

Foreword

Mutations with an effect on coat color or behavior were recorded by mouse fanciers well before the science of Genetics was established. They were curiosities, occurring by chance at very low frequency, and their main advantage was to make the mouse an even more interesting pet animal with many phenotypic variations that could be produced in different combinations by breeding.

Over the last century, mouse geneticists, especially the most sagacious, collated and studied a great variety of mutations, many of them exhibiting neuromuscular, eye or skeletal defects and abnormal fur or coat colors. These mutations were discovered either spontaneously, in the nucleus of inbred strains, or as side-products of the many experiments that were undertaken to assess the genetic risks associated with the use of nuclear radiations. All have been extremely helpful, contributing for example, to the development of the genetic map. A few of these mutants have also been used as animal models for human diseases while others, such as the nude or SCID mice, which can both permanently accept a variety of grafts including xenografts, were and still are used as tools for research in immunology or oncology.

More recently, by taking advantage of the exceptional mutagenic activity of ethylnitroso-urea, programs aimed at the mass-production of new mutations have been undertaken in several laboratories worldwide. With such on-going programs, mutations are no longer rare events occurring spontaneously, but random hits still occur in the mouse genome. Their phenotypes can be studied in great detail but the characterization of the molecular defect will necessarily follow (forward genetics).

With the development of highly efficient techniques of genetic engineering in pluripotent embryonal stem cells (ES cells) and the availability of a nearly complete sequence of the mouse genome, the situation has changed dramatically. Here again, large projects involving a network of laboratories have been undertaken with the aim of producing a very large number of knockout mutations, ideally one in every gene of the mouse genome, and it is likely that these projects will reach their goal within the next five years. Geneticists will then have at their disposal a collection of ES cell libraries, with up to 20,000 genes potentially inactivated. Any genetic defect can then be accurately identified but the main problem will then be to describe the phenotype of the mutant mice as precisely and comprehensively as possible.

The reason for developing such projects is quite clear: producing many mutations and phenotyping them very precisely is the best and most logical way of as-

sessing the function of the genes in the mouse genome. Indeed, when a gene becomes non-functional after a mutation has occurred, careful comparison of the mutant and normal phenotype in addition to taking into account the molecular defect generated by the mutational event, is an excellent (not to say the best) method of assessing the function(s) of the gene in question. In short, the production of new mutations and the precise phenotyping of the mutant genotype are the two sides of the same coin.

Geneticists have worked out many strategies for the efficient production of new mutations in the mouse genome using either chemical mutagenesis or gene trapping or gene targeting but until recently phenotyping was not their main concern and as a result has received less attention. In other words, whilst it was technically possible to inactivate almost any gene in the mouse genome, there was limited scientific interest in the subsequent analysis of the resulting phenotype as the relevance of any data thus obtained was thought to be questionable. Indeed, many knockouts produced over the last 10 years in genes which were thought to be extremely important, were reported to be phenotypically "normal" to the great surprise of their creators!

There is a wide range of difficulties associated with the process of phenotyping. Although it is easy to detect and describe a cerebellar defect in the mouse or a disorder which leads to the animal losing its fur after a few days, it is more difficult to detect an inner ear defect with a relatively late onset or a very subtle degenerative disorder of the retina and it is virtually impossible to detect the phenotype of certain mutations in genes involved in the innate mechanisms of defense unless a specific challenge test is carried out to reveal the mutation. The situation is even more complex when modifier genes in the genetic background interact with the pathology of the mutant allele.

This book, edited by Professor Martin M. Hrabe de Angelis in cooperation with Steve Brown and Pierre Chambon, is original and the timing of its publication is opportune. It consists of 13 chapters, all written by expert scientists who are members of the EUMORPHIA consortium and work in different research institutes across Europe. This volume describes in detail a series of screens known as EMPReSS (European Mouse Phenotyping Resource for Standardized Screens) that encompass more than 150 standard operating procedures (SOPs) covering all the main body systems of the mouse.

This book will definitely be of major interest to those creating or using a variety of mutant mice and the authors must be warmly thanked for this initiative.

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Jean Louis Guénet

Preface

The speed with which information regarding mammalian genomes has accumulated over the last few years is remarkable. Yet, despite this wealth of information, its immediate use in the diagnosis and therapy of human diseases is limited since only a small fraction of mutations causing congenital malformations or other human diseases has been identified.

Animal models are essential to the understanding of the genetics and pathogenesis of human diseases. The mouse is intensively used as a model system because of its similarity to humans in genome organization, development, biochemical pathways, and physiology. Mouse models have been the key to unraveling several fundamental scientific findings which are important for understanding the molecular mechanisms underlying human diseases in addition to the development and testing of drugs and therapies. Specific advantages of the mouse as a model system include:

- The genome is 90% identical to the human genome.
- It is possible to alter the genome in the mouse using gene-driven and phenotype-driven approaches and to produce models of human diseases, including genetic diseases.
- Alteration of the mouse genome may also produce changes in the normal functioning of organs, systems, and behavior, giving insight into the mechanisms behind their normal function and possible treatments for malfunction.
- The mouse model is used for drug screening and testing of therapies, including gene delivery and gene therapy.

The bottleneck in the process of establishing suitable mouse models is quite often appropriate phenotyping. From my own experience as a postdoctoral fellow, phenotypic analysis of "my" mouse mutants were focused on very specific organ systems and their function.

This strategy was successful and unraveled several important functional aspects of genes but at the same time I was not able to detect additional phenotypic alterations in the very same mouse model. These additional alterations were caused by the pleiotropic effect of the gene of interest. I simply missed additional alterations because I did not look for them or because of the lack of equipment and experience in specific methods used in other areas of research.

Triggered by this experience and the expertise in phenotype-driven forward genetics screens the idea of the German Mouse Clinic was born. The German

Mouse Clinic is a unique platform for the comprehensive standardized phenotyping of mouse lines. Fourteen laboratories specializing in different areas of research, work under one roof and measure over 240 parameters in every mouse line and as a result many new findings have emerged. For almost all lines, including well-known mutant mouse lines, new phenotypes have been identified. This confirms the power and feasibility of standardized comprehensive phenotyping.

The German Mouse Clinic works in close collaboration with pan-European projects such as EUMORPHIA and EUMODIC. Together with my colleagues Steve Brown and Pierre Chambon we were able to bring together experts in the field of mouse functional genomics to assemble a book that presents a wide set of standardized phenotyping assays in 13 research fields.

This book should be seen as a starting point rather than as an end-product since mouse phenotyping will be developed further over the coming years and additional chapters and research fields such as "genome-environment interaction" might be added in future editions.

Implementation of the "German Mouse Clinic" led to a unique platform for comprehensive phenotyping. Baselines for more than 240 parameters have been established, and "Proof of Principle" has been shown in several mouse lines; for example, through the GMC an additional severe metabolic disorder was demonstrated in the mouse line ABE17 which was previously known only as a neurological model for prion disease. Comprehensive phenotyping was essential in the discovery of this additional feature, which will impact upon the interpretation of affected pathways. Japan, the USA, and other countries in Europe are implementing organizations similar to the GMC, underlining the need for these enterprises. The realization of the GMC was only possible with substantial financial support from the NGFN and the GSF. The GMC has already produced important scientific results through the isolation and characterization of various mouse models. In the lung function screen, we have built a unique database of reference values regarding the phenotypic variance of respiratory function in inbred mouse strains. We have been able to detect strong inter-strain variance (e. g. a factor of 3 for lung compliance), and a high genetic-to-total variance suggests a significant genetic contribution to phenotypic variability. Mouse strains with an obviously unfavorable lung function, which should be prone to lung diseases, may serve as ideal animal models.

However, the phenotypic analysis of mouse mutants is often focused on the individual research interests of the particular laboratory or limited to specific tests because of the lack of equipment and experience of specific methods in other research areas.

In order to take better advantage of the existing mutant mouse lines and to provide the scientific community with a platform for systematic, standardized, and comprehensive phenotyping of mouse models, we have established the German Mouse Clinic (GMC) at the GSF in Munich. We have brought together experts from different institutions (Universities of Bonn, Marburg, Munich (TU and LMU), GBF) to work side by side in one building. Within NGFN 1, the coordinating team of the GMC and the GMC staff built up the German Mouse Clinic in a concerted effort (the set-up of the laboratories, establishment of a unique and comprehensive

primary screen, standardization of methods, etc). Because the GMC is unique in its concept and organization, it sets standards for SOPs and comprehensive analysis of mouse models. The phenotyping platform covers the research areas of dysmorphology, behavior, neurology, ophthalmology, clinical chemistry, immunology, allergy, nociception, molecular phenotyping, lung function, energy metabolism, and pathology and is well equipped with the newest technologies (e.g. microcomputer tomography, blood auto-analyzer). We offer phenotypic analysis on the basis of scientific collaboration and have the facilities to house guest scientists in dedicated guest laboratories.

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Martin Hrabé de Angelis