

Animals with Novel Genes. Edited by N. Maclean, Cambridge University Press, Cambridge, 1995; x + 266 pp. \$49.95 (hb). ISBN 0-521-43256-1.

In 1980 Gordon et al. (Proc. Natl. Acad. Sci. USA 77, 7380) demonstrated that newborn mice, when microinjected at the pronuclear stage with recombinant DNA, harbored the new DNA in their tissues. Gordon and Ruddle (Science 214, 1244, 1981) subsequently coined the term 'transgenic' to describe these mice. Although some efforts had been made prior to that time to establish a system for gene transfer into the germ lines of intact organisms, these results clearly demonstrated the biological practicability of such experimental strategies, and established an approach to creating transgenic animals that still predominates today. It is from these experiments, and the profoundly important information obtained from subsequent studies wherein genes were efficiently expressed in transgenic mice (Brinster et al., Cell 27, 223, 1981) that a veritable explosion in transgenic research took place, setting the stage for publication of this book entitled 'Animals with Novel Genes'. The book reviews approaches to production of transgenic mice, livestock, birds, fish, insects, and even, briefly, relatively exotic species such as arachnids and *C. elegans*. Also discussed in several places in the book are the potential ecological and extant ethical problems posed by transgenic research. Given the scope of the material discussed in this volume, as well as the rapid progress of this area of research, the book is remarkably successful and readable.

Success is achieved by focusing attention on the way in which the reproductive characteristics of these various organisms impose upon the technical approaches to gene transfer. In the course of these discussions, the interesting differences in reproductive physiology are underscored, and if nothing else, this book presents an entertaining lesson in reproductive biology. Of course, each chapter mentions the rationale for attempting transgenesis in the species under discussion, and also reviews some of the more successful experiments. However, in general, the volume is pragmatically superficial in this area. Thus, while the reader is acquainted with the scientific goals that drive the research, he is not burdened with the scientific minutiae of particular experiments.

Some important general principles can be developed from reading this book. First, it appears that nuclear or pronuclear injection leads to DNA concatamerization, regardless of the species involved. This implies that the processes leading to concatamer formation, whether they involve homologous recombination between injected fragments, or repair mechanisms invoked by the embryo, are fundamental. Second, it is clear that production of valuable transgenic animals of any class can be greatly facilitated if a model species, highly suitable to transgenic manipulation, is chosen for development of the technology. This strategy not only allows for efficient testing of recombinant constructs, it inevitably leads to assembly of a genetic map which can be highly useful for future basic or applied study. Thus the mouse for mammals, *Drosophila* for insects, and the zebra fish for fish have become, or are becoming, predominant in their respective classes. A final important generalization is that mammals appear uniquely amenable to transgenic manipulation. In insects, fish and avian species it is either quite difficult to penetrate the early embryo, or, it is not possible to identify a single pronucleus for microinjection. Fortunately in *Drosophila* transposable elements have done much to overcome this problem.

An important point relevant to this issue is that many problems producing transgenics in non-mammalian species would be overcome if DNA could be carried to the embryos in spermatozoa. It is therefore not surprising that the work of Lavitrano et al. (Cell 57, 717, 1989) is frequently cited in the book. These authors reported a high frequency of gene integration into mice after exposure of sperm to DNA. However, despite the fact that this paper was hailed as a major breakthrough at the time of publication, the work has been difficult to repeat (Brinster et al., Cell 59, 239, 1989 and our unpublished observations), and the original authors have published only minimal follow-up data (e.g., Gandolfi et al., J. Reprod. Fert. Abstr., No. 4, 21, 1989). Moreover, close examination of the original publication reveals some egregious omissions and inconsistencies. These problems, in conjunction with the aforementioned difficulties in repeating the findings, places the onus on these investigators to demonstrate the reproducibility of their technique or clarify their original published findings with definitive follow-up data. This book serves as a sobering reminder of the thousands of man hours of research spent in frenzied efforts to extend sperm-mediated gene transfer to other species.

This volume also clearly illustrates how poorly developed embryonic stem cell technology is for most species. This approach would certainly facilitate efforts to engineer desirable traits into livestock, fish, or even some insects. It should be quite interesting to follow progress in this area, which, even in mice, could still be improved substantially.

Turning to the specific chapters of the book, the opening chapter functions as an introduction, and covers a broad range of topics. Some of the assertions made are difficult to understand. For example, it is stated on pp. 10 that transmission of transgenes may be complicated by gender imprinting, which is not the case. Rather, transgene expression is affected by imprinting. Also, on the same page, difficulties in defining promoter/enhancer elements for potential transgenes are perhaps overstated. Concerning the release of transgenic organisms into the wild, it is of course wise to be careful, but at present no data exist to indicate that a genetically engineered organism would not be at a significant disadvantage in the wild. Introductions of transgenics should not be considered equivalent to introductions of new species, as suggested on pp. 19, but rather, as introductions of mutant members of extant species. Since speciation is defined as loss of the capacity of two organisms to produce fertile hybrids after cross-breeding, a transgenic mouse cannot be considered a new species of mouse. The statement on pp. 16, that transgenics must be monitored as potential weapons of war, also appears a bit melodramatic, and it hardly appears appropriate to single out Russia and China as potential sources of trouble.

The chapter on transgenic insects is quite interesting, and familiarizes the reader with the well established use of *P* elements in *Drosophila*. Quite an extensive discussion is made of efforts to transform mosquitos, with the space taken perhaps not justified by the success thus far obtained. This material is quite interesting because it highlights the obstacles to efficient transgenic insect production. An important potential suggested in this exposition is that of controlling infectious disease by introducing transgenic mosquitos into the environment. This is certainly an exciting prospect, though again, it is intuitively difficult to believe that such altered organisms would compete effectively in the wild.

For similar reasons the chapter on transgenic fish is also quite absorbing. The problems of reproductive management, and molecular biology are quite well delineated. As previously stated, the discussion here indicates that some effort should perhaps be made to establish the zebra fish as an experimental model. Again, as with the chapter on insects, it is difficult to believe that transgenic fish, regardless of the new phenotypes they exhibit, will compete effectively against their feral counterparts. The end of this chapter contains a nice summary of fish genes cloned, and of efforts to date to produce transgenic fish.

The following chapter on transgenic birds is authoritative and quite informative as well. The problems with production of transgenic birds are quite formidable, though retroviral vectors appear to work well. How well recombinant retroviruses will express in transgenic fowl of course remains to be learned. An interesting section is presented on the use of disabled sperm as vectors for gene transfer. Since parthenogenetic birds are viable, it is at least theoretically possible to activate development with inviable sperm which nonetheless carry new genetic traits. If this strategy were perfected, it might be further developed such that new DNA could be introduced into the sperm prior to their use. The authors also entice the reader with the prospect that primordial germ cells might be isolated, genetically altered, and then returned to embryos.

The chapter on transgenic rodents is necessarily incomplete. The authors do a nice job of explaining some of the emerging approaches to targeted gene modification via embryonic stem cells. The review of previous findings with transgenic mice could have been improved somewhat. Understated is the fact that mice allowed the demonstration of the relative importance of *cis*-acting elements, *trans*-acting factors, and chromosomal position in determining patterns of differential gene expression. The section on oncology also fails to discuss salient differences between transgene-induced tumors, which are polyclonal and generally non-metastatic, and cancers that arise by more conventional means. Virtually nothing is said of the use of transgenic mice in studies of immunology. A brief review of this area would have tied in quite well with the succeeding chapter, where transgenic

livestock are discussed, because the introduction of rearranged immunoglobulin genes into livestock is a major area of research. All in all, however, the explanation of knockout and replacement technology is quite valuable for the newcomer.

The production of transgenic livestock is an exciting area, and it is handled very well in the next chapter. The current status of this field is well described, and the management of some of these larger species, even if a bit more detailed than necessary, makes entertaining reading. Obviously, the enormous cost of producing these animals is a major problem. The perfection of other methods of gene transfer, and/or of embryonic stem cell technology, would contribute significantly to the further advancement of this field, though microinjection clearly works well.

The final chapter, on 'minor transgenic systems' was perhaps included for the sake of completeness. These systems are quite disparate, and relatively little work has yet been done in many of them.

Nonetheless this information is useful because it satisfies potential curiosity and provides a reference base in the event one or more of these areas develops substantially.

Overall, this book covers a very large area of science surprisingly well. For an individual with extensive knowledge of one transgenic field but little understanding of others, it makes enjoyable reading. It also would serve as nice introductory reading for the graduate student or advanced undergraduate. Where possible the book is comprehensive, but where this is not possible, the book does not lapse into prosaic, detailed discussions of specific experiments that would inevitably lead to an imbalanced presentation. That a readable, informative book could be distilled from the mass of information available in this field is a credit to the authors and editor.

Jan. W. Gordon

Patch-Clamp Applications and Protocols, edited by A.A. Boulton, G.B. Baker and W. Walz, 1995. Humana Press Inc. Totowa, NJ, USA, 1995. viii + 316 pp. \$89.50. ISBN 0-89603-311-2.

Written by leading experts from around the world, this book comprehensively reviews the latest on the patch-clamp technique. Contributors offer practical advice on how to overcome the problems encountered in performing and analyzing patch-clamp experiments. Most of the different chapters provide all the information needed to set up and carry out any of the procedures without having to use other resource books.

This book can be divided into three sections. The first section summarizes some basic concepts on the fabrication and properties of patch pipets, cell-free ion channel recordings and on whole-cell patch-clamp recordings. The chapter on 'Patch-Clamp Electrode Technology' reviews, in great detail, the methods used to obtain low-noise whole-cell or single channel recordings. A particular emphasis is given to the choice of the glass used in electrode fabrication. The chapter on 'Cell-free Ion-Channel Recording' gives an excellent description of the methods and problems encountered in performing inside-out membrane patches (like problems of channel rundown and problems of diffusion to the ion channel in the patch); it also provides a list of tips and hints for performing a successful experiment.

The second section contains more specialized applications of the patch-clamp technique. There are three chapters that illustrate the use of different methods of controlling the composition and concentration of the solutions inside and outside the patch-pipet. The chapter on the perforated patch-clamp technique gives a brief but informative review on the different protocols (ATP, nystatin, amphotericin) used to

permeabilize the membrane patch. Hamill and McBride describe a novel variant of the patch-clamp technique: the pressure clamp method. This method allows the application of precise and rapid suction or pressure steps to membrane patches and whole cells and has been recently used to study mechano-gated ion channels. The chapter on the 'Loose Patch-Clamp technique' gives a useful overview on the technique, showing its unique features over other methods and pointing out possible sources of errors and artifacts. There is also a chapter devoted to the use of the patch-clamp technique on more intact preparations such as brain slices.

The last section contains protocols on the combination of patch-clamp and molecular biological techniques. The chapter on single-cell RT-PCR gives a detailed description of the technical steps involved in this procedure. The authors show that the presence of different types of mRNA (and splice variants) can be analyzed within a cell after patch-clamp recording and, in some instances, correlated with the functional properties of the cell. The chapter on receptor/ion-channel expression gives hands-on procedures on oocyte DNA or RNA microinjection, two-electrode voltage clamp and oocyte patch-clamp.

Overall, this book will be an extremely useful tool for electrophysiologists and advanced students who wish to acquire practical skills and first-hand experience in the patch-clamp technique.

Andrés F. Oberhauser

Introduction to Biocatalysis Using Enzymes and Micro-organisms; Edited by S.M. Roberts, N.J. Turner, A.J. Willetts and M.K. Turner, Cambridge University Press, Cambridge, 1995, xii + 197 pp. ISBN 0-521-43070-4.

This timely and comprehensive introduction to biocatalysis not only provides the reader with historical perspective and the current state of the art, but addresses issues and challenges pertinent to the practitioner. Biocatalysis has evolved as a very interdisciplinary field, encompassing microbial biochemistry, enzymology, organic chemistry, and chemical engineering. More recently, the tools of recombinant DNA technology have added significantly to the field. This development has greatly expanded the range of enzymes available for study, the ease of studying new enzymes, and the potential to engineer novel features into existing biocatalysts. Overviewing this interdisciplinary and rapidly expanding field for the non-practitioner presents a unique challenge to the authors. Furthermore, since academic and industrial practitioners bring different perspectives and focus to this field, a comprehensive treatise needs to balance contributions from these sectors. These four experienced and well known scientists have handled this challenge very well.

The text consists of six chapters divided into four sections: a historical perspective (Chapter 1); an introduction to biocatalytic systems available and how to choose them (Chapter 2); reviews of biocatalyzed reactions arranged by three general reaction types –

hydrolytic, redox, and C–X bond forming (Chapters 3–5); and industrial scale applications of biocatalysis (Chapter 6).

In their introductory chapter, the authors place the recent surge of interest in biocatalysis (since the mid 80's) in proper historical perspective as a 'reconvergence' of the fields of synthetic organic chemistry and biochemistry which had diverged earlier to pursue separate agendas. Various examples of key biochemical technology developed early in the century are highlighted, including several which integrate biotransformations and organic synthesis. The five-step Reichstein process, developed in the 1930's for the synthesis of ascorbic acid, remains today as the primary industrial process for this important vitamin.

The second chapter addresses many of the practical aspects of biocatalysis, and is geared towards the 'non-microbiologist'. Topics include enzyme classification, enzyme cofactors and cofactor recycling, criteria for choosing between a whole cell versus 'free' enzyme system, and immobilization of enzymes and cells. The authors use the well studied Baeyer–Villiger type biooxidation to outline how a biocatalyst is chosen, where microorganisms can be obtained, and how they are grown, induced, and optimized for a given biotransformation. Since