REVIEW

Mouse models of human disease. Part I: Techniques and resources for genetic analysis in mice

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The mouse is an ideal model organism for human disease. Not only are mice physiologically similar to humans, but a large genetic reservoir of potential models of human disease has been generated through the identification of >1000 spontaneous, radiation- or chemically induced mutant loci. In addition, a number of recent technological advances have dramatically increased our ability to create mouse models of human disease. These technological advances include the development of high-resolution genetic and physical linkage maps of the mouse genome, which in turn are facilitating the identification and cloning of mouse disease loci. Furthermore, transgenic technologies that allow one to ectopically express or make germ-line mutations in virtually any gene in the mouse genome have been developed, as well as methods for analyzing complex genetic diseases. In Part I of this review, we summarize some of the classical and modern approaches that have fueled the recent dramatic explosion in mouse disease model development. In Part II of this review, we list >100 mouse models of human disease where the homologous gene has been shown to be mutated in both human and mouse (Bedell et al., this issue). In the vast majority of these models, the mouse mutant phenotype very closely resembles the human disease phenotype and these models therefore provide valuable resources to understand how the diseases develop and to test ways to prevent or treat these diseases. Additionally, we highlight a number of areas of this research where significant progress has been made in the past few years.

Strain development

Formal mouse genetics began in the early part of this century with the development of the first inbred strain, DBA (Morse 1981; Hogan et al. 1994). Since this initial report, the number of inbred strains created has risen to

>465 (Festing 1994). Inbred strains are produced by at least 20 consecutive generations of brother-sister mating and were developed originally for the study of cancer (Morse 1981). However, inbred strains of mice differ in hundreds of other ways besides tumor susceptibility and they thus provide models for many other diseases besides cancer.

In the 1940s, George Snell pioneered the development of a new kind of inbred strain, the congenic strain, to isolate loci affecting tissue transplantation (for review, see Flaherty 1981; Frankel 1995). Like the inbred strains, congenic strains have proven extremely useful for disease research. Congenic strains are constructed by transferring a chromosome segment carrying a locus, phenotypic trait, or mutation of interest from one strain to another by 10 or more successive backcross matings, coupled with selection for the donor chromosome segment at each generation. After 10 backcross matings, the new congenic strain differs from its parent strain by an average of only an ~20-cM region representing the donor chromosome segment. In some cases, the phenotype of the congenic strain may differ significantly from the donor strain. For example, the NOD.B10-*H2^b* congenic strain was constructed by transferring the *H2^b* region of the major histocompatibility (MHC) locus from the C57BL/10SnJ (B10) strain into the nonobese diabetic (NOD) strain. Because the NOD strain develops diabetes, but neither the B10 nor the NOD.B10-*H2^b* strain develop diabetes, these results indicated that the MHC locus is essential for disease susceptibility (for review, see Wicker et al. 1995). However, the reciprocal congenic strain, B10.NOD-*H2^s*, also did not develop diabetes indicating that other, non-MHC linked genes are also required. Backcrosses of these and other congenic strains, coupled with molecular analysis using polymorphic markers, have been used to localize at least 14 insulin dependent diabetes susceptibility loci (Wicker et al. 1995).

Recombinant inbred (RI) strains have also proven useful for disease research (for review, see Justice et al. 1992). These strains are derived from the systematic inbreeding of randomly selected pairs of the F₂ generation of a cross between two different inbred strains of mice

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(Bailey 1981). During inbreeding, the genes from the progenitor strains segregate and randomly reassort, and are fixed in various combinations in the RI strain family members. A crucial advantage of studying diseases in RI strains is that the study is not limited to the lifespan of a single mouse. Because RI strains are inbred, they provide unlimited material for analysis. A major advantage of RI strains for the study of polygenic diseases is that the multiple loci associated with the disease are partially segregated in advance. In fact, a number of polygenic diseases have been studied successfully in RI strains, including epilepsy (Frankel et al. 1994), atherosclerosis (for review, see Justice et al. 1992), and substance abuse (for review, see Crabbe et al. 1994).

Although RI strains have been very useful for analyzing polygenic diseases, they do have certain disadvantages that limit their widespread applications. These include the small number of RI strains available for analysis, the length of time required to construct a new series of RI strains, and the large number of loci that are often involved in disease development. The latter limitation has been somewhat circumvented by the development of a modified type of RI strain called a recombinant congenic (RC) strain (for review, see Justice et al. 1992). RC strains are produced by limited backcrossing between two inbred strains before subsequent brother-sister mating. In this way, a series of strains is created, each of which carries a small fraction of the genome from one of the strains (the donor strain) on the genetic background of the other strain (the background strain). Whereas RI strains carry a 50% contribution from each parent, RC strains typically carry 12.5% of the genome of the donor strain and 87.5% of the background strain. Thus, the probability that a disease locus will be segregated away from the other disease loci in an RC strain increases compared with RI strains. Although the currently available RC strains have been developed primarily for the study of cancer (Fijneman et al. 1995; Lipoldova et al. 1995; Moen et al. 1996), they can also be used to study numerous other diseases as well. It is important to note, however, that genetic analysis in RC strains still suffers many of the same limitations as analysis of RI strains, that is, the development of RC strains is time-consuming, few family members are available for each RC series, and the genetic resolution of mapping in RC strains is still limited in cases where there are very large numbers of segregating disease loci.

Genetic mapping

Recent advances in mapping techniques have greatly facilitated disease gene identification and model development. Until a few years ago, gene mapping in the mouse was done mainly using crosses of inbred laboratory strains or RI strains. These methods were limited, however, by the low degree of polymorphism observed among laboratory strains and RI progenitors and the associated difficulty in finding a polymorphism needed for mapping. In the mid-1980s this problem was overcome through the development of interspecific crosses, which

involve crosses between a laboratory strain and a distantly related species of *Mus* (Avner et al. 1988). The high degree of genetic polymorphism present between the parents of such a cross makes it possible to map virtually any gene in a single cross. The most commonly used parents for interspecific backcrosses (IB) are C57BL/6J (the prototypic inbred strain) and *Mus spretus*, the most distantly related mouse species that will still form fertile hybrids with laboratory mice. A number of IB mapping panels have been developed for large-scale gene mapping in mouse (Table 1). Two of these mapping panels, the Jackson Laboratory Interspecific Backcross (JLIB) and the European Collaborative Interspecific Backcross (EUCIB) panels, are publicly available, and at least three are available on a collaborative basis.

Marker development

Another major advance in mapping has been the development of markers that can be typed by PCR and are highly polymorphic, even in inbred strain crosses. The

Table 1. Commonly used interspecific mapping panels

Publicly Available Panels

1. The Jackson Laboratory Interspecific Backcross (JLIB) Mapping Panel (Rowe et al. 1994)
 - (a) Two 94 animal reciprocal interspecific backcrosses
 - (1) [C57BL/6J $\times$ *M. spretus*] $\times$ C57BL/6J (BSB panel)
 - (2) [C57BL/6J $\times$ *M. spretus*] $\times$ *M. spretus* (BSS panel)
 - (b) Both Southern filters and PCR templates available
 - (c) Access through MGD (<http://www.informatics.jax.org/mgd.html>)
2. The European Collaborative Interspecific Backcross (EUCIB) Mapping Panel (Breen et al. 1994)
 - (a) Two reciprocal [C57BL/6J $\times$ *M. spretus*] interspecific backcrosses (982 animals total)
 - (b) Both Southern filters and PCR templates available
 - (c) Access through MRC-MGD (<http://www.mgc.har.mrc.ac.uk/about-mgc.html>)

Collaboratively Available Panels

1. Frederick Interspecific Backcross Mapping Panel (Copeland et al. 1993)
 - (a) 205 animals from a [C57BL/6J $\times$ *M. spretus*] $\times$ C57BL/6J interspecific backcross
 - (b) Contact Neal Copeland (copeland@ncicrf.gov) or Nancy Jenkins (jenkins@ncicrf.gov)
2. NIH Interspecific Backcross Mapping Panel (Danciger et al. 1993)
 - (a) Panel generated from three different crosses
 - (1) NFS/N or C58/J $\times$ *M.m musculus*] $\times$ *M.m musculus*
 - (2) NFS/N $\times$ *M. spretus*] $\times$ *M. spretus*
 - (3) NFS/N $\times$ *M. spretus*] $\times$ C58/J
 - (b) Contact Christine Kozak (ckozak@nih.gov)
3. Seldin Interspecific Backcross Mapping Panel (Seldin et al. 1988)
 - (a) ~450 animals from a [C3H/HeJ-*gld/gld* $\times$ *M. spretus*] $\times$ C3H/HeJ-*gld/gld* interspecific backcross
 - (b) Contact Mike Seldin (mfseldin@ucdavis.edu)

most widely used markers of this class are the simple sequence length polymorphisms (SSLP) or microsatellite markers (see Copeland et al. 1993). These polymorphisms occur at sites of di-, tri-, or tetranucleotide repeat sequence that have variable length in different mouse strains. Using primers that flank the repeats, PCR analysis is used to rapidly determine the segregation patterns of these markers in the progeny of various backcrosses. By analyzing 92 meioses from an intersubspecific cross between a laboratory strain and *Mus musculus castaneus*, a subspecies of *Mus*, Dietrich and colleagues at the Whitehead Institute/MIT Center for Genome Research have defined and mapped 6336 SSLP markers (Dietrich et al. 1996) (data available at <http://www-genome.wi.mit.edu/cgi-bin/mouse/index>). The MIT SSLP map has also been integrated with the Frederick IB map (Table 1), a gene-based map that includes >2300 genes. In addition, Dietrich and colleagues have defined 224 additional SSLPs using the Frederick IB, bringing the total number of SSLPs mapped in the two crosses to 6560. The SSLPs are well distributed across the autosomes, but are under-represented on the X chromosome (57% of expected). Whereas 50% of all SSLPs tested are polymorphic between various inbred strains, this frequency increases to 95% between wild mouse strains (*M. spretus* and *M. musculus castaneus*) and inbred strains. Thus, it is now possible to rapidly map the chromosomal location of a new mutation or disease trait, even using a cross of two inbred strains, and identify the known genes located in the vicinity.

A related, collaborative project between the Human Genome Mapping Project Resource Center in the UK and the Pasteur Institute in France, that is headed by Steve Brown, will be to map the SSLPs defined by Dietrich et al. (1996) on the EUCIB panel comprised of 982 meioses (Breen et al. 1994). This will provide a considerably higher map resolution (0.3 cM) than is now available and will increase the utility of these markers even further. Information on the current status of the EUCIB mapping effort is available at <http://www.hgmp.mrc.ac.uk/MBx/MBxHomepage.html>.

Mutant loci

Over the past century, >1000 spontaneous, radiation- or chemically induced mutations have been identified in the mouse (see Doolittle et al. 1996 or the Mouse Locus Catalog at <http://www.informatics.jax.org/locus.html>). Many of these mouse mutants recapitulate phenotypes seen in human diseases and, in a number of cases, mutations in homologous genes of both species have been identified (for review, see Bedell et al., this issue). Additional disease genes are therefore likely to be identified as more genes encoded by these mutant loci are identified and their human counterparts cloned. There are three general strategies that have been used to identify the genes encoded by these mutant loci: the candidate gene approach, positional cloning, and molecular tagging. To date, nearly three-quarters of all cloned mouse mutations have been identified using the candidate gene

approach. In this approach, known mutant loci in the vicinity of a newly mapped gene are reviewed to determine if any cause a phenotype that might be expected from an alteration in the mapped gene. Examples of mouse genes cloned by the candidate approach include *Pax6* and *Kit*, which are encoded by the *small eye* and *Dominant spotting* loci, respectively, and are models for aniridia and piebald trait in humans (for review, see Bedell et al., this issue). Positional cloning entails physically walking from closely linked molecular markers by using yeast or bacterial artificial chromosomes (YACs or BACs) or phage clones that contain murine chromosomal DNA. Once an interval that is likely to contain the gene of interest is identified, exon trapping, cDNA selection, or sequencing are used to identify candidate genes. The product of the *shaker 1* locus in the mouse, *Myo7a*, was positionally cloned and is homologous to the Usher syndrome 1 gene (for review, see Bedell et al., this issue). The final approach utilizes a known molecular tag, such as a transgene or retrovirus, that causes a mutant phenotype when integrated into a gene. The gene in question can then be cloned using probes that flank the integration site as starting points. For example, the *Mitf* gene, which is encoded by the *microphthalmia* (*mi*) locus and is homologous to the gene involved in Waardenburg syndrome type II in humans, was cloned as the result of a transgene integration (for review, see Bedell et al., this issue).

The further development of dense genetic linkage maps for the mouse and the SSLP markers described by Dietrich et al. (1996) will undoubtedly increase the rate of gene identification using either positional cloning or the candidate gene approach. A YAC-based physical map of the mouse genome should also have a profound impact on our ability to positionally identify disease genes. One such map is being constructed at the MIT Mouse Genome Center by screening a large insert (~1 Mb), 10-genome equivalent mouse YAC library with the >6000 SSLP markers developed by Dietrich et al. and another ~4000 random PCR markers. To date, 4200 SSLP markers have been screened against the YAC library (data available at <http://www-genome.wi.mit.edu/cgi-bin/mouse/index>) and it is anticipated that this project will be completed in the near future.

The development of a set of expressed sequence tags (ESTs) for the mouse and their positioning on mouse linkage maps will also greatly impact on our ability to positionally identify disease genes. For example, a \$2.3 million 2-year project is being initiated at the Washington University School of Medicine, St. Louis, with funds provided by the Howard Hughes Medical Institute. This project is under the direction of Drs. Robert H. Waterston and James S. McDonnell and will generate 400,000 partial gene sequences expressed in mice using normalized cDNA libraries developed by Dr. Bento Soares of Columbia University. To survey the entire spectrum of mouse genes that may be involved in disease, cDNA libraries from different stages of development will be constructed and analyzed. Once the sequences are completed and verified, they will be made available immedi-

ately via the Internet (see <http://medinfo.wustl.edu/mstp/mousegenes.html>).

Similar efforts for physical and EST mapping of the mouse genome are being accomplished by the United Kingdom Medical Research Council Human Genome Mapping Project Resource Center (HGMP) and information on these projects is available at <http://www.hgmp.mrc.ac.uk/MBx/MBxHomepage.html>.

Comparative maps

Of the >5000 genes that have been mapped in mouse, ~1800 have also been mapped in humans (up-to-date list available at <http://www.informatics.jax.org/reports.html>). These 1800 genes define >180 conserved linkage groups between mouse and human (DeBry and Seldin 1996) and make it possible to predict, with 80–90% accuracy, the chromosomal location of a gene in one species based solely on its map location in the other species. Comparative maps have many important applications for disease gene identification and model development. In some cases, information gained from studies of the mouse have led directly to the identification of human disease genes. Examples of this include the identification of *MITF* mutations in Waardenburg syndrome (Tassabehji et al. 1994) and *LYST* mutations in Chediak-Higashi syndrome (Barbosa et al. 1996). The murine homologs of these genes were first shown to be mutated in *mi* mice (Hodgkinson et al. 1993) and *beige* (*bg*) mice (Barbosa et al. 1996; Perou et al. 1996), respectively, and their localization in the mouse was used to predict where the human genes would map. Mouse-human comparative maps can also be exploited in the reverse manner, where the localization of a human disease gene is used to predict a gene responsible for a mouse mutant phenotype. One example of this is the *Btk* gene that was first associated with X-linked agammaglobulinemia, Bruton type in humans (Tsukada et al. 1993; Vetrie et al. 1993) and subsequently with *X-linked immune deficiency* (*xid*) in mice (Rawlings et al. 1993). Although *mi*, *bg*, and *xid*, as well as numerous other mouse mutants, were proposed as models based on phenotypic similarities with human diseases, the results of comparative mapping and molecular analysis validated the usefulness of the models.

Manipulation of the mouse genome: transgenic mice

In the early 1980s, methods were developed that allowed foreign DNA to be introduced into the germ line of mice by pronuclear injection (see Hogan et al. 1994). It then became possible to create transgenic mice carrying various forms of genes associated with human disease, such as oncogenes or tumor suppressor genes, and provide conclusive evidence that the genes in question caused the disease. The transgenic mice also serve as valuable models to study the pathogenesis of the disease and to test various treatment regimes. In addition, the transgene may be introduced into different inbred strain backgrounds to look for extragenic enhancers or suppressors

of the disease or bred to transgenic mice carrying other disease genes to look for cooperation between gene products.

There are some limitations, however, to the use of transgenic mice in developing disease models. One serious limitation is that sequences required for normal expression of a gene may be located within introns, in the 5' or 3' untranslated sequences or at a great distance (downstream or upstream) from the gene. Achieving normal regulated transgene expression can therefore be a daunting task. Transgenes also integrate at variable copy number into random sites within the genome and often are subject to position effects, whereby sequences that flank the integration site can positively or negatively affect expression of the transgene (for review, see Wilson et al. 1990). Thus, even when a transgene construct contains all of the sequences required for proper gene expression in some chromosomal locations, the transgene may be misexpressed because of position effects in other locations. In rare instances, transgenes can also integrate into genes and disrupt their function. Although this can be a problem, it can also be an advantage as the transgene provides a molecular tag for cloning the mutant gene (see above).

A recent development that may alleviate some of the problems of transgene expression is the ability to create transgenic mice carrying large DNA fragments, such as those cloned in YACs, BACs, or P1s (see Lamb and Gearhardt 1995). Large DNA fragments are much more likely to contain distal regulatory sequences necessary for normal gene expression and their large size makes them less susceptible to position effects. Like small constructs propagated in plasmids, large DNA fragments also integrate as multiple copies into random sites, but the copy numbers of the latter are often lower. Expression levels of large transgenes are also more comparable to the endogenous gene and are often copy number-dependent (Lamb et al. 1993; Peterson et al. 1993; Schedl et al. 1993).

The ability to create transgenic mice carrying large DNA inserts offers a number of important applications for disease research. For example, Smith and colleagues (1995) have used this approach to develop a transgenic mouse model for Down syndrome. Down syndrome is caused by trisomy of human chromosome 21 and is associated with a range of defects in addition to mental retardation. The defects found in Down syndrome patients are likely to result from gene dosage effects mediated by the overexpression of many different genes on chromosome 21. Smith et al. have produced transgenic mice that carry a contiguous 2-MB set of YAC and P1 clones from human chromosome 21q22.2, a known Down syndrome critical region. Transgenic mice carrying these YAC and P1 clones can now be screened for the different defects seen in Down syndrome patients and the sequences, and ultimately genes, responsible for these defects identified. The ability to create transgenic mice carrying large DNA inserts has important applications for the positional cloning of disease genes. This is especially true for polygenic disease loci where the ge-

netic, and thus physical, intervals containing these loci are often large and the nature of the mutation responsible for the disease trait is likely to be quite subtle (see below). A prerequisite to these studies is the availability of YAC or BAC libraries that are prepared from mice of specific strain backgrounds or genotype.

Manipulation of the mouse genome: gene knockouts

A major advance in our ability to create mouse disease models was the development of technology that makes it possible to introduce specific mutations into endogenous genes and then transmit these through the mouse germ line (for review, see Hogan et al. 1994; Melton 1994). The desired mutations are first created via homologous recombination in embryonic stem (ES) cells, which contribute to all cell lineages when injected into blastocysts. Just about any kind of desired mutation can now be introduced into a mouse gene including null or point mutations, as well as complex chromosomal rearrangements such as large deletions, translocations, or inversions (Hasty et al. 1991; Valancius and Smithies 1991; Wu et al. 1994; Ramirez-Solis et al. 1995). Once a human disease gene is cloned, the construction of mice that contain mutations in the corresponding gene is quite straightforward. In many cases where this has been accomplished the mice have a similar, if not identical, phenotype as the human patients (for review, see Bedell et al., this issue) and the mice then become an excellent model for the human disease.

Most of the ES cell lines currently in use are derived from a congenic 129/SvJ strain of mice carrying wild type alleles at the *albino* (*c*) and *pink eyed dilution* (*p*) coat color loci. The mice derived from these cells are thus agouti in coat color. Most host blastocysts used for ES cell microinjection are derived from C57BL/6J mice that are nonagouti. Thus, the mice that are produced by injection of blastocysts with ES cells are chimeric for the desired mutation and for pigmentation, and must be bred further to test for germ-line transmission of the mutant allele and to generate mice heterozygous and homozygous for the mutation. In the case of mutations that were made in 129/SvJ-derived ES cells, breeding the chimeras to 129 mice results in a coisogenic strain after only one generation, and the mutation is immediately present on a congenic 129 background. However, the use of the 129 strain for phenotypic analysis has significant limitations compared with other strains: The reproductive performance of 129 mice is poor; phenotypic comparisons to other genetic loci is limited because the vast majority of inbred strains in the mouse are derived from strains other than 129; and 129 mice are not a good choice for some studies, particularly of behavioral traits, because of major anatomical differences in the brain and severely impaired spatial learning (see Livy and Wahlsten 1991; Gerlai 1996).

The choice of strain may have significant impact on the phenotype as unlinked genes contained in the strain background can have a dramatic effect on the disease

phenotype. An extreme example of this was reported by Threadgill et al. (1995) for null mutations in the epidermal growth factor receptor (*Egfr*) gene. On an outbred CF-1 background, *Egfr* mutant embryos have defects in the inner cell mass that cause peri-implantation lethality; on the 129/SvJ inbred background, these mutants have placental defects that cause mid-gestation lethality; and on a congenic C57BL/6J background, defects in various organs cause juvenile lethality. Although such variability in phenotype can confound studies aimed at elucidating mutant phenotypes, it affords an excellent opportunity to identify unlinked modifiers that affect the phenotype of interest. This is extremely important if the mice are a model for a human disease and the unlinked modifiers suppress the disease phenotype. By mapping and cloning these unlinked modifier genes it may be possible to identify ways to suppress the corresponding human disease. Toward this end, as well as to circumvent problems associated with the 129/SvJ strain, it would be desirable to determine the phenotype of a given mutation on different genetic backgrounds, such as an inbred strain like C57BL/6J or an outbred strain like CF-1. One way to achieve this is to develop ES cell lines from different inbred strain backgrounds and some success with ES cell lines from C57BL/6J (Kontgen et al. 1993), DBA/1LacJ (Roach et al. 1995), and BALB/c (Noben-Trauth et al. 1996) has been reported. Another way to achieve the same goal is to create congenic lines by backcrossing the knockout mutation onto different inbred strain backgrounds by 10 or more successive backcross matings. The major disadvantage of this approach is the time involved in creating congenic mice. On average, one is lucky to achieve three to four backcross generations per year so it may take three or more years before a congenic line is fully constructed. It is important to keep in mind that the progeny from the first several generations of these crosses may exhibit wide variation in phenotype resulting from segregation of modifier loci in the different strains. Furthermore, the sequences immediately surrounding the mutation in congenic strains are still derived from the strain from which the ES cells were made.

The time involved in creating congenic mice can be shortened greatly through the use of marker assisted selection (so-called speed congenics). Mice at the second backcross generation are typed for markers distributed across the entire mouse genome and only those mice that carry the highest percent of the genome of the desired strain are used to produce the next backcross generation. This process is again repeated at each successive backcross generation. In this way, it is possible to create congenic lines in only three to four backcross generations (~1 year) as opposed to 10 backcross generations for normal congenic strains.

For many genes, germ-line mutations cause early embryonic lethality, making it impossible to study the effects of the mutations at late stages of embryonic development or in the adult. A recently developed strategy, using the Cre-lox system of site-specific recombination of bacteriophage P1, now makes it possible to generate

somatic knockout mutations that are tissue-specific and inducible at different stages in embryonic or adult development (for review, see Chambers 1994). In this system, the recognition sequences for Cre recombinase (*loxP* sites) are introduced into chosen sites of the mouse genome using homologous recombination in ES cells, for example at sites flanking an entire gene or an exon encoding a particular domain of a protein. Mice containing the desired *loxP* sites are generated as described above, and are then crossed to transgenic mice that express Cre recombinase under the control of tissue-specific or inducible regulatory elements. When Cre is expressed, recombination occurs at the *loxP* sites resulting in deletion of the intervening sequences and the resulting mutation occurs in a lineage-specific or conditional fashion. The Cre-*lox* system has also been used to induce large deletions, specific inversions, duplications, and translocations in the mouse (Ramirez-Solis et al. 1995; Van Deursen et al. 1995). Such manipulations should have a major impact on developing accurate models of the often complex diseases of humans that are caused by chromosomal rearrangements.

In attempting to engineer a mouse model for a human disease, it is important to know what kind of mutation causes the disease (i.e., null mutation, hypomorphic mutation, or dominant negative mutation) and, if possible, introduce the same kind of mutation into the mouse gene. For example, point mutations in the collagen type X, $\alpha 1$ gene (*COL10A1*) cause Schmid metaphyseal chondrodysplasia in humans (Warman et al. 1993), and transgenic mice with analogous *Col10a1* mutations have a phenotype very similar to the human patients (Jacenko et al. 1993). However, mice that are homozygous for a germ-line null mutation in the *Col10a1* gene are phenotypically normal (Rosati et al. 1994). These results provide strong genetic evidence that Schmid metaphyseal chondrodysplasia results from dominant negative mutations in the *COL10A1* gene and not an overall deficiency in type X collagen. Mice carrying a null mutation in the *Col10a1* gene are therefore not appropriate models for the human disease, whereas transgenic mice that carry mutant *Col10a1* genes are an appropriate model.

Manipulation of the mouse genome: large-scale mutagenesis

Although gene-driven approaches, such as transgenics and targeted mutations, are useful for understanding gene function, it is likely that phenotype-driven schemes will be necessary to fully understand the functional complexities of the mouse genome. The precedence for this may be found in studies of *Drosophila*, where saturation mutagenesis coupled with phenotypic screens have had an enormous impact on gene identification and dissection of specific developmental pathways. Such large-scale mutagenesis schemes are possible in the mouse, albeit at a significantly higher cost in time and money than in lower eukaryotes. Two of the most effective germ-cell mutagens in the mouse are X-rays and *N*-ethyl-*N*-nitrosourea (ENU) (for review, see Rinchik 1991). X-

rays most often cause large lesions, such as deletions and translocations that can involve multiple genes, whereas ENU treatment is frequently associated with intragenic mutations, such as point mutations. Estimates of the mutation rates per locus with either of these treatments in the mouse range from 13×10^{-5} to 150×10^{-5} per gamete, in comparison to a spontaneous mutation rate of 0.5×10^{-5} to 1.0×10^{-5} . However, the mutation frequencies are low enough that only large mouse facilities can routinely generate new mutants in this manner.

The most extensively used procedure for the generation of new mutations in the mouse is the specific locus test (SLT), developed by Dr. William L. Russell more than 40 years ago (for review, see Rinchik and Russell 1990). Large-scale mutagenesis schemes using the SLT have been carried out at several laboratories, under the direction of Dr. Liane B. Russell (Oak Ridge National Laboratory, Oak Ridge, TN), Dr. Bruce M. Cattaneach (the MRC Radiobiology Unit, Chilton, Didcot, UK) and Dr. Jack Favor (GSF-Institut für Saugtiergenetik, Neuherberg, Germany). In the SLT, mutagenized mice are mated to a tester strain that is homozygous for recessive mutations at seven loci, each of which causes a visible, viable phenotype. Thus, new mutations in the F_1 progeny of these matings are easily detected and large numbers of different alleles of each of these loci have been identified. Importantly, four of the seven loci used in the standard SLT [*c*, *p*, *brown* (*b*), and *piebald* (*s*)] encode genes homologous to those affected in four human disorders (oculocutaneous albinism types I, II, and III, and Hirschprung's disease, respectively) (for review, see Bedell et al., this issue). Other large-scale ENU mutagenesis experiments have screened for mutations that cause specific phenotypes, such as cataracts (Favor et al. 1990), phenylketonuria (Shedlovsky et al. 1993), and circadian behavior (Vitaterna et al. 1994). In addition, saturation mutagenesis of specific chromosomal regions of the mouse has been accomplished, such as the regions of chromosome 17 and chromosome 7 that surround the *Brachyury* (*T*) and *c* loci, respectively (for review, see Rinchik 1991).

Genetic screens for new recessive mutations are greatly expedited by utilizing tester strains that carry chromosomal deletions. These deletions represent regions of segmental hemizygosity that allow recessive mutations to be detected in breeding schemes that use two generations, as opposed to three generations required for homozygosity. The feasibility of such an approach has been demonstrated by Rinchik and coworkers (for review, see Rinchik 1991), who used a 6–11 cM deletion at the *c* locus and saturation mutagenesis with ENU to identify new loci and new alleles of previously known loci in the region that flanks the *c* gene. Recently, defined deletions of up to 3–4 cM have been constructed in ES cells using the Cre-*loxP* system and shown to be transmitted through the germ line (for review, see Ramirez-Solis et al. 1995). This procedure could be used to generate strains of mice that carry defined deletions at virtually any chromosomal location, and could then be used in genome-wide mutagenesis screens.

Polygenic diseases

Many of the most important diseases affecting humans, such as diabetes, cancer, epilepsy, and obesity, are not caused by single-gene mutations, but rather by the cumulative effect of mutations at several different loci. Furthermore, some of these diseases reflect a predisposition that is genetically inherited but is under significant influence from somatically acquired mutations or environmental influences. The genes that cause or predispose to such complex diseases, called quantitative trait loci (QTLs), can be dominant or recessive and act additively or epistatically to induce disease. Each QTL by itself may have only a weak effect and it is only when several QTLs are inherited by a single individual that disease or disease predisposition ensues. The challenge for the geneticist has been to devise ways to identify, map, and eventually clone QTLs (Lander and Botstein 1989; Lander and Schork 1994). In general, QTL analysis requires screening hundreds, if not thousands, of individuals and scoring them for markers scattered across the entire genome. Here the mouse has obvious advantages over humans as many inbred strains are available for analysis and thousands of progeny can easily be produced by programmed breeding.

Although QTL analysis shows great promise for dissecting complex traits, there are some major problems associated with identifying and characterizing the genes involved. Because QTLs are frequently not fully penetrant, one cannot rely on individual crossovers for localization and the mapping resolution attainable with QTLs is thus often limited compared with monogenic traits. Another major problem with QTLs lies in the nature of the nucleotide alterations that cause them. Often, these alterations result in seemingly subtle amino acid substitutions or may be in regulatory sequences; in both of these situations, possible effects on gene function may not be easily predicted or tested. In addition, many QTLs are allelic variants that segregate in normal populations, rather than mutations that cause loss- or gain-of-function. Thus, the challenge is to identify which polymorphisms are causally related to the disease and which are silent. In principle, the mouse may offer a distinct advantage in both of these areas. In mapping studies, QTLs identified in classical genetic crosses can be confirmed and more finely localized by establishing congenic strains for each QTL and analyzing its effects either alone or in combination with other QTLs (made possible by crossing congenic strains that harbor different QTLs). Once a QTL is finely localized and a physical map for the region generated, clones from the region can be introduced into transgenic mice and their phenotypic effects measured. If a phenotype is seen, then smaller and smaller clones can be introduced until a biologically active clone is found that contains a single gene. In this way the gene can be identified without the need for mutational analysis. One caveat to extrapolation of mouse QTLs to human disease is that, because of subtle differences in biochemical pathways or environmental influences, genes involved in the former may not necessarily

be the same as those involved in humans. Nonetheless, studies of mouse QTLs are an important first step toward understanding complex diseases in humans.

Given the extreme importance of polygenic diseases to human populations, it seems likely that much of the future in mouse disease research lies in the study of these complex diseases (see Avner 1994; Frankel 1995). Although such studies are only in their infancy, a number of QTLs already have been identified in the mouse that produce phenotypes similar to human diseases, such as airway hyperresponsiveness (De Sanctis et al. 1995), alcohol and morphine preference (Berretini et al. 1994; Crabbe et al. 1994), atherosclerosis (Hyman et al. 1994), epilepsy (Frankel et al. 1994), and obesity (West et al. 1994). The ability to approach such complex problems has only recently become possible because of the efforts of the human genome project and the dense genetic and physical maps that are now available for mouse. Ultimately, the complete sequence of the mouse genome will be known, mutations in every mouse gene will have been created, and traits associated with mutations or misexpression of individual genes will be determined. However, we will still not be able to predict how these mutations are likely to interact in vivo. Programmed breeding, such as to create double and triple mutants, and reverse genetics in the mouse will still be needed to approach an understanding of the complexities of human genetic diseases.

Mice on the Web

There are numerous resources and services available for mouse geneticists that are accessible over the World Wide Web (WWW). As the amount of information available on the WWW is expanding rapidly, here we describe just a few of the sites that contain information of particular significance to mouse models of human disease. Many of these sites are linked, so it is possible to gain access to a large number of resources from just one site.

The Jackson Laboratory (<http://www.jax.org>) maintains a number of databases including the Mouse Genome Database (MGD), Gene Expression Database, and Encyclopedia of the Mouse Genome. One may access MGD and search the Genetic Markers and Mouse Locus Catalog for all of the genetic mapping, probe, mammalian homology, phenotypic and expression information available for a specific gene. Alternatively, searches may be conducted for all mapped genes on an entire chromosome or portion of a chromosome, or for all genes or loci associated with a specific phenotypic class (such as hearing, immunological, neurological and neuromuscular or skeletal defects), or type of gene product (such as enzymes, homeoboxes, oncogenes or receptors). Linkage maps of seven different mouse backcross panels, including the Frederick, MIT, and EUCIB panels described above, and the strain distribution patterns of markers in 24 different RI sets are also accessible through MGD. The Chromosome Committee Reports, that are published annually in *Mammalian Genome* and describe all of the mouse genome mapping and analysis efforts

worldwide, are also available electronically by accessing MGD. Similarities between mouse and human phenotypes may be obtained by doing integrated searches of the Mouse Locus Catalog and Online Mendelian Inheritance in Man (OMIM; see below). In addition, various mouse resources that may be obtained from The Jackson Laboratory are listed including the Induced Mutant Resource (a repository of transgenic and targeted mutant mice), Mouse Mutant Resource (a repository of classical mouse mutants), Mouse DNA Resources (genomic DNA from various inbred and mutant strains), and the JLIB Backcross Mapping Panel. An electronic bulletin board service (mgi-list) for the world-wide mouse genetics research community is maintained by the Mouse Genome Informatics project of The Jackson Laboratory.

OMIM contains a catalog of human genes and genetic disorders compiled at the Johns Hopkins University and prepared for the WWW by the National Center for Biotechnology Information. The home page for OMIM is at <http://www3.ncbi.nlm.nih.gov/omim/> and searches of the database may be performed. In addition, this site is linked to the Seldin/DeBry Human/Mouse Homology Map, that compares chromosomal regions of synteny between the two species. TBASE (at <http://www.gdb.org/Dan/tbase/tbase.html>) is a database for genetically altered organisms, including the mouse, that was compiled at the Division of Biomedical Information Science at Johns Hopkins University. Searches may be conducted in TBASE for targeted mutations in all mouse genes that cause specific phenotypes. Descriptions of the map location of the gene, methods for constructing the mutation, the mutant phenotype, possible human homologies, related references, and names of contact persons for each gene can then be obtained. Based on the map location of a particular mouse gene in that list, the dysmorphic human-mouse homology database may then be searched for human homologies to that region of the mouse chromosome, and direct access to both MGD and OMIM provides complete descriptions of the murine gene and possible human syndromes. An additional database for targeted mutations that is based on a compilation of data by Brandon et al. (1995) is available at <http://biomednet.com>.

A collection of rodent genome databases maintained by the UK HGMP is available at <http://www.hgmp.mrc.ac.uk/Public/rodent-gen-db.html>. This site is linked to both of the EUCIB and MIT mouse backcross maps and to MGD at JAX. Additional databases at the HGMP site include the dysmorphic human and mouse homology database (that allows malformation syndromes in each species to be linked through chromosomal synteny) and mouse cytogenetic maps. This site also contains information about a radiation hybrid panel. A variety of Internet resources for rodent research are available at the Mouse and Rat Research Home Page (at http://www.cco.caltech.edu/~mercer/htmls/rodent_page.html). Some of the many resources accessible through this site are: links to various genome informatics databases; information on texts and guides for development, anatomy, and physiology of mice; veterinary resources

and legal issues relating to the care and use of mice in research; and E-mail lists, courses, and conferences of importance to mouse researchers. In addition, large-insert libraries of mouse DNA are publicly available from the Resource Centre/Primary Database (RZPD) of the German Human Genome Project (<http://www.rzpd.de>) and are commercially available from Research Genetics in Huntsville, Alabama (<http://www.resgen.com/>) or Genome Systems, Inc., of St. Louis, Missouri (<http://www.genomesystems.com/>).

Concluding remarks

In the last decade we have seen a dramatic explosion take place in genome research. In the mouse this has meant the development of detailed linkage maps, the development of markers that can be typed by PCR and are amenable to automation, and new methods for manipulating the mouse genome including methods for introducing germ-line mutations into virtually any gene in the mouse. These unparalleled advances have already had a profound effect on our ability to generate new mouse disease models (as detailed here and in Part II of this review). Given the expected advances of genome research in the future, it will not be surprising if this pace accelerates even more dramatically.

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