CAPITALIZING ON LARGE-SCALE MOUSE MUTAGENESIS SCREENS

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Variation is the crux of genetics. Mutagenesis screens in organisms from bacteria to fish have provided a battery of mutants that define protein functions within complex pathways. Large-scale mutation isolation has been carried out in *Caenorhabditis elegans*, *Drosophila melanogaster* and zebrafish, and has been recently reported in the mouse in two screens that have generated many new, clinically relevant mutations to reveal the power of phenotype-driven screens in a mammal.

GENE TRAPPING
A mutation strategy that uses insertion vectors to trap or isolate transcripts from flanking genes. The inserted sequence acts as a tag from which to clone the mutated gene.

ALLELIC SERIES
An array of possible mutant forms of a gene, which usually cause multiple phenotypes.

HYPOMORPH
A mutant allele that does not eliminate the wild-type function of a gene and may give a less severe phenotype than a loss-of-function mutant.

COMPLEMENTATION
When two mutations are combined in an organism and the phenotype is wild type, the mutations are said to complement each other.

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The completion of the human genome sequence promises a new era for biology. Although many approaches to functional studies will provide new information, the approach that has defined function in many organisms is mutagenesis. Recently, the mouse has joined other model organisms, such as *C. elegans*, *Drosophila* and zebrafish, in large-scale mutation isolation. This review summarizes the current mouse mutagenesis efforts and compares them with programmes in other organisms. It also discusses the mutagenesis efforts that are underway and the need for infrastructure to support the analysis, maintenance, archiving and distribution of the new mutants.

Mouse mutagenesis

The biological similarity and extensive comparative genetic linkage map between mouse and human makes the mouse an ideal model organism for defining mammalian gene function and modelling human disease. A large array of genetic tools with which to investigate the mouse genome is available, and all are being used. These genetic approaches can be genotype-driven, as gene disruptions are, or phenotype-driven, as traditional genetic screens are. For example, by using embryonic stem (ES) cell technology, any gene involved in human disease can be disrupted, providing valuable information about the consequences of mutation in the whole animal[1]; and to investigate gene functions through expression patterns, enhancer-driven GENE-TRAP approaches have been devised[2]. More recently, gene-trap vectors that provide a valuable sequence tag to identify the interrupted gene have been used to speed up the gene disruption process[3].

Mutation isolation by the mouse supermutagen *N*-ethyl-*N*-nitrosourea (ENU) has the potential to generate an abundance of mutations in phenotype-driven screens (BOX 1). Mutations induced by ENU provide a unique mutant resource. Although loss-of-function mutations are valuable, an important advantage of chemical mutagenesis is that it generates ALLELIC SERIES. These provide a range of phenotypes in which a gene's function can be affected in different ways. Loss-of-function mutations, viable HYPOMORPHS of lethal COMPLEMENTATION groups, ANTIMORPHS, and gain-of-function mutations have been isolated in mouse mutagenesis screens. MISSENSE MUTATIONS in coding regions are common after ENU treatment, giving many different phenotypes and providing a fine-structure dissection of protein function and complex mammalian isoforms. Furthermore, phenotype-driven mutagenesis offers many advantages in speed of generation and removal of investigator bias over allelic series generated by homologous recombination.

Dominant mutations: large-scale isolation

Two recent papers[4,5] published in *Nature Genetics* illustrate the power of phenotype-driven mutagenesis approaches in the mouse. One screen, directed by Steve Brown at Harwell, UK, is being carried out by a consortium of the Medical Research Council and Smith Kline Beecham Pharmaceuticals (UK ENU Mutagenesis programme). The second, led by Rudi Balling and Martin Hrabe de Angelis at the GSF Forschungszentrum für

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ANTIMORPH
A mutant allele that antagonizes gene function and acts in a semi-dominant manner.

MISSENSE MUTATION
A mutation that results in the substitution of an amino acid in a protein.

NONSENSE MUTATION
A mutation that results in the introduction of a stop codon to cause the premature termination of the protein.

HAPLOINSUFFICIENT
A phenotype that arises in diploid organisms owing to the loss of one functional copy of a gene.

Box 1 | ENU, a powerful mutagen in mice

***N*-ethyl-*N*-nitrosourea (ENU) is an alkylating agent that causes random single-base-pair mutations in a wide variety of organisms. The ethyl group of ENU can be transferred to oxygen or nitrogen molecules in DNA, causing an ethylated base to be mistakenly identified during DNA replication. Mispairing and base-pair substitution will result if the mismatch is not repaired. In the mouse, the highest mutation rates occur in pre-meiotic spermatogonial stem cells, with single-locus mutation frequencies of 6×10^{-3} to 1.5×10^{-3} per mutagenized genome[35,36], equivalent to isolating a new functionally defective allele in 175 to 655 screened gametes.**

Mouse mutagenesis with ENU is simple. Male mice are injected intraperitoneally to mutagenize their spermatogonial stem cells. Males go through a sterile period, but mutagenized spermatogonia eventually repopulate the testis to produce clones of mutagenized sperm. Males are mated in breeding schemes to isolate mutations[37]. In dominant screens, many of the observed phenotypes (up to 50%) are not inherited, so phenotypes that result from mutation must be confirmed by breeding.

The primary lesions induced by ENU in the mouse germline are A•T to T•A transversions and A•T to G•C transitions. Of the sequenced mutations, 64% cause missense mutations, 10% cause NONSENSE MUTATIONS and 26% cause splicing errors[38]. ENU's power lies in its efficiency in inducing functionally defective mutations. So it is the mutagen of choice in phenotype-driven screens. However, disadvantages to its use include the difficulty of locating and isolating the DNA lesion responsible for the desired phenotype, and its ability to make extraneous or undesired lesions elsewhere in the genome.

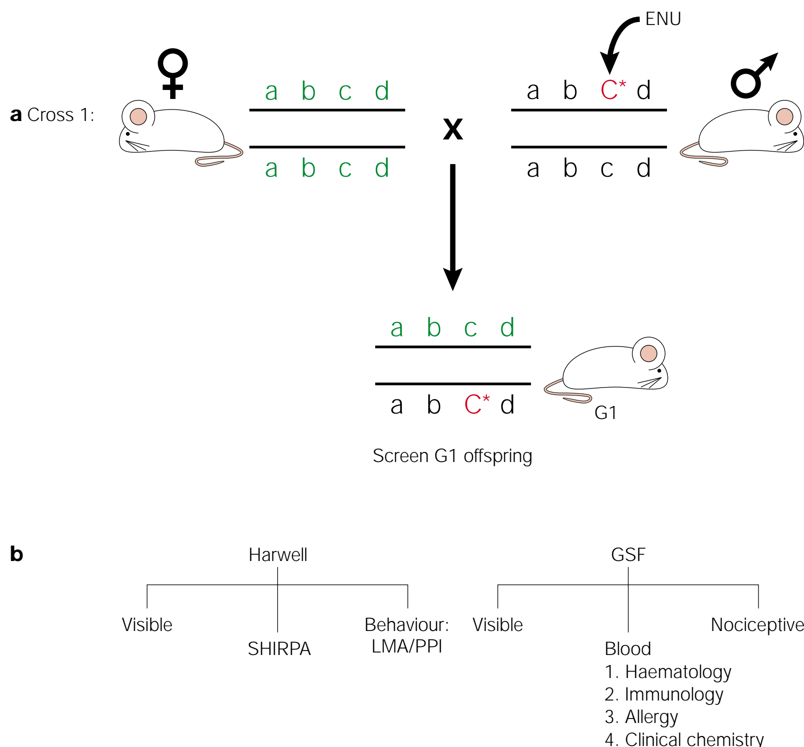

Figure 1 | **Dominant screens to isolate new human disease models. a** | Male mice were mutagenized with ENU and mated to wild-type females. Loci in a region of a chromosome are designated 'a b c d' (shown as black in the ENU mutagenized male and as green in the wild-type female). A new dominant mutation (C*, in red) is shown in the G1 offspring. **b** | G1 progeny were screened by both groups for visible and specialized phenotypes. Harwell included screens for neurological disorders using the SHIRPA protocol. SHIRPA is a comprehensive stepwise assessment of many parameters, designed to detect sensory, neuromuscular and neurological defects[6]. The locomotor activity (LMA) test measures the number of times that a mouse splits an electronic beam by rearing and the number of times that it crosses the cage over a 30-minute period. The pre-pulse inhibition of the acoustic startle response (PPI) test takes place in a sound-proof chamber with calibrated noises delivered in random order over a time period. Startle responses are measured after a single pulse alone, or after pre-pulses and a pulse. The GSF programme included screens for haematological, immunological and allergy defects, as well as for nociception (pain response). Both groups included clinical chemistry screens; however, these screens were carried out on only a subset of animals.

Umwelt und Gesundheit is a consortium of investigators from around Munich, Germany (German ENU-mouse mutagenesis screen project). Together, these groups have isolated over 300 confirmed mouse mutations and, by estimation from the number of inherited phenotypes, probably around 1,000 to 1,500 new mutations. Both groups used a simple breeding scheme to isolate dominant mutations (FIG. 1), screening 26,047 and 14,000 mice, respectively, for visible phenotypes. A commonly isolated class of mutations in a screen for dominant phenotypes is the HAPLOINSUFFICIENT CLASS, and these loci yield numerous alleles in such a screen. Mutants that produce a visible phenotype by dominance or haploinsufficiency may already be present in the mutant database. However, each group used a unique screen on a subset of these animals to isolate new classes of mutant phenotypes. The Harwell group focused on sensory, neurological and neuromuscular phenotypes by using an assay called SHIRPA[6], as well as behavioural assays such as locomotor activity (LMA) and pre-pulse inhibition of the acoustic startle response (PPI). The German group focused on blood assays for haematological, clinical chemistry, immunological and allergy defects. Each group therefore identified a number of phenotypes relevant to human disease (TABLE 1).

A large proportion of the new mutations cause visible defects. These include mutations that affect the mouse hair and skin, pigmentation, skeletal morphology and eyes. The most common phenotype falls into the pigmentation class. This is not surprising, as a mouse coat colour is an easy phenotype to score. However, it is not clear how many of these mutations will be unique because mouse coat-colour variation has been the pursuit of hobbyists and experimentalists for over 200 years. Notably, the Harwell group complementation tested and mapped some of the mutations that seemed to be repeated phenotypes, and showed that two mutations were alleles of the Kit oncogene (*Kit*), and four were alleles of the Kit ligand (*Kitl*). The first allele of *Kit*, dominant white spotting (*KitW*), was first reported in 1937 (REF. 7) and repeat mutations in *Kit* have occurred

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Table 1 | **Mutant phenotypes confirmed in recent mouse ENU screens**

Phenotype classification	Skin/ coat	Sensory organs	Skeletal	Size	Neurological/ behavioural	Haematological	Immunological	Allergy	Clinical chemistry	Nociceptive
Harwell*	27	26	15	17	34	0	0	0	6	0
Germany‡	31	18	40	4	21	15	30	9	8	9
Total	**58**	**44**	**55**	**21**	**55**	**15**	**30**	**9**	**14**	**9**

In addition, Harwell* confirmed one mutation that caused abdominal swelling owing to peritoneal ascites, and the German group‡ confirmed one mutation that affected the teeth.

frequently[8]. The first allele of *Kitl* was observed as a semi-dominant steeloid coat[9], and mutations at this locus have also appeared frequently. So their isolation in this new mutant screen is not surprising.

The Harwell consortium confirmed more mutations in the visible neurological class, whereas the German group confirmed more mutations in the skeletal dysmorphology class, possibly reflecting the primary interests of the two groups. Each group included a hearing test and isolated a similar number of mutations affecting hearing per mutagenized genome. Six of the Harwell circling, deaf mutations were mapped to the proximal end of mouse chromosome 4, near the mouse mutation Wheels (*Whl*), and all may be alleles of the same locus. This implies that certain loci may be easily detected in a screen for dominant phenotypes, and raises the possibility that this locus is haploinsufficient. It also implies that this screen may already be approaching saturation for some of the visible phenotypes.

Perhaps the most intriguing mutations are those that were isolated by the unique phenotype screens, designed to model human diseases. Harwell confirmed a total of 50 neurological mutations: 12 that were classified as neurological and neuromuscular, 16 that caused circling and deafness, and 22 that produced behavioural phenotypes. The 22 behavioural mutations would not have been isolated without the more thorough phenotypic assessments by the SHIRPA, LMA and PPI tests. High or low LMA is associated with neurobehavioural abnormalities, and deficits in PPI are seen in several human psychiatric disorders, such as schizophrenia. The German screen confirmed 54 haematological mutations: 15 that were found by complete blood counts, 30 that caused immunological defects, and 9 that resulted in high or low serum immunoglobulin E (IgE) levels. The most frequent type of allergic reaction, immediate type I hypersensitivity, is associated with elevated IgE levels. The immunological mutations were identified by screens that utilized ELISA for immunoglobulins and antibodies against DNA, and by flow cytometry on peripheral blood to measure T- and B-cell populations. Clinical chemistry screens by both groups may have also isolated models of kidney disease. It is likely that many of these mutations have identified novel gene functions or novel genes.

Each group has a huge number of unconfirmed mutations and numerous unmapped mutations, suggesting that mutation confirmation and chromosomal localization are bottlenecks in this mutation-isolation process. To be valuable, new ENU-induced 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 in meiotic crosses using phenotypic information, as a molecular tag is not available with this strategy. The Harwell consortium tackled the mapping problem by incorporating polymorphisms into their initial breeding strategy, and by using speed BACKCROSSES generated by *in vitro* fertilization (IVF), along with DNA POOLING strategies. Speed backcrosses use sperm from an affected male to fertilize eggs obtained from superovulated wild-type females, a quick and efficient way to obtain numerous progeny for mapping dominant mutations. DNA pooling of mutant DNAs and wild-type DNAs eliminates the need to do molecular genome scans on individual backcross offspring[10,11]. These methods improve the efficiency of mapping but do not eliminate the bottleneck of mutations that remain to be mapped. The development of mapping techniques that do not require extensive breeding, or mutation-isolation schemes that use a mutation 'tag', would help to speed up the steps between identifying a mutant phenotype and isolating the underlying mutation.

The mouse already has a rich resource of visible dominant and recessive mutations from historical collections, spontaneous events, and smaller mutagenesis screens. So it is unclear how many of these dominant ENU-generated mutations will identify novel loci, and how many represent alleles of known genes. After the loci have been mapped, identifying them should be made simpler by the availability of the complete sequence of the mouse and human genomes. Although some hurdles remain, these mutagenesis efforts show the efficacy of ENU in producing numerous new mutations in the mouse genome, especially if new phenotype screens are incorporated. These single-gene mutations will also be valuable for dissecting multigenic and multifactorial traits[12]. Some of the mutations, for example, may represent a more penetrant allele of a locus that contributes to a multifactorial phenotype, but the monogenic trait will be easier to access molecularly. Placing the new mutations on different genetic backgrounds may also reveal different phenotypes, which could lead to the discovery of interacting loci in new developmental, biochemical or physiological pathways.

Mutagenesis screens in other species

Large-scale mutation isolation has been carried out in worms, flies and zebrafish. Chemical mutagenesis in all organisms is plagued by extraneous mutations, and so

ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY.)
A sensitive antibody-based method for the detection of an antigen such as a protein.

BACKCROSS
The mating of an individual with its parent, or with an individual of the same genotype as its parent, to follow the inheritance of alleles and phenotypes.

DNA POOLING
A mapping strategy that pools DNA from phenotypically distinct backcross or intercross progeny to identify marker alleles that are linked to the genes that determine phenotype; it reduces the time and expense of genotyping individual mice from linkage crosses.

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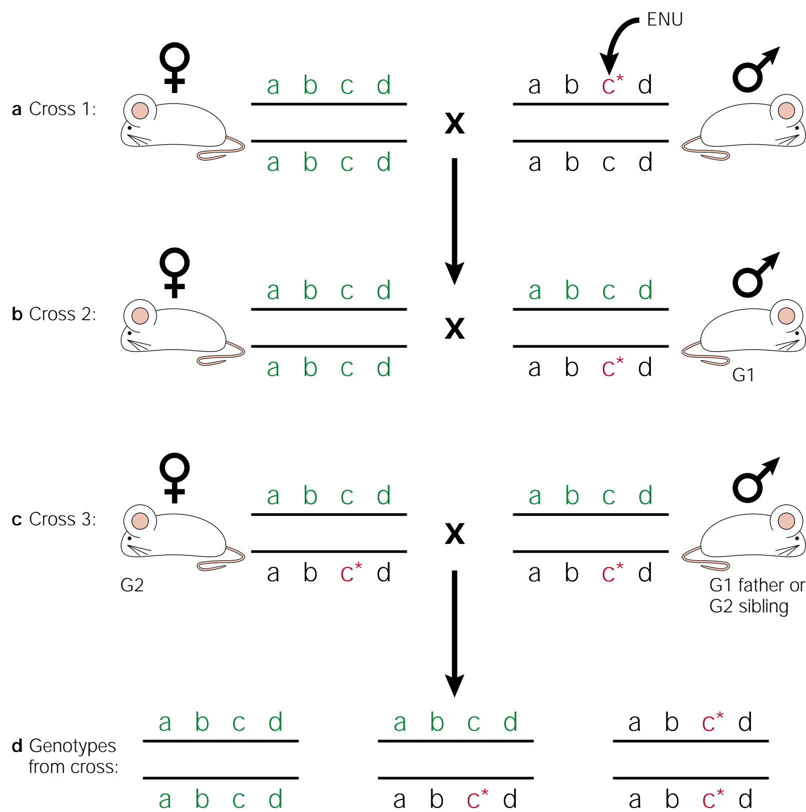

Figure 2 | **A three-generation breeding scheme without genetic markers to isolate recessive mutations.** Loci in a region of a chromosome are designated 'a b c d' (shown as black in the ENU-mutagenized male and as green in the wild-type female). **a** | Fertile ENU-mutagenized males are mated to wild-type females. **b** | G1 males carrying a recessive mutation (c*, in red) are mated to wild-type females. **c** | G2 siblings are intercrossed, or G2 daughters, which may be wild type or carriers of a new recessive mutation, are mated back to their fathers. **d** | New recessive mutations (c*/c*) can be identified in the G3 offspring.

FORWARD GENETICS
A genetic analysis that proceeds from phenotype to genotype by positional cloning or candidate-gene analysis.

REVERSE GENETICS
A genetic analysis that proceeds from genotype to phenotype by gene-manipulation techniques, such as homologous recombination in ES cells.

PARTHENOGENESIS
The production of offspring by a female with no genetic contribution from a male.

large-scale efforts have used transposable elements when possible. The worm genome has been elegantly tackled by both FORWARD and REVERSE GENETIC approaches that have yielded millions of mutations[13,14]. Chemical mutagenesis in fly and fish using ethyl methane sulphonate (EMS) or ENU, respectively, has been the method of choice in many phenotype-driven screens to address gene function.

In 1984, the large-scale isolation of *Drosophila* mutations by chemical mutagenesis was reported by Eric Wieschaus and Christine Nusslein-Volhard[15–17]. These screens capitalized on methodologies pioneered by Madeleine Gans[18] and used a quick-screening assay to look at *Drosophila* embryo morphology. Males with marked chromosomes were treated with EMS and were used in mating schemes designed to isolate recessive mutations, one chromosome at a time. The use of balancer chromosomes to isolate recessive lethal mutations was key to their success (BOX 2). Over 20,000 lethal mutations on the X, second and third chromosomes were retrieved, and the embryonic-lethal group of mutants was screened to reveal nearly 600 mutations that affected the patterning of the larval cuticle. Using the power of *Drosophila* genetics, each of the mutations was mapped and tested for complementation. The utility of these mutations in dissecting the principles of embryonic pattern formation has been unsurpassed[19]. Many other mutagenesis screens have now been carried out in the fly and, at present, the *Drosophila* stock centre houses over 8,000 stocks that include deletions, inversions, duplications, marked chromosomes, and insertional and point mutations.

It has been argued that detailed studies in comparatively simple model organisms, such as *Drosophila*, could condense the function of all human genes into a few hundred biological processes[20]. However, although studies in *Drosophila* will help to elucidate many genetic pathways, flies are limited as a model organism for analysing human gene function. There are two key reasons for this. First, health-related genes, such as those involved in arthritis, cardiovascular disease, asthma, inflammation, obesity, osteoporosis, fetal–maternal circulation and other uniquely mammalian diseases, cannot be easily studied in lower organisms. Second, the network of interactions may be different between flies and mammals because of the many tissues that distinguish the two species.

In 1996, large-scale mutagenesis programs reported recessive mutations that affected developmental processes in the zebrafish, *Danio rerio*. Together, two groups identified a total of 6,647 mutations[21,22]. These programmes screened for embryogenesis defects and essential functions, phenotypes that are largely ignored in the current mouse screens. Fish have the advantage of PARTHENOGENETIC activation of embryos, eliminating the requirement for complex breeding schemes to characterize some of the mutations. Furthermore, because zebrafish eggs develop externally, they can be easily visualized, unlike the development of mice and humans, which takes place in an uterine environment. Zebrafish embryos are also relatively transparent , which aids the detection of phenotypic abnormalities. Mapping crosses in zebrafish are simple and cost effective because many fish can be reared in a single tank. However, the zebrafish does not have an extensive comparative molecular genetic linkage map with the human genome, although mapping efforts reveal that linkage groups are highly conserved between the two species[23]. Unfortunately, so many mutations were isolated in these initial screens that many of the new mutants without obvious morphological defects were

Box 2 | Balancer chromosomes

- **An ideal balancer chromosome suppresses recombination over the length of a chromosome. Many balancers are constructed by making several inversions on a single chromosome.**
- **The balancer chromosome should be easily identified in crosses by using a dominant marker.**
- **In self-sustaining crosses, balancer chromosomes should be lethal when homozygous. Therefore, by maintaining a lethal mutation *in trans* to the balancer, the only offspring that will be viable from an intercross will be those that carry the mutation and are heterozygous for the balancer chromosome.**

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Table 2 | **Large-scale chemical mutagenesis in model organisms**

	Drosophila	Zebrafish	Mouse
Chemical	EMS	ENU	ENU
Mutants isolated	>20,000	>6,500	>1,000
Mutants confirmed	~600	>2,000	>300
Class	Recessive	Recessive	Dominant
Advantages	Genetic reagents	Non-uterine development	Comparative linkage map
	Generation time	Parthenogenesis	Clinically relevant phenotypes
	Maintenance	Ease of rearing	Sperm freezing
		Sperm freezing	

The fly screens represent saturation mutagenesis of three chromosomes: X, second and third. 'Mutants isolated' represents the number of lethal mutations, and the mutants confirmed were those that died before hatching and had patterning defects. Numerous mutations were isolated in zebrafish screens; however, only about 2,000 lines were maintained as stocks.

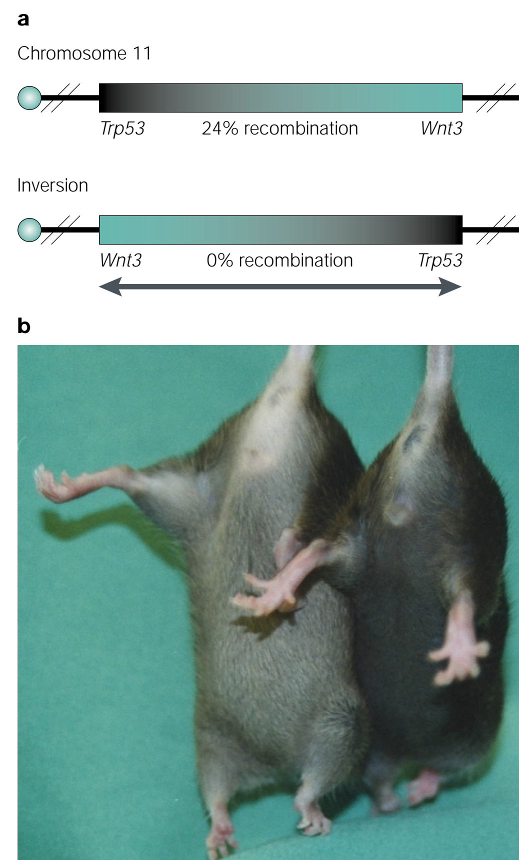

Figure 3 | **A mouse balancer chromosome. a** | The first balancer chromosome was engineered to disrupt *Trp53* and *Wnt3* on chromosome 11, which causes embryos that are homozygous for the balancer to die during embryonic development. This balancer suppresses recombination over 1/3 of the chromosome, is dominantly marked with the yellow coat conferred by the *K14-agouti* transgene, and is lethal when homozygous. **b** | A wild-type mouse (right) with a mouse heterozygous for the balancer chromosome (left). Note that the mice are not completely yellow, but have a yellowish ventrum, as well as yellow ears and tail (not shown).

discarded, leaving only about 30% of the original mutant collection. Still, the remaining collection of over 2,000 mutations has been extremely valuable for studying developmental processes in vertebrates, and further mutagenesis efforts using insertional mutagens are continuing[24].

The current mouse mutagenesis efforts pale in comparison with these model organisms (TABLE 2). However, the mouse is a complex mammal, and unique recessive phenotypes will probably be discovered more slowly, even though many represent disease phenotypes. For example, the mouse progressive ankylosis mutation (*ank*) causes a generalized, incremental form of arthritis, accompanied by mineral deposition, bony outgrowths and joint destruction[25]. The *ank* gene encodes a multipass transmembrane protein that controls pyrophosphate levels, providing functional insight into the development of arthritis[26]. Although the *ank* gene is highly conserved in vertebrates, including zebrafish, rats, mice, cows and humans, it is not found in bacteria, yeast, worms or flies. Mutations affecting skeletal development and maintenance can be easily seen in the mouse as a dysmorphology or an abnormal gait, and many will have direct relevance to debilitating human diseases.

In any ENU screen, mutation isolation relies on the phenotypic assay, requiring that the mutant phenotype must vary significantly from the background. In mouse screens, there should be an emphasis on assays for clinical phenotypes that model human diseases; clinical testing of mouse blood, for example, can yield a considerable array of phenotypes relevant to human disease. A complete blood count (CBC) 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, as was carried out in the GSF screen. Clinical chemistry tests can diagnose several organ system anomalies, including liver, pancreatic, heart and kidney disorders. Urine analysis on mice can reveal increased levels of glucose or other abnormal by-products of disease. High-throughput biochemical analysis, such as tandem mass spectrometry, can detect various metabolic disorders that affect lipids, fatty acids or amino acids. Through grants issued by the United States National Institutes of Health (NIH), more phenotypic assays are being developed to identify new neurological, cardiovascular, haematopoietic, ageing and immune phenotypes using high-throughput technologies.

Although mouse stocks can be quickly archived as frozen sperm, the logistics of breeding, complementation testing and mapping in the mouse may prove limiting. Furthermore, characterizing the pathology of mouse mutants to make them relevant to human disease can be a daunting task. Recognizing this fact, the NIH has established a Mouse Genomics and Genetics Resources Committee to suggest and implement strategies for improving the infrastructure for mouse genetics[27,28]. These initiatives include training in pathobiology, improving genomic resources, improving sperm-freezing methods, and establishing new mutant archiving and distribution centres.

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Future approaches to mouse mutagenesis

Isolating recessive mutations genome-wide. Several new mutagenesis centres are being established in the United States through cooperative agreements with the NIH, and have already been established in Australia, Japan and Canada. To identify novel loci and further define gene function, some of these groups are beginning the large-scale isolation of recessive mutations using genome-wide and regional screens. A genome-wide strategy for recessive mutations requires a three-generation breeding scheme (FIG. 2). This strategy is being carried out in parallel to the dominant screen by the GSF consortium, which is planning to screen 800 mutagenized genomes for recessive mutations. Similarly, Chris Goodnow at Australian National University in Canberra has focused on isolating recessive mutations genome-wide (FIG. 2), incorporating specialized phenotypic screens for immune defects[29].

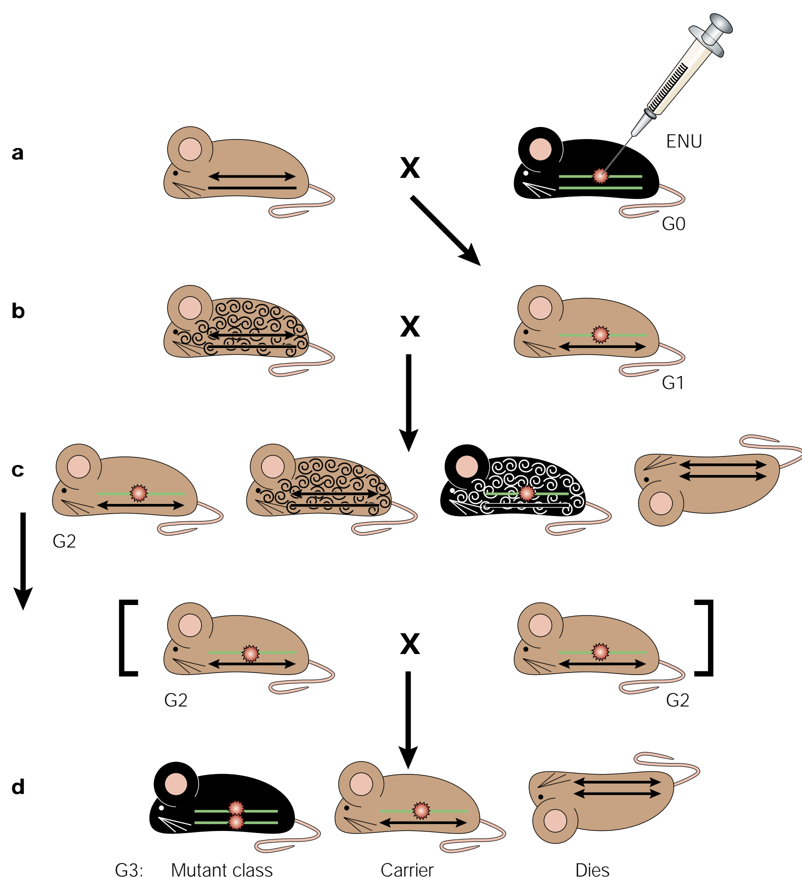

Figure 4 | **Isolating recessive detrimental mutations with a marked balancer chromosome.** This approach is being used for the saturation mutagenesis of mouse chromosome 11 (REF. 4). In this scheme, an inversion generated by Cre/*loxP* is tagged with a *K14-agouti* transgene to produce mice with a dominant yellow coat. Black coat coloured C57BL/6J males are treated with a 3 x 100 mg kg^{-1} dose of ENU and are allowed to recover fertility (G0). **a** | Fertile mutagenized males are mated to females carrying a balancer chromosome (shown as a double-headed arrow) to generate G1 animals that may carry a new mutation (shown in red). **b** | G1 animals (males or females) are mated to animals carrying both a balancer chromosome and a marker for the non-mutagenized chromosome (such as the *Rex* allele, which gives mice a dominant curly coat, as shown by the curly lines). **c** | Informative G2 animals are identified by their yellow coat colour and are intercrossed to look for new mutations in the G3 test class of offspring. **d** | Any pedigrees with greater than 24 offspring and no black animals must carry a new lethal mutation on chromosome 11. Lethal or detrimental mutations can be rescued by the yellow-marked carriers. Mutations are mapped if they segregate *in trans* to the yellow coat-colour marker. Visible mutations mapping genome-wide may also be isolated if they are detected in both test and carrier offspring.

Balancer chromosomes: isolating recessive mutations. A second approach to isolating recessive-lethal and detrimental mutations is being carried out by this author and Allan Bradley at Baylor College of Medicine, USA (Baylor mouse genome project). Using Cre/*loxP* chromosome engineering, inversions are being engineered on individual mouse chromosomes. To make these balancer chromosomes (BOX 2) similar to those that are constructed in flies, *loxP* sites are placed in opposite orientations at two different locations on a mouse chromosome in ES cells. On the introduction of Cre recombinase, a recombination event produces an inverted chromosomal rearrangement. One end of the inversion is targeted with a vector that contains the *K14-agouti* transgene. This construct expresses the *agouti* gene under the control of the skin-specific promoter, *keratin 14* (REFS 30,31). The first balancer chromosome has been engineered on chromosome 11, in which one endpoint in the first inversion breaks in *Wnt3*, a gene that is essential for early embryonic development (FIG. 3a). Disruption of this gene in mice homozygous for the balancer chromosome causes them to die during embryogenesis. The balancer chromosomes are used in schemes that are designed to isolate lethal or detrimental mutations by screening for the presence or absence of the coat-colour phenotype that they confer (FIG. 3b). This means that the mutations that are linked to the balancer can be easily scored in a few crosses, eliminating the need to map them. Finally, the balancers are useful for the long-term maintenance and characterization of the new mutations. Because the mutations are isolated in a three-generation pedigree mating scheme (FIG. 4), recessive lethal and visible mutations linked to the balancer, and which are therefore mapping on the chromosome of interest, are isolated at a high frequency. However, recessive viable mutations located elsewhere in the genome are also observed, and need to be mapped in the traditional way.

Gene-based screens. An alternative approach to screening for desired phenotypes is to screen for DNA lesions in a gene of interest and to determine a subsequent phenotypic effect. Two different groups, using ENU and EMS, have shown that this approach can be effectively carried out in mouse ES cells[32,33]. ES cells are treated with the chemical in culture, and the genes of interest are screened for point mutations. Alternatively, the cultured cells can be screened for a phenotype. Any ES cell that contains a desired mutation can be clonally expanded and used to generate chimeric mice that can transmit the mutation in the germ line. This may be the most powerful approach for saturating a single gene or locus with mutations. These studies have the added benefit that they will determine which changes in the gene are neutral and fail to cause any noticeable phenotype in the whole organism. Using this strategy, identifying the DNA lesions in the whole organism as well as in ES cells should be robust and cost-effective. Further technological developments

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from the Human Genome Project may also facilitate mutation detection, including DNA sequencing by hybridization to DNA chips, and high-efficiency single nucleotide polymorphism (SNP) detection.

Mutagenesis and the community. A large number of ENU-induced mutations have already been produced that represent only a fraction of the potential of the mammalian genome, and further mutagenesis experiments will generate new human disease models and reveal new mammalian developmental pathways. In addition to the programmes reported here, several new mutagenesis centres are being established in the US, Japan and Canada[28]. The mutants will soon be available to numerous smaller laboratories, whose specialization, for example in physiology or endocrinology, will enable further dissection of the biological outcome of novel mutations. The mutagenesis centres could also aid smaller laboratories by providing ENU-treated males or their G1 offspring for smaller-scale experiments. Furthermore, mutagenesis centres could act as hosts to visiting scientists from more specialized laboratories. These scientists could help to develop new phenotype assays to isolate their favourite mutants, which could then be taken away for further investigation.

The large-scale comparative analysis of mouse and human diseases requires the generation of phenotype databases, which are being developed using standardized phenotype vocabularies to make descriptions and search terms uniform (The gene ontology project)[34]. Community cooperation is required to manage and distribute stocks, characterize phenotypes and functionally annotate the genome.

The addition of a complex mammal, such as the mouse, to the list of informative genetic model organisms will allow an analysis of conserved and divergent evolution among species. ENU mutagenesis could also be used to dissect physiological parameters in other complex mammals, such as the rat, for which pilot screens are being considered. Further mutagenesis efforts in mammals are likely to produce phenotypes that will have an enormous effect on drug development and human health.

Links

DATABASE LINKS ***Kit* | *Kitl* | *Whl* | *ank* | *Wnt3***
FURTHER INFORMATION UK ENU mutagenesis programme | German ENU-mouse mutagenesis project | *Drosophila* stock centre | Mouse Genomics and Genetics Resources Committee | Australian mutagenesis centre | Canadian mutagenesis centre | Chris Goodknow at Australian National University | Baylor Mouse Genome Project | The Gene Ontology Project | Mouse Genome Database (MGD)

1. Capecchi, M. R. Altering the genome by homologous recombination. *Science* **244**, 1288–1292 (1989).
2. Bellen, H. J. Ten years of enhancer detection: Lessons from the fly. *The Plant Cell* **11**, 2271–2281 (1999).
3. Zambrowicz, B. P. *et al.* Disruption and sequence identification of 2,000 genes in mouse embryonic stem cells. *Nature* **392**, 608–611 (1998).
4. Nolan, P. M. *et al.* A systematic genome-wide phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nature Genet.* **25**, 440–443 (2000).
5. Hrabe de Angelis, M. *et al.* Genome wide large scale production of mutant mice by ENU mutagenesis. *Nature Genet.* **25**, 444–447 (2000).
References 4 and 5 describe the first large-scale ENU mutagenesis screens in the mouse.
6. Rogers, D. C. *et al.* SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm. Genome* **8**, 711–713 (1997).
7. Dunn, L. C. Studies on spotting patterns. II. Genetic analysis of variegated spotting in the house mouse. *Genetics* **22**, 43–64 (1937).
8. Blake, J. A., Richardson, J. E., Davisson, M. T. & Eppig, J. T. The Mouse Genome Database (MGD): Genetic and genomic information about the laboratory mouse. The Mouse Genome Database Group. *Nucleic Acids Res.* **27**, 95–98 (1999).
This paper describes the features of the Mouse Genome Database (MGD).
9. Sarvella, P. A. & Russell, L. B. *Steel,* a new dominant gene in the house mouse. *J. Hered.* **47**, 123–128 (1956).
10. Taylor, B. A., Navin, A. & Phillips, S. J. PCR-amplification of simple sequence repeat variants from pooled DNA samples for rapidly mapping new mutations of the mouse. *Genomics* **21**, 626–632 (1994).
11. Taylor, B. A. in *Mouse Genetics and Transgenics: A Practical Approach* (eds Jackson, I. J. & Abbott, C. M.) 87–120 (Oxford Univ. Press, Oxford, 1999).
12. Nadeau, J. H. & Frankel, W. N. The roads from phenotypic variation to gene discovery: mutagenesis versus QTLs. *Nature Genet.* **25**, 381–384 (2000).
13. Anderson, P. Mutagenesis. *Methods Cell Biol.* **48**, 31–58 (1995).
14. Jansen, G., Hazendonk, E., Thijssen, K. L. & Plasterk, R. Reverse genetics by chemical mutagenesis in *Caenorhabditis elegans. Nature Genet.* **17**, 119–121 (1997).
15. Nusslein-Volhard, C., Wieschaus, E. & Kluding, H. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* I. Zygotic loci on the second chromosome. *Roux's Archives Dev. Biol.* **193**, 267–282 (1984).
16. Wieschaus, E., Nusslein-Volhard, C. & Jurgens, G. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* III. Zygotic loci on the X-chromosome and fourth chromosome. *Roux's Archives Dev. Biol.* **193**, 296–307 (1984).
17. Jurgens, G., Wieschaus, E., Nusslein-Volhard, C. & Kluding, H. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* II. Zygotic loci on the third chromosome. *Roux's Archives Dev. Biol.* **193**, 283–295 (1984).
18. Gans, M., Audit, C. & Masson, M. Isolation and characterization of sex-linked female-sterile mutants in *Drosophila melanogaster. Genetics* **81**, 683–704 (1975).
19. Nusslein-Volhard, C. & Wieschaus, E. Mutations affecting segment number and polarity in *Drosophila. Nature* **287**, 795–801 (1980).
20. Miklos, G. L. & Rubin, G. M. The role of the genome project in determining gene function: Insights from model organisms. *Cell* **86**, 521–529 (1996).
21. Haffter, P. *et al.* The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio. Development* **123**, 1–36 (1996).
22. Driever, W. *et al.* A genetic screen for mutations affecting embryogenesis in zebrafish. *Development* **123**, 37–46 (1996).
23. Postlethwait, J. H. *et al.* Vertebrate genome evolution and the zebrafish gene map. *Nature Genet.* **18**, 345–349 (1998).
24. Amsterdam, A. *et al.* A large-scale insertional mutagenesis screen in zebrafish. *Genes Dev.* **13**, 2713–2724 (1999).
25. Hakim, F. T. *et al.* Hereditary joint disorder in progressive ankylosis (*ank/ank*) mice. *Arthritis Rheum.* **27**, 1411–1420 (1984).
26. Ho, A. M., Johnson, M. D. & Kingsley, D. M. Role of the mouse *ank* gene in control of tissue calcification and arthritis. *Science* **289**, 265–270 (2000).
27. Battey, J., Jordan, E., Cox, D. & Dove, W. An action plan for mouse genomics. *Nature Genet.* **21**, 73–75 (1999).
This paper explains the rationale for the trans-NIH mouse initiatives.
28. Denny, P. & Justice, M. J. Mouse as the measure of man? *Trends Genet.* **16**, 283–287 (2000).
29. Finkel, E. Australian 'ranch' gears up to mass-produce mutant mice. *Science* **288**, 1572–1573 (2000).
30. Zheng, B., Mills, A. A. & Bradley, A. A system for rapid generation of coat color-tagged knockouts and defined chromosomal rearrangements in mice. *Nucleic Acids Res.* **27**, 2354–2360 (1999).
31. Zheng, B. *et al.* Engineering a balancer chromosome in the mouse. *Nature Genet.* **22**, 375–378 (1999).
A seminal paper that describes chromosomal engineering techniques to generate a coat-colour tagged balancer chromosome for genetic studies in the mouse.
32. Munro, R. J. *et al.* Mouse mutants from chemically mutagenized embryonic stem cells. *Nature Genet.* **24**, 318–321 (2000).
33. Chen, Y. *et al.* Genotype-based screen for ENU-induced mutations in mouse embryonic stem cells. *Nature Genet.* **24**, 314–317 (2000).
References 32 and 33 describe the use of point mutagens for gene-based screens in mouse embryonic stem cells.
34. Eppig, J. T. Algorithms for mutant sorting: The need for phenotype vocabularies. *Mamm. Genome* **11**, 584–589 (2000).
35. Hitotsumachi, S., Carpenter, D. A. & Russell, W. L. Dose-repetition increases the mutagenic effectiveness of *N*-ethyl-*N*-nitrosourea in mouse spermatogonia. *Proc. Natl Acad. Sci. USA* **82**, 6619–6621 (1985).
36. Shedlovsky, A., McDonald, J. D., Symula, D. & Dove, W. F. Mouse models of human phenylketonuria. *Genetics* **134**, 1205–1210 (1993).
37. Justice, M. J. in *Mouse Genetics and Transgenics: A Practical Approach* (eds Jackson, I. J. & Abbott, C. M.) 185–215 (Oxford Univ. Press, Oxford, 1999).
38. Justice, M. J., Noveroske, J. N., Weber, J. S., Zheng, B. & Bradley, A. Mouse ENU mutagenesis. *Human Genet.* **8**, 1955–1963 (1999).

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