Human Molecular Genetics, 1999, Vol. 8, No. 10 Review **1955–1963**

Mouse ENU Mutagenesis

Monica J. Justice[+], Janice K. Noveroske, John S. Weber, Binhai Zheng and Allan Bradley[1]

Department of Molecular and Human Genetics and [1]Howard Hughes Medical Institute, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77096, USA

Received July 14, 1999; Accepted July 20, 1999

The progress of human genome sequencing is driving genetic approaches to define gene function. Strategies such as gene traps and chemical mutagenesis will soon generate a large mutant mouse resource. Point mutations induced by *N*-ethyl-*N*-nitrosourea (ENU) provide a unique mutant resource because they: (i) reflect the consequences of single gene change independent of position effects; (ii) provide a fine-structure dissection of protein function; (iii) display a range of mutant effects from complete or partial loss of function to exaggerated function; and (iv) discover gene functions in an unbiased manner. Phenotype-driven ENU screens in the mouse are emphasizing relevance to human clinical disease by targeting cardiology, physiology, neurology, immunity, hematopoiesis and mammalian development. Such approaches are extremely powerful in understanding complex human diseases and traits: the base-pair changes may accurately model base changes found in human diseases, and subtle mutant alleles in a standard genetic background provide the ability to analyze the consequences of compound genotypes. Ongoing mouse ENU mutagenesis experiments are generating a treasure trove of new mutations to allow an in-depth study of a single gene, a chromosomal region or a biological system.

INTRODUCTION

Manipulative genetic tools in the mouse are extensive and powerful. Mouse geneticists can eliminate or overexpress genes in the whole animal or in a specific tissue, introduce large pieces of self or foreign DNA into the genome and engineer whole chromosomes. These techniques, combined with the genetic, developmental, pathologic and physiologic similarities between mouse and human, have established the laboratory mouse as a primary model organism for human disease research. Inbred strains of mice provide the opportunity to study a disease trait in a defined genetic background, allowing distinction between the phenotypes conferred by a single mutation versus the contributions of other genetic modifiers.

The ability to engineer loss of function mutations by homologous recombination in embryonic stem cells pioneered a revolution in mouse biology. Using embryonic stem cell technology, any gene involved in human disease can be disrupted, providing valuable information about the consequences of mutation in the whole animal. While null mutations are necessary, subtle mutations induced by *N*-ethyl-*N*-nitrosourea (ENU) are invaluable for examining the full range of gene function. Mutations in coding regions and regulatory elements can yield vastly different phenotypes while they provide a fine-structure dissection of protein function and gene regulation. Series of alleles may be generated by homologous recombination, but phenotype-driven mutagenesis offers many advantages in speed of generation and removal of investigator bias. Therefore, a knockout database for the mouse genome should be considered only as a starting point; additional alleles and tissue specificities are mandatory to complete the functional analysis.

MUTAGENIZING THE MOUSE GERMLINE

ENU can transfer its ethyl group to oxygen or nitrogen radicals in DNA, resulting in mispairing and base-pair substitution if not repaired. The highest mutation rates occur in pre-meiotic spermatogonial stem cells, with single locus mutation frequencies of 6–1.5 $\times$ 10^{-3} (1–3), equivalent to obtaining a mutation in a single gene of choice in one out of every 175–655 gametes screened. Because of its power in isolating mutations in any gene or region of interest, ENU has been used in the mouse to: (i) obtain multiallelic series of single genes to further define function; (ii) dissect biochemical or developmental pathways; (iii) obtain new recessive mutations on a single chromosome or genome-wide; and (iv) saturate regions of the mouse genome that are uncovered by deletions (4–10). The analysis of 62 sequenced germline mutations from 24 genes reveals that ENU predominantly modifies A/T base pairs, with 44% A/T→T/A transversions, 38% A/T→G/C transitions, 8% G/C →A/T transitions, 3% G/C→C/G transversions, 5% A/T→C/G transitions and 2% G/C→T/A transitions (Table 1; Fig. 1). When translated into a protein product, these changes result in 64% missense mutations, 10% nonsense mutations and 26% splicing errors (Table 1; Fig. 1).

Because it is a point mutagen, ENU can induce many different types of allele. Loss-of-function mutations, viable hypomorphs of lethal complementation groups, antimorphs and gain-of-function mutations have been isolated in mouse

[+]To whom correspondence should be addressed. Tel: +1 713 798 5440; Fax: +1 713 798 1489; Email: mjustice@bcm.tmc.edu

Table 1. Summary of sequenced ENU-induced mutations

Locus	Number of ENU mutations	Number sequenced	Mutation	Functional classification	References
Agouti (*a*)	8	1	AT-GC	Missense	(41,42)
Adenomatous polyposis coli (Apc^{Min})	1	1	AT-TA	Nonsense	(43)
Bone morphogenetic protein-5 (*Bmp5*)	17	11	4 AT-GC 4 AT-TA 2GC-AT 1 unknown	6 missense 2 nonsense 2 splicing with deletion 1 splicing with deletion	(44)
Clock	1	1	AT-TA	Splicing with deletion	(29)
γ-Crystallin (*Cryge*)	?	1	AT-GC	Missense	(45)
Dystrophin (*Dmd*)	4	4	3 AT-TA 1 GC-AT	3 splicing with frameshift 1 nonsense	(46)
Embryonic endoderm (*eed*)	3	2	AT-GC AT-TA	2 missense	(10,16)
Connexin 50 (*Gja8*)	1	1	AT-CG	Missense	(47)
Glucose phosphate isomerase (*Gpi1*)	5	4	3 AT-GC 1 unknown	Missense Splicing with deletion	(48)
Glucose 6 phosphate dehydrogenase (*G6pdx*)	1	1	AT-TA	Splicing with reduced protein	(49)
Hemoglobin alpha (*Hba*)	1	1	AT-TA	Missense	(50)
Hemoglobin beta (*Hbb*)	2	2	AT-GC AT-TA	2 missense	(14,51)
Kreisler (*krm1*)	1	1	AT-GC	Missense	(52)
Lactate dehydrogenase (*Ldh1*)	5	5	4 AT-GC 1 AT-CG	4 missense Splicing with reduced protein	(53) (54)
Mast cell growth factor (*Mgf*)	?	1	AT-TA	Splicing with frameshift	(55)
Micropthalmia (*Mitf*)	?	1	AT-TA	Missense	(56)
Myosin VA (*Myo5a*)	50	10	2 GC-CG 5 AT-TA 2 AT-GC 1 GC-AT	8 missense 2 splicing, 1 protein product from 1 of 3 isoforms	(57,58) (42)
Myosin VIIA (*Myo7a*)	7	5	3 AT-TA 1 AT-GC 1 GC-AT	1 missense 1 splicing with deletion 1 splicing with frameshift 2 nonsense	(10,12,59)
Phenylalanine hydroxylase (*Pah*)	3	2	2 AT-GC	2 missense	(2,60)
Paired box homeobox 6 (*Pax6*)	6	1	GC-TA	Splicing with frameshift	(67)
Quaking (*qk*)	5	3	2 AT-GC 1 AT-TA	2 missense 1 disruption of one isoform	(11,19)
Triosephosphate isomerase (*Tpi*)	4	4	3 AT-TA 1 AT-CG	3 missense 1 Stop to C	(61)
Tyrosinase related protein 1 (*Tyrp1*)	40	1	AT-GC	Missense	(62)

The number of mutations induced by ENU and those that have been sequenced are shown in columns 2 and 3. The number of lesions of each type are shown in column 4; these do not correlate with the functional change in column 5. Note that the lesion was not found in two of the mutations listed as splice mutations, so these two are not included in the numbers given in the text. ?, the number of ENU-induced mutations is not known.

mutagenesis screens (4,9–16). Series of alleles at single loci are extremely powerful when analyzed together to define function. Allelic series at complex loci can disrupt individual protein isoforms, leading to the discovery of distinct functions in various tissues throughout the life of an organism (17). A striking example of such an allelic series is the quaking (*qk*) locus. Prior to the isolation of the ENU-induced alleles, the quaking locus was defined by a single spontaneous allele, qk^{v}, with a homozygous phenotype of seizures and quaking caused by severe central nervous system (CNS) dysmyelination (18). The ENU-induced alleles, however, reveal that quaking also functions during embryogenesis (7,13). Homozygotes of four of five ENU-induced alleles (qk^{l-1}, qk^{kt1}, qk^{k2}, $qk^{kt3/4}$) die at embryonic days (E) 8.5–10.5 with CNS defects, whereas only one is viable with quaking and seizures (qk^{e5}) (19; J.K. Noveroske and M.J. Justice, unpublished data). Although the quaking alleles can be grouped based on embryo lethality or viable dysmyelination, each allele deviates in some significant way from these general phenotypes to make it a unique and valuable resource for fine-structure/function studies and modeling human neural tube defects, as well as seizure and myelination disorders (Table 2). Allelic series generated by

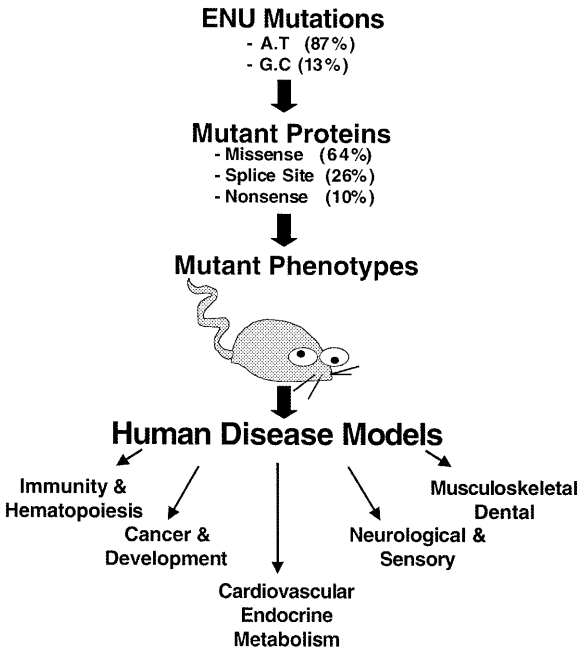

Figure 1. ENU mutagenesis to isolate mutations. ENU induces lesions in the DNA of mouse spermatogonial stem cells, primarily affecting AT base pairs. These lesions are present in the sperm of the male, and after their isolation through genetic and phenotypic screens, give rise to a variety of phenotypic mutations. The mutant protein products are primarily missense mutations, a valuable class of mutations for dissecting protein structure and function. Mutagenesis in the mouse will emphasize modeling human diseases through phenotype-driven assays.

ENU or gene targeting at other complex loci will be valuable tools for dissecting protein function (17).

ISOLATING DISEASE MODELS USING ENU

Different genetic screens can be used to isolate ENU-induced mutations. A single generation screen can rapidly generate viable and fertile mutants that represent allele series, modifiers or dominant mutations. Two-generation deletion screens can identify recessive lethal mutations in a defined region of the genome. Three-generation pedigree screens may be used to scan the entire genome for a viable mutation of interest or, in combination with linked markers or balancer chromosomes, to isolate lethal or sterile alleles (see below).

Large-scale screens

Several large-scale mutagenesis screens have already been funded internationally. The key features of these screens are summarized in Table 3. Each of these screens uses a different genetic strategy to isolate mutations: some screens target dominant mutations, whereas others are designed to isolate recessive mutations. Some groups are screening for recessive lethal and detrimental mutations with regional mutagenesis to address gene function in parallel with the Human Genome Project. Other groups are scanning large numbers of mutagenized genomes for dominant neurological and clinical hematology or biochemical variants.

Small-scale screens

Screening for mutations does not have to be carried out on a large scale. Two cost-effective strategies for the small laboratory are allelic series and sensitized pathway screens. A sensitized pathway screen targets a single biological or biochemical pathway, and exploits non-allelic non-complementation to isolate mutations in the first generation offspring of mutagenized males. In such a screen, an induced mutation (*) at a locus that interacts with the locus of interest (*m*) may fail to complement (*/+;+/*m*), yet yield a phenotype reminiscent of the original homozygous mutant (*m*/*m*). Some sensitized screens may be carried out in the background of a drug or environmental modification, instead of a genetic modification. For example, using a phenylalanine injection as a sensitizer, a number of mutations affecting the phenylalanine metabolism pathway were isolated as heterozygotes (20,21).

Combining gene-driven and phenotype-driven approaches

A unique approach towards isolating mutations combines gene-based targeting in embryonic stem cells with phenotype-driven ENU mutagenesis. Powerful genetic strategies in *Drosophila* rely on the availability of genetic reagents such as deletions, duplications and inversions to facilitate genetic screens and provide simple, cost-effective stock maintenance. Deletions are useful for generating haploidy in genetic screens, as well as for mapping using non-complementation strategies (Fig. 2B) (22). Inversions that carry a dominant marker and are homozygous lethal are ideal balancer chromosomes to suppress recombination

Table 2. Unique features of quaking alleles

Allele[a]	Homozygous phenotype	Unique phenotype	Lesion
qk^{v}	Viable	Sterile males	1 Mb deletion/loss of brain-specific regulatory region
qk^{kt1}	Embryo lethal	Spontaneous seizures in aged heterozygotes	Unknown
qk^{k2}	Embryo lethal	Earlier and more severe embryo lethality with semi-dominant reduction of adult brain myelin lipids	V→G change in conserved RNA binding domain
$qk^{kt3/4}$	Embryo lethal	Null phenotype: CNS, brain, heart defects	E→G change/loss of protein dimerization
qk^{l-1}	Embryo lethal	Transient quaking in compound heterozygotes qk^{l-1}/qk^{v}	Splice defect/loss of nuclear localized protein isoform
qk^{e5}	Viable	Fertile males. Extremely severe quaking and seizures	Unknown

[a]Each allele arose in an ENU experiment except for the spontaneous qk^{v} allele.

Table 3. Funded large-scale ENU screens

Location	Primary investigators	Genetic screen	Mouse region	Human homologue	Phenotypes	References	Web site
Mouse Mutagenesis Programme, Mouse Genome Centre, Medical Research Council, Harwell, UK	Steve Brown Jo Peters	Dominant Recessive	Genome-wide M13	 H6p22–p23	Neurological	(63)	http://www.mgu.har.mrc.ac.uk/mutabase/
GSF-National Research Center for Environment and Health, Neuherberg, Germany	Rudi Balling Martin Hrabe de Angelis	Dominant Recessive	Genome-wide Genome-wide		Clinical hematology/chemistry, dysmorphology, allergy, lysosomal	(64)	http://www.gsf.de/isg/groups/enu-mouse.html
Mouse Genome Center, Baylor College of Medicine, Houston, Texas	Allan Bradley Monica Justice	Recessive	M11 M4	H17 H9p/9q/1p	Gene function/development/human disease	(22,65)	http://www.mouse-genome.bcm.tmc.edu
The Jackson Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania	John Schimenti Maya Bucan	Recessive	M5	H4p16–4q12 H7q36	Gene function/behavior	(66)	http://lena.jax.org/~jcs/
Oak Ridge National Laboratory, Oak Ridge, Tennessee	Eugene Rinchik Dabney Johnson	Recessive	M7	H11p H15q	Gene function/development	(10)	http://www.bio.ornl.gov/htmouse
Medical Genome Centre, Australia National University, Canberra, Australia	Chris Goodnow	Recessive	Genome-wide		Visibles/immunity, aging	C. Goodnow, personal communication	http://jcsmr.anu.edu.au/group_pages/mgc/MedGenCen.html
Mouse Functional Genomics Research Group, RIKEN Genomic Sciences Center, Yokohama, Japan	Toshihiko Shiroishi Tetsuo Noda Yoichi Gondoh	Dominant Recessive	Genome-wide Genome-wide		Late onset	T. Shiroishi, personal communication	Available in October

and readily identify classes of offspring for the isolation and maintenance of lethal or detrimental mutations (Fig. 2C). The ability to engineer whole chromosome regions using Cre/*loxP* technologies allows the creation of these genetic reagents in a two-step gene targeting process (Fig. 2A) (23,24). The approach is gene-based because the endpoints of the deletion or inversion are known, minimizing the amount of effort required to characterize the rearrangements. Engineering techniques allow for the rearrangements to be tagged with a dominant yellow coat color marker, *K-14 agouti*, providing a resource for simple mapping, stock maintenance and genetic screens (22,24,25). The creation of mouse genomic libraries containing the constructs necessary for the targeting events provides a rapid system for their generation anywhere in the mouse genome (Fig. 2A) (25).

An initial mutagenesis effort, designed to isolate recessive mutations of many phenotypic classes, is targeting mouse Chromosome 11, which is a gene rich chromosome highly conserved with human Chromosome 17. The goal is to saturate the chromosome with mutations to define gene function, then use linkage conservation between the mouse and human to predict gene function in the human. Using the genetic reagents generated by Cre/*loxP* engineering, two mutagenesis schemes are being carried out: two-generation deletion pedigrees (Fig. 2B) and three-generation pedigrees using balancer chromosomes (Fig. 2C). Both schemes take advantage of the yellow coat color conferred by the *K14-agouti* transgene. The deletion scheme can be used only for large deletions that are not detrimental to the animal, limiting the screen to certain regions, but allowing mutations to be isolated in only two generations. The inversion scheme allows a larger portion of the chromosome to be screened for mutations, but requires three generations of breeding.

IDENTIFYING POINT MUTATIONS

To be valuable, new mutations must be localized so that candidate genes and relevant human disease models can be identified. Point mutations isolated by their phenotype must be mapped using phenotype information, since a molecular tag is not available. Traditionally, these mutations are mapped in meiotic backcrosses segregating the phenotype relative to multiple molecular polymorphisms (for a review see ref. 26). To map to a resolution of 10 cM requires analyzing 100 meioses. Thus, 100 new mutations isolated genome-wide would require the analysis of 10 000 mice. High resolution mapping strategies for positional

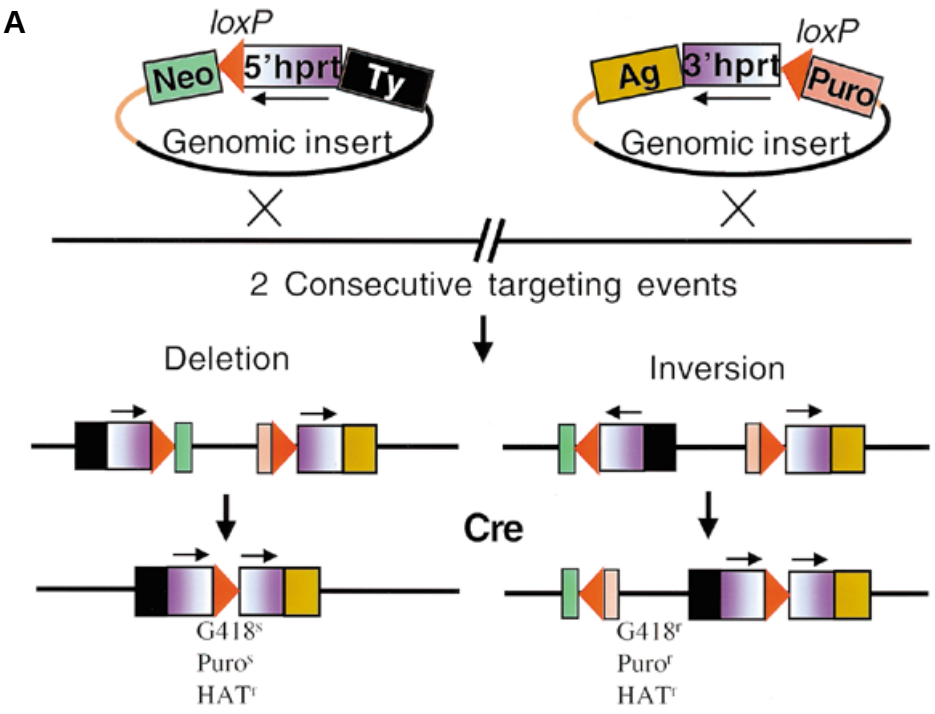

Figure 2. (**A**) Generation of chromosome rearrangements using Cre/*loxP*. Two lambda mouse genomic libraries have been constructed that contain the selectable markers required for two step targeting events. One contains the selectable marker neomycin (*Neo*), the 5′ end of hypoxanthine phosphoribosyltransferase (*Hprt*), a *loxP* site and the *Tyrosinase* minigene (*Ty*). The second library contains the selectable marker puromycin (*Puro*), the 3′ end of *Hprt*, a *loxP* site and the *K14-Agouti* transgene (*Ag*). If the *loxP* sites are inserted *in cis* in the same orientation, recombination after Cre transfection will produce a deletion, and HAT resistant, Puro sensitive, Neo sensitive ES cells. If the *loxP* sites are inserted in opposite orientation, recombination after Cre transfection will result in an inversion, with HAT resistant, Puro resistant, Neo resistant ES cells. (B and C) Mutagenesis schemes for mouse Chromosome 11 using yellow coat color-tagged chromosomal rearrangements. The deletion or inversion is tagged with a dominant yellow coat color marker, *K-14-agouti* (yellow). Each scheme uses the dominant Rex (*Re*, and indicated by the black chromosome) mutation on Chr 11, which causes curly fur (mottled). In each scheme, wild-type males (C57BL/6J, black) are injected with a 3 × 100 mg/kg dose of ENU. (**B**) The deletion scheme. After regaining fertility, ENU-treated males are mated to homozygous *Re/Re* females. The *Re* mutation marks the non-mutagenized chromosome, with the caveat that recombination can occur between a new linked mutation and *Re*. G1 animals, heterozygous for ENU mutagenized chromosomes and *Re* are mated to mice hemizygous for a yellow-tagged deletion. The resulting classes of offspring can be readily identified: (i) the mutant class is yellow and straight-haired and, if missing, indicates the likelihood of a lethal mutation; (ii) a carrier class that is wild-type, and can be used to recover any lethal mutations; (iii) two curly-haired classes of mice (black and yellow) that are uninformative and can be immediately discarded. (**C**) The inversion scheme. The balancer chromosome contains an inversion that suppresses recombination over a reasonable interval, 20–30 cM, is marked with the dominant *K14-agouti* transgene conferring yellow coat color, and is homozygous lethal due to disruption of one or more lethal genes at its endpoints. After regaining fertility, ENU-treated males are mated to females carrying the balancer chromosome (yellow). G1 animals that are yellow are mated with animals heterozygous for the balancer chromosome and *Re* (yellow mottled). Three classes of offspring can be identified in the second generation, and the fourth class, which is homozygous for the balancer chromosome, dies (upside down). The useful G2 animals are the yellow, straight-haired animals, which are brother–sister mated. The G3 offspring are easily classified as: (i) the wild-type mutant class, which if missing indicates the likelihood of a linked lethal mutation and (ii) a carrier class used to rescue any lethal mutations, which carries the balanced point mutation, ideal for stock maintenance.

cloning usually involve the analysis of 1000–2000 meioses per mutation. A multitude of dominant and recessive mutations can be isolated in any ENU screen, making mapping the bottleneck in the process, and requiring simpler mapping technologies such as DNA pooling (27). The coat color tagged screens described above are unique in that the mutation is isolated linked to visible chromosomal markers, so its chromosome location is known upon isolation, eliminating the need for meiotic mapping. Furthermore, the Cre/*loxP* generated deletions can be used to localize the mutations to a sub-chromosomal region by non-complementation (22,28). Because the sequence of the mouse genome will soon be available, many mutations will be assigned to genes based on positional candidacy after their localization. In addition, BAC complementation can be used to identify mutation–gene correlations (29).

As new technologies are being developed for high-throughput single nucleotide polymorphism detection, technologies for the detection of point mutations will become simpler (30,31). Unlike humans, naturally occurring polymorphisms are rare in inbred strains of mice, in particular within coding regions. Thus, the detection of point mutations is possible in an inbred strain background, making mutation detection with mismatch repair enzymes a potential approach to quickly map and identify the lesions.

SCREENING FOR DISEASE PHENOTYPES

In any screen for mutations using ENU, mutation isolation relies upon the phenotype assay, requiring that the mutant phenotype must vary significantly from the background. However, screening for mutations involves the analysis of many animals, so phenotype screens must be broad and inexpensive. Visible phenotypes that affect the eye, coat, size or neurological behavior are simple to identify, and such screens often yield novel mutations. Behavior and sensory organ phenotypes can be screened for using standard tests for reflex, sight or hearing loss, motor development, balance

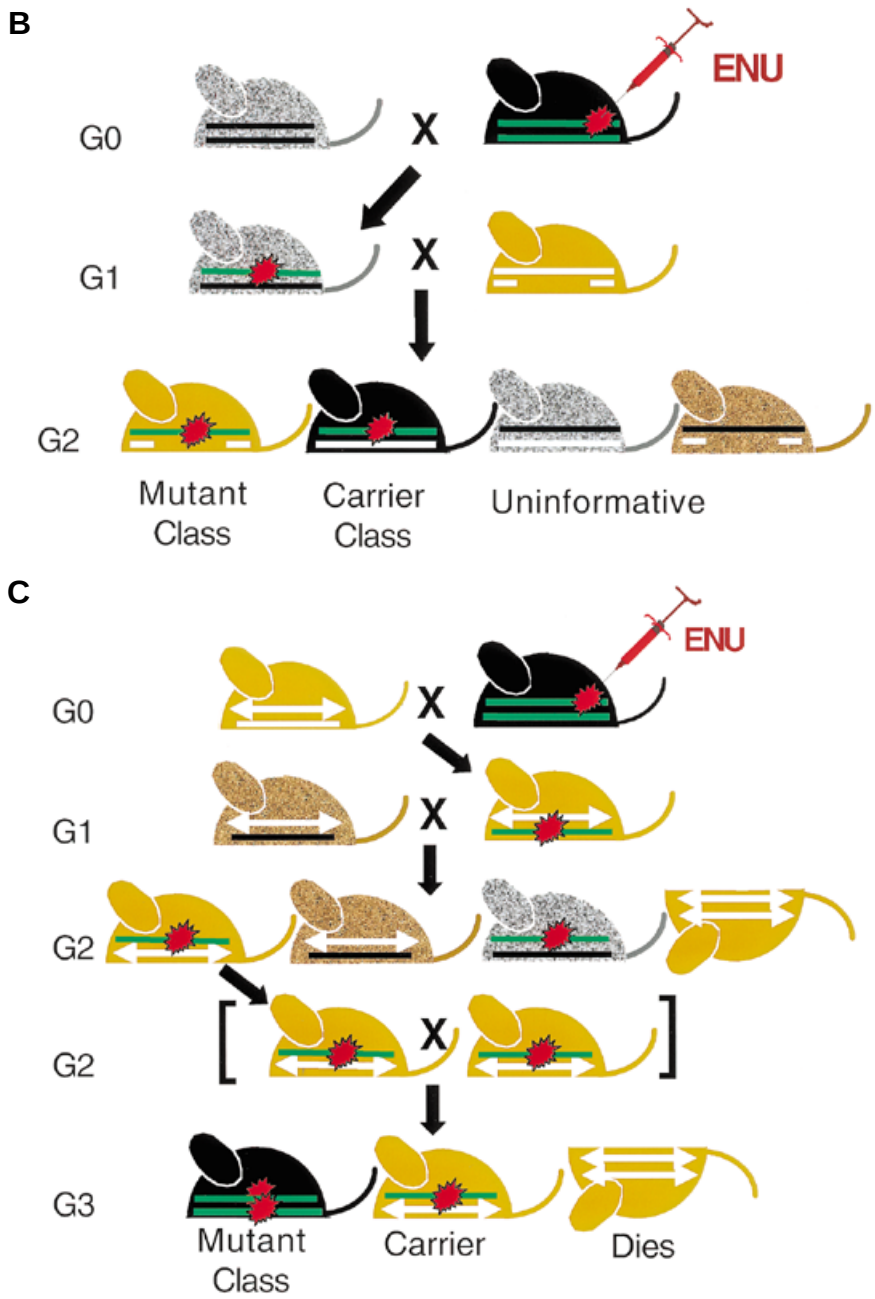

Figure 2. *Continued*

and coordination, as well as learning and memory. Skeletal development and soft tissue morphology can be examined using high resolution, low energy X-ray analysis. Clinical tests performed on mouse blood can yield a vast array of phenotypes relevant to human clinical disease even though tests performed on mouse body fluids must be performed on a microscale. Because of existing microscale tests for human infants, many clinical tests are already available. A complete blood count with microscopic differential analysis can identify abnormalities in red blood cell and white blood cell numbers or morphology, as well as platelet abnormalities. Extending the analysis of blood cells with flow cytometry may uncover other immunological defects. Anti-nuclear antibody quantitation can detect serum autoantibodies in a large variety of autoimmune disorders, including systemic lupus erythematosus. Clinical chemistry tests can diagnose multiple organ system anomalies, including liver, pancreatic, heart and kidney disorders. Urinalysis on mice reveals increased levels of protein or other abnormal by-products of disease. Tandem mass spectrometry can detect a variety of metabolic disorders affecting lipids, fatty acids or amino acids. Additional assays are being

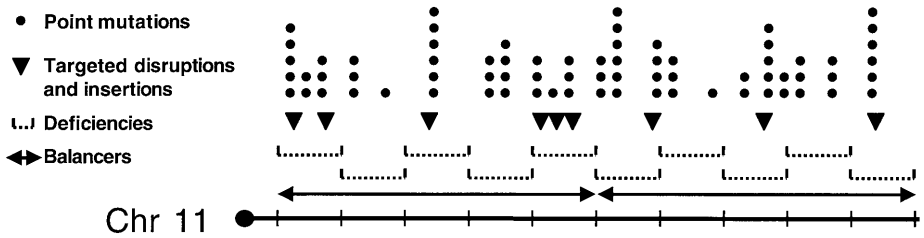

Figure 3. A mutant mouse resource. Initially, a mutant mouse resource at Baylor College of Medicine will be developed for mouse chromosome 11, and is used here as a demonstration of the types of genetic resource that will be available in the mouse. Ideally, such resources will be developed for other mouse chromosomes. The mutant resource will consist of a variety of mutations, including point mutations induced by ENU, targeted gene disruptions and insertions, as well as genetic reagents such as deletions and inversions. These mutations will constitute a functional map of the chromosome, and will be localized in molecular intervals, so they can be anchored to the physical map consisting of BAC and YAC contigs. When the mouse genome is sequenced, the mutations can be correlated with candidate genes that lie within the molecular intervals.

developed to identify neurological, cardiovascular, hematopoietic and immune phenotypes using high-throughput technologies such as microarrays.

GENERATING RESOURCES FOR MUTANT MICE

The generation of large numbers of mutations will create new ethical and scientific issues within the mouse community: new databases and phenotype assays will be required, cost effective mutation recovery from cryopreserved or freeze–dried sperm will become essential, and animal care and cost issues will arise. These efforts require a tremendous investment in mouse resources.

Genetic resources

One of the most powerful genetic resources currently available in the mouse are the inbred strains. Further defining genetic diversity in these strains is one of the priorities of the mouse community, with efforts underway to develop a strain characteristics database. This requires analyzing the inbred strains for many phenotypic parameters, including clinical hematology and chemistry, pathology, behavior and physiology.

The maintenance, analysis and mapping of large numbers of mouse mutations will require the generation of genetic resources to lower costs and reduce mistakes. Genetic reagents such as deletions are being developed by a variety of methods including Cre/*loxP* and radiation (22,23,25,32,33). Balancer chromosomes can currently be generated quickly and efficiently using Cre/*loxP* approaches (24,25). Transgenic mice containing human YACs or BACs can be useful in complementation and rescue studies (34). Because mutant phenotypes can vary on different inbred strain backgrounds, genetic reagents should also be maintained on standard genetic backgrounds.

Archiving

The generation of large numbers of mouse stocks requires efficient archiving and reconstitution of stocks. Storing mouse embryos by cryopreservation is an effective technique that has already been used for over a decade. Recently, successes in sperm cryopreservation have made it a tractable technique (35–37). In particular, mouse sperm can be used to reconstitute stocks by a number of assisted reproductive technologies: artificial insemination, *in vitro* fertilization and intracytoplasmic sperm injection (ICSI) (38). The successful reconstitution of mouse strains from freeze-dried sperm using ICSI may provide another method for archiving and reviving mouse strains (39).

Databases

Databases are already required for handling mouse genetic and phenotypic data. The primary mouse database is the Mouse Genome Database housed at the Jackson Laboratory (Bar Harbor, ME; http://www.informatics.jax.org), which includes a Mouse Locus Catalogue describing existing mouse mutants, and containing extensive map information describing their locations. To simplify the search for mutant mice, the International Mouse Strain Resource has recently been developed (40), which is mirrored at two Web sites: the Jackson Laboratory at http://www.jax.org/pub-cgi/imsrlist and the MRC, Harwell, at http://imsr.har.mrc.ac.uk/ . In addition, large amounts of phenotype data are likely to accumulate on large numbers of mutant mouse strains, creating the need for phenotype databases that can be linked with mapping and mutagenesis databases.

Distribution

To provide a genetic resource to the community, mutant mice must be readily available. Although many mutant stocks are available from commercial vendors or from the Jackson Laboratory, additional distribution centers are required. To meet this demand, several NIH-funded Mutant Mouse Resource Centers will serve as stock archives and distribution centers for the various regions of the USA. The European Mutant Mouse Archive with nodes in Monteretondo (Roma, Italy), the CNRS (Orleans, France), the Gulbenkian Institute (Lisbon, Portugal) and the Karolinska Institute (Huddings, Sweden) is a resource for the European efforts. These resource centers are designed to distribute all types of mutant mouse strain.

THE FUTURE LOOKS BRIGHT

A large number of mutant mouse stocks will be generated over the next decade that will include mice carrying deletions, duplications, balancer chromosomes, targeted disruptions, gene trap, retroviral or transgenic insertions, and point mutations. The compilation of these mutations into a mutant mouse resource will require refining their map locations in the mouse and predicting their locations in the human. The mutations will generate a functional map of the genome that can be correlated with the sequence and transcript map to provide a rich resource of functional information (Fig. 3). A large number of ENU-induced

mutations have already been produced that represent only a fraction of the mutational potential of the mammalian genome, and additional experiments will generate new human disease models and reveal new mammalian developmental pathways. Our view of gene function in mammals will be dramatically and permanently changed by these experiments, which will greatly impact drug development and human health.

ACKNOWLEDGEMENTS

The authors thank Yin-Chai Cheah for assistance in finalizing the manuscript. The Chromosome 11 work is funded by US Department of Public Health grant P01 CA75719. A.B. is an investigator of the Howard Hughes Medical Institute.

REFERENCES

1. Hitotsumachi, S., Carpenter, D.A. and Russell, W.L. (1985) Dose-repetition increases the mutagenic effectiveness of *N*-ethyl-*N*-nitrosourea in mouse spermatogonia. *Proc. Natl Acad. Sci. USA*, **82**, 6619–6621.
2. Shedlovsky, A., McDonald, J.D., Symula, D. and Dove, W.F. (1993) Mouse models of human phenylketonuria. *Genetics*, **134**, 1205–1210.
3. Hong, H.-K., Noveroske, J., Justice, M. and Chakravarti, A. (1999) The winged-helix transcription factor is mutated in satin mice. *Nature Genet.*, in press.
4. Bode, V.C. (1984) Ethylnitrosourea mutagenesis and the isolation of mutant alleles for specific genes located in the *T* region of mouse chromosome 17. *Genetics*, **108**, 457–470.
5. Justice, M.J. and Bode, V.C. (1986) Induction of new mutations in a mouse t-haplotype using ethylnitrosourea mutagenesis. *Genet. Res.*, **47**, 187–192.
6. Shedlovsky, A., Guenet, J.-L., Johnson, L.L. and Dove, W.F. (1986) Induction of recessive lethal mutations in the *T/t-H-2* region of the mouse genome by a point mutagen. *Genet. Res.*, **47**, 135–142.
7. Shedlovsky, A., King, T.R. and Dove, W.F. (1988) Saturation germ line mutagenesis of the murine *t* region including a lethal allele at the quaking locus. *Proc. Natl Acad. Sci. USA*, **85**, 180–184.
8. Kasarkis, A., Manova, K. and Anderson, K.V. (1998) A phenotype-based screen for embryonic lethal mutations in the mouse. *Proc. Natl Acad. Sci. USA*, **95**, 7485–7490.
9. Rinchik, E.M., Carpenter, D.A. and Handel, M.A. (1995) Pleiotropy in microdeletion syndromes: neurologic and spermatogenic abnormalities in mice homozygous for the p^{6H} deletion are likely due to dysfunction of a single gene. *Genetics*, **92**, 6394–6398.
10. Rinchik, E.M. and Carpenter, D.A. (1999) *N*-ethyl-*N*-nitrosourea mutagenesis of a 6- to 11-cM subregion of the *Fah-Hbb* interval of mouse chromosome 7: completed testing of 4,557 gametes and deletion mapping and complementation analysis of 31 mutations. *Genetics*, **152**, 373–383.
11. Ebersole, T.A., Chen, Q., Justice, M.J. and Artzt, K. (1996) The *quaking* gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. *Nature Genet.*, **12**, 260–265.
12. Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K.A., Antonio, M., Beisel, K.W., Steel, K.P. and Brown, S.D.M. (1995) A type VII myosin encoded by the mouse deafness gene *shaker-1*. *Nature*, **374**, 62–64.
13. Justice, M.J. and Bode, V.C. (1988) Three ENU-induced alleles of the murine *quaking* locus are recessive embryonic lethal mutations. *Genet. Res.*, **51**, 95–102.
14. Peters, J., Andrews, S.J., Loutit, J.F. and Glegg, J.B. (1985) A mouse β-globin mutant that is an exact model of hemoglobin Rainier in man. *Genetics*, **110**, 709–721.
15. Vitaterna, M.H., King, D.P., Chang, A.M., Kornhauser, J.M., Lowrey, P.L., McDonald, J.D., Dove, W.F., Pinto, L.H., Turek, F.W. and Takahashi, J.S. (1994) Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science*, **264**, 719–725.
16. Schumacher, A., Faust, C. and Magnuson, T. (1996) Positional cloning of a global regulator of anterior–posterior patterning in mice. *Nature*, **383**, 250–253.
17. Davis, A.P. and Justice, M.J. (1998) Mouse alleles: if you've seen one, you haven't seen them all. *Trends Genet.*, **14**, 438–441.
18. Sidman, R.L., Dickie, M.M. and Appel, S.H. (1964) Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. *Science*, **144**, 309–311.
19. Cox, R.D., Hugill, A., Shedlovsky, A., Noveroske, J.K., Best, S., Justice, M.J., Lehrach, H. and Dove, W.F. (1998) Contrasting effects of ENU-induced embryonic lethal mutations of the *quaking* gene. *Genomics*, **57**, 333–341.
20. McDonald, J.D., Bode, V.C., Dove, W.F. and Shedlovsky, A. (1990) Pah^{hph5}: a mouse mutant deficient in phenylalanine hydroxylase. *Proc. Natl Acad. Sci. USA*, **87**, 1965–1967.
21. Symula, D.J., Shedlovsky, A., Guillery, E.N. and Dove, W.F. (1997) A candidate mouse model for Hartnup disorder deficient in neutral amino acid transport. *Mamm. Genome*, **8**, 102–107.
22. Justice, M.J., Zheng, B., Woychik, R.P. and Bradley, A. (1997) Using targeted large deletions and high-efficiency *N*-ethyl-*N*-nitrosourea mutagenesis for functional analyses of the mammalian genome. *Methods*, **13**, 423–436.
23. Ramirez-Solis, R., Liu, P. and Bradley, A. (1995) Chromosome engineering in mice. *Nature*, **378**, 720–724.
24. Zheng, B., Sage, M., Cai, W.W., Thompson, D.M., Tavsanli, B.C., Cheah, Y.C. and Bradley, A. (1999) Engineering a balancer chromosome in the mouse. *Nature Genet.*, **22**, 375–378.
25. Zheng, B., Mills, A.A. and Bradley, A. (1999a) A system for rapid generation of coat color-tagged knockouts and defined chromosomal rearrangements in mice. *Nucleic Acids Res.*, **27**, 2354–2360.
26. Copeland, N.G., Jenkins, N.A., Gilbert, D.J., Eppig, J.T., Maltais, L.J., Miller, J.C., Dietrich, W.F., Weaver, A., Lincoln, S.E., Steen, R.G., Stein, L.D., Nadeau, J.H. and Lander, E.S. (1993) A genetic linkage map of the mouse: current applications and future prospects. *Science*, **262**, 57–66.
27. Taylor, B.A., Navin, A. and Phillips, S.J. (1994) PCR-amplification of simple sequence repeat variants from pooled DNA samples for rapidly mapping new mutations of the mouse. *Genomics*, **21**, 626–632.
28. Rinchik, E.M., Carpenter, D.A. and Long, C.L. (1993) Deletion mapping of four loci defined by *N*-ethyl-*N*-nitrosourea-induced postimplantation-lethal mutations within the pid-Hbb region of mouse chromosome 7. *Genetics*, **135**, 1117–1123.
29. King, D.P., Zhao, Y., Sangoram, A.M., Wilsbacher, L.D., Tanaka, M., Antoch, M.P., Steeves, T.D.L., Vitaterna, M.H., Kornhouser, J.H., Lowrey, P.L., Turek, F.W. and Takahashi, J.S. (1997) Positional cloning of the mouse circadian *Clock* gene. *Cell*, **89**, 641–653.
30. Cotton, R. (1997) Slowly but surely towards better scanning for mutations. *Trends Genet.*, **13**, 43–46.
31. Gradia, S., Subramanian, D., Wilson, T., Acharya, S., Makhov, A., Griffith, J. and Fishel, R. (1999) *hMSH2-hMSH6* forms a hydrolysis-independent sliding clamp on mismatched DNA. *Mol. Cell*, **3**, 255–261.
32. You, Y., Bergstrom, R., Klemm, M., Lederman, B., Nelson, H., Ticknor, C., Jaenisch, R. and Schimenti, J. (1997) Chromosomal deletion complexes in mice by radiation of embryonic stem cells. *Nature Genet.*, **15**, 285–288.
33. Thomas, J.W., LaMantia, C. and Magnusson, T. (1998) X-ray-induced mutations in mouse embryonic stem cells. *Proc. Natl Acad. Sci. USA*, **95**, 1114–1119.
34. Smith, D.J. and Rubin, E.M. (1997) Functional screening and complex traits: human 21q22.2 sequences affecting learning in mice. *Hum. Mol. Genet.*, **6**, 1729–1733.
35. Nakagata, N. and Takshima, T. (1993) Cryopreservation of mouse spermatozoa from inbred and F1 hybrid strains. *Exp. Anim.*, **42**, 317–320.
36. Sztein, J.M. (1997) Motility of cryopreserved mouse spermatozoa affected by temperature of collection and rate of thawing. *Cryobiology*, **35**, 46–52.
37. Songsasen, N. and Leibo, S. (1997) Cryopreservation of mouse spermatozoa. *Cryobiology*, **35**, 255–269.
38. Marschall, S. and Hrabe de Angelis, M. (1999) Cryopreservation of mouse spermatozoa: double your mouse space. *Trends Genet.*, **15**, 128–131.
39. Wakayama, T. and Yanagimachi, R. (1998) Development of normal mice from oocytes injected with freeze-dried spermatozoa. *Nature Biotechnol.*, **16**, 639–641.
40. Eppig, J.T. and Strivens, M. (1999) Finding a mouse: the International Mouse Strain Resource (IMSR). *Trends Genet.*, **15**, 81–82.
41. Hustad, C.M., Perry, W.L., Siracusa, L.D., Rasberry, C., Cobb, L., Cattanach, B.M., Kovatch, R., Copeland, N.G. and Jenkins, N.A. (1995) Molecular genetic characterization of six recessive viable alleles of the mouse *agouti* locus. *Genetics*, **1995**, 255–265.

42. Russell, L.B., Russell, W.L., Rinchik, E.M. and Hunsicker, P.R. (1990) Factors affecting the nature of induced mutations. In Allen, J.W., Bridges, B.A., Lyon, M.F., Moses, M.J. and Russell, L.B. (eds), *Banbury Report.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, NY, Vol. 34, pp. 271–289.
43. Su, L.K., Kinzler, K.W., Vogelstein, B., Preisinger, A.C., Moser, A.R., Luongo, C., Gould, K.A. and Dove, W.F. (1992) Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science*, **256**, 668–670.
44. Marker, P.C., Seung, K., Bland, A.E., Russell, L.B. and Kingsley, D.M. (1997) Spectrum of Bmp5 mutations from germline mutagenesis experiments in mice. *Genetics*, **145**, 435–443.
45. Klopp, N., Favor, J., Loster, J., Lutz, R.B., Neuhauser-Klaus, A., Prescott, A., Pretsch, W., Quinlan, R.A., Sandilands, A., Vrensen, G.F.J.M. and Graw, J. (1998) Three murine cataract mutants (*Cat2*) are defective in different γ-crystallin genes. *Genomics*, **52**, 152–158.
46. Im, W.B., Phelps, S.F., Copen, E.H., Adams, E.G., Slightom, J.L. and Chamberlain, J.S. (1996) Differential expression of dystrophin isoforms in strains of mdx mice with different mutations. *Hum. Mol. Genet.*, **5**, 1149–1153.
47. Steele, E.C., Lyon, M.F., Favor, J., Guillot, P.V., Boyd, Y. and Church, R.L. (1998) A mutation in the connexin 50 (*Cx50*) gene is a candidate for the *No2* mouse cataract. *Curr. Eye Res.*, **17**, 883–889.
48. Pearce, S.R., Peters, J., Ball, S., Morgan, M.J., Walker, J.I.H. and Faik, P. (1995) Sequence characterization of ENU-induced mutants of glucose phosphate isomerase in mouse. *Mamm. Genome*, **6**, 858–861.
49. Sanders, S., Smith, D.P., Thomas, G.A. and Williams, E.D. (1997) A glucose-6-phosphate dehydrogenase (G6PD) splice site consensus sequence mutation associated with G6PD enzyme deficiency. *Mutat. Res.*, **374**, 79–87.
50. Popp, R.A., Bailiff, E.G., Skow, L.C., Johnson, F.M. and Lewis, S.E. (1983) Analysis of a mouse α-globin gene mutation induced by ethylnitrosourea. *Genetics*, **105**, 157–167.
51. Jones, J. and Peters, J. (1991) The molecular characterization of an A:T to G:C transition in the *Hbb-b1* gene of the murine homologue of hemoglobin Rainier. *Biochem. Genet.*, **29**, 617–626.
52. Cordes, S.P. and Barsh, G.S. (1994) The mouse segmentation gene kr encodes a novel basic domain-leucine zipper transcription factor. *Cell*, **79**, 1025–1034.
53. Sandaluche, R., Pretsch, W., Chatterjee, B., Gimbel, W., Graw, J. and Favor, J. (1994) Molecular analysis of four lactate dehydrogenase-A mutants in the mouse. *Mamm. Genome*, **5**, 777–780.
54. Pretch, W., Chatterjee, B., Favor, J., Merkle, S. and Sandulache, R. (1998) Molecular, genetic and biochemical characterization of lactate dehydrogenase-A enzyme activity mutations in *Mus musculus*. *Mamm. Genome*, **9**, 144–149.
55. Brannan, C.I., Bedell, M.A., Resnick, J.K., Eppig, J.J., Handel, M.A., Williams, D.E., Lyman, S.D., Donovan, P.J., Jenkins, N.A. and Copeland, N.G. (1992) Developmental abnormalities in *Steel*^{17H} mice result from a splicing defect in the steel factor cytoplasmic tail. *Gene Development*, **6**, 1832–1842.
56. Steingrimsson, E., Favor, J., Ferre-D'Amara, A.F., Copeland, N.G. and Jenkins, N.A. (1998) *Mitfmi-enu122* is a missense mutation in the HLH dimerization domain. *Mamm. Genome*, **9**, 250–252.
57. Huang, J.-D., Cope, M.J.T.V., Mermall, V., Strobel, M.C., Kendrick-Jones, J., Russell, L.B., Mooseker, M.S., Copeland, N.G. and Jenkins, N.A. (1998) Molecular genetic dissection of mouse unconventional myosin-VA: head region mutations. *Genetics*, **148**, 1951–1961.
58. Huang, J.-D., Mermall, V., Strobel, M.C., Russell, L.B., Mooseker, M.S., Copeland, N.G. and Jenkins, N.A. (1998) Molecular genetic dissection of mouse unconventional myosin-VA: tail region mutations. *Genetics*, **148**, 1963–1972.
59. Mburu, P., Liu, X.Z., Walsh, J., Saw, D.J., Cope, M.J., Gibson, F., Kendrick-Jones, J., Steel, K.P. and Brown, S.D.M. (1997) Mutation analysis of the mouse myosin VIIA deafness gene. *Gene Func.*, **1**, 191–203.
60. McDonald, J.D. and Charlton, C.K. (1997) Characterization of mutations at the mouse phenylalanine hydroxylase locus. *Genomics*, **39**, 402–405.
61. Zingg, B.C., Pretsch, W. and Mohrenweiser, H.W. (1995) Molecular analysis of four ENU-induced triosephosphate isomerase null mutants in *Mus musculus*. *Mutat. Res.*, **328**, 163–173.
62. Zdarsky, E., Favor, J. and Jackson, I.J. (1990) The molecular basis of brown, an old mouse mutation, and of an induced revertant to wild-type. *Genetics*, **126**, 443–449.
63. Rogers, D.C., Fisher, E.M., Brown, S.D., Peters, J., Hunter, A.J. and Martin, J.E. (1997) SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm. Genome*, **8**, 711–713.
64. Hrabe de Angelis, M. and Balling, R. (1998) Large scale ENU screens in the mouse: genetics meets genomics. *Mutat. Res.*, **400**, 25–32.
65. Justice, M.J. (1999) Mutagenesis of the mouse germline. In Jackson, I. and Abbott, C. (eds), *Mouse Genetics and Transgenics: A Practical Approach.* Oxford University Press, Oxford, in press.
66. Schimenti, J. and Bucan, M. (1998) Functional genomics in the mouse: phenotype-based mutagenesis screens. *Genome Res.*, **8**, 698–710.
67. Hill, R.E., Favor, J., Hogan, B.L.M., Ton, C.C., Saunders, G.F., Hanson, I.M., Prosser, J., Jordan, T., Hastie, N.D. and van Heyningen, V. (1991) Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature*, **354**, 522–525.