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New approaches for modelling sporadic genetic disease in the mouse

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Sporadic diseases, which occur as single, scattered cases, are among the commonest causes of human morbidity and death. They result in a variety of diseases, including many cancers, premature aging, neurodegeneration and skeletal defects. They are often pathogenetically complex, involving a mosaic distribution of affected cells, and are difficult to model in the mouse. Faithful models of sporadic diseases require innovative forms of genetic manipulation to accurately recreate their initiation and pathogenesis. Such modelling is crucial to understanding these diseases and, by extension, to the development of therapeutic approaches to treat them. This article focuses on sporadic diseases with a genetic aetiology, the challenges they pose to biomedical researchers, and the different current and developing approaches used to model such disorders in the mouse.

Introduction

Sporadic diseases are defined epidemiologically as those that arise in the absence of evidence for an environmental or heritable aetiology. Inevitably, many apparently sporadic diseases will have an environmental or genetic basis, including instances of new dominant or rare recessive germline mutations, as well as diseases with a cryptic infectious or other environmental cause. Additionally, several diseases that would otherwise fit the definition of sporadic occur more frequently in some populations or in individuals with certain inherited polymorphisms, thus blurring the distinction between sporadic and non-sporadic illness. This Perspective will focus on sporadic diseases that arise as a result of somatic mutation and modern approaches for modelling them in the mouse.

The diseases

Cancer and premalignant clonal disorders are the archetypal diseases resulting from somatic mutations, however they are not unique in this respect: another group of diseases is caused by somatic mutation occurring in the genome of a single cell in the early embryo and is therefore associated with somatic mosaicism. Although mosaicism per se need not be associated with illness (Yu et al., 2002), mutational mosaicism can lead to diseases that are normally associated with the equivalent germline mutations, albeit with a delayed onset. For example, one individual carrying a mutation in presenilin 1 in 8% of peripheral lymphocytes and in 14% of cells in the cerebral cortex presented with Alzheimer's disease later than would be seen with a similar inherited mutation (Beck et al., 2004). Human mosaics have also been described for disorders caused by aneuploidy, such as trisomy 21 (Pangalos et al., 1994). Furthermore, acquired aneuploidy arising through somatic mutation is widespread in the normal human brain, although the functional or pathological consequences of this remain unknown (Westra et al., 2008).

An intriguing form of mosaicism involving somatic mutation is observed with genes for which germline mutation is lethal. Mitchell and colleagues estimate that ~30% of the genes in the mouse genome are essential for development (Mitchell et al., 2001; Sun et al., 2008) and inheriting a homozygous null mutation, or in some cases a heterozygous gain-of-function mutation, in these genes will often result in death in utero or early in postnatal life. However, the effects of such lethal mutations can be tolerated better if they are not present in all cells. McCune-Albright syndrome, or polyostotic fibrous dysplasia, is an example of a disease that arises from an activating somatic mutation in the embryo in the gene *GNAS* (guanine nucleotide-binding protein, alpha-stimulating activity polypeptide 1), thus, patients have a single clonal population of mutant cells (Schwindinger et al., 1992; Weinstein et al., 1991). The syndrome is characterised clinically by abnormal skeletal development, café-au-lait pigmentation of the skin and peripheral precocious puberty, occasionally in association with other endocrine abnormalities.

A contrasting form of mosaicism, which can also moderate the phenotype of severe disease, is sometimes observed with inherited recessive mutations that impair growth and proliferation of tissue stem cells. Under these circumstances, somatic reversion of the mutation on one allele back to the wild-type generates a 'rescued' population of cells that reduces the severity of the clinical phenotype. Examples of such 'reverse mosaicism' have been observed in haematological and lymphoreticular diseases such as Fanconi anaemia (Waisfisz et al., 1999), X-linked severe combined immunodeficiency (Stephan et al., 1996) and adenosine deaminase deficiency (Hirschhorn et al., 1996).

The accumulation of somatic mutations throughout life, in both the nuclear and the mitochondrial genomes, is likely to be responsible for aspects of ageing (Khrapko and Vijg, 2009). Mitochondrial DNA has a higher mutation rate than nuclear DNA and this can be increased further in the presence of high oxidative stress. Conventional gene targeting of nuclear genes has been

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undertaken to produce an increased susceptibility to mitochondrial mutations. For example, deleting the nuclear gene *Ant1* (also known as *Slc25a4*) in mice prevents ADP/ATP exchange in the inner mitochondrial membrane, causing an increase in reactive oxygen species production and DNA rearrangements (Esposito et al., 1999). More recently, mice carrying mutant DNA polymerase γ, which lacks proofreading activity, have been generated and these show accelerated aging (Kujoth et al., 2005; Trifunovic et al., 2004).

Some germline nuclear mutations predispose to somatic mutations. Heritable mutations in the DNA repair machinery cause susceptibility to somatic mutation, higher rates of cancer and, sometimes, premature ageing including neurodegeneration. These mutations manifest as diseases such as Bloom's syndrome, which arises from a defect in the RecQ-like helicase *BLM* (Bohr, 2008), and xeroderma pigmentosum, which arises from defects in genes involved in the nucleotide excision repair system (Dworaczek and Xiao, 2007). Such syndromes can be modelled in mice using standard gene targeting approaches to create the equivalent germline mutations in DNA repair genes, although embryonic lethality can be a problem (Andressoo et al., 2009; Chester et al., 2006).

Challenges to modelling sporadic disease

The unique advantage of animal models as systems for investigating human disease is that they enable studies at the level of the whole organism. Genetic mouse models in particular are powerful tools for generating and studying the effects of precise genetic manipulations of a mammalian genome. Mouse models have been very successful in the study of inherited diseases, for which the pathogenetic process operates through the detrimental effects of specific genetic mutations that are present from the time of conception onwards. However, sporadic diseases usually have a much more complex pathogenesis that can be difficult to model accurately.

In trying to create mouse models of sporadic human disease, we have to use genetic systems to model stochastic non-heritable processes. The advent of molecular tools that enable the expression of genetic mutations in specific organs or cells types, and that can also allow temporal control of this process, go some way towards this goal. However, we are still limited in our ability to recapitulate diseases such as cancer that start in a single cell and develop through a stochastic acquisition of serial mutations. Nevertheless, recent groundbreaking advances that are discussed later in this Perspective promise to address such complexities.

Modelling sporadic disease by manipulating the nuclear genome

We need to be able to model sporadic diseases to understand their pathogenesis and elucidate why, in some cases, the effects of somatic mutation are different from the effects of germline mutation in the same gene (Tidyman and Rauen, 2008). Older approaches to modelling somatic mutation in the nuclear genome involved creating chimeras, in which two independent cell lineages are present in one animal. For the last few years we have had technologies that allow temporal and spatial control of gene expression in a single tissue or cell type. Most recently, researchers are developing systems to model the process of somatic mutation itself.

Chimeras

A chimeric animal is one that is derived from two or more zygotes. The use of chimeras has a long and valuable record in research into development and cellular interactions (Rossant and Spence, 1998). Chimeras were made originally by the aggregation of two pre-implantation embryos. Currently, chimeras are usually generated by either injection of embryonic stem (ES) cells into host blastocysts or by aggregating ES cells with morula-stage embryos (Draper and Nagy, 2007).

Chimeras have been particularly helpful in defining cell-autonomous and non-cell-autonomous effects, which is important for understanding pathogenesis and designing therapies. Cleveland and colleagues created mice that were chimeras for wild-type cells and for cells carrying a dominant mutation in superoxide dismutase 1 (*Sod1*), which causes a neurodegenerative disease (amyotrophic lateral sclerosis, ALS) in humans. By carefully studying cell death in the chimeric mice, they showed that ALS is not simply the result of the cell-autonomous death of motor neurons, but that expression of mutant *Sod1* in glial cells also contributes to disease (Clement et al., 2003). This has important implications for therapy, suggesting that drugs that are able to support glial cells may help prolong life in human ALS patients.

Another use of chimeras is in the study of otherwise lethal mutations. The rescue of mutant cells in chimeras can allow an examination of possible later roles for the gene in question. For example, mutation of *Gata2*, a haematopoietic transcription factor, causes lethality in mid-gestation because of severe anaemia, suggesting that *Gata2* is required for yolk sac haematopoiesis. However, the embryos die too early to reveal any effects on definitive haematopoiesis. Chimeras made with homozygous mutant cells allowed for such an analysis, and showed that definitive haematopoiesis was severely affected too, implicating *Gata2* as a crucial gene in the proliferation of haematopoietic progenitors (Tsai et al., 1994). The main disadvantages of working with chimeras for studies of somatic mutations are, first, the difficulty in generating homozygous mutations in ES cells and, second, the lack of control over the level of colonisation of the different tissues; so, for most applications, they have been superseded by more controllable and definable systems that create conditional and inducible mutations.

Conditional and inducible systems to model somatic mutation

For several years it has been possible to create mouse models in which gene expression may be switched on or off in specific tissues at specific times, thus enabling the study of genetic mutations in different tissues or at different stages of development. These systems, developed primarily to understand the function of single genes, can effectively model aspects of somatic mutation, including the introduction of mutations into single cells, such as those responsible for cancer. There are two broad approaches for targeted introduction of genetic mutations in mouse tissues: 'conditional' and 'inducible'.

Conditional systems rely on the deployment of one of two DNA recombinase systems that have been developed for use in the mouse: the Cre-*loxP* system of the P1 bacteriophage, and the Flp-*FRT* system encoded by the 2μ plasmid of *Saccharomyces cerevisiae*. In these systems, the recombinase proteins Cre and Flp (Flippase) mediate site-directed DNA recombination between two unique 34-

base pair sequences composed of two 13-base pair inverted repeats, separated by an asymmetric 8-base pair spacer sequence (Broach et al., 1982; Broach and Hicks, 1980; Hoess et al., 1982; Sternberg and Hamilton, 1981). The recombinase binds specifically to a pair of its cognate sites, known as *loxP* for Cre and *FRT* for Flp, and brings them together for cleavage (Hamilton and Abremski, 1984; Hoess and Abremski, 1985). The relative orientation of the two sites, dictated by their asymmetric spacer regions, determines whether the recombination event results in excision, inversion or translocation of host DNA (Hoess et al., 1986).

To introduce genetic mutations conditionally, DNA targeting in mouse ES cells is used to generate mouse strains in which the *loxP* or *FRT* recombination sites are placed in the same orientation either side of a gene, exon or other locus of interest ('floxing' or 'flirting' the sequence, respectively). These floxed or flirted mice are then crossed to a transgenic mouse line in which the expression of the relevant recombinase (Cre or Flp) is driven by a cell lineage-specific promoter so that recombination leading to deletion or mutation of the locus occurs only in the cell type expressing the recombinase (Gu et al., 1994; Orban et al., 1992; Tsien et al., 1996) (Fig. 1A). Such systems have been used widely to model a variety of human diseases and have proved to be particularly helpful in studies of cancer biology (Marino et al., 2000). The cell specificity of Cre expression need not come just from crosses to transgenic mice. For example, the Cre-*loxP* system has been used to examine the relationship between Rb1 and Trp53 in lung cancer by administering Cre directly to the target site using adenovirus delivery by intratracheal injection (Meuwissen et al., 2003).

The Cre-*loxP* and Flp-*FRT* systems can also be used to activate gene expression if a transcriptional stop sequence is placed after a promoter or a splice acceptor site. The stop cassette can then be excised by the recombinase, and expression of the gene can be selectively activated in a specific cell population (Lakso et al., 1992). This approach can be used to model somatic acquisition of oncogenic mutations, for example by using adenovirus expressing Cre to induce expression of an oncogenic allele of Kras in the lung, leading to the development of lung adenocarcinomas (Jackson et al., 2001). Other adaptations of the system enable the expression of reporter genes, the generation of chromosomal translocations, and many other genomic modifications.

A further level of control over the expression of mutant alleles can be achieved by using mouse models carrying an inducible Cre-*loxP* system, which can be activated in the presence of an exogenous inducer and, thus, switched on or off in specific lineages at specific times. Two types of inducer are used widely, one that relies on the oestrogen receptor and another that employs the tetracycline regulatory system.

In the first, the Cre recombinase is fused to the ligand-binding domain of a mutated oestrogen receptor (ER) that has lost the ability to bind to endogenous oestrogen, but can still bind to its analogue, tamoxifen. In the presence of tamoxifen, the Cre fusion protein translocates into the nucleus so that Cre gains access to the floxed target locus within the genome of the cell, and recombination occurs (Fig. 1B) (Brocard et al., 1998; Danielian et al., 1993; Metzger et al., 1995; Zhang et al., 1996). There are numerous studies in which this inducible system has been used to model somatic diseases. A recent study generated prostate-specific antigen (PSA)-Cre-ER^{T2} mice that express a tamoxifen-dependent Cre-ER^{T2} recombinase in the prostate epithelium, enabling ablation of a floxed tumour suppressor gene to model prostate cancer (Ratnacaram et al., 2008). However, it should be noted that the use of Cre-ER fusion proteins was shown recently to have direct toxic effects, at least on the haemopoietic system, which have to be distinguished from the effects of any somatic mutation studied (Higashi et al., 2009).

The tetracycline (Tet) regulatory system can be used to switch gene expression on or off by using constructs based on the tetracycline repressor protein TetR, which interacts with a DNA sequence known as the tetracycline response element (TRE) within the promoter of the bacterial tetracycline resistance operon. The Tet-off system uses the tetracycline transactivator (tTA) protein, which is a fusion between TetR and the transcriptional activation domain of VP16 (Fig. 1C) (Gossen and Bujard, 1992). In the absence of tetracycline, or its analogue doxycycline, tTA binds to TRE sequences and, if these are adjacent to a mammalian promoter, activates gene transcription. Addition of tetracycline in this system causes tTA to dissociate from the TRE and, thus, turns off gene expression. Conversely, the Tet-on system uses a mutated version of tTA termed reverse tTA (rtTA), which only binds to TRE sequences in the presence of tetracycline; therefore, administration of the drug can be used to induce gene expression (Kistner et al., 1996). These Tet systems can be used to switch the expression of any gene on or off, including Cre and FLP recombinases. Thus, the Tet systems can be used to deliver temporal and tissue-specific control of gene expression, depending on when tetracycline or doxycycline are administered. The Tet systems have proved to be useful in a variety of studies of somatic disorders, from prion diseases (Tremblay et al., 1998) to the role of oncogenes such as *Ras*, *Myc* and *Bcr-Abl1* in tumourigenesis and tumour regression (Chin et al., 1999; Felsher and Bishop, 1999; Huettner et al., 2000).

Finally, it is worth mentioning that some recombinase alleles are, inherently, both conditional and inducible. A widely used example of this is the *Mx-Cre* allele, which places the cDNA for Cre under the control of the interferon-responsive *Mx1* promoter. Expression is induced by the administration of interferon (IFN) or by stimulation of its endogenous secretion in response to polyinosinic-polycytidylic acid (pIpC). In this context, Cre is expressed in tissues where the IFN-inducible *Mx1* gene is normally expressed, including hepatocytes and haemopoietic cells (Kuhn et al., 1995).

Modelling somatic mutation with insertional mutagens

Insertional mutagenesis using retroviruses is another approach to modelling somatic mutation and has been particularly useful in identifying novel tumour suppressors and oncogenes. A retrovirus inserts into the genome as a provirus with two long terminal repeats (LTRs), which contain strong promoter and enhancer elements. If the retrovirus disrupts a tumour suppressor gene or activates an oncogene, tumourigenesis may result. This type of modelling of somatic mutation has been used in genetic screens to identify novel cancer genes (Suzuki et al., 2002). However, retroviruses have a number of functional limitations, such as the ability to infect only dividing cells, integration biases and effects on distant genes that can be difficult to decipher. As a result, retroviral insertional mutagenesis has so far been limited to the study of haematopoietic and mammary cancers (Collier and Largaespada, 2005).

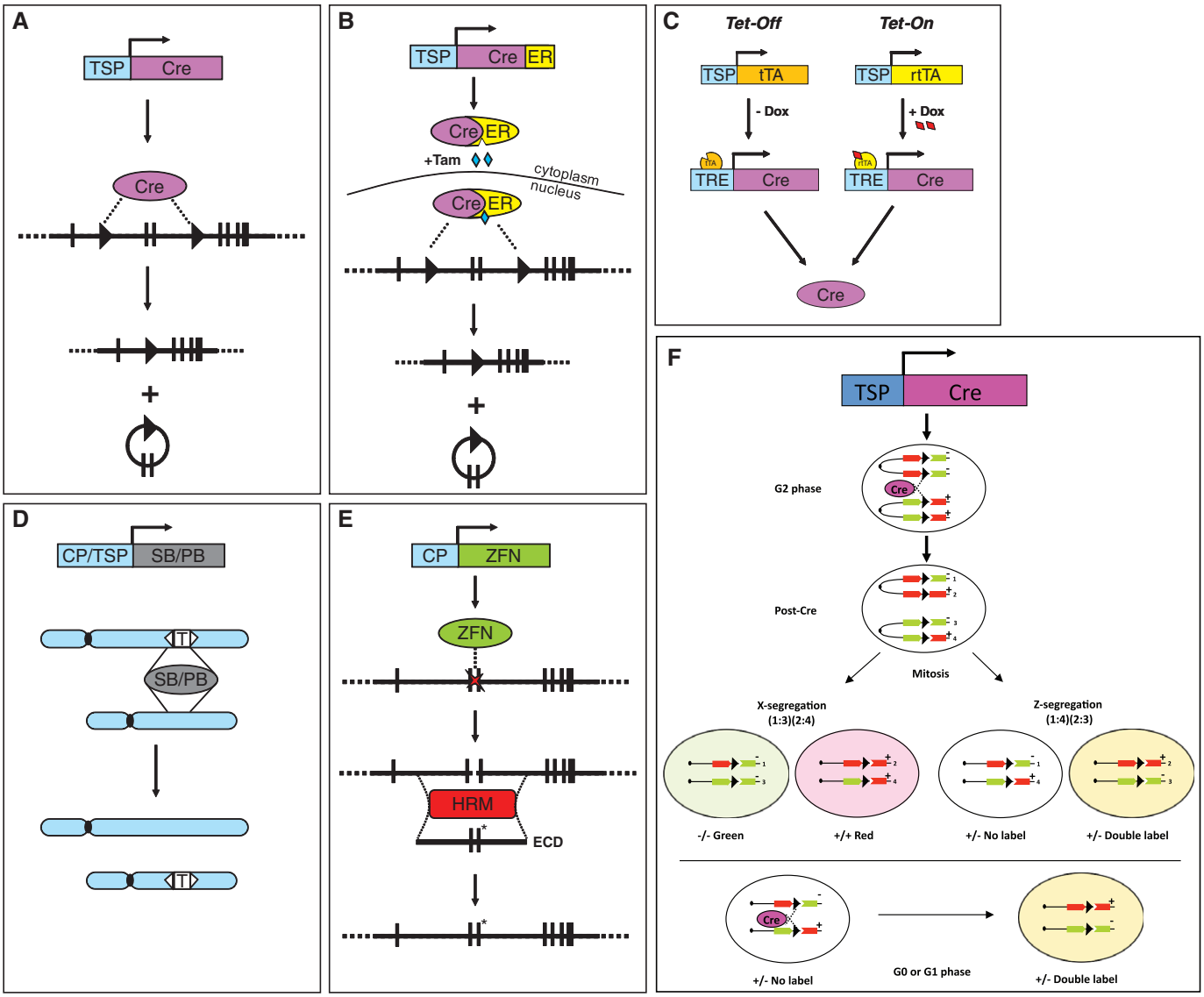

Fig. 1. Approaches to modelling somatic mutation in the mouse. (A) Conditional system. The gene encoding Cre recombinase is expressed from a tissue-specific promoter (TSP) and Cre catalyses the recombination between *loxP* sites (black arrowheads). As a result, the intervening sequence containing key exons (vertical black lines) is circularised and excised from the genome, so that it is lost later with cell division. (B) Tamoxifen-inducible system. A fusion gene that encodes Cre and a modified oestrogen ligand-binding domain (ER) is expressed from a TSP. The Cre-ER chimeric protein translocates to the nucleus in the presence of tamoxifen (Tam), resulting in recombination between *loxP* sites. (C) Tet-on/Tet-off inducible system. Tet-off: In the absence of doxycycline (Dox), the transcriptional activator tTA, which is expressed from a tissue-specific promoter, binds to the TRE to activate transcription of the downstream *Cre* transgene. If doxycycline is present, it binds to tTA preventing TRE binding and *Cre* transcription. Tet-on: In the presence of doxycycline, rtTA binds to TRE and activates *Cre* transcription. This binding does not happen in the absence of doxycycline. (D) Transposition. *Sleeping Beauty* (SB) or *PiggyBac* (PB) transposase expressed from a constitutive promoter (CP) or a TSP mobilises a transposon (T) from a chromosomal donor site to a distant site on another chromosome where it can disrupt a gene (insertional mutagenesis). (E) Zinc finger nuclease (ZFN) system. ZFN cDNA is delivered by a retrovirus and expressed from a CP. The ZFN protein generates a targeted double-strand break, which is then repaired by the homologous recombination machinery (HRM) of the cell using a co-delivered extra-chromosomal DNA sequence (ECD) that carries a mutant or reverted exon (*). The mutation or reversion is thus incorporated into the cellular genome. (F) Mosaic analysis with double markers (MADM). Two reciprocally chimeric marker genes (GFP-RFP and RFP-GFP) are targeted to the same locus on homologous chromosomes. One is associated with a wild-type allele (+, GFP-RFP in this example) and one with a knockout allele (–, RFP-GFP in this example). In cells that are heterozygous for the two chimeric markers, Cre mediates recombination between non-sister chromatids at *loxP* sites during the G2 phase of the cell cycle. When this is followed by X segregation (where sister chromatids segregate into two different daughter cells), it generates singly labelled cells (green or red) with an altered genotype (–/– or +/+). When G2 recombination is followed by Z segregation (where sister chromatids segregate into the same daughter cell), or after G0 or G1 recombination, either colourless or double-coloured cells are generated whose genotype is unaltered (+/–). GFP, green fluorescent protein; RFP, red fluorescent protein.

A more flexible group of insertional mutagens are the transposons, which are mobile genetic elements that were first discovered in maize (McClintock, 1950). Transposons proved very useful as functional genomic tools in plants and lower eukaryotes before being adapted recently for use in mammalian cells. Transposon systems are bipartite and consist of a transposase enzyme and a DNA transposon vector flanked by binding sites for the former. The transposase can mobilise the transposon from its position within the genome and cause its reintegration elsewhere by a cut-and-paste mechanism (Fig. 1D). There are several advantages of using transposons for insertional mutagenesis, including the ability of transposons to integrate throughout the mouse genome, the minimal requirements for host factors, the capacity to carry DNA fragments that are able to alter gene expression, integration near to their target genes and, perhaps most importantly, the fact that transposition can be controlled temporally and targeted to the cell or tissue of interest (Weiser and Justice, 2005).

Accumulated mutations have rendered resident transposons inactive in vertebrates. However, the *Sleeping Beauty* transposon system of salmonid fish has been carefully restored by phylogenetics and site-directed mutagenesis (Ivics et al., 1997). This system has since been used in insertional mutagenesis screens for cancer gene discovery. In these studies, the DNA transposon construct contained splice acceptor sites and bidirectional polyadenylation signals to terminate gene transcription, as well as a viral LTR to activate gene transcription. Mice bred to carry both the *Sleeping Beauty* transposase and DNA transposon developed tumours after a short latency. When tumour DNA was analysed for the sites of integration, both known and novel oncogenes were identified (Collier et al., 2005; Dupuy et al., 2005; Dupuy et al., 2006; Ivics et al., 1997). Use of the *Sleeping Beauty* transposon system in *Blm* knockout mice, which have an increased rate of mitotic recombination, has allowed the method to be extended to the identification of tumour suppressor genes (Dupuy et al., 2006).

The flexibility of the *Sleeping Beauty* system is leading to the development of a large number of novel applications. For example, *Sleeping Beauty* transposons were engineered to carry promoterless candidate oncogenes and then mobilised by the transposase to new sites within the host genome. A chance insertion downstream of a promoter may activate transcription of the oncogene and lead to the onset of tumours, thus allowing the genetic environment for individual oncogenes to be examined (Su et al., 2008). The *Sleeping Beauty* system has also been used to model somatic mutation in specific tissues. Wiesner and colleagues co-injected transposase-expressing plasmids and transposon elements with a cargo of candidate oncogenes into the mouse brain to induce brain tumours (Wiesner et al., 2009). This approach allowed combinations of oncogenes to be tested for tumour formation and progression. In investigating the genetic mutations leading to colorectal cancer, Starr and colleagues crossed mice with mutagenic *Sleeping Beauty* transposons to mice expressing the transposase in the gastrointestinal tract epithelium; analysis of tumours in the offspring has identified almost 80 new candidate genes involved in this type of cancer (Starr et al., 2009).

However, although *Sleeping Beauty* is clearly an important system for modelling somatic mutation, it has some limitations: transposon re-integration can have a predilection for sites close to the original site (local hopping), the efficiency of jumping is affected by cargo size, and a 7-base pair footprint is usually left behind after mobilisation (Collier and Largaespada, 2005; Izsvak et al., 2000; Wu et al., 2007). *PiggyBac* is an alternative, more active, transposon from the cabbage looper moth *Trichoplusia ni* that has been shown to transpose efficiently in mammalian cells and in the mouse germline, and to carry up to 9.1 kb of cargo (Ding et al., 2005; Wu et al., 2007). Cadinanos and Bradley have recently engineered a mouse codon-optimised inducible *PiggyBac* transposase by fusion to the ligand-binding domain of the oestrogen receptor. This system allows tight temporal control of transposition and overcomes problems with chromosomal instability and embryonic lethality (Cadinanos and Bradley, 2007). *PiggyBac* has also been used in conjunction with the Cre-*loxP* system to scatter *loxP* sites through the genome and create random large-scale deletions and duplications for mutagenesis screens (Wu et al., 2007).

One salient feature of insertional mutagens is that, within the tissues that they target, they generate a patchwork of cells each carrying a different mutation or combination of mutations, as well as many cells without any mutations. This achieves a closer approximation of human carcinogenesis than most recombinase-based systems, which produce the same mutation in a very large proportion of cells, sometimes approaching 100% of all cells in a tissue.

Novel approaches for modelling somatic mutations

An important type of somatic mutation involves chromosomal deletion or duplication during mitotic recombination between paired homologous chromosomes during the G2 phase of the cell cycle. This event results in a mosaic animal carrying homozygous mutant cells in a non-mutant background. Modelling of this type of somatic mutation was first undertaken in Drosophila, in which mitotic recombination occurs at a higher frequency than in mammals because homologous chromosomes pair at interphase (Liu et al., 2002; Zong et al., 2005). To induce mosaicism through aberrant mitotic recombination, Xu and Rubin generated fly strains carrying an *FRT* sequence near the centromere of a major chromosome, as well as either a cell-autonomous marker or a mutation of interest at a distal location (Xu and Rubin, 1993). FLP recombinase was induced by heat shock, and simple reciprocal recombination between *FRT* sites in a cell that was heterozygous for the marker gene and the mutation produced one daughter cell with two copies of the marker and one daughter cell with no copies of the marker, but that was homozygous for the mutation. Lee and Luo extended this system in Drosophila to produce the mosaic analysis with a repressible cell marker (MARCM) system in which all homozygous mutant cells are also labelled (Lee and Luo, 1999). These fly systems allow genome-wide functional analysis of somatic mutations, in vivo tracking of biological processes, and identification of new genes by mosaic screening.

A number of approaches have been developed to facilitate the study of homozygous somatic mutations. One approach is to generate mice from $Blm^{-/-}$ ES cells carrying targeted heterozygous mutations. The markedly increased rate of mitotic recombination in these mice leads to an increased rate of conversion of heterozygous to homozygous mutations (loss of heterozygosity, LOH) (Luo et al., 2000). The mosaic analysis with double markers (MADM) system allows simultaneous labelling and gene targeting

in clones of somatic cells, enabling the creation of conditional knockouts in small populations of labelled cells. This requires the targeting, in ES cells, of two reciprocally chimeric marker genes to the same locus on homologous chromosomes. The chimeric genes are composed of the N-terminal of one fluorescent protein (GFP or RFP) and the C-terminal of the other (RFP or GFP), separated by a *loxP* site. When gene targeted animals are crossed to Cre transgenics, Cre recombination between the *loxP* sites restores the functional GFP and RFP expression cassettes and, after segregation in which recombinant chromatids segregate to different daughter cells, a green daughter cell that is homozygous for the mutation (located downstream of GFP) and a red cell that is homozygous for the wild-type allele are generated (Zong et al., 2005) (Fig. 1F). This technique has been used to model sporadic LOH by mutating p27 using the Cre-*loxP* system. It also provides a useful tool in the understanding of the function of tumour suppressor genes showing that, for example, sporadic loss of p27 generates a marked cell expansion phenotype in hepatocytes and cerebellar granule cells (Muzumdar et al., 2007). Using mitotic recombination in combination with the Flp-*FRT* system, Wang, Bradley and colleagues created a mouse harbouring a p53 mutation in a small population of cells and found that p53-mitotic recombination mice developed twice as many carcinomas and had greater diversity in this tumour set (Wang et al., 2007). Recently, mitotic recombination between homologous chromosomes has been carried out in B and T lymphocytes in the mouse, a system that allows the generation of somatic homozygous mutations in the immune system (Sun et al., 2008).

Mosaicism was also used recently as a means for studying whether detrimental mutations act in a cell-autonomous manner and how they contribute to the development of complex organs. The new technique, known as mosaic complementation, involves the introduction of a bacterial artificial chromosome (BAC) transgene into the mouse X chromosome so that, as a result of X-chromosome inactivation, only half of all cells of female mice express genes from the BAC. Prosser et al. used this technique to express myosin VIIa from the mouse X chromosome in myosin VIIa null mice, and showed that the gene encoding myosin VIIa acts in a cell-autonomous manner to facilitate the development of sensory hair cells in the inner ear (Prosser et al., 2008).

For the study of genomic mutations in the mouse, large-scale chromosomal rearrangements (deletions, duplications and inversions) have been created by Cre-mediated recombination between a pair of *loxP* sites. The location and relative orientation of the *loxP* sites dictates the type of chromosomal rearrangement generated. This form of mouse 'chromosome engineering' was first used to generate germline chromosomal rearrangements (Ramirez-Solis et al., 1995; Tybulewicz and Fisher, 2006) but is now being extended to model somatic mutation in the mouse in the presence of Cre recombinase (Otsuji et al., 2008; Zheng et al., 2001).

Another recently developed system for targeted genomic modification utilises chimeric proteins known as zinc finger nucleases (ZFNs), which are composed of a zinc-finger DNA-binding domain and a DNA-cleavage domain, and which were designed originally as gene therapy tools (Urnov et al., 2005). ZFNs can be designed to create a double-strand break at a specific genomic locus, which can then be repaired by the homologous recombination machinery of the cell using a co-delivered extra-chromosomal DNA donor sequence (Fig. 1E). In this manner, any specific mutation or reversion carried by the donor sequence is introduced into the genome. Unlike DNA recombinases, these molecules can introduce targeted modifications into previously intact somatic genomes, including those of primary human cells, without the need for prior introduction of target sites such as *loxP* or *FRT* (Sera, 2009).

Conclusion

Sporadic diseases that are secondary to somatic mutations are a major cause of human mortality and morbidity. Our ability to model these diseases in mice is improving rapidly, owing to the advent of sophisticated molecular tools that allow us to delve deeper into their pathobiology. The approaches described in this article represent the current state-of-the-art in mouse genetic engineering and have produced models that are able to recapitulate many of the events underlying different types of sporadic genetic disease, from the initiation of somatic mutation to the study of its evolution and the understanding of its consequences. Despite such significant advances, many challenges lie ahead before we can fully recapitulate the complexity of naturally occurring pathological processes such as cancer. Nevertheless, progress in the field continues inexorably with novel technologies and mouse resources for somatic mutation modelling being developed continuously (Nguyen and Xu, 2008), and it is widely expected that these efforts will give us unprecedented insight into the somatic genome and the pathogenesis of sporadic diseases.

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COMPETING INTERESTS

The authors declare no competing financial interests.

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