

Discovery Genetics: Serendipity in Basic Research

Muriel T. Davisson

Abstract

The role of serendipity in science has no better example than the discovery of spontaneous mutations that leads to new mouse models for research. The approach of finding phenotypes and then carrying out genetic analysis is called forward genetics. Serendipity is a key component of discovering and developing mice with spontaneous mutations into animal models of human disease. In this article, the role of serendipity in discovering and developing mouse models is described within a program at The Jackson Laboratory that capitalizes on serendipitous discoveries in large breeding colonies. Also described is how any scientists working with mice can take advantage of serendipitous discoveries as a research strategy to develop new models. Spontaneous mutations cannot be planned but happen in all research mouse colonies and are discovered as unexpected phenotypes. The alert scientist or technician can rationally exploit such chance observations to create new research opportunities.

Key Words: forward genetics; model; mouse; mutation; resource; spontaneous

Introduction

Serendipity is key to discovering mice with new spontaneous mutations. Such mutations can then be developed into new mouse models for human disease and for basic and biomedical research by genetic and phenotypic analysis or characterization. Spontaneous mutations are random genetic events that happen by chance. They occur in mice in all research and resource mouse colonies and are discovered as unexpected phenotypes that deviate from the normal. Although naturally occurring mutations cannot be planned, the alert animal care or research technician can discover them by careful observation during routine operations, and the astute scientist can rationally exploit such chance observations to create new research opportunities from something not sought.

At The Jackson Laboratory (TJL¹), the juxtaposition of large-production mouse breeding colonies maintained by genetically defined breeding protocols and a faculty of basic research scientists provide a unique opportunity to discover spontaneous mutations of biomedical significance. The animal care technicians in TJL's Production and Repository breeding colonies are crucial to this new mutant discovery program. The successful, sometimes serendipitous results of these combined factors are described below.

Value of Spontaneous Mutations

Despite advances in molecular technologies that have increased our ability to create mouse models of inherited human conditions through targeted mutagenesis and transgenesis, spontaneous mutations continue to provide valuable models of human disease, as well as basic research systems for understanding mammalian biology. Spontaneous mutations identify novel genes and provide potential models for human inherited disorders for which the mutated gene has not yet been identified. In addition, the genetic defects in most spontaneous mutations of human beings and other mammals are usually confined to a single gene (i.e., small insertions or deletions or single base pair changes [Grompe et al. 1989]), whereas genetically engineered models often involve more than one molecular change. Finally, spontaneous mutations often produce a phenotype that more closely resembles the human disorder than do targeted mutations for the same gene. For example, a spontaneous mutation of the homeobox D13 (*Hoxd13*) gene is the result of a polyalanine repeat as in human synpolydactyly (Bruneau et al. 2001), and the resulting phenotype much more closely resembles that of the human disorder than the phenotype resulting from a targeted mutation of *Hoxd13* (Dollé et al. 1993). A short list of selected examples of spontaneous mutation mouse models may be found in Table 1.

The development of inherited spontaneous mutation model systems requires two steps: discovery and analysis. TJL has an established program for discovering and analyzing spontaneous mutation mouse models that has been highly successful because of the juxtaposition of research

Muriel T. Davisson, Ph.D., is Director of Genetic Resources and a Senior Staff Scientist at The Jackson Laboratory, Bar Harbor, Maine.

¹Abbreviations used in this article: MGI, Mouse Genome Informatics database; MMR, Mouse Mutant Resource; NCRR, National Center for Research Resources; NIH, National Institutes of Health; SNP, single nucleotide polymorphism; TJL, The Jackson Laboratory.

Table 1 Selected examples of spontaneous mouse mutations that provide models for human disorders and for which the genes in both species have been cloned^a

Mouse				Human			
Gene/allele symbol	Chr	Mutant allele name	Gene name	Gene symbol	Chromosomal location	Human disorder (OMIM ^b)	OMIM #
<i>Ap3b1^{P^e}</i>	13	pearl	adaptor-related protein complex AP-3, beta 1 subunit	<i>AP3B1</i>	5 (p15.2-p14.1)	Hermansky-Pudlak syndrome 2	#203300
<i>Ar^{Tfm}</i>	X	testicular feminization	androgen receptor	<i>AR</i>	X (q11.2-q12)	androgen insensitivity syndrome (AIS)	#300068
<i>Atp7a^{Mo-br}</i>	X	brindled	ATPase, Cu ⁺⁺ transporting, alpha polypeptide	<i>ATP7A</i>	X (q13.2-q13.3)	Menkes syndrome	*300011 ^b ; #309400
<i>Atp7b^{bx-J}</i>	8	toxic milk Jackson	ATPase, Cu ⁺⁺ transporting, beta polypeptide	<i>ATP7B</i>	13 (q14.3-q21.1)	Wilson disease	*606882; #277900
<i>Cacna1a^{tg}</i>		tottering	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	<i>CACNA1A</i>	19 p13.1	episodic ataxia, type 2 (EA2) spinocerebellar ataxia 6 (SCA6)	#108500; #183060
<i>Cacnb4^{lh}</i>	2	lethargic	calcium channel, voltage-dependent, beta 4 subunit	<i>CACNB4</i>	2 (q22-q23)	myoclonic epilepsy, juvenile, 1 (EJM1)	#183086
<i>Cdh23^{v-2J}</i>	10	waltzer 2 Jackson	cadherin 23 (otocadherin)	<i>CDH23</i>	10 (q21-q22)	Usher's syndrome, type ID (USH1D)	#601067
<i>Clcn1^{adr-mto}</i>	6	myotonia	chloride channel 1	<i>CLCN1</i>	7 (q32-qter)	myotonia congenita, autosomal dominant	#160800
<i>Cln6^{ncif}</i>	9	neuronal ceroid lipofuscinosis	ceroid-lipofuscinosis, neuronal 6	<i>CLN6</i>	15 (q21-q23)	ceroid lipofuscinosis, neuronal, late-infantile, variant (vLINCL)	*606725; #601780
<i>Col1a2^{oim}</i>	6	osteogenesis imperfecta	procollagen, type I, alpha	<i>COL1A2</i>	7 (p21.3-q22.1)	osteogenesis imperfecta (various)	*120160
<i>Col2a1^{sed}</i>	15	spondyloepiphyseal dysplasia	procollagen, type II, alpha 1	<i>COL2A1</i>	12 (q12-q13.2)	spondyloepiphyseal dysplasia, congenital	#183900
<i>Eda^{Ta}</i>	X	tabby	ectodysplasin-A	<i>ED1</i>	X (q12-q13.1)	ectodermal dysplasia 1, anhidrotic (ED1)	*305100
<i>Eya1^{bor}</i>	1	branchio otorenal syndrome	eyes absent 1 homolog (<i>Drosophila</i>)	<i>EYA1</i>	8 q13.3	branchiootorenal dysplasia syndrome	#113650
<i>Fbn1^{tsk}</i>	2	tight skin	fibrillin 1	<i>FBN1</i>	15 q21.1	Marfan's syndrome	#154700
<i>Fbxw4^{Dac}</i>	19	dactylaplasia	F-box and WD-40 domain protein 4	<i>FBXW3</i>	22 q11	split-hand/foot malformation 3 (SHFM3)	*600095
<i>Galc^{twi}</i>	12	twitcher	galactosylceramidase	<i>GALC</i>	14 q31	Krabbe's disease	#245200
<i>Ghrh^{lit}</i>	6	little	growth hormone releasing hormone receptor	<i>GHRHR</i>	7(p15-p14)	growth hormone deficiency, isolated	*139191
<i>Gli3^{Xt-J}</i>	13	extra toes Jackson	GLI-Kruppel family member GL13	<i>GL13</i>	7 p13	Greig cephalopolysyndactyly syndrome (GCPS)	*165240; #175700
<i>Gus^{mps}</i>	5	mucopolysaccharidosis VII	beta-glucuronidase	<i>GUSB</i>	7 q22	mucopolysaccharidosis type VII	*253220
<i>Hbb^S</i>	7	s allele; [hemoglobin single]	hemoglobin beta chain complex	<i>HBB</i>	11 p15.4		
<i>Hoxd13^{spdh}</i>	2	synpolydactyly homolog	homeo box D13	<i>HOXD13</i>	2 q31-q32	syndactyly, type II	#186000
<i>Lep^{ob}</i>	6	obese	leptin	<i>LEP</i>	7 q32.1	obesity, leptin deficiency, hypogonadism	*164160

Table 1 Selected examples of spontaneous mouse mutations that provide models for human disorders and for which the genes in both species have been cloned^a (Continued)

Mouse			Human				
Gene/allele symbol	Chr	Mutant allele name	Gene name	Gene symbol	Chromosomal location	Human disorder (OMIM ^b)	OMIM #
<i>Leprd</i>	4	diabetes	leptin receptor	<i>LEPR</i>	1 p31	obesity, morbid, with hypogonadism	*601007
<i>Matp^{uw-d}</i>	15	underwhite dense	membrane-associated transporter protein	<i>MATP</i>	5 (p13.3)	oculocutaneous albinism type IV (OCA4)	#606574
<i>Mitt^{Mi}</i>	6	microphthalmia	microphthalmia-associated transcription factor	<i>MITF</i>	3 (p14.11-p12)	Waardenburg syndrome, type IIA (WS2A)	*156845
<i>Myo7a^{sh1}</i>	7	shaker 1	myosin VIIa	<i>MYO7A</i>	11 q13.5	Usher's syndrome, type IB; deafness, neurosensory, AR 2 (DFNB2)	*276903 #600060
<i>Npc1^N</i>	18	Niemann-Pick type C1 NIH	Niemann-Pick type C1	<i>NPC1</i>	18 q11	Niemann-Pick disease, type C	*257220
<i>pⁱ</i>	7	pink-eyed dilution Jackson	pink-eyed dilution	<i>OCA2</i>	15 (q11.2-q12)	oculocutaneous albinism, type II (OCA2)	*203200
<i>Pax3^{Sp}</i>	1	splotch	paired box gene 3	<i>PAX3</i>	2 q36	Waardenburg syndrome type (WS1)	#193500
<i>Pax6^{Sey-Dey}</i>	2	small eye, Dickie	paired box gene 6	<i>PAX6</i>	11 p13	anidia, type II (AN2)	#106210
<i>Pcdh15^{av-3J}</i>	10	Ames waltzer 3 Jackson	protocadherin 15	<i>PCDH15</i>	10 (p11.23-q21.1)	Usher's syndrome, type IF	#602083
<i>PheX^{hyp}</i>	X	hypophosphatemia	phosphate regulating gene with homologies to endopeptidaseson	<i>PHEX</i>	X (p22.2-p22.1)	hypophosphatemia, X-linked	*307800
<i>Pit1^{dw}</i>	16	dwarf	pituitary-specific transcription factor 1	<i>POU1F1</i>	3p11	pituitary hormone deficiency (CPHD)	#173110
<i>Plp^{jp}</i>	X	jimpy	proteolipid protein (myelin)	<i>PLP1</i>	X (Q21.33-q22)	Pelizaeus-Merzbacher disease (PMD)	*300401
<i>Pmp22^{Tr-J}</i>	11	trembler Jackson	peripheral myelin protein, 22 kDa	<i>PMP22</i>	17 (p12-p11.2)	Charcot-Marie-Tooth disease, type 1A (CMT1A);	*601097 #118220
<i>Rab27a^{ash}</i>	9	ashen	RAB27A, member RAS oncogene family	<i>RAB27A</i>	15 (q15-q21.1)	Griscelli syndrome	#214450
<i>Sgsh^{mps3a}</i>	11	mucopoly-saccharidosis IIIA	N-sulfoglucosamine sulfohydrolase (sulfamidase)	<i>SGSH</i>	17 q25.3	mucopolysaccharidosis type IIIA	*605270 #252900
<i>Tc1rg1^{oc}</i>	19	osteosclerotic	T-cell, immune regulator 1	<i>TCIRG1</i>	11 (q13.4-q13.5)	osteopetrosis, autosomal recessive	*604592 #259700
<i>Tgn^{cog}</i>	15	congenital goiter	thyroglobulin	<i>TG</i>	8 (q24.2-q24.3)	goiter, familial, with hypothyroidism AR	*188450
<i>Tshr^{hyt}</i>	12	hypothyroid	thyroid-stimulating hormone, receptor	<i>TSHR</i>	14 q31	hypothyroidism, thyrotropin resistant	*603372
<i>Twist1^{Pde}</i>	12	polydactyly EMS ^b	twist gene homolog (Drosophila) 1	<i>TWIST</i>	7 p21.2	Saethre-Chatzen syndrome (SCS)	#101400

^aCitations for this information may be found by searching on the mouse gene symbol in the Mouse Genome Database (MGD 2005).

^bOMIM, On-line Mendelian Inheritance in Man <http://www3.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>; EMS, ethylmethanesulfonate.

scientists and large-production breeding colonies and because of a large mutant resource development program funded by the National Institutes of Health (NIH¹) National Center for Research Resources (NCRR¹). It is important to

note, however, that any scientist who maintains a mouse colony for research or any organization with a large mouse breeding program can capitalize on spontaneous mouse mutations to develop new mouse models. The only require-

ments are astute observation to identify abnormal phenotypes and the capability to follow up on the observations with mutant analyses.

The mouse has long been the mammal of choice for studying the role of genes in development and in normal biological functions, and for providing experimental models of human inherited diseases. Because mice have close metabolic and internal anatomical similarities to humans, mutant genes in mice frequently produce syndromes similar to human inherited conditions. For example, the mouse eye and inner ear are almost identical in structure to the human eye and ear, and many mouse models closely resemble human deafness disorders (Zheng et al. 1999) and ocular disorders (Chang et al. 2002).

The short life span of the mouse enables the study of the entire course of a disease over a relatively short period of time. Because of their small size and relative ease of maintenance, mice are economical to maintain. Mouse mutations can be maintained on controlled genetic backgrounds, so that mutant and control mice differ only by the mutated gene being studied. The short generation time and availability of inbred strains make it possible to study the effect of modifying genes by placing mutations on different genetic backgrounds and observing alterations in phenotypes. Finally, the mouse genome is the best genetically mapped of any experimental mammal, and it was the first mammalian model organism genome to be sequenced. Importantly, the protein coding sequence of DNA is 85 to 95% conserved between the mouse and human genome (e.g., Consortium 2002; Makalowski and Boguski 1998). This genomic conservation provides additional evidence of potential benefits to be gained from using mouse models of human diseases.

Discovery of Spontaneous Mutations

Natural Occurrence

Spontaneous mutations are accidents of nature that occur during DNA replication or damage repair. Average mutation rates are approximately 10^{-5} to 10^{-7} events per gene per generation (Melvold and Kohn 1975; Melvold et al. 1997; Schlager and Dickie 1971). Thus, the probability of finding such events is greater as the size of the breeding colony increases. The probability of finding recessive mutations is greater with increased inbreeding.

Mutation rates may vary, however, from one gene to another. For example, nearly 100 independent spontaneous mutations have been reported in the *Kit* oncogene (*Kit*) locus, also known as the dominant spotting gene (MGD 2005). Mutations in individual genes that cause skeletal, neurological, endocrinological, and metabolic defects are much less common; frequently only one or two mutant alleles of a single gene have been discovered. The type of mutant genes found by spontaneous mutation is biased by their phenotypic visibility and viability. Mutations that cause internal metabolic defects may manifest only as “fail-

ure to thrive” phenotypes. A good rule of thumb is that a single sickly pup in a litter may carry a mutation, whereas failure to thrive in all pups in a litter is more likely to be a maternal or nutritional effect. At least one study suggests, however, that metabolic mutations that are compatible with postnatal survival will be detected by phenotypes other than simply failure to thrive (Wood et al. 1988). However, mutations that cause prenatal lethality are more difficult to discover. They may manifest only as reduced litter size and are expected to go undetected in most strains of mice.

Mutation Rate Enhancement by Mutagenesis

Many radiation-induced mutations were created after World War II and the dropping of the first atomic bomb in studies to assess radiation risk levels. Most of these studies were performed at the Oak Ridge National Laboratory in the United States and the Medical Research Council at Harwell in the United Kingdom. Sometimes radiation-induced mutations are the result of chromosomal abnormalities disrupting genes and complicating their genetic molecular analysis. For example, the radiation-induced mutation hairy ears (*EH*) was subsequently shown to result from a chromosomal inversion (Davisson et al. 1990), and radiation-induced shaker with syndactylism (*sy*) was shown to be a contiguous gene syndrome due to a chromosomal deletion spanning several genes (Johnson et al. 1998a).

In recent years, mutagenesis programs have been developed to enhance the probability of discovering random mutations in novel genes. The theory behind such programs is that increasing the mutation rate makes it feasible to screen for subtle phenotypes. Such screens are often too expensive or labor intensive to carry out on mice from unmutagenized populations because of the large numbers of mice that would need to be examined. Ethylnitrosourea (the most potent and commonly used chemical mutagen), when injected into progenitor mice increases the mutation rate by about 10- to 20-fold to an average of 10^{-3} events per gene per generation and increases the probability of finding mutant mice among their progeny (Hitotsumachi et al. 1985; Russell et al. 1979).

Mutagenesis programs focused on neurological disorders and heart, lung, blood, and sleep disorders (e.g., Goldowitz et al. 2004; Svenson et al. 2003) were funded in the United States during the 1990s and 2000s by NIH. Other more general mutagenesis programs were established in North America and Europe (e.g., Herron et al. 2002; Hrabe et al. 2000; Nolan et al. 2000).

Chemically induced mutations from such programs occur randomly and must be analyzed in the same way as spontaneous mutations (see the T3L Discovery Program, below). Gene traps that target and disrupt gene-specific sequences also can be used to increase the mutation rate and give the advantage that the mutated gene is tagged with the gene trap sequence (Skarnes et al. 1992, 2004).

The Jackson Laboratory's Mutation Discovery Program

TJL is an ideal location to search for, develop, and maintain new spontaneous mutation mouse models for biomedical research. The large Production and Repository breeding colonies and the fact that all strains are maintained by defined genetic breeding protocols enhance the probability of discovering rare recessive mutations. TJL has an established Phenotypic Deviant Search Program for identifying potential new mutations within the Production colonies. Animal care technicians are encouraged to watch for any phenotype not characteristic of a particular strain while providing routine animal care. Deviant mice, together with their parents and siblings, are submitted to a biweekly clinic. Individual TJL investigators take advantage of this opportunity to observe the mice carefully for the potential importance of any deviations in appearance or behavior. In addition, TJL has an established Mouse Mutant Resource (MMR¹) in which the genetics and pathological effects of new mutations are systematically characterized. It is recognized that many spontaneous mouse mutations resemble human mutations that cause severely debilitating genetic diseases (Grompe et al. 1989), and the resulting disorders may more closely resemble human disorders than those caused by targeted mutations in the same genes (e.g., Bruneau et al. 2001; Johnson et al. 1998b).

Often new mutations occur on controlled genetic backgrounds (e.g., inbred strains), which enhances their value for research. A single mutation segregating on an inbred background provides an experimental system in which differences between mutant and control mice can be attributed to the mutant gene. Although mutations can be backcrossed onto an inbred genetic background, resulting in a so-called congenic strain, spontaneous occurrence on an inbred background both obviates the need and time this process requires and eliminates the possibility of a change in the original phenotype due to the new genetic background. Because the family history of all mice in the breeding facility can be traced, it is almost always possible to recover new mutations identified through the Phenotypic Deviant Search Program and to establish and reproduce indefinitely the strains bearing them. TJL's animal caretakers are skilled at recognizing deviants. The sound husbandry practices and regular animal health and genetic quality control monitoring programs ensure that deviants are likely to be mutations rather than the result of environmental changes. Finally, TJL's Cryopreservation Resource offers a secure and economical means to ensure against the inadvertent loss of valuable mutations and to preserve mutations with future potential economically.

The MMR at TJL evolved from a long tradition of studying and maintaining mutant mice. The first mutant colonies were established by Drs. Elizabeth S. Russell and George D. Snell in the 1930s and early 1940s. The tradition continued from the 1940s to 1960s, with Drs. Margaret Dickie, Margaret C. Green, and Eva M. Eicher; and Priscilla

“Skippy” Lane, Hope Sweet, Jan Southard, and Linda Washburn. The first institutional predecessor of the current MMR was funded in 1959 by the National Science Foundation and has been supported continuously since the early 1970s by NCCR. TJL's current MMR has the following three vital functions: (1) to identify and characterize new mouse mutations for biomedical research, (2) to propagate and cryopreserve new and established mouse mutations in genetically defined stocks, and (3) to distribute mice carrying these mutations to other scientists world wide. The functions are described briefly below.

Discovery and Characterization of New Mutations

The first step in this program is to identify new mutations that may have biomedical significance. More than 3 million mice are produced each year in TJL's Production and Repository colonies, as well as in the colonies of individual research staff members. Experienced animal care technicians who are trained to screen for any deviant phenotypes transfer all of the mice to clean cages once a week. The Phenotypic Deviant Search Program provides research staff with biweekly displays of phenotypic deviants from Production.

Genetic Analysis

The most important feature of mutations is that they are genetically transmitted and can be propagated. No matter how valuable a new deviant may appear, it is useless unless it is reproducible and can be maintained and made available to other scientists. If viable and fertile, a new deviant is crossed to an unrelated strain to determine whether the characteristic is transmitted to offspring and, if it is, the mode of inheritance. If the characteristic appears among the F1 progeny, it is inherited as a dominant or X-linked mutation. If the original abnormal phenotype does not appear among the F1 progeny, they are intercrossed, because a recessive character will reappear in approximately one fourth of the F2 progeny. A semidominant gene will produce an intermediate phenotype in F1 progeny, and both the intermediate and original phenotypes will be recovered in the F2 generation. When mutations cause lethality or sterility, the same breeding process can be carried out using heterozygotes instead of homozygotes. X-linkage will be detected by sex-related transmission patterns; X-linked mutations are transmitted only to female progeny from sires and usually cause a more severe phenotype in hemizygous males than heterozygous females. Female heterozygotes often display a variegated or variable phenotype due to Lyonization (Lyon 1963).

New mutations are often tested for allelism if there is a previously identified gene mutation that produces a similar phenotype by making direct crosses between mice carrying the two mutations. Note that a new allele of an already known gene also is valuable for helping identify the gene

mutated and for studying the function of the gene product (Lorenz-Depiereux et al. 2004). With the increased efficiency of linkage testing strategies, however, it is sometimes easier to establish chromosomal location of a new gene first and then test for allelism with genes in the chromosomal region if any produce a similar phenotype.

Once a deviant phenotype is shown to be an independent new mutation, the second step in genetic analysis is to determine the chromosomal location of the mutant gene. Determining the chromosomal location is the first step toward identifying the mutated gene by the positional candidate gene approach. Once a mutant gene is localized to a chromosomal region, the public mouse sequence is examined for known and predicted genes in the region where alteration might cause the mutant phenotype. If obvious candidates are not found in the mouse sequence, the public human sequence is examined for potential candidates and the mouse sequence is analyzed for the conserved mouse genes. The more genes that exist in the chromosomal interval to which the mutated gene maps, the more progeny must be produced in the linkage cross. This process, often referred to as a high resolution cross, reduces the size of the interval and, hence, the number of candidates. To identify the mutated gene, one then either sequences candidate genes in DNA from mutant mice or performs expression analysis to look for reduced or lack of expression.

Because each new spontaneous or chemically induced mutation is a unique event, efficient methods used for mapping polymorphic loci (e.g., recombinant inbred strains and DNA preserved from interspecific backcrosses) are not useful for their genetic mapping. Instead, a new cross must be set up involving each new mutation. The most common mapping strategy uses an inbred strain derived from *Mus musculus castaneus*, CAST/Ei, as a linkage testing stock and widely dispersed DNA sequence variations as genetic markers (Johnson et al. 1993, 1994). Examples are simple sequence length polymorphisms that can be amplified by polymerase chain reaction (e.g., simple sequence length polymorphisms; Dietrich et al. 1992) or, more recently, single nucleotide polymorphisms (SNPs¹; Petkov et al. 2004). Crosses with CAST are not as advantageous for SNP analysis because there is much variation among standard inbred strains. For this reason, the number of varying markers is comparable in crosses between standard strains and crosses with CAST.

The efficiency of the approach described above is enhanced by performing an intercross (each mutant F2 mouse represents two potentially recombinant F1 chromosomes) and pooling 15 to 20 DNAs from mutant mice for the initial screen (Taylor et al. 1994). Once the chromosomal location of the new mutation is detected, its position on the chromosome is determined by genotyping individual linkage cross DNAs for other DNA markers along the chromosome. DNAs from the same linkage cross are saved to test for recombination between the new mutation and any nearby potential candidate loci.

Basic phenotypic characterization is carried out simultaneously with genetic mapping. Basic characterization of the effects of a new mutation includes determining anatomical and physiological defects by careful observation during development of the mutant, by autopsy, and by external examination. All tissues and organs are screened histologically for pathological changes. Serum and urine samples are analyzed for abnormal clinical biochemistry. More in-depth analysis may be performed depending on the nature of the phenotype. For example, mutants with skeletal abnormalities are analyzed using X-ray, bone densitometry, and alizarin-stained skeletal preparations.

Maintenance of Mutant Stocks

A “model” should be reproducible, readily available, economical to maintain, and relatively easy to handle. In addition, a mutant gene is most useful for research when it is on a defined genetic background. For this reason, mutations should be maintained whenever possible on an inbred genetic background, usually the one on which the mutation arose. Such strains may be maintained by mating homozygote to heterozygote, or by mating progeny-tested heterozygotes in the case of recessive mutations. These breeding systems produce mutant mice with nonmutant “wild type” littermate controls. One must be aware, however, that all or some of the wild type controls are heterozygotes and may have subtle or nonvisible mutational effects. Maintenance of strains with dominant mutations is often accomplished by mating a mouse carrying the mutation (heterozygotes) to mice from the strain of origin.

Deleterious mutations that cause postnatal lethality or functional sterility may be maintained by ovary transplantation, as long as the ovary is fertile. The ovaries from homozygous recessive mutant females are transplanted into a histocompatible host that is mated to a +/+ male, producing obligate heterozygous progeny. The progeny are intercrossed to produce homozygous mutant and +/? control mice. In the MMR, we use a “color-coded” system for mutations with recessive coat colors. Using this scheme, hosts with a dominant coat color are transplanted with mutant ovaries carrying a recessive coat color and mated to a male from the mutant or background strain; offspring with the recessive coat color are at least obligate heterozygotes for the transferred mutation.

Some deleterious mutations must be maintained on hybrid backgrounds to provide affected mice that survive long enough to be useful in research, to provide breeding stock that produces large enough litters to ensure occurrence of sufficient affected offspring, and to conserve space. For example, the hybrid breeding procedure is used to maintain the spastic mutation (*Glr^b^{spa}*). The homozygous (*spa/spa*) female is mated to a hybrid B6C3FeF1/J-*a/a* F1 male. The offspring of this cross are all known heterozygotes (*spa/+*).

and are mated together to produce more *spa/spa* females to be mated back to a hybrid male again to produce the next N generation. This procedure maintains the vigor of the stock and makes available to other investigators both spastic mutants with controls and breeding pairs.

Finally, a traditional but still useful method for some mutant genes is to maintain “balanced” stocks in which the mutant gene is linked to (coupling) or balanced against (repulsion) a closely linked, easily detected second gene. Examples of such strains are the following two diabetes strains maintained at TJL: B6.Cg-*m* *+/+* *Leprd*/*J* and B6.Cg-*m* *Leprd*/*+* *+/J*. In the first case (repulsion), mating heterozygotes produces *Leprd*/*Leprd* (diabetes) homozygotes, *m/m* (misty) homozygotes, and more heterozygous breeders. In the second case (coupling), all diabetes homozygotes are also homozygous for misty and are recognized by their misty gray coat color.

All mutant strains maintained in an investigator’s or breeder’s colony should be protected against loss by cryopreserving embryos or gametes. Loss of a characterized strain not only wastes the money spent in the genetic and phenotypic characterization analysis but also prevents the use of a valuable strain by other investigators. For a repository such as that at TJL, cryopreservation also provides cost-effective colony management. Cryopreserved strains can be removed from the breeding colony when demand diminishes to make room for new strains. Strains should never be removed from the breeding colony until live mice have been successfully reconstituted from the frozen state.

Distribution of Mice and Information

A new mutation is of no use unless it is made known to potential users in the scientific community. In addition, investigators funded by NIH must make their strains available to meet the established NIH sharing policy. Publication in the scientific literature is the most obvious method for individual investigators. It is important to register all new mutants or newly discovered genes with the Mouse Genome Informatics database (MGI¹; <http://informatics.jax.org>) and to obtain proper genetic nomenclature. Registering a new mutation with MGI will automatically make public even new mutant strains that may not be sufficiently analyzed to warrant publication. Mutant strains may be submitted to a centralized repository (e.g., TJL) if investigators cannot distribute mice themselves.

Training for Discovery

Serendipity awareness should be included in the standard training for all animal care technicians because they are in the “front lines” for finding new, potentially valuable mutations. Animal care training programs should include lectures on being observant and discussing possible types of abnormalities that are likely to represent important new mutations. For example, small size may indicate an endocrinological or growth factor defect; fur loss often indicates an

immunological deficit. Well-trained animal care technicians can play a major role in discovering new spontaneous mutation mouse models.

Summary

Serendipity plays a major role in discovering new mouse models for human genetic disorders and for basic biomedical and biological research. Proper training of animal care technicians greatly increases the probability of discovering new spontaneous mutations that cause visible phenotypes in mice. Capitalizing on such discoveries requires genetic and phenotypic analysis of the new potential mutant models discovered in this way. The established new mutant analysis and development program in the TJL MMR provides evidence that serendipitous observation of new deviant phenotypes can be exploited to develop new mouse models.

The approach used by the MMR has contributed to progress in biomedical and basic research in the following ways:

- Providing mutant genes and mouse models to research scientists;
- Contributing to basic knowledge on the genetics of the mouse;
- Serving as a source of information on mouse mutations and genetics;
- Economically preserving mutations; and
- Distributing the mutant mice carrying these mutations to scientists in the United States and around the world.

New mutations discovered and characterized by the MMR team are announced on TJL’s web site (<http://www.jax.org>) “Mouse Resources” web page (http://www.jax.org/resources/mouse_resources.html) as soon as basic genetic and phenotypic characterization has been completed.

Acknowledgments

The basic spontaneous mutation development program in the Mouse Mutant Resource at The Jackson Laboratory (TJL) is supported by the following grants from the National Institutes of Health: National Center for Research Resources grant P40 RR01183, National Eye Institute grants EY015073 and EY07758, National Institute for Child Health and Human Development contract HD53230, and Cancer Center Core grant CA34196; as well as TJL institutional funds and revenues generated by the distribution of mice to other scientists. The author gratefully acknowledges the assistance of Leah Rae Donahue and Kenneth R. Johnson for critically reviewing the manuscript before submission.

References

- Bruneau S, Johnson KR, Yamamoto M, Kuroiwa A, Duboule D. 2001. The mouse *Hoxd13*(*spd*) mutation, a polyalanine expansion similar to hu-

- man type II synpolydactyly (SPD), disrupts the function but not the expression of other *Hoxd* genes. *Dev Biol* 237:345-353.
- Chang B, Hawes NL, Hurd RE, Davisson MT, Nusinowitz S, Heckenlively JR. 2002. Retinal degeneration mutants in the mouse. *Vision Res* 42: 517-525.
- Consortium [Mouse Genome Sequencing Consortium]. 2002. Initial sequencing and comparative analysis of the mouse genome. *Nature* 420: 520-562.
- Davisson MT, Roderick TH, Akeson EC, Hawes NL, Sweet HO. 1990. The hairy ears (*Eh*) mutation is closely associated with a chromosomal rearrangement in mouse chromosome 15. *Genet Res* 56:167-178.
- Dietrich W, Katz H, Lincoln SE, Shin H-S, Friedman J, Dracopoli NC, Lander ES. 1992. A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* 131:423-447.
- Dollé P, Dierich A, LeMeur M, Schimmang T, Schuhbauer B, Chambon P, Duboule D. 1993. Disruption of the *Hoxd-13* gene induces localized heterochrony leading to mice with neotenic limbs. *Cell* 75:431-441.
- Goldowitz D, Frankel WN, Takahashi JS, Holtz-Vitaterna M, Bult C, Kibbe WA, Snoddy J, Li Y, Pretel S, Yates J, Swanson DJ. 2004. Large-scale mutagenesis of the mouse to understand the genetic bases of nervous system structure and function. *Brain Res Mol Brain Res* 132:105-115.
- Grompe M, Gibbs RA, Chamberlain JS, Caskey CT. 1989. Detection of new mutation disease in man and mouse. *Mol Biol Med* 6:511-521.
- Herron BJ, Lu W, Rao C, Liu S, Peters H, Bronson RT, Justice MJ, McDonald JD, Beier DR. 2002. Efficient generation and mapping of recessive developmental mutations using ENU mutagenesis. *Nat Genet* 30:185-189.
- Hitotsumachi S, Carpenter DA, Russell WL. 1985. Dose-repetition increases the mutagenic effectiveness of N-ethyl-N-nitrosourea in mouse spermatogonia. *Proc Natl Acad Sci U S A* 82:6619-6621.
- Hrabe de Angelis MH, Flaswinkel H, Fuchs H, Rathkolb B, Soewarto D, Marschall S, Heffner S, Pargent W, Wuensch K, Jung M, Reis A, Richter T, Alessandrini F, Jakob T, Fuchs E, Kolb H, Kremmer E, Schaeble K, Rollinski B, Roscher A, Peters C, Meitinger T, Strom T, Steckler T, Holsboer F, Klopstock T, Gekeler F, Schindewolf C, Jung T, Avraham K, Behrendt H, Ring J, Zimmer A, Schughart K, Pfeiffer K, Wolf E, Balling R. 2000. Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nat Genet* 25:444-447.
- Johnson KR, Cook SA, Ward-Bailey P, Bustin M, Davisson MT. 1993. Identification and genetic mapping of the murine gene and 20 related sequences encoding chromosomal protein HMG-17. *Mamm Genome* 4:83-89.
- Johnson KR, Cook SA, Davisson MT. 1994. Identification and genetic mapping of 151 dispersed members of 16 ribosomal protein multigene families in the mouse. *Mamm Genome* 5:670-687.
- Johnson KR, Cook SA, Zheng QY. 1998a. The original shaker-with-syndactylism mutation (*sy*) is a contiguous gene deletion syndrome. *Mamm Genome* 9:889-892.
- Johnson KR, Sweet HO, Donahue LR, Ward-Bailey P, Bronson RT, Davisson MT. 1998b. A new spontaneous mouse mutation of *Hoxd13* with a polyalanine expansion and phenotype similar to human synpolydactyly. *Hum Mol Genet* 7:1033-1038.
- Lorenz-Depiereux B, Guido VE, Johnson KR, Zheng QY, Gagnon LH, Bauschatz JD, Davisson MT, Washburn LL, Donahue LR, Strom TM, Eicher EM. 2004. New intragenic deletions in the *Phex* gene clarify X-linked hypophosphatemia-related abnormalities in mice. *Mamm Genome* 15:151-161.
- Lyon MF. 1963. Lyonisation of the X chromosome. *Lancet* 12:1120-1121.
- Makalowski W, Boguski MS. 1998. Evolutionary parameters of the transcribed mammalian genome: An analysis of 2,820 orthologous rodent and human sequences. *Proc Natl Acad Sci U S A* 95:9407-9412.
- Melvold RW, Kohn HI. 1975. Histocompatibility gene mutation rates: *H-2* and *non-H-2*. *Mut Res* 27:415-418.
- Melvold RW, Wang K, Kohn HI. 1997. Histocompatibility gene mutation rates in the mouse: A 25-year review. *Immunogenetics* 47:44-54.
- MGD [Mouse Genome Database]. 2005. <http://www.informatics.jax.org>.
- Nolan PM, Peters J, Strivens M, Rogers D, Hagan J, Spurr N, Gray IC, Vizor L, Brooker D, Whitehill E, Washbourne R, Hough T, Greenaway S, Hewitt M, Liu X, McCormack S, Pickford K, Selley R, Wells C, Tymowska-Lalanne Z, Roby P, Glenister P, Thornton C, Thaug C, Stevenson JA, Arkell R, Mburu P, Hardisty R, Kiernan A, Erven A, Steel KP, Voegelings S, Guenet JL, Nickols C, Sadri R, Nasse M, Isaacs A, Davies K, Browne M, Fisher EM, Martin J, Rastan S, Brown SD, Hunter J. 2000. A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nat Genet* 25:440-443.
- Petkov PM, Ding Y, Cassell MA, Zhang W, Wagner G, Sargent EE, Asquith S, Crew V, Johnson KA, Robinson P, Scott VE, Wiles MV. 2004. An efficient SNP system for mouse genome scanning and elucidating strain relationships. *Genome Res* 14:1806-1811.
- Russell WL, Kelly EM, Hunsicker PR, Bangham JW, Maddux SC, Phipps EL. 1979. Specific-locus test shows ethylnitrosourea to be the most potent mutagen in the mouse. *Proc Natl Acad Sci U S A* 76:5818-5819.
- Schlager G, Dickie MM. 1971. Natural mutation rates in the house mouse, estimates for five specific loci and dominant mutations. *Mut Res* 11: 89-96.
- Skarnes WC, Auerbach BA, Joyner AL. 1992. A gene trap approach in mouse embryonic stem cells: The lacZ reporter is activated by splicing, reflects endogenous gene expression, and is mutagenic in mice. *Genes Dev* 6:903-918.
- Skarnes WC, von Melchner H, Wurst W, Hicks G, Nord AS, Cox T, Young SG, Ruiz P, Soriano P, Tessier-Lavigne M, Conklin BR, Stanford WL, Rossant J; International Gene Trap Consortium. 2004. A public gene trap resource for mouse functional genomics. *Nat Genet* 36:543-544.
- Svenson KL, Bogue MA, Peters LL. 2003. Invited review: Identifying new mouse models of cardiovascular disease: A review of high-throughput screens of mutagenized and inbred strains. *J Appl Physiol* 94:1650-1659.
- Taylor BA, Navin A, Phillips SJ. 1994. PCR-amplification of simple sequence repeat variants from pooled DNA samples for rapidly mapping new mutations of the mouse. *Genomics* 21:626-632.
- Wood PA, Armstrong D, Sauls D, Davisson MT. 1988. Screening mutant mice for inborn errors of metabolism. *Lab Anim Sci* 38:15-19.
- Zheng QY, Johnson KR, Erway LC. 1999. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. *Hear Res* 130:94-107.