns to the elements that determine

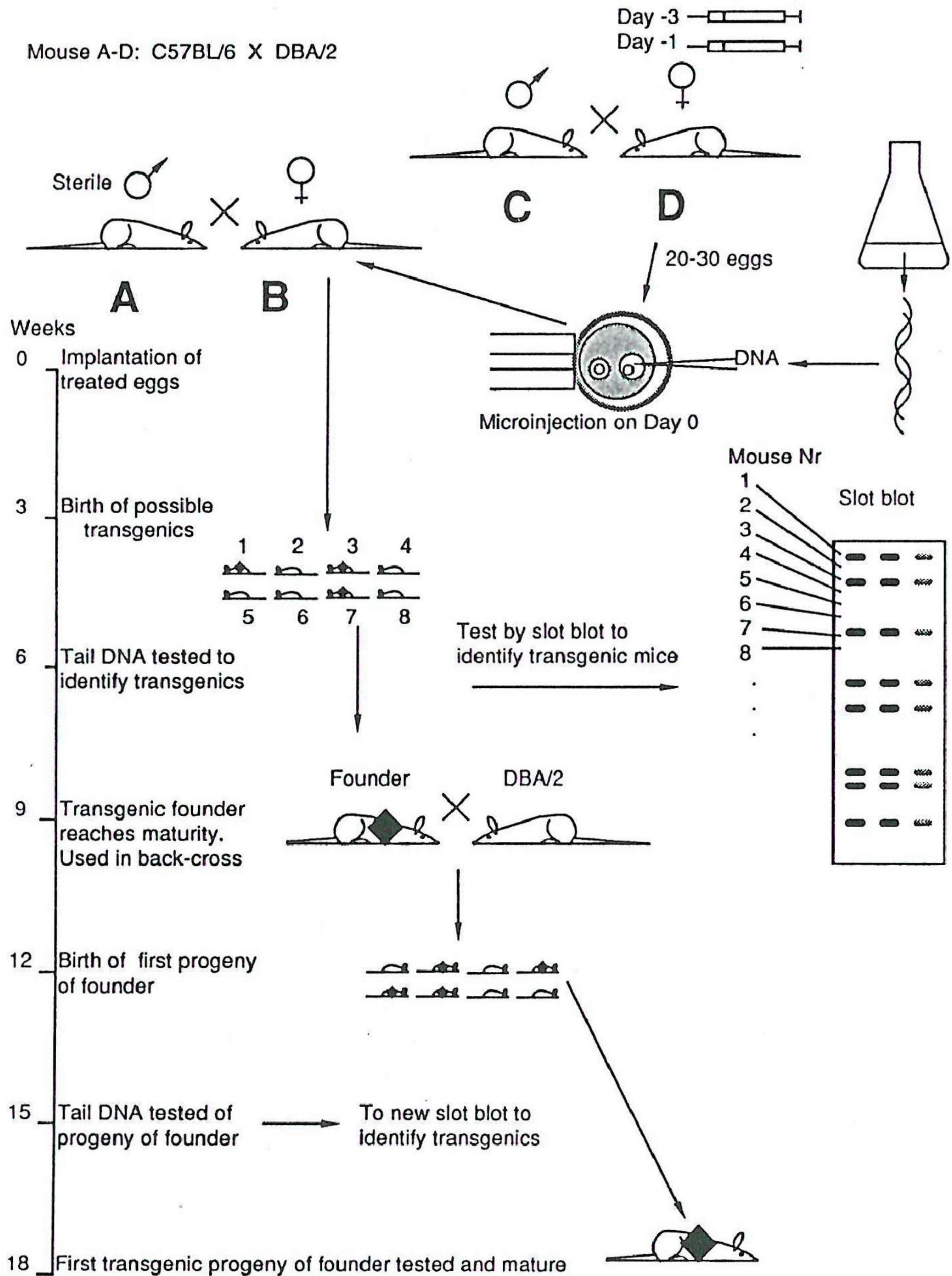

Figure 1. The creation of a transgenic line of mice. Slot blots are used as tests for the presence of the new DNA in the DNA of the mice. It involves the following procedure: A small DNA sample from each tested mouse is transferred to a membrane filter. To detect transgenic DNA the DNA bound to the filter is then hybridized to radioactive »probe DNA«, which contains the same DNA as that which was injected. The transgenic DNA recognizes and binds the probe, and the bound radioactivity is detected by letting the irradiation act upon an X-ray film. After development, black spots on the film reveal which DNA samples contain the foreign DNA. Each row on the film represents DNA from one mouse. The diamond signs in this figure indicate transgenic mice.

when and where the gene shall function. The zooming in on a specific gene and its immediate control elements includes the isolation and multiplication of a piece of DNA that contains the DNA region under scrutiny. The enrichment process, usually performed in *Escherichia coli,* gives easy access to sufficient amounts of DNA for the physical characterization and testing in biological systems. If the piece of DNA is of mammalian origin it will most often be tested for its biological activity in mammalian cultured cells. This type of experiment has provided much information on what parts of the DNA direct the functioning of the structural gene.

Control of RNA synthesis

Transcription, i.e. converting the information stored in the DNA into RNA, is intricately controlled. A very important part of the regulation of gene expression is exerted as control of the rate of RNA formation. Here we shall only discuss the synthesis of mRNA, the RNA directing the synthesis of protein. To concentrate on this topic we shall deliberately steer around other interesting aspects of the information flow from DNA, such as the processing of the original RNA transcript needed to prepare it for its translation into protein.

The known DNA elements that control transcription of genes coding for proteins in mammals can crudely be divided into two categories, promoters and enhancers. The word *promoter* strictly refers to the genetic entity that provides a start point for RNA polymerase, the enzyme that copies the DNA template into an RNA transcript. In higher eucaryotes a region of DNA up to approximately 100 base pairs before the actual transcription start is important for the transcription start; we shall include this whole region in the term promoter. The promoter determines the start point and direction for the transcription, so its position and direction are important. An *enhancer* is a genetic element that stimulates transcription from a promoter, often very strongly. It can stimulate a promoter from a distance of up to several thousands of base pairs. However, it has to reside on the same DNA molecule as the promoter it affects. Enhancers can also be positioned after the gene they control, and are unaffected by being turned around. Elements similar to enhancers but with a subduing effect on neighbouring genes have also been identified; they are called *silencers* (*Brand et al.* 1985). *In vitro* experiments show that promoters, enhancers, and silencers provide biding sites for a number of proteins. These proteins contribute to transcription, exerting both stimulatory and inhibitory effects. Different cell types have different sets of DNA-binding proteins. Enhancers and other protein-binding DNA elements control gene activity through their interactions with protein complexes (*Ephrussi et al.* 1985, *Sassone-Corsi & Borrelli* 1986). Combinations of different proteins may determine the activity of each gene by binding to single or multiple DNA elements flanking the gene, thus directing it to be active under certain specific conditions only.

Cells in tissues

Each cell continuously receives chemical signals from the outside containing information concerning its position in the body and the status of the organism. These signals may for example order the cell to grow or to produce excretory products. Some of the signals that reach the cell are hormones, others are known as growth factors, identified by their ability to confer upon cultured cells the ability to grow in synthetic media. Receptors on the envelope of the cell determine which signals it will recognize. The signals are further transmitted inside the cell along pathways that often involve phosphorylation of regulatory proteins and whose final step can be a protein-DNA interaction.

Many aspects of gene regulation in animals have been investigated by the use of cell cultures. There are limitations to the use of cell cultures, however. One limitation is that not all cell types can be easily grown in culture, and those that grow usually represent only one particular stage in the differentiation process. Cultures of cells generally have limited life spans when they

originate from normal tissue. Established *cell lines,* i.e. clones of cells that can be perpetuated for very many generations in culture, often deviate from their cellular counterpart in important ways. Typically they have properties in common with tumour cells, and more often than not their chromosome number is aberrant or even varying. Finally, cells in culture are deprived of the interplay with their native surroundings; in their tissues of origin they grow under the influence of many interactions with neighbouring and more distant cells. Therefore, certain important problems of gene regulation implicitly involve the whole organism.
Transgenic techniques provide an exceedingly powerful tool here. In particular, they are invaluable in the analysis of complex problems that involve cells in the context of the organism, such as the differentiation of cells during embryonic development. We shall present here a few illustrations of uses of transgenic mice to solve problems in molecular biology.

FIRST CASE HISTORY

Studying the control of gene specificity using elastase I.

Several aspects of specific gene expression have been investigated by means of mice transgenic for the elastase I gene or its control region. Elastase I is a digestive enzyme produced only in the acinar cells of the exocrine pancreas. It has been studied as an example of a gene whose activity is very strictly limited to one cell type. With the object to identify the DNA sequences that determine specificity, transgenic mice carrying the entire rat elastase I gene and the surrounding DNA region were produced. The piece of DNA included 7000 base pairs before the gene and 5000 base pairs after it (*Swift et al.* 1984). The functioning of the rat gene was followed by specific detection of its mRNA in the tissue by hybridization tests using a radioactive DNA probe. The first finding was that the rat gene was indeed active in the transgenic mice. Moreover, it was functional exclusively in the pancreas, indicating that the regulatory apparatus of the mouse cells recognized regulatory sequences of the rat gene well enough to allow tissue-specific control.

From a quantitative view point the situation was less straightforward. First of all, the number of copies of the DNA fragment inserted at the integration site varied wildly. Four mice had the following copy numbers of the elastase I gene per cell: 2, 7, 9, 100. Such integration of transgenic DNA in multiple copies is much more common than integration of just one copy. The amount of elastase mRNA produced per cell was only moderately related to the gene copy number. Thus the mouse with two genes per cell produced normal amounts of mRNA but the mouse with 100 copies produced only 12-fold more than normal. The integration site appeared to have been of little importance.
To focus on the sequences responsible for tissue-specific expression all unnecessary parts of surrounding DNA were removed (*Hammer et al.* 1987). It was found that the only part needed for tissue-specificity were 134 base pairs lying between 205 and 72 base pairs before the transcription start point. In this search for the minimal determinant of tissue-specificity even the structural gene itself and its normal transcription start were replaced. The human growth hormone gene and its transcription start were used instead. In this context the growth hormone gene serves as a »reporter gene«, in other words a gene whose expression tests the ability of the elastase I sequence connected to it to direct tissue-specificity. This construct directed the synthesis of human growth hormone specifically in the acinar cells of transgenic mice. Interestingly, the short control region contained information also for the temporal specificity of the rat elastase I gene. Production of the human growth hormone was thus turned on around day 16 in the transgenic fetuses as is normally elastase I production in rats and in mice transgenic for the rat gene.
Incidentally, the growth hormone was secreted into the alimentary canal, and was without effect on the animals. In contrast, the same growth hormone gene when governed by a control region that directs expression in many cell types allows the hormone to diffuse into the blood stream (*Palmiter et al.* 1983). The effect then is exaggerated growth as with the mouse on the Nature cover.

The same principle as in this example has been used with many other genes to study their regulation as determined by tissue and time: a reporter gene is coupled to a regulatory sequence whose output is to be monitored. Any gene can serve as a reporter gene, provided that its products can be measured in the mouse tissues without being confused with products normally made there. Often the regulatory sequence is then modified to analyse the effect of the modification on gene expression.
The next step in the exploration of what makes genes active in specific cells is to identify the regulatory proteins that recognize the specificity-determining DNA sequences. Later steps in the process are concerned with what in turn controls the synthesis of the regulatory proteins. For the next section we have chosen our examples from a class of regulatory proteins and their genes. These proteins play a role so high up in the regulatory cascade that they profoundly influence cell proliferation.

SECOND CASE HISTORY
Of viruses and oncogenes.
During the last few years there has been much excitement among scientists investigating how perfectly normal cells in the body turn into menacing tumour cells. The increasing understanding of the mechanism of oncogenesis comes in particular from studies of how oncogenic viruses cause cancer (For review see Varmus 1987). The emerging picture reveals more and more of a well safe-guarded regulatory network and of how much it takes to destroy it.
An oncogenic virus entering a cell may contribute to a deregulation of the cellular mechanisms ensuring that the cell grows only in obedience to the rules appropriate to its role in the organism. Sometimes the virus brings into the cell a regulatory gene, sometimes it affects preexisting cellular genes. Work with oncogenic retroviruses has defined a number of genes that are involved in the process by which these genes contribute to tumorigenesis. Many such retroviruses carry among their genes a gene responsible for the oncogenic activity of the virus. In several instances such genes have been shown to be variants of normal cellular genes. The viral version is called an oncogene and the cellular version is called a proto-oncogene.
The normal role of some of the protein products of certain protooncogenes has been partially characterized. Some such proteins are growth factors, i.e. signals that reach the cell from the outside; others are growth factor receptors that extend across the cell membrane, receiving the signal from the outside and delivering it on the inside by interacting with a cytoplasmic cell components; others again are part of a signal transducing chain that carries the message of the signal from the cell periphery to the nucleus; finally others are proteins in the nucleus which interact with DNA or with proteins binding to the DNA to produce the final effect. This final effect may be synthesis of DNA or of a gene product important for cell proliferation.

C-myc as a transgene
One of the genes identified as a proto-oncogene is called c-*myc*. Its protein product binds DNA (*Donner et al.* 1982; *Persson & Leder* 1984) and appears to be somehow involved in the initiation of DNA synthesis (*Iguchi-Ariga* et al. 1987). the murine c-*myc* gene has been used to create transgenic mice. In one set of experiments the c-*myc* gene was brought under the control of different enhancers of immunoglobulin genes (*Adams et al.* 1985), which become active in B-type lymphocytes. The result in short was that the transgenic mice produced in these experiments developed lymphomas, tumours of B-cell origin, within few months after birth. The illicit expression of the c-*myc* gene due to the unrelated enhancer was necessary but not sufficient for the oncogenesis. Another, unknown, event was required in addition to c-*myc* expression to cause the formation of a tumour. This was evidenced by the observation that not all B-cells in the animal behaved as tumour cells. Rather, it could be shown that all cells in each tumour represented a clone, which had arisen from one progenitor cell and had characteristics that made it distinguishable from other B-cells.

The oncogenic activity of c-myc steered to different cell types.

Another investigation (*Stewart et al.* 1984, *Leder et al.* 1986) provides an example of c-*myc* coupled to another control region. In this study a promoter-enhancer piece is obtained from mouse mammary tumour virus (MTV), a retrovirus. The expression of this viral promoter is known to be stimulated by glucocorticoid hormones. Several transgenic lines were found to express the c-*myc* primarily in breast tissue and in salivary glands; they developed mammary tumours during pregnancies. One line expressed the transgene in several types of tissues and also developed tumours in a broader range of tissues. Again, the presence of the transgene was necessary but not sufficient to cause cancer. A further genetic event and hormone stimulation appeared necessary to cause cancer formation.

Two simultaneous oncogenes

A different oncogene, the *ras* gene, was used in a similar experiment (*Sinn et al.* 1987). The protein corresponding to *ras* is membrane-bound and most likely has a role in the chain of interactions whose first step is the recognition of a protein signal at the exterior of the cell. The *ras* gene was brought under the control of the MTV promoter-enhancer region and transgenic mice were prepared. Mice transgenic for this construction were predisposed to getting tumours in a variety of tissues.

In search for the combination of events needed for the oncogenic conversion, mice carrying either c-*myc* or *ras* under the control of the MTV promoter-enhancer were crossed (*Sinn et al.* 1987). Progeny carrying both transgenes were identified. They were extremely prone to tumours, and the tumours formed earlier than in the two parental lines. However, the conversion into tumour cells still was not an inherent quality of all cells of the affected tissues, but tumour formation had to be preceded by an unknown genetic event also in these, doubly transgenic, mice.

Other investigations have used a number of other determinants of tissue-specificity in conjunction with oncogenes to probe their role in normal and perturbed control of growth and proliferation. It has become abundantly clear that transgenic techniques have taken the exploration of specific oncogenes a long step further than cell culture. They let one observe the oncogenic process as it progresses, subject to influences exerted by the blood supply, by the neighbouring cells, and by the immune system.

THIRD CASE HISTORY

Myelination as a test of gene function.

The underlying cause of heritable diseases in man and animals is often difficult to pin-point. Even if a mutation appears reliably correlated to a visible malfunction and even if mutant individuals can be shown to lack a certain protein, a causal relationship can be hard to establish. For instance, it is not easy to exclude that other proteins than that shown to be missing are also affected.

The function of many genes that are active in all types of cells can be studied in cell cultures. However, when the gene products perform functions specific to highly differentiated tissues, cell cultures may be out of the question. The great and increasing number of mouse mutants offers great possibilities for approaches based on the introduction of DNA into embryos. This may be especially important in tissues where specialization occurs after birth. The nervous system is matured postnatally, and we shall take an example from neurobiology to illustrate how transgenic mice can be used to study gene function where cell cultures necessarily fail to be of use.

Many mutants with defects of the nervous system are viable, surviving at least for a short period after birth. Of the more than 700 murine mutants known today at least 150 are neuromutants (*Bauman & Lachapelle* 1982). Some very well characterized neuromutants, defective in myelination in the brain and in the peripheral nerves, have been analyzed thoroughly with the aid of transgenes.

Of myelin and mutations affecting it

Meylin is the insulating material that surrounds

axons, facilitating the transmission of nerve impulse. In the brain, myelin constitutes a large fraction of the mass, and its proteins are produced from very active brain-specific genes (for reviews see Sutcliffe 1987, 1988). Despite its complex structure, myelin consists of only a few abundant proteins. In the myelin of the central nervous system (CNS) the major protein component, constituting 30-40% of the total protein, is called myelin basic protein (MBP).

Most mutants of the MBP gene appear normal, unless they are homozygotic, i.e. both chromosomes in the chromosome pair carry the defective version of the gene. Two different dysmyelinating mutations, *shi* for »shiverer« and *mld* for »myelin-deficient« affect the MBP gene. In homozygotic »shiverer« (*shi/shi*) mice no MBP gene expression can be detected, and CNS axons are devoid of myelin sheaths; in homozygotic »myelin-deficient« (*mdl/mld*) mice MBP is much lower than normal and its developmental timing is anomalous (*Popko et al.* 1987).

Investigation at the DNA level in the *shi* mutants showed that they lacked most of the MBP gene, so that no MBP protein could be produced (*Sutcliffe* 1987). In the *mld* mice, in contrast, the structural gene is normal. Its function is however disturbed by a rearrangement of nearby DNA sequences (*Okano et al.* 1988).

Although the *mld* mice have a complete structural gene, they are virtually as severely affected as the *shi* mice. In the two mutants the disease pattern is the same: generalized tremors upon movement, beginning at the age of two weeks and becoming more prominent with age, with tonic seizures (convulsions) at a few months; the mice have a shortened life span of maximally 90-150 days. Can the lowered capacity to synthesize MBP explain the defect in the *mld/mld* mice or is it necessary to consider the involvement of other genes? A quantitative analysis of different types of transgenic mice provided an answer.

Mice transgenic for the MBP genes

The formal proof that the reduced synthesis of MBP is actually the cause of the syndrome came from experiments in which 37,000 base pairs including an intact MBP gene were used to establish transgenic mice (*Readhead et al.* 1987; *Popko et al.* 1987). The mice were bred to have either one copy of a transgene on the chromosome where it had integrated or to have it on both members of the corresponding chromosome pair. The effect of one or two copies was investigated in *shi/shi* mice, in *shi/mld* mice, and in *mld/mld* mice. In this way a whole series of animals with increasing ability to make MBP was created. In the *shi/shi* mice the syndrome was not improved by a single copy of the transgene. In contrast, both in *mld/mld* mice with one copy of the transgene and in *shi/shi* mice with two copies of the transgene the condition was greatly improved: They were normal at six weeks, never developed convulsions, and lived for more than seven months. Anatomical results showed precisely what could be expected from the condition of the animals and the assumed role of the gene: *shi/shi* mice with two transgenes had a greater number of sufficiently myelinated axons in the CNS than had *shi/shi* mice with one copy of the transgene but less than normal mice. Thus, not only was the lack of MBP firmly established to be the cause of the syndrome, but a great deal of very precise information was assembled concerning the minimal MBP synthesis needed to cause decisive improvement.

The usefulness of transgenic mice in the investigation of gene function is by no means unique to neurobiology. Whenever cultured cells cannot reproduce the condition in which the gene investigated is important, transgenic mice may provide an invaluable tool.

PROBLEMS RELATED TO INTEGRATION

The tendency of the added DNA to integrate as blocks containing many, occasionally thousands, of copies is sometimes a disturbing factor. The reason for multiple integration may be that pronuclear microinjection induces random breaks in the DNA. The injected DNA

may then integrate at these break points (*Palmiter & Brinster* 1986).
The effect of the chromosomal integration site on transgene expression sometimes must be taken into account. While it is clear that strongly expressed genes can function efficiently at many sites, weakly expressed genes may be more sensitive to the position of their site of integration (*Allen et al.* 1988, *Kothary et al.* 1988). Their expression might for instance be unduly stimulated by fortuitous integration within the activity range of a strong, unrelated enhancer. In such cases the expression of the particular integrate of the transgene may be largely dictated by the chromosomal domain into which it has integrated. These problems and others not discussed here make it difficult to predict with certainty the properties of any contemplated transgenic construction. Nonetheless, these difficulties can be handled in well-controlled experiments.

SOME OTHER ASPECTS

Occasionally the injected DNA may integrate into a gene or into the surrounding DNA required for its normal functioning, thereby causing insertional mutation (For review see Gridley et al. 1988). Mutations of this type will most often be recessive and detectable only in homozygotes. Using the integrated sequences as molecular marker can facilitate cloning and characterization of the affected gene. (*Schnieke et al.* 1983).
An efficient technique to *direct* mutation to specific genes has recently been devised (*Mansour et al.* 1988). It uses integration of a foreign DNA fragment into a *predetermined* gene and takes advantage of a combination of cell culture, involving ingenious selection of rare cells, and microinjection and should be applicable to any mouse gene for which the DNA of the gene in question has been isolated.
Transgenic techniques have become a tool to get to grips with the esoteric phenomenon of imprinting, which is still very poorly understood. Imprinting in the present context means that genes inherited from one parent are somehow labeled as coming from that parent. Differential expression of certain parental genes is a prerequisite for normal embryonic development (for review see Monk 1988). Embryos manipulated to contain either two paternal or two maternal pronuclei fail to develop to term. In eggs containing two paternal pronuclei the extraembryonic membranes and the placenta developed normally, but the fetus developed poorly. In the situation with two maternal pronuclei the opposite was true. Introduced transgenes have given some clues to the imprinting mechanism. In some lines, transgene expression was dependent on whether its origin was maternal or paternal (*Swain et al.* 1987). Methylation of some bases in DNA is known from other contexts and has been suggested as part of the explanation of imprinting. It is therefore interesting that a great difference could be detected in the degree of methylation of the transgenic DNA contributed by the father and that contributed by the mother (*Hadchouel et al.* 1987, *Monk* 1988).

OUT-LOOK

The experiments that we have sketched are just examples of how transgenic techniques can serve the investigation of fundamental processes in the mammalian organism. Transgenic techniques have enormous potential in this area. Yet, anyone who, like ourselves, comes to this field with knowledge derived from DNA-based molecular biology using simpler systems must feel truly humble when confronted with the many possibilities of this new and fascinating technique. It certainly invites multidisciplinary cooperation with people from the fields of mouse genetics, embryology, physiology, animal pathology, immunocytology, and many others. In molecular biology and in these adjoining fields very central questions remain unanswered. Like always when a genuinely new tool brings together fields with burning questions it is hard to divine what will be the most valuable fruits of the encounter. We look with particular curiosity at the integration of knowledge within fields that deal with embryonic development and cell proliferation.
It should be abundantly clear from our exam-

ples that it is in basic research that we see the great value of transgenic techniques today. We will, however, comment shortly on the possible direct use of transgenic techniques for practical purposes.

The »oncomouse«, designed to allow carinogen testing with fewer mice than previously existing mouse strains has acquired some fame as the object of a recently granted U.S. patent (See Editorial Nature, *336*, 293, 1988). The patent is quite controversial as the first patent of a living animal and as a possible hindrance to free research and information flow in this important area.

The application of transgenic techniques to common domestic animals is not simple: Technical obstacles include both a different time scale than with mice and difficulties to retrieve embryos at a defined stage and to microinject DNA into them. Worse, the very objects of applications to livestock may be forbiddingly difficult to achieve. The goals, healthier and more productive animals, may be much easier to reach with classical breeding, since good health and high productivety are not determined by single genes but rather by a delicate balance between many genes.

One application to a practical problem deserves special mention, however: While gene cloning in bacteria has allowed microbial production of human polypeptide hormones, the synthesis of medically important glycosylated polypeptides and proteins such as blood coagulation factors is a much more difficult task.

Transgenic techniques may offer a solution here. It has already been possible to direct the production of chosen proteins to the mammary glands of mice (*Pittius et al.* 1987). It will no doubt be possible to apply this technique to e.g. sheep without compromising their wellbeing in the process. The milk from such farm animals may well become a rich source of lifesaving biopreparations in the not too distant future.

Summary

Our intention with this paper is to explain why transgenic mice have become an indispensable tool to molecular biology. The central parts of the paper are: a description of how transgenic mice are made by microinjection of DNA into the nucleus of an early embryo, a background orientation about molecular biology, and three examples of uses of transgenic mice. The examples illustrate how transgenic mice can be used to help in the answering of the following grand questions: 1. How is an individual gene regulated during the embryological development? 2. How is cell proliferation normally controlled, and how does the control become defective when normal cells turn into cancer cells? 3. How can a gene defect be related to function as seen in the individual and in an affected tissue? Furthermore, we discuss some difficulties that concern the making of transgenic animals and finally comment briefly on a few further developments, present and immediately foreseen.

Sammendrag

Vor hensigt er at gøre rede for hvorfor transgene mus er blevet til et uundværligt værktøj indenfor molekylær biologi. Artiklens centrale afsnit er: en beskrivelse af hvordan man fremstiller transgene mus ved mikroinjektion af DNA i kernen på det tidlige embryo, en baggrunds orientering om molekylær biologi, og tre eksempler på anvendelser af transgene mus. Eksemplerne belyser hvorledes transgene mus kan bruges som et af hjælpemidlerne til at besvare følgende store spørgsmål: 1. Hvordan reguleres et gen under embryonaludviklingen? 2. Hvordan kontrolleres cellers vækst og formering normalt, og hvordan ødelægges kontrollen, når normale celler bliver til cancerceller? 3. Hvordan kan, ved en arvelig sygdom, den defekte funktion hos det syge individ og i det ramte væv blive sat i forbindelse med et beskadiget gen? Endvidere diskuterer vi vanskeligheder ved fremstilling af transgene dyr og berører til slut i korthed nogle videre udviklinger, aktuelle og forudsete indenfor den nærmeste fremtid.

Sammanfattning

Vår avsikt är att redogöra för hur transgena möss har blivit ett oundgängligt redskap i molykylärbiologin. Artikelns centrala delar är: en beskrivning av hur man framställer transgena möss genom att mikroinjicera DNA i kärnan hos det tidiga embryot, en bakgrundsorientering om molekylärbiologi och tre exempel på användningar av transgena möss. Exemplena belyser hur transgena möss kan användas som hjälpmedel vid besvarandet av följande stora frågor: 1. Hur regleras en bestämd gen under embryonalutvecklingen efter tidpunkt och vävnad? Hur kontrolleras cellers växt och mångfaldigande normalt, och hur störs kontrolen, när normala celler blir till cancerceller? 3. Hur kan man se sammanhanget mellan en gen, som är skadad vid en ärftlig sjukdom och en drabbad funktion i individen och i den berörda vävnaden? Vidare diskuterar vi svårigheter vid framställningen av transgena djur och kommenterar till slut kort ett par utvecklingsmöjligheter inom området, aktuella och omedelbart väntade.

Yhteenveto / K. Pelkonen

Artikkelin tarkoituksena on selostaa miksi transgeenisistä hiiristä on tullut korvaamaton tutkimusmenetelmä molekyylibiologiassa. Artikkelissa kuvataan: miten transgeenisiä hiiriä tehdään varhaisen alkion tumaan tapahtuvien DNA-mikroinjektioiden avulla, molekyylibiologista taustaa sekä annetaan kolme esimerkkiä transgeenisten hiirien käytöstä. Esimerkit valaisevat miten transgeenisiä hiiriä voidaan käyttää seuraavien perustavien kysymyksien vastaamiseen: 1. Miten yksittäisen geenin toimintaa säädellään sikiökehityksen aikana ajallisesti ja kudoskohtaisesti? 2. Miten solunjakautumisen normaali säätely tapahtuu ja miten se häiriintyy solun muuttuessa syöpäsoluksi? 3. Miten geenipuutos liittyy yksilössä ja kudoksessa havaittavaan toimintahäiriöön? Lopuksi pohditaan eräitä transgeenisten eläinten tekemisessä olevia vaikeuksia sekä kommentoidaan muutamia jo olemassaolevia ja lähitulevaisuudessa odotettavissaolevia kehityssuuntia.

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