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Mini Review

Mouse phenogenomics, toolbox for functional annotation of human genome

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Mouse models are crucial for the functional annotation of human genome. Gene modification techniques including gene targeting and gene trap in mouse have provided powerful tools in the form of genetically engineered mice (GEM) for understanding the molecular pathogenesis of human diseases. Several international consortium and programs are under way to deliver mutations in every gene in mouse genome. The information from studying these GEM can be shared through international collaboration. However, there are many limitations in utility because not all human genes are knocked out in mouse and they are not yet phenotypically characterized by standardized ways which is required for sharing and evaluating data from GEM. The recent improvement in mouse genetics has now moved the bottleneck in mouse functional genomics from the production of GEM to the systematic mouse phenotype analysis of GEM. Enhanced, reproducible and comprehensive mouse phenotype analysis has thus emerged as a prerequisite for effectively engaging the phenotyping bottleneck. In this review, current information on systematic mouse phenotype analysis and an issue-oriented perspective will be provided. [BMB reports 2010; 43(2): 79-90]

INTRODUCTION

The laboratory mouse (*Mus musculus*) is a powerful tool for basic research and plays a key role in human genetics. It has become one of the best animal model species in biomedical research today because of its similarity to humans in anatomy, physiology and genetics (1). Indeed, over 95% of the mouse genome is similar to our own, making mouse genetic research particularly applicable to human disease. Furthermore a great advantage using mouse as model system is easy mutagenesis using transgenic and gene knockout technology with abundant genomic information (1, 2). Mice have a short generation time with an accelerated life span, they are prolific breeders and can be maintained in a laboratory situation. These advantages can allow us to save the costs, space and time required to perform research manageable including control of the environment, which is a major contributory factor that is extremely difficult to control for in studies of complex human diseases (3). It was already well known that gene function and physiology are so well conserved between mice and humans, as they are both mammals and contain similar numbers of genes, which are highly conserved between the species. Despite these clear benefits, the greatest issue associated with using the mouse is the availability of pure, inbred lines and the ability to develop new strains by genetically engineering technology (3, 4). There is a wide range of inbred strains of mice available that allows rigorous genetic control within a strain yet genetic variability between strains. Genetically engineered mice (GEM) have become essential tools in both mechanistic studies and drug development (5).

Many human diseases can be modeled through the alteration of a specific gene central to a normal biological process. Despite advances in molecular technologies that have increased our ability to create mouse models of inherited human conditions through targeted mutagenesis and transgenesis, the way finding interesting biological changes in GEM is fragmented, and much is not publicly available. Rapid and systematic phenotype validation of mutant mouse can allow us to understanding the function of human genes. This review provides an overview of the available resources and tools in mouse genetics and systematic mouse phenogenomic approach for functional annotation of human genome.

History of mouse genetics

Laboratory mouse

Mouse fanciers of the late 19th and early 20th centuries in Asia and, later, Europe and America were the origin of most laboratory mice of today. The history of mouse genetics might have begun in the late 1800. DBA (Diluted Brown), the first inbred strain of laboratory mice was created in 1903 by Castle and his colleagues. (6, 7). Little also founded the C57/C58 BL

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strains and these strains were moved to and developed in The Jackson Laboratory in 1929 (8). Because of their origins in the mouse fancy trade, laboratory mouse strains are a genetic mix of four different subspecies: *Mus musculus musculus* (eastern Europe), *Mus musculus domesticus* (western Europe), *Mus musculus castaneus* (Southeast Asia), and *Mus musculus molossinus* (Japan) (9-11). In 1916, Taking advantage of partially inbred lines, Little and Tyzzer found that grafts from parent to F1 were accepted, whereas reciprocal grafts were rejected (12). Sub-sequent crosses gave a minimum estimate of the number of histocompatibility loci. Out of this grew the great work on the H-2 loci, leading to a Nobel Prize for Snell (7, 13).

Genetic mutants

Since the first establishment of inbred mouse, DBA/2, several inbred mice have continuously developed. They showed very unique phenotypes responding to human disease such as DBA, NOD, NZB, and NZO. Specific phenotypes found in inbred mice might be due to their polygenic genetic trait. They have provided us useful information to identify disease candidate genes from human (14). Also monogenic mutants in mice have been used to identify genes that elucidate biochemical pathways pertinent to common human diseases. The best example of these is the cloning of the genes underlying single-gene mouse models of obesity : obese and diabetes (4, 15-17). Since Cuénot reported that the mouse mutant Agouti yellow was based on a Mendelian trait in 1905, a collection of approximately 1600 spontaneous mutations (covering 800 genes) in the mouse are available, which are mainly maintained and disseminated by the Jackson Laboratory, USA (18, 19).

In order to identify a specific gene, genetic marker is essential. Every genetic system ultimately rests on the availability of a useful set of "markers," genes or DNA sequences whose alleles can be conveniently typed in crosses to track the inheritance of chromosomal regions. The first genetic markers of mouse were morphological mutants whose changes in coat color or skeletal characteristics were obvious to the naked eye. And then, DNA sequence-based technology was introduced as a molecular marker in mouse genetics. The first molecular markers were restriction fragment length polymorphisms (RFLPs), simple sequence length polymorphisms (SSLPs), and single nucleotide polymorphisms (SNPs). Now several million SNPs are available in the mouse and human genomes, providing an inexhaustible supply of densely spaced markers. It can allow us to know their physical as well as genetic location. With the development of powerful genetic markers, mutant genes in mice could be cloned and identified (4, 7, 20). Since Coleman and his colleagues found obese mutant in mouse during their breeding experiments in 1952, however we should wait for more than 40 years to make a cloning real proteins coding obese (leptin) by Friedman (15, 16). Douglas Coleman and Katrina Hummel showed the importance of the genetic background of the modifier genes in inbred mice. They found that the *obese* (*Lepob*) and *diabetic* (*LepR*) mutations, which produced quite different phenotypes in the two inbred strains in which they arose, actually produced the same phenotype if they were present in the same genetic background. It may contain on the expression of a single major locus determining a pathological trait (4, 7, 21).

Genetically engineered mice

As the human genome project approaches completion, the challenge for biologists is to develop approaches for the systematic annotation of human genome function. The analysis of organisms that carry genetic mutations has been fundamental to research into human disease states. There are two types of ways to induce mouse mutagenesis (Fig. 1). This concept will be introduced and discussed with more detail information in next chapter. Methods for engineering the mouse genome have been developed for all fundamental mechanisms of mutagenesis, accessing the germline genome through gametes, zygotes and embryonic stem (ES) cells. The Nobel Prize in physiology and medicine in 2007 was awarded to Drs Mario Capecchi, Martin Evans and Oliver Smithies for their work on genetic modifications in mice using embryonic stem cells. They have developed technologies that allow any gene in the nucleus to be either partly deleted (knockout) or inserted (knockin) whilst in the germline, and have gone on to generate mice that carry and express the engineered gene (4, 5, 22).

Current technology also permits insertion of 'reporter' genes into the knocked-out gene, which can then be used to determine the temporal and spatial expression pattern of the knocked-out gene in mouse tissues. Such marking of cells by a reporter gene facilitates the identification of new cell types according to their gene expression patterns and allows further characterization of marked tissues and single cells. Tissue-specific regulation of gene expression including transgenesis and knockout is possible with Cre-loxP recombination system. Cre is an enzyme that catalyzes the removal of a DNA segment that lies between two specific 34 base-pair sequences, termed *loxP*. The system involves creation of two separate lines of

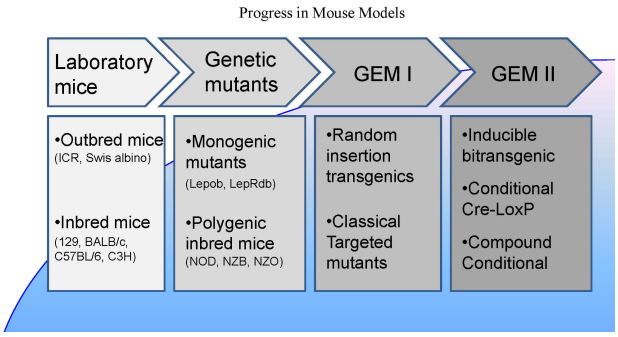

Fig. 1. Progress in mouse models.

mice. In one line, the gene to be knocked out is bracketed with two *loxP* sequences by homologous recombination. The other line has Cre sequences targeted to tissue specific promoters inserted into the genome of the cells of interest. The two lines are then cross-mated to produce a line that has knock out gene sequence in the cells of interest. The gene sequence remains present and functional in the other tissues, although it continues to be bracketed by two *loxP* sequences (23). Also targeted gene expression could be regulated in mouse by turning them on and off for specific time periods with inducible promoters such as tetracycline. Manifestation of a genetic alteration is needed only for the period of time necessary to accomplish a study. By using a tetracycline promoter, interested gene expression can be normal while the animal does not receive tetracycline. Gene expression can be stopped by feeding the animal tetracycline and restored by discontinuing administration of the tetracycline (24). Now other systems that allow the controlled expression of transgenes are also available such as doxycycline-inducible promoter and ecdysone-inducible system (25, 26). Currently to meet the growing demand for conditional mutant models, internationally coordinated initiatives have been established for the systematic generation of mouse mutants on a large scale using various strategies (27). Detail knowledge and information would be available from several articles (3, 5, 22, 23, 28).

Two major strategies for developing mouse mutagenesis

Reverse genetics

A major challenge in post-genomics is the systematic determination of mammalian gene function. There are two ways to define *in vivo* gene function in mouse: reverse and forward genetics (Fig. 2). A variety of mouse mutagenesis technologies, both reverse and forward genetic approaches, are being used to underpin systematic and comprehensive approaches to mammalian gene function studies. These two concepts share to annotate the single gene function under the condition of loss or gain of gene function. Reverse genetics is an approach to discovering gene function by analyzing the phenotypic effects from altered gene sequence. This investigative process proceeds in the opposite direction of so-called forward genetic screens of classical genetics. Reverse genetics refers to the process by which a gene is modified or altered first, after which the phenotype that results from this genetic changes is studied. This was also called as genotype-driven approach. While reverse genetics seeks to find what phenotypes arise as a result of specific gene, forward genetics seeks to find the genetic alterations causing phenotype changes (4). At present, a number of mutagenesis strategies based on ES cells are used, all of which use homologous recombination to modify genes in their original location, producing either 'knock outs' to cripple gene function or 'knock ins' to introduce an altered gene version (27). Gene-targeting technology based on ES cell biology is the most powerful technology for making knockout mice. Gene trap technology has been used for its speed and powerful gene silencing effect from large scale GEM production program and consortium. Gene trapping is the most cost-effective way to create gene-specific mutations. Various gene trapping laboratories have coordinated their activities in the International Gene Trap Consortium (http://www.igtc.org.uk/) and mutations in more than one-third of genes are now available (28-32). Recently RNAi technology is widely employed for making a gene knock down. The gene targeting and gene trap methodology, though important, is beyond the scope of this article and is reviewed elsewhere (28-32). Reverse genetic strategies have been truly powerful in understanding molecular pathways, since the phenotypes induced by gene ablation or knockout experiments represent the most effective route to acquire information on gene function.

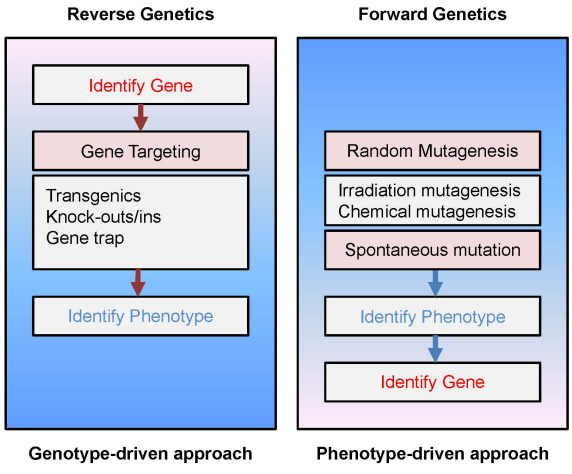

Fig. 2. Forward and reverse genetic approaches in mouse.

Forward genetics

In forward genetics, the starting point is typically a mutant phenotype which molecular basis of the underlying genetic alteration should be revealed. In reverse genetics, the gene is already known and its function is elucidated by its or over-expression or depletion and the subsequent phenotyping mutant mice (19). A limitation of the gene driven approach for making knockout or knockin mouse is that it can only examine genes which is already thought to regulate the physiological process under study. If the assumptions about the gene function are not correct then much time and money would be spent producing a mutant mouse. It cannot be a good model for the human disease under investigation. To address this problem, a scientist can start to identify the interesting phentypes first and then isolated the altered gene or genes responsible for the phenotypes second without making any a pre-assumptions on gene function (4). This way is very similar with physicians for making a diagnosis for disease. Despite the power of the tar-

geted mutation technology, genotype-driven approach, this approach has limitations for large-scale screening. The creation of targeted mutations is still laborous way and needs a long time for successful production of "live mutated mouse". More importantly, if a scientist is primarily interested in a certain phenotype, a phenotypic screen should be appropriate (33). Initially, a large number of these mouse models resulted from spontaneous mutations that yielded striking phenotypes, such as the obesity-associated metabolic problems, caused by mutations in the genes encoding leptin and its receptor (15, 16). However, due to the low rate of spontaneous mutations of 1 x10-6 per locus, the number of interesting and relevant mutant mouse lines was restricted, and for this reason different natural or synthetic compounds such as X-radiation and a large number of chemicals were systematically tested for their efficacy to induce mutations at a higher frequency (19). Before the advent of molecular biology, many important discoveries were derived from mutations that arrived spontaneously in inbred mouse colonies. However, to generate significant numbers of mutant mice more efficiently, a number of large-scale N-ethyl-N-nitrosourea (ENU) mutagenesis programs were instigated ten years ago (34, 35). This forward genetic approach became possible once the mouse genome had been sequenced and the identification of Nethyl-N-nitrosurea (ENU) had been identified as a potent means of inducing random point mutations across the genome (36). In an effort to provide a broader supply of spontaneous-like mutations, strategies including chemical and radiation mutagenesis of sperm or ES cells have been adopted, with ethylnitrosourea (ENU) being the mutagen of choice (37, 38). The potential of ENU mutagenesis in the mouse has been widely accepted as one of the major strategies of classical forward genetics regarding the systematic analysis of gene function (19). Although with ENU mutagenesis the generation of mutant mice is fast, the real success of this technique rests in the implementation of validated phenotyping resources relevant to the trait/disease of interest. Once a mutant phenotype has been identified, then the underlying gene should be identified to make the connection between gene and function (38). Gene-driven mutagenesis technologies along with ENU phenotype-driven mutagenesis approaches provide the possibility of delivering a mutant for every gene in the mouse genome.

Systematic screening system for mouse phenotype

The ultimate purpose of making mutant mice is to discover the function of human genome through modeling human disease. The phenotype analysis of each mutant mouse can allow us to define the function of human genome. Determining and measuring phenotype has been a goal of all biologists, as the phenotype is an indicator of how an organism functions in different environments and under various challenges and insults (39). Phenogenomics is the functional annotation of human genome by the phenotype assessment of mutant mice. The recent improvement in mouse genetics has now moved the bottleneck in mouse functional genomics from the production of GEM to the systematic phenotyping analysis of GEM. Enhanced, reproducible and comprehensive mouse phenotype analysis has thus emerged as a prerequisite for effectively engaging the phenotyping bottleneck. The challenge for mouse geneticists is to develop approaches that will provide comprehensive phenotype datasets for these mouse mutant libraries (38, 40). To make an animal model for human disease, many of the same clinical diagnostic assays that would be carried out on the human should be carried out on mice. A careful necropsy, including histology when gross lesions are found, should be a component of any basic phenotyping protocol. This is particularly important for animals that are observed to be ill or die unexpectedly. It is possible to follow these methods with more specialized testing to assess particular abnormalities or to assess the potential utility of the mutant line for a particular research area (29). Large-scale mutagenesis screens were carried out in the mouse, which required phenotype screening assays that were rapid, robust and cost-effective. Such screens allowed for the assessment of a wide variety of phenotypes in many mouse strains and mouse mutants. The information from studying these GEM can be shared through the International Collaborative Program and Consortium for Large Scale Production of GEM. However, there are many limitations in utility because not all human genes are knocked out in mouse and they are not yet phenotypically characterized by standardized ways which is required for sharing and evaluating data from GEM (41). If a mutant mouse doesn't have the phenotype that the laboratory expected, it might be useless job. This approach would have high risk because GEM production needs long time and big money. However every single human gene has an impact on regulating normal physiology. A broad phenotype analysis in mutant mice can provide us to define "invisible" gene function. Bcl-X homozygote KO mice are dead at the mid-gestation stages result from massive cell death of hematopoietic cells and neurons. A broad phenotype assessment of the Bcl-x mutant mouse would have detected the platelet abnormalities. The identification of this abnormal response has led to a field dedicated to understanding the role of Bcl-xL isoform on regulating clock for platelet lifespan (42). Typically knockout mice for a specific gene are generated in a laboratory with restricted expertise and scientific interests and in many cases mice will, understandably, only be examined in certain phenotypic areas. When nothing abnormal is detected, it is difficult to draw any conclusions (43). However, most labs are interested in genes involved in specific processes or diseases. They are unlikely to invest effort to find a phenotype for a mutant that is outside of their area of expertise or interest, with neither the time, money nor scientific interest to justify the effort. One way to solve this is to create large-scale phenotyping platforms for mice that are accessible to the scientific community but accomplished by specialists. With improved phenotyping tools, new phenotypes can be found even in heterozygous mutants (41, 44).

To develop and offer a large scale standardised and comprehensive phenotypic analysis of mouse mutants from various sources (transgenic mice, knockin/knockout mice, spontaneous mutants and ENU-mutagenesis). Standardised phenotypic screening tools should be provided with large scale. There are several large-scale mouse phenotype center including German Mouse Clinic (Germany), Institute for Mouse Clinics (France), and Toronto Center for Phenogenomics (Canada). They are designated to the areas of behaviour, bone and cartilage development, neurology, clinical chemistry, eye development, immunology, allergy, steroidmetabolism, energy metabolism, lung function, vision and pain perception, molecular phenotyping, cardiovascular analyses and pathology (Table 1) (39, 43, 45, 46). It is impossible to list all the phenotypic measurements that can be evaluated in mice and interested readers should consult several web pages of the large scale phenotype center for the assays currently in use (Table 2). In general, phenotyping assays can be classed as basic and disease-related phenotypes. The goal of basic assays is to uncover traits of interest in a relatively easy, reliable, noninvasive and efficient way. Basic assays identify outliers that can then be subject to more intensive in-depth secondary screens (disease-related phenotypes), which is closely related with human disease and might require minimally invasive instrumentation (29). Standard protocols for phenotyping mice have been developed in Europe through a program called EUMORPHIA (European Union Mouse Research for Public Health and Industrial Applications, www.eumorphia.org). In addition to providing detailed phenotype information for a number of mouse strains, groups within EUMORPHIA compared assays across laboratories for their replicability and robustness, to develop a database of over 100 standard operating procedures (SOPs) that can be used for phenotyping every major organ system, performing pathology, and assessing gene expression. (47-49). German Mouse Clinic has offered a standardized broad phenotyping platform for mutant mice for the last few years, and they have found new phenotypes in 40% of the lines (43, 50). Despite all efforts listed above in detecting a phenotype, there are numerous cases of gene inactivation where mice appear healthy and normal, although one might have expected a significant effect on phenotype based on the known function of the gene and its expression. In this case, we may apply a phe-

Table 1. Mouse phenotype assessment platform

Classification	Parameter and assays
Hematology and clinical chemistry	Complete and differential blood cell count with flow cytometer Urinalysis, Hormone assay, ELISA assays, Tandom mass spectrometry
Morphology	Gross anatomy, Histology Immunohistochemistry, in situ hybridization Micro-ultrasound, MRI, Micro-CT/Micro-PET Opthalmoscope
Metabolism/Physiology	Basal body temperature Body mass index and Bone Mineral Density by DEXA Food intake/output, Calorimetry Urine volume (24 hrs) O2/CO2 consumption, Breathing rate, GTT (Glucose tolerance test), ITT (Insulin tolerance test), Insulin clamp Cold test Meal tolerance test
Behavior/Neurobiology	Rotarod/Treadmill Light/dark transition test (anxiolytic behavior) Home-cage activity Tremor monitor, Grip strength apparatus Elevated plus mazes and O-rings, Morris water maze Open field activity, Passive avoidance test, Learning and memory paradigms Tail suspension test, Depressive behavior Tail flick test, Hot plate test, Pain responses Prepulse inhibition test, Sensory responses Conditioned fear test Auditory clickbox, Auditory brainstem response, Visual tracking drum, Visual cliff test (depth perception) Electroencephalogram (EEG) SHIRPA (SmithKlein Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment)
Cardiovascular	Invasive and non-invasive blood pressure, Heart rate, Invasive LT-ventricular haemodynamics Electrocardiogram (EKG), Echocardiogram

Table 2. International resources and information on mouse genetics

GEM strain resources and archive	
IMSR, International Mouse Strain Resources	http://www.informatics.jax.org/imsr/index.jsp
FIRMe, Federation of International Mouse Resources	http://www.fimre.org
TBASE, DB for transgenic and knockout mice	http://tbase.jax.org
EMMA, European Mouse Mutant Archive	http://www.emma.rm.cnr.it
AMMRA, Asia Mouse Mutagenesis and Resources Association	http://www.ammra.info
CMMR, Canadian Mouse Mutant Repository	http://www.cmmr.ca/index.html
MMRRC, Mutant Mouse Regional Resource Centers	http://www.mmrrc.org
MMHCC, Mouse Model for Human Cancer Consortium	http://mouse.ncifcrf.gov
UK Mouse Locator Network	http://bioinformatics.cancerresearchuk.org/mouse_locator/mouse_locator.html
MGI, Mouse Genome Informatics	http://www.informatics.jax.org
Mouse Book, DB all the data from MRC Harwell	http://www.mousebook.org/index.php
CMGCC, Comparative Mouse Genomics Centers Consortium	http://www.niehs.nih.gov/cmgcc
MICER, Mutagenic Insertion and Chromosome Engineering Resource	http://www.sanger.ac.uk/PostGenomics/mousegenomics/
GEM production	
IKMC, International Knock-out Mice Consortium	http://www.knockoutmouse.org
KOMP, Knock Out Mouse Project	http://www.knockoutmouse.org/about/komp
EuCOMM, European Conditional Mouse Mutagenesis	http://www.eucomm.org/
NorCOMM, North American Conditional Mouse Mutagenesis	http://www.norcomm.org/index.htm
TIGM, Texas A&M Institute for Genomic Medicine	http://www.tigm.org/
IGTC, International Gene Trap Consortium	http://www.igtc.org.uk/
Mouse phenotype analysis consortium & center	
IMPC, International Mouse Phenotype Consortium	
EuMODIC, European Mouse Disease Clinic	http://www.eumodic.org
EuMORPHIA, European Union Mouse Research for Public Health and Industrial Applications	http://www.eumorphia.org/
EMPReSS, EU Mouse Phenotyping Resource of standardized screens	http://www.empress.har.mrc.ac.uk/
Infrafrontier-The European infrastructure in phenotyping and archiving of model mammalian genomes	http://www.infrafrontier.eu
NorIMM, Nordic Infrastructure for Mouse Models	http://www.norimm.org/
MPD, Mouse Phenome Database	http://www.jax.org/phenome
MTB, Mouse Tumor Biology Database	http://tumor.informatics.jax.org
MMPC, Mouse Metabolic Phenotyping Centers	http://www.mmpc.org
TCP, Toronto Center for Phenogenomics	http://www.phenogenomics.ca/
ICS, Insitut Clinique de la Souris	http://www-mci.u-strasbg.fr/
GMC, German Mouse Clinics	http://www.mouseclinic.de/
JMC, Japanese Mouse Clinics	http://www.brc.riken.go.jp/lab/jmc/mouse_clinic/en/index.html
GEM informatics	
I-DCC, International Knockout Mouse Consortium Data Coordination Center	http://www.i-dcc.org/I-DCC
KOMP-DCC, Knockout Mouse Project Data Coordination Center	http://www.knockoutmouse.org/about/komp-dcc
CASIMIR, Coordination and Sustainability of International Mouse Information Resources	http://www.casimir.org.uk
InterPhenome, Mouse Phenotype Database Integration Consortium	http://www.interphenome.org/

notype assessment under the changes of environments such as physical training, diet-restriction or overfeeding, irradiation and circadian rhythm (Fig. 3) (50). Currently molecular imaging can allow the non-invasive assessment of biological and biochemical processes in living subjects. Such technologies therefore have the potential to enhance our understanding of disease and drug activity during preclinical and clinical drug development (51). In mice there are numerous examples where the phenotypic effects of a mutation are influenced by the genetic background (11, 43). The various inbred strains of laboratory mice show considerable genotypic and, as a result, phenotypic variation. This can be clearly seen in the enormous amount of variation for phenotypes already analysed in inbred strains. The genetic backgrouds of GEM should be carefully considered for phenotype assessment such as physical environments (52). It was also reported that animal handling and housing conditions have an important impact on most variables measured, also provide a value range for these parameters. Minor changes in procedures can profoundly affect biological variables, underscore the importance of establishing uniform and validated animal procedures to improve reproducibility of mouse phenotypic data (53).

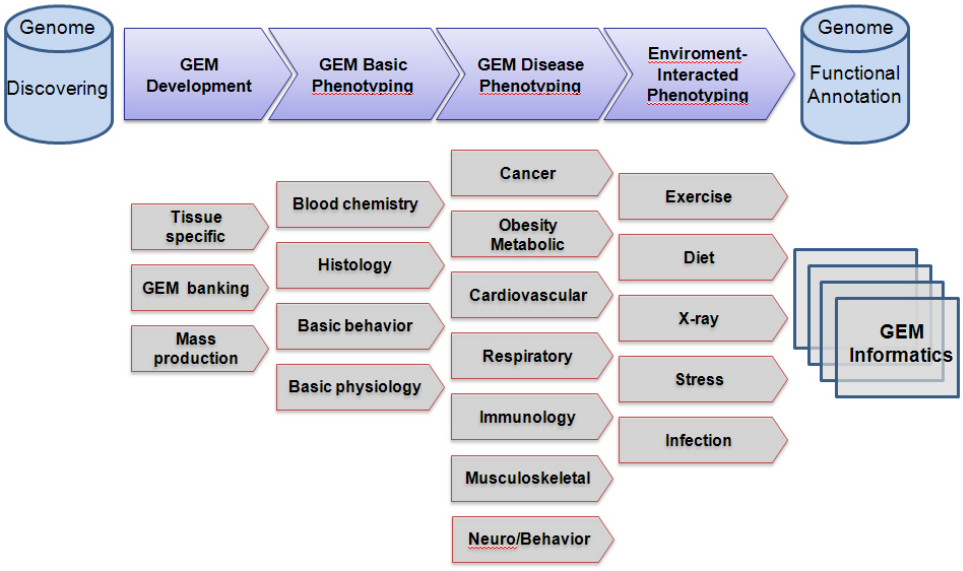

Fig. 3. Systematic phenotyping approaches for functional annotating of human genome in mouse biology.

International collaboration of mouse genetics

Currently many resources on mouse genetics are available listed in Table 2 and Fig. 4.

Large scale production of GEM

Three major mouse knockout programs are underway worldwide, working together to mutate all protein-encoding genes in the mouse using a combination of gene trapping and gene targeting in mouse embryonic stem (ES) cells. KOMP (*KnockOut Mouse Project*, http://www.knockoutmouse.org), funded by the NIH, encompasses a number of efforts that all aim to enhance the availability and utility of mouse knockout strains. The program involved the acquisition of 251 knockout strains extensive associated phenotype data, supporting the deposition of additional mouse knockout strains produced by academic researchers into repositories. The major effort was that aim to create 8500 targeted mutations in ES cells in genes that have not yet been knocked out (54). NorCOMM (*North* American *C*onditional *M*ouse *M*utagenesis Project, http://norcomm.phenogenomics.ca/index.htm) is a Canadian project, funded by Genome Canada and partners, whose mission is to contribute to the generation of a mouse ES cell resource with characterized mutations in every gene in the genome. The project's goals include the generation of 10,000 gene trap insertion clones in ES cells derived from the 129 mouse strain using conditional-ready gene trap vectors, 2000 targeted conditional mutant ES cell lines, and a toolbox of vectors and associated molecular reagents under development (54). EUCOMM (EUropean Conditional Mouse Mutagenesis Program, http://www.eucomm.org), funded by the FP6 program of the EC, combines the expertise of 11 research institutions across Europe and is coordinated by the GSF-.National Research Center for Environment and Health and the Wellcome Trust Sanger Institute. The goals of the EUCOMM project are to produce 12,000 conditional gene trap mutations and 8000 conditional targeted mutations in mouse C57BL/6N ES cells using conditional gene trapping and gene targeting approaches (55, 56). KOMP, EUCOMM, and NorCOMM have agreed to share gene lists and data to help coordinate these international efforts. The complexity and cost of such an undertaking required the deployment and coordination of resources on a global scale and close coordination of the programs is essential to achieve the desired efficiency of scale. To that end, the International Knockout Mouse Consortium (IKMC) has been officially launched based on three major global mutant mouse consortium Texas Institutefor Genomic Medicine (TIGM), has joined the IKMC. The IKMC subscribes to the principles of free and open release of data, the sharing of new mouse knockout technologies, the coordination of production plans, the coordination of public communications regarding the international effort, and the coordination of issues related to the efficient archiving and distribution of resources to the scientific community. Increased international cooperation among mouse mutagenesis programs will significantly enhance the mouse genetic resources available to the scientific community and will further our understanding of human biology and disease (57).

Archiving and banking

The Federation of International Mouse Resources (FIMRe) is a collaborating group of Mouse Repository and Resource Centers worldwide whose collective goal is to archive and provide strains of mice as cryopreserved embryos and gametes, ES cell lines, and live breeding stock to the research community (58).

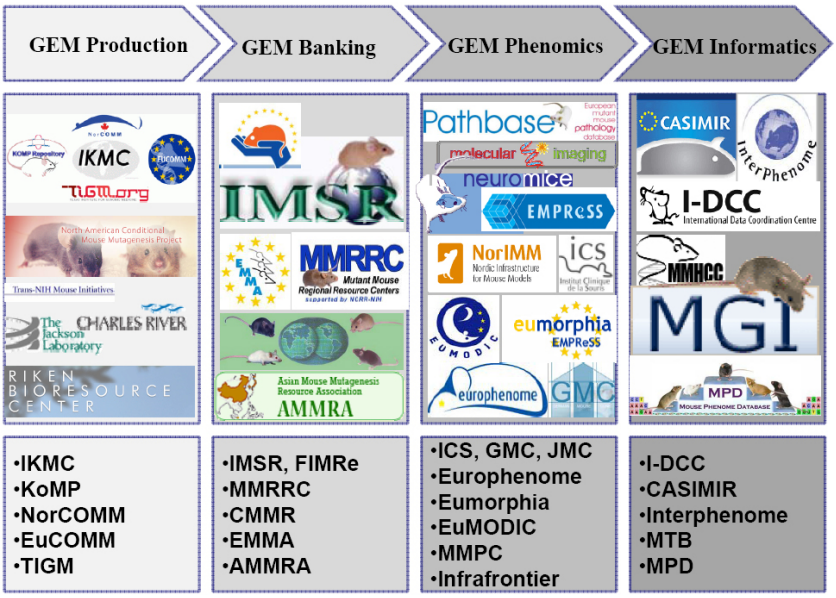

Fig. 4. International efforts for rapid development and sharing mouse resources and information.

The European Mouse Mutant Archive (EMMA) is a non-profit repository for the collection, archiving (via cryopreservation) and distribution of relevant mutant strains essential for basic biomedical research in EU. The laboratory mouse is the most important mammalian model for studying genetic and multi-factorial diseases in man. Thus the work of EMMA plays a crucial role in exploiting the tremendous potential benefits to human health presented by the current research in mammalian genetics (59). In Asia, AMMRA (Asian Mouse Mutagenesis Resources Association) was organized and shared their mice resources and information (60).

Large scale phentotyping center and consortium

There has already been considerable focus and investment on the development and standardisation of phenotyping approaches, particularly broad based primary screens, by a number of consortia including EUMORPHIA (http://www.eumorphia.org/) and EUMODIC (http://www.eumodic.org/). EUMODIC is a first step towards tackling the need for large-scale phenotyping in the mouse and the comprehensive study of mammalian gene function and its role in disease. EUMODIC will undertake a primary phenotype assessment of up to 650 mouse mutant lines. In addition, a number of these mutant lines will be subject to a more in depth secondary phenotype assessment. The EUMODIC consortium is made up of 18 laboratories across Europe who are experts in the field of mouse functional genomics and phenotyping and have a track record of successful collaborative research in EUMORPHIA. The challenge will be to use assessment tools efficiently to uncover a large range of phenotypes, and then to direct information and mutants to disease-focused groups. An International Mouse Phenotyping Consortium (IMPC) has been formed to discuss how scientists might prepare for this onslaught of mouse strains and conditions. Representatives of phenotyping centers worldwide met in Rome in December 2007 to consider the formation of an IMPC and to outline both early and longer term goals for a global phenotyping effort. The current phenotyping centers perform a dual role: firstly, undertaking systematic phenotyping of the mouse mutant catalogue; secondly, operating as clinics and providing services (at cost) to the wider biomedical sciences community in characterising mouse mutants (27, 40). EMPReSS (European Mouse Phenotyping Resource for Standardized Screens) is the product of EuMORPHIA (http://www.eumorphia.org), the largest programme to date to develop standardized phenotyping protocols. EMPReSS is a comprehensive database of validated Standard Operating Procedures (SOPs) for screens to determine the phenotype of a mouse (27, 40). The European Mouse Phenotyping Resource for Standardized Screens (EMPReSS) constitutes the first phase of this initiative and consists of a set of first line SOPs: SOPs that are simple to apply, require little specialist equipment and can be used for preliminary screening of mutant phenotypes. It incorporates 96 SOPs that cover all of the main body systems including: clinical chemistry, hormonal and metabolic systems, cardiovascular, allergy and infection, sensory function, neurological and behavioural function, cancer, and bone and cartilage systems. In addition, there are generic SOPs for histology, necropsy, pathology and gene expression. EMPReSS is a platform of individual tests, but these can also be grouped together into phenotyping pipelines. EuroPhenome (http://www.europhenome.org) was instigated as an online mouse phenotyping resource to store baseline data generated from the application of EMPReSS SOPs (47-49). More recently new mouse phenotype centers are launched even in Asia : Japane Mouse

Clinics and Taiwan Mouse Clinics (61).

Data coordination center and database consortium

The Mouse Phenome Project is an international collaboration representing five countries in both the academic and corporate sectors. Comprehensive phenotypic information on inbred mouse strains is urgently needed because the laboratory mouse, with its hundreds of inbred, specialized, and mutant strains, serves as the primary animal model for exploring genetic variation and human biology. Reliable phenotypic data are essential for realizing the full utility of genomic information that will emerge from sequencing the mouse genome. The scope of this large-scale collaborative project requires international cooperation and both academic and industrial participation (14, 62). The goal of the I-DCC is the creation of a single point of access to information on the entire mutant mouse ES collection. The I-DCC will benefit the user community by ensuring data is compiled in a standard format accessible through a single web portal. To achieve this, ambition the I-DCC brings together leading scientists in mouse genetics and genome informatics who have devoted much of their career to the generation of resources for the mouse genetics (www.i-dcc.org). MICER (The Bradley Lab - Mutagenic Insertion and Chromosome Engineering Resource MICER) provide information on mutagenic insertion and chromosome engineering resource in mice. The aim of this resource is to provide vector sequences and information on using MICER vectors for generating knockout mice, and for chromosome engineering (63).

Ethical issues in GEM

The number of GEM used in biomedical field has been significantly rising and so sharing animals and information from animal experiments are becoming an increasingly important means of both reducing and refining animal use to minimise suffering. The increase in use of GEM presents many challenges in reviewing protocols and providing care. Identification and resolution of any welfare problems is a responsibility that should be shared by institutional animal care and use committee (IACUC), veterinarian and research scientists. To share information and resources of GEMSs may help reduce the increasing number of GEM used in experiments. The new guidance in the form of a booklet "*Sharing and archiving of genetically altered mice: Opportunities for reduction and refinement*" were launched by MRC, Cancer Research UK aimed at scientists working with animals. The number of GEM used in scientific procedures has risen consistently over recent years and this trend appears to be continuing. Whilst the use of these mice has enabled significant advances in science, this increasing use raises practical, scientific and ethical issues. As more novel GEM strains are produced, and their use becomes more widespread, the sharing and archiving of information and material related to these animals is becoming an increasingly important means of both reducing animal use and of refining procedures to improve animal welfare (64).

Application of GEM for drug development and safety test

Prospective mining of the druggable genome is being catalysed by large-scale mouse knockout programs combined with phenotypic screens focused on identifying targets that modulate mammalian physiology in a therapeutically relevant manner (65-68). Gene targeting approaches where gene activation is depleted by homologous recombination have become the main technologies for target discovery and validation in the last couple of years (19). Also Carcinogenicity testing increasingly uses GEM which offer improved systems for evaluation of compounds. Mice with activated *myc, ras*, and *neu* oncogenes provide test systems with increased sensitivity for the detection of carcinogenic chemicals (64). Big advance in genetic manipulation of mouse can be applied to study the genetic and molecular basis for carcinogenesis and may contribute to improving the scientific basis for human cancer risk assessment. Several transgenic and knock-out mice in oncogenes and tumor suppressor genes showed dramatic cancer-related phenotypes. Routine carcinogenicity testing in mice is typically performed in Swiss-derived CD mice and F1 hybrid mice (so called NTP mouse) obtained from crossing female C57BL/6 and male C3H/HeN mice. However it needs long time and many resources including animals and test samples and so it might be one of bottle for developing new drugs. Four strains of GEM have been proposed for use as complements to or alternatives for the conventional 2-year rodent bioassay. The lines proposed bear altered forms of genes that are known to be mutated in certain human cancers. The Tg.AC line carries an activated form of the v-Ha-ras oncogene, the p53 heterozygote KO possesses an inactivated copy of the p53 tumor suppressor gene, the rasH2 transgenic mouse bears the human c-Ha-ras gene, and the XPA heterozygote KO mouse is deficient in nucleotide excision repair (69). The ras family of oncogenes is activated by point mutations, and such activation has been implicated in various human and animal cancers (70). RasH2 transgenic mice exposed to numerous genotoxic carcinogens for ~6 months exhibited a more rapid onset and higher incidence of malignant tumors than did control non-transgenic mice (69, 71). The Tg.AC mouse was constructed by zygote injection of a gene construct containing the z-globin promoter region fused to an activated form of the ras oncogene (v-Ha-ras) and a 3'' SV40 polyadenylation signal (72, 73). TPA-induced skin papillomas and squamous cell carcinomas (SCC) in Tg.AC mice were significantly malignant in the absence of an initiating carcinogen exposure suggested that the initiation/mutation stage of skin multistage carcinogenesis is replaced by the presence of the transgene and that the tumor response can be driven by the application of a promoter. Mutation of the p53 gene is one of the more common genetic alterations found in a variety of human cancers, including those of the colon, lung, esophagus, liver, and breast. p53 KO

mouse appears to be susceptible to tumor induction by mutagenic but not by nonmutagenic carcinogens possibly reflecting the role that the p53 gene product plays in responding to DNA damage (74). The p53 heterozygous KO mice are predisposed to spontaneous and chemical-induced tumors. The p53 heterozygous KO mice could have been used as alternative screening system for cancer risk assessment (75). Patients with the hereditary disorder xeroderma pigmentosum (XP) are predisposed to develop skin cancer where the skin is exposed to sunlight. A defect in the nucleotide excision repair process is responsible for this cancer-prone phenotype. XPA KO mice have an increased susceptibility to ultraviolet light-induced and dimethylbenzanthracene-induced skin cancers (76). The use of GEM for carcinogenicity testing should be thoroughly evaluated by examining their responses to well-characterized reference compounds under standardized conditions in many laboratories. Also new GEM strain should be applied for carcinogenesis test to compensate the weakness of data obtained from previous GEM including Tg.AC, rasH2, p53 heterozygote KO, XPA heterozygote KO mice.

Summary

Understanding the functions encoded in the mouse genome will be significantly essential to an understanding of the genetic basis of human disease. To achieve this, it will be essential to be able to characterize the phenotypic consequences of variation and alterations in individual genes. Phenogenomics is the functional annotation of human genome by the phenotype assessment of mutant mice. The recent improvement in mouse genetics has now moved the bottleneck in mouse functional genomics from the production of GEM to the systematic phenotyping analysis of GEM. Enhanced, reproducible and comprehensive mouse phenotype analysis has thus emerged as a prerequisite for effectively engaging the phenotyping bottleneck. Systematic phenotype assessments in mutant mouse allow us to find new function of human gene leading to development new drugs. Mouse genetics is a high cost work. Infrastructure for supporting a scientist who wants to employ mouse biology should be well organized and provided from large scale GEM production to more comprehensive and improved systematic phenotype analysis (Fig. 5).

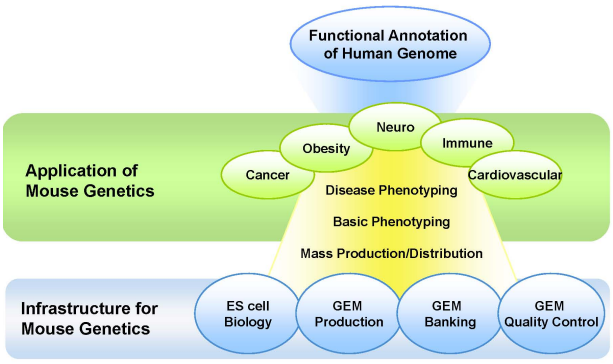

Fig. 5. Infrastructure for mouse genetics.

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