

Review

Mutagenesis in rodents using the L1 retrotransposon

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Abstract

LINE1 (L1) retrotransposons are genetic elements that are present in all mammalian genomes. L1s are active in both humans and mice, and are capable of copying themselves and inserting the copy into a new genomic location. These *de novo* insertions occasionally result in disease. Endogenous L1 retrotransposons can be modified to increase their activity and mutagenic power in a variety of ways. Here we outline the advantages of using modified L1 retrotransposons for performing random mutagenesis in rodents and discuss several potential applications.

Introduction

LINE1 (long interspersed nucleotide element 1 [L1]) elements are important genome modifiers, altering mammalian genomes in many ways. L1 elements have contributed to new gene formation by inserting their own coding sequences into genes, by shuffling other coding sequences through L1 mediated transduction, and by creating processed pseudogenes. They have created deletions both during their retrotransposition (by their mechanism of integration) and after insertion through unequal homologous recombination. They have affected gene expression by altering both transcription and translation of nearby genes. They have expanded the genome by their continued retrotransposition and by mediating retrotransposition of non-autonomous retrotransposons (for reviews [1-6]). L1s have also been a source of insertional mutagenesis, occasionally inserting into genes and disrupting their function. In this review, we discuss a practical application of L1 retrotransposons, namely harnessing their mutagenic power by modifying endogenous elements and using them to create mammalian animal models.

A full-length active L1 retrotransposon is approximately 6,000 nucleotides long and encodes the proteins necessary to mobilize itself to a new genomic location. The L1 retrotransposon moves by a 'copy and paste' mechanism, which

means that the parent element remains in its original genetic location, and a copy (*de novo* insertion) is inserted somewhere else in the genome (as opposed to the 'cut and paste' mechanism of DNA transposons, in which the parent element is removed from its original genetic location and inserted somewhere else). An L1 element is transcribed into RNA and the bicistronic mRNA is exported from the nucleus to the cytoplasm, where it is translated into open reading frame (ORF)1 and ORF2 proteins. At least one L1 RNA molecule, one ORF2 molecule, and one or more ORF1 molecules may assemble into a ribonucleoprotein (RNP) complex. Then, both the ORF2 protein and associated L1 RNA must gain access to the nucleus, where the L1 RNA is reverse transcribed and integrated into a new genomic location by a process called target primed reverse transcription (TPRT; Figure 1; for review [6]).

Advantages of using retrotransposons for performing random mutagenesis Genes can be disrupted completely and in a stable manner

Retrotransposons can be used to deliver a gene trap that efficiently splices into genes and disrupts their function. This means that up to 30% of insertions (the percentage of the genome that comprises gene exons and introns) may disrupt

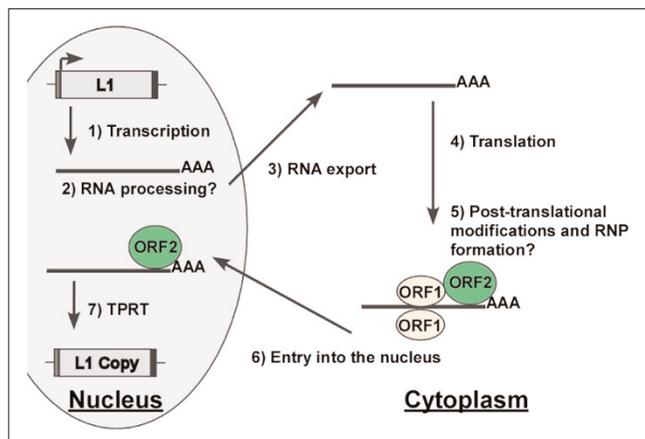


Figure 1
Steps in L1 retrotransposition. L1, long interspersed nucleotide element 1 (LINE1); ORF, open reading frame; TPRT, target primed reverse transcription.

a gene. This is a major improvement on chemical mutagenesis, which creates point mutations that often do not affect gene function. Also, unlike DNA transposon insertions, L1 insertions are permanent and stable, eliminating the possibility of losing the mutation over time.

Genes are randomly disrupted throughout the entire genome

A complete genome-wide mutagenesis strategy requires unbiased gene disruption. Although retrovirus-based mutagenesis strategies result in ‘hotspots’ of mutation [7], L1 retrotransposons insert into the genome without apparent site specificity. The L1 element contains an apurinic/apyrimidinic endonuclease with an inexact target site preference for 3'-AA/TTTT-5' [8-10], which allows the L1 retrotransposon to insert randomly throughout the entire genome. Recent human L1 insertions (within the past few million years) have been found on every chromosome and are present in introns, in intergenic regions, and in DNA regions of high and low GC content [11]. The randomness of *de novo* L1 insertions is well established by the study of recent insertions from the various genome sequencing projects, and we and others have confirmed random L1 insertion in mouse mutagenesis models [12,13]. Note that the random pattern of *de novo* insertions is distinct from the pattern that occurs after post-insertion selection bias has taken place, which causes L1 elements to accumulate into AT-rich and gene-poor regions. Post-insertion selection bias occurs over millions of years and does not affect the randomness of mutagenesis systems. Therefore, retrotransposon based mutagenesis offers a significant advantage over retrovirus based mutagenesis. It also offers an advantage over insertional mutagenesis using DNA transposons such as *Sleeping Beauty* (SB; a member of the *Tc1/mariner* transposon family) [14] and *piggyback* (a DNA transposon from the cabbage looper moth) [15], which insert non-

randomly when used for *in vivo* mutagenesis. The majority of *de novo* transposon insertions occur within 3 megabases of their original genomic location and 80% occur on the same chromosome [16-19], a phenomenon termed ‘local hopping’.

Genes are disrupted at a high frequency

L1 retrotransposons have no limit on the number of mutations that they can cause because they replicate by a ‘copy and paste’ mechanism. This is in contrast to DNA transposons, which move by a ‘cut and paste’ mechanism and are limited by the number of transposons in the transgenic founder [20]. In an ideal germline mutagenesis strategy, each germ cell would contain a single mutation that disrupts a single gene that leads to a phenotype. Fewer mutations introduce inefficiency, although more than one mutation per germ cell is not necessarily ideal because matching mutations to phenotypes becomes problematic. L1 retrotransposon based mutagenesis has a high frequency of mutation, approaching the ideal of one mutation per germ cell.

The genomic locations of mutations are easily mapped

L1 retrotransposons demonstrate *cis* preference, which means that the retrotransposition proteins almost exclusively mobilize the RNA that encoded them [21]. Therefore, when inserting a tagged L1 retrotransposon into an animal, one can be sure that all *de novo* insertions will be from tagged L1 retrotransposons and not from retrotransposition of other cellular mRNAs. Any new phenotypes will be due to a tagged insertion that can be easily mapped, as opposed to an untagged insertion that cannot be easily mapped. Unlike *N-ethyl-N-nitrosourea* (ENU) base substitutions, *de novo* retrotransposon insertions are large, unique sequences that are relatively easy to map using inverse polymerase chain reaction (PCR) or thermal asymmetric interlaced PCR. Although easy mapping of mutations is a feature shared with mutagenesis using transposons, the SB system has a drawback. Recent experiments demonstrated that *de novo* SB insertions occasionally occur via multiple sequential ‘hops’ from one chromosomal region to another [22,23], a process that can result in large deletions. After SB excision three terminal base pairs of the transposon remain, and resolution of the double strand break occurs via the nonhomologous end joining repair pathway, resulting in a net 3 base pair insertion. Occasionally, through unknown mechanisms, deletions occur at SB excision sites at lengths of up to 100 base pairs [24,25]. Deletions created in this manner are not associated with the transposon that caused them because the transposon is excised during the event (cut and paste mechanism), and so these mutations cannot easily be mapped, which confounds attempts to link interesting phenotypes with the causative mutation. L1s can cause genomic deletions at insertion sites [26,27], but these deletions remain associated with the L1 element that caused them because the retrotransposon is not subsequently excised (copy and paste mechanism) and so these mutations can be identified more easily.

Table 1**Comparison of gene knockout strategies in rodents**

Criteria	Retroviral	ENU	Retrotransposon
Disrupt genes completely	Yes	No	Yes
Random mutation	No	Yes	Yes
High frequency mutation	Yes	Yes	Yes
Quickly map mutations	Yes	No	Yes
Efficiently create knockout animals	No	Yes	Yes

ENU, N-ethyl-N-nitrosourea.

Animals that contain disrupted genes can be created efficiently

Direct mutation in the germline produces offspring with gene disruptions by natural breeding. This is important because the use of embryonic stem cells can be costly, time consuming, and technically impossible in most mammalian models, including rat [28].

Table 1 provides a summary of the various methods of mutagenesis in rodents. Many of the advantages of mutagenesis using retrotransposons are shared with DNA transposons, except as noted above, and their use is discussed in other reviews in this supplement.

Mouse mutagenesis using L1 retrotransposons
Germline mutagenesis

The first description of an endogenous L1 causing a disease in humans by insertional mutagenesis was reported in 1988. In that instance, an L1 had inserted into the gene encoding coagulation factor VIII, thereby causing hemophilia A [29]. Since that time, there have been more than 20 descriptions of disease causing *de novo* L1 insertions in humans and mice [2,6]. It is not apparent whether these endogenous L1 insertions integrated directly into the germline during spermatogenesis or oogenesis, or whether they integrated early enough in development that they were later incorporated into the germ cells, where they were passed on to future generations. However, it is clear from the many instances of insertional mutagenesis by endogenous L1s and by the hundreds of thousands of L1 elements that have accumulated in mammalian genomes that at least some *de novo* insertions make their way into the germline and are stably transmitted to offspring.

Proof-of-principle for germline mutagenesis in the mouse using L1 retrotransposons was demonstrated in animals that contained a transgene consisting of a highly active human L1 element tagged with a modified version of an enhanced green fluorescent protein (EGFP) retrotransposition cassette [30]. Several lines of transgenic mice were created. In some lines the L1 element used its endogenous promoter for

transcription, and in others the L1 was driven by the addition of a heterologous promoter. As expected, the endogenous L1 promoter was active in the germ cells. The addition of the heterologous promoter increased transcript levels in the germ cells and allowed for low levels of transcription in multiple somatic tissues. Mouse lines that contained either type of transgene demonstrated retrotransposition, detected by reverse transcription PCR and PCR of *de novo* insertions. Two germline insertions, one of which had inserted into a gene intron, were cloned from mice that had the heterologous promoter transgene. However, the transgenes in these initial mice did not contain gene traps and were not expected to disrupt genes when inserting into introns. The frequency of insertion was estimated at approximately one *de novo* insertion in every 70 sperm for mice with the heterologous promoter, and lower for mice containing the transgene that used only the endogenous promoter. More refined estimates using real-time PCR have demonstrated that some of these lines have germline insertion frequencies as high as one insertion in every 20 sperm.

Subsequent attempts at germline mutagenesis using L1 involved strategies to increase the frequency of retrotransposition, including the use of a more active human L1 element, a different heterologous promoter, and a more efficient retrotransposition cassette [13]. The highest reported frequency of germline transmission of *de novo* L1 insertions was estimated at one insertion in every three sperm [12]. These transgenic mice contained a codon optimized mouse L1 [31]. Although L1-based germline mutagenesis will probably play a role in the creation of a genome-wide collection of mouse knockouts, the existence of other already well established methods for creating germline mutations in mice (knockouts, knockins, and conditional mutations, among others) may limit its usefulness.

Somatic mutagenesis

L1 elements are also capable of somatic mutagenesis. However, little is known about the regulation, frequency, and relevance of such retrotransposition. Two examples of disease-causing somatic L1 insertions have been described in humans, one associated with a case of colon cancer and the other associated with a breast cancer. An L1 insertion into exon 16 of the *APC* tumor suppressor, predicted to be an inactivating mutation, was discovered in a human colon cancer and was not present in adjacent normal tissue [32,33]. An L1 insertion between exons 2 and 3 at the *myc* locus was also identified in a ductal carcinoma of the breast, although the functional implication of this mutation was not determined [34]. These findings indicate that L1 elements are occasionally active in somatic tissue and are capable of driving carcinogenesis. However, the relative contribution to oncogenic transformation is largely unknown. Growing evidence that L1 elements become hypomethylated within cancers suggest that L1 expression may be reactivated in

somatic tissue and that retrotransposition may play a significant role as an insertional mutagen, thereby contributing to genomic instability [35-39]. Whether global L1 hypomethylation augments L1 expression and retrotransposition requires more rigorous investigation.

Human and synthetic mouse L1 sequences have proven to retrotranspose in somatic tissue in transgenic mice [12,13,40]. In one transgenic model, a highly active human L1 element (L1_{RP}) [41], which was tagged with an EGFP retrotransposition cassette [42] and was transcribed using its endogenous promoter, exhibited somatic retrotransposition within neuronal precursors [40]. However, it is not known whether retrotransposition from endogenous L1 elements occurs in neuronal precursor cells in mice or humans. In other transgenic mice, a native human L1 element (L1_{LRE3}), tagged with a markerless retrotransposition cassette and driven by the mouse *Hspa2* promoter, demonstrated somatic retrotransposition into sites throughout the genome, including transcriptional units, with 14.6% of insertions found within RefSeq genes [13]. A synthetic, codon optimized mouse L1 (*ORFeus*) tagged with an EGFP retrotransposition cassette and driven by the CAG promoter (a hybrid between the human cytomegalovirus immediate early promoter and chicken β -actin promoter) was also capable of a high level of somatic retrotransposition in transgenic mice [12]. Because both high level ubiquitous expression (with CAG) and codon optimization (*ORFeus*) would both probably enhance retrotransposition, the relative contributions of each of these factors in this transgenic mouse is unknown.

The most practical application of L1-based somatic mutagenesis in mice would be a screen for tumor suppressors and oncogenes. A tissue specific promoter could direct L1 mediated mutagenesis to a particular organ to drive the formation of different types of cancers. The incidence of loss of function mutations could be enhanced by tagging the L1 with a gene trap cassette containing strong splice acceptors. For some trapped tumor suppressors a loss of heterozygosity may be rate limiting for carcinogenesis, but could be expedited by using a *Blm* mutant background. Germline mutations in the RecQ-like helicase *Blm* causes a profound increase in sister chromatid exchange, which is mediated by homologous recombination, resulting in widespread cancer [43]. *Blm* inactivation causes accelerated loss of heterozygosity of the wild-type *Apc* allele in *Apc*^{min/+} mice, causing nearly four times as many polyps [43]. Because *Blm* appears to repress cancer in multiple tissues, *Blm* mutants may be useful for somatic mutagenesis in many different cell types.

Rat mutagenesis

Without question, the mouse is and will continue to be an important animal model for performing functional genomics. However, there are circumstances in which mouse

models are limited. The great majority of common human diseases remain un-modeled in the mouse. Published knockouts exist for approximately 10% of mouse genes. Although efforts are now underway to create publicly available genome-wide collections of mouse knockouts, it will take many years to achieve this goal, and it seems unlikely that every gene in the mouse will be amenable to disruption [44,45]. Furthermore, there are many instances in which disrupting a gene in the mouse leads to no observable phenotype. Rat models are an alternative to mice that may enable the creation of new gene disruptions that are unavailable in the mouse, and that can complement existing transgenic mouse models. The evolutionary distance between rats and mice, some 12 to 24 million years [46,47], is about the same as that between humans and new world monkeys [48]. Comparing mouse and rat models with known human diseases can allow the distinction between rodent specific phenotypes and those that may be general to all mammals.

In many applications the rat is a better animal model for human disease. Although mice have been the animal model of choice for most geneticists, the rat has traditionally been favored by physiologists and pathologists. Their larger size make rats more conducive to study by instrumentation, and facilitates manipulations such as blood sampling, studying nerve conduction, or performing surgery. In many ways, rats are physiologically more similar to humans than are mice. For example, rats have a heart rate similar to that of humans, whereas mice have a heart rate nearly ten times as fast [28]. Rats have been used as important models for human cardiovascular disease, diabetes, arthritis, and many other autoimmune and behavioral disorders [28]. Rat models are superior to mouse models for testing the pharmacodynamics and toxicity of potential therapeutic compounds, partially because the number and type of many of their detoxifying enzymes are very similar to those in humans [49].

Most techniques for genetic manipulation, including random mutagenesis with a gene trap (both retrovirus-based and non-retrovirus-based), gene knockouts, gene knockins, and conditional mutations, depend upon embryonic stem (ES) cells [50]. However, for unknown technical reasons, rat ES cells cannot be isolated and used to create a viable organism [51]. Consequently, many genetic manipulation techniques widely used in the mouse have not been possible in the rat. There are currently only two technologies that can be used to produce rat models of human disease: cloning and chemical mutagenesis using ENU. Although cloning could be used to create rats with specific genetic modifications, by first creating mutations in mitotic cells and then using the mutated cells to clone a rat, this approach is extremely inefficient. The first published attempt at cloning a rat had a success rate of less than 1% [52]. Alternatively, ENU mutagenesis is a common random mutagenesis gene knock-

out strategy in the mouse that can also be used in the rat. However, only a very small number of the total mutations created by ENU mutagenesis have an observable phenotype [53], and mapping mutations responsible for interesting phenotypes is typically difficult and time consuming.

There is a need for new techniques that can rapidly create and map gene knockouts in rats for the creation of new models of human disease. Mutagenesis using transposable elements is an attractive option. Indeed, a recent report demonstrated the feasibility of using SB for mutagenesis in rats [54]. We recently showed the feasibility of using L1 for mutagenesis in the rat (Sprague-Dawley strain) and demonstrated a high level of somatic retrotransposition with occasional germline transmission of *de novo* insertions (Kano H, Ostertag EM, Kazazian HH Jr, unpublished data).

Factors affecting the frequency and tissue specificity of retrotransposition

When designing an L1 mutagenesis system, it is important to consider the factors that will determine the frequency and tissue specificity of retrotransposition. The following are important considerations.

Choice of L1 element

At present, the human genome contains 80 to 100 endogenous full-length active L1s. Although they have similar nucleic acid sequence, they are known to exhibit very different levels of activity. A recent survey of the retrotransposition capability of elements in the human genome demonstrated there are just a few elements with very high activity. These elements were called 'hot' L1s [55]. Another study found that more active elements tend to insert larger amounts of DNA during retrotransposition [56]. Therefore, hot L1 elements such as L1_{RP} and L1_{LR3} are a good choice for use in random mutagenesis because they are highly active and better equipped to deliver gene traps. The L1_{RP} element was used in the initial studies of mouse models of L1 retrotransposition [30,57] and in a transgenic mouse model that demonstrated somatic retrotransposition in neuronal precursor cells [40]. A more recent study that demonstrated L1 retrotransposition events at relatively high frequency and characterized more than 50 *de novo* L1 insertions used the L1_{LR3} element [13].

Most mouse L1 elements cloned to date have exhibited poor retrotransposition activity in a cultured cell assay when compared with the hot human elements [58,59]. However, the poor retrotransposition capability of mouse L1 elements in cultured cells may be due to poor translation [60]. To overcome this problem, Han and Boeke [31] designed a synthetic mouse L1 that codon optimized the open reading frames of an endogenous mouse L1 element without changing the amino acid sequence. The codon optimized L1 (*ORFeus*) was used to create transgenic mice, in which it

demonstrated high activity [12]. In this model, all transgene positive progeny demonstrated somatic insertions and some insertions were passed through the germline.

Choice of retrotransposition cassette

The L1 element may be tagged with a retrotransposition cassette to facilitate detection of *de novo* L1 insertions. Retrotransposition cassettes are disrupted by an intron sequence and are cloned into the 3' untranslated region of an L1 element. The intron is removed by splicing during a retrotransposition event, allowing the differentiation between a *de novo* L1 insertion and the L1 element(s) present in the transgene. The cassette can contain a gene that is disrupted by the intron and activated upon retrotransposition, thereby allowing positive or negative selection. For example, the EGFP cassette allows detection of cells that contain a retrotransposition event by fluorescence [30,42]. A negative selection strategy could rescue cells that would otherwise die by the expression of a rescue gene upon retrotransposition. There are also cassettes that contain no selectable marker. In these 'markerless' cassettes, retrotransposition can be detected by PCR detection of loss of the intron [13]. The main advantage of using a markerless cassette is that it facilitates detection of smaller insertions. The majority of L1 insertions are 5' truncated during the integration process. Therefore, a smaller cassette permits detection of more insertions. Recent L1 transgenic mouse studies demonstrated that more than 90% of *de novo* insertions were 5' truncated, and some of them were less than 500 nucleotides [12,13].

Gene trapping technology can be used to maximize the rate at which retrotransposition insertions interrupt coding exons. Only about 1% of the genome is composed of exons. Therefore, only one in every 100 random insertions is expected to disrupt the coding sequence of a gene. A gene trap that disrupts the normal splicing sequence of genes during transcription can be included in the L1 retrotransposon, such that retrotransposons that land within gene introns in the correct orientation will disrupt gene expression. Because approximately 30% of the mouse genome consists of exons and introns [61], and new insertions are likely to be in the correct orientation 50% of the time, this strategy should increase the incidence of gene disruptions resulting from retrotransposition events from 1% to approximately 15%. To increase this rate of disruption even further, we designed a unique bi-directional gene trap that disrupts gene expression when inserted into an intron in either orientation (and that can therefore be expected to increase the rate of gene disruptions to approximately 30% of all retrotransposition events) [13]. The gene trap that we chose utilizes the very strong splice acceptor from the human *BCL2* gene, which is able to splice into exons located more than 100 kilobases away without alternative splicing [62]. This particular gene trap demonstrated 100% efficiency (complete disruption of a gene with no alternative splicing)

when tested in a cultured cell system [63] and has successfully been used to disrupt genes in the mouse [64]. We have demonstrated an L1 element tagged with the bi-directional gene trap retrotransposition cassette can disrupt genes in cultured cells and have used the cassette in transgenic animals [13].

Choice of promoter

L1 elements contain an endogenous promoter [65], which is sufficient to direct expression of the L1 transgene during spermatogenesis and oogenesis in transgenic animals [30]. Transcripts produced by the endogenous promoter are integrated either in the germline or early during development, so the endogenous promoter can be used for germline mutagenesis. The endogenous promoter has also been used in transgenic mice that demonstrated retrotransposition in neuronal precursor cells [40]. Because L1 retrotransposition appears to be limited by the steady-state levels of the processed L1 mRNA [66], a reasonable method to increase retrotransposition frequency is by incorporating a strong heterologous promoter. We and others have demonstrated that a heterologous promoter can boost retrotransposition for both germline [30] and somatic mutagenesis [12,13].

A feasible somatic mutagenesis screen in mice would hinge on robust and reliable expression in the desired tissues. This can be achieved by removing the endogenous L1 promoter and adding a strong tissue specific promoter. Unless the promoter possesses a locus control region, expression of the tagged L1 transgene may be affected significantly by the genomic integration site. Ideally, L1 transgenes designed for mutagenesis should have a promoter/enhancer that is resistant to position effect silencing, such as the albumin, α -fetoprotein, elastase, or β -globin promoters [67-72]. Alternatively, L1 transgenes can be targeted to specific loci in mouse ES cells to recapitulate the tissue-specific expression of that gene entirely. Recombinase mediated cassette exchange is a useful method that is increasingly being used to target particular loci, such as the β -actin locus [73]. Another alternative would be the incorporation of insulators into the transgene, such as a chicken β -globin insulator [74-76].

Choice of genetic background

Mouse strain

From the large number of endogenous long terminal repeat (LTR) retrotransposons that have inserted in mice, such as intracisternal A particles (IAP) and early transposons (ETn), it is known that mouse strain can affect the activity of LTR retrotransposons [6,77]. Most IAP insertions have been identified in C3H mice. ETn elements tend to retrotranspose in A/J, SELH/Bc, and MRL/MpJ mice. It is unknown whether genetic background is also important for L1 retrotransposition, because the number of endogenous L1 insertions characterized in mice is much fewer than LTR retrotransposon insertions. Characterized L1 insertions include

the spastic mouse in the SPE strain [78,79], the Orleans reeler mouse in BALB/c [80], the black eyed white mouse in C3H [81], the Chediak-Higashi mouse in C3H (with radiation treatment) [82], the med mouse in PCT [83], and the retinal degeneration mouse (strain not specified) [84]. All the L1 transgenic mice created to date were created in hybrid strains, such as B6SJLF1 [12,13,30,57] and B6D2F1 [40].

Host factors

Little is known about the mechanisms by which the host genome suppresses L1 retrotransposition, but several have been reported as possibly involved. The L1 transgene on the background of a mouse with retrotransposon defense mechanisms knocked out or knocked down could potentially produce more insertions.

DNA methylation has been proposed to be a major defense mechanism against transposable elements, not only inhibiting transcription but also assembling sequences into the condensed state to prevent recombination [85]. Both human and mouse L1s contain CpG islands in the 5' untranslated region, which contains the L1 promoter activity [65,86]. It is believed that the CpG island of L1 is maintained in heavily methylated status most of the time. It is noteworthy that the human L1 promoter is heavily methylated even in the mouse genome, and the retrotransposition activity of human L1 transgene seems to be correlated with its methylation status (Kano H, Ostertag EM, Kazazian HH Jr, unpublished data). The L1 transgene flanked by an insulator sequence retains unmethylated CpGs in its 5' untranslated region and shows higher retrotransposition activity than that of methylated L1 transgene without the insulator sequence (Kano H, Ostertag EM, Kazazian HH Jr, unpublished data).

DNA methyltransferase 3L (encoded by *Dnmt3L*) is required for the *de novo* methylation of retrotransposons. *Dnmt3L* is expressed in testis, and *Dnmt3L* knockout in mice causes high levels of transcription of retrotransposons in spermatogonia and spermatocytes [87]. Male mice that are homozygous for the *Dnmt3L* knockout allele are unable to produce sperm. Therefore, mutagenesis in *Dnmt3L* deletion males would need to be performed by using conditional mutations of *Dnmt3L* or by using a retrotransposition cassette that expresses a copy of *Dnmt3L* upon retrotransposition, which might restore spermatogenesis only in those spermatogonia that contain a *de novo* insertion. Lymphoid-specific helicase (*Lsh*) is a member of the SNF2 family of chromatin remodeling proteins. Contrary to the retrotransposon reactivation in male germ cells of *Dnmt3L* knockout, *Lsh* knockout mice exhibit demethylation and transcriptional reactivation of repetitive elements in the female germline. *Lsh* is essential in epigenetic silencing of retrotransposons in the female meiosis [88,89]. *Lsh* deletion mice exhibit severe oocyte loss and lack of ovarian follicle formation, making mutagenesis on this background difficult.

In humans, the apolipoprotein B mRNA editing complex (APOBEC) family consists of *APOBEC1*, *APOBEC2*, and *APOBEC3A, B, C, D/E, F, G*, and *H*. Among them, *APOBEC3G* is the best characterized and is known to catalyze C to U deamination of the minus strand during reverse transcription. Thus, *APOBEC3G* produces inactivated copies of LTR retrotransposons, but not *L1*, by introducing mutations. *APOBEC3A, B, C*, and *F* have been reported to inhibit *L1* retrotransposition in the cultured cells [90-92]. However, the precise mechanism of *L1* suppression by these *APOBECs* is not well understood, because they do not increase the number of mutations in retrotransposed copies of *L1*. Mice and rats have the *APOBEC1* and *APOBEC2* genes and only a single *APOBEC3* gene. The mouse *APOBEC3* may not be involved in repression of retrotransposons because the *APOBEC3* knockout mouse unexpectedly demonstrated normal development, survival, and fertility. In addition, wild-type mice exhibit poor expression of *APOBEC3* in testis [93]. Therefore, it is unclear at this point whether the *APOBEC* genes affect *L1* retrotransposition *in vivo* or whether the *APOBEC* knockout mice could be used to boost retrotransposition from an *L1*-containing transgene.

Conclusion

Endogenous *L1* elements have caused both germline transmissible and somatic insertions in humans and mice. We and other groups have demonstrated that modified *L1* elements can be used to perform random mutagenesis in mice or rats. Depending on the promoter used to drive transcription, either germline or somatic mutagenesis is feasible. Given the urgent need for rat models of human diseases and the limited methods for performing mutagenesis in rats, *L1* based mutagenesis is an attractive option. When designing an *L1* transgene for mutagenesis, it is important to consider the activity of the retrotransposon, the type of retrotransposition cassette used to detect insertions, the strength and tissue specificity of the promoter used to drive transcription of the *L1*, and the genetic background of the animal model.

Competing interests

EMO has been awarded grants from the NIH for the commercial development of *L1*-based mutagenesis technology. HK and BBM declare that they have no competing interests.

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References

- Babushok DV, Ostertag EM, Kazazian HH Jr: **Current topics in genome evolution: molecular mechanisms of new gene formation.** *Cell Mol Life Sci* 2007, **64**:1-13.

- Chen JM, Stenson PD, Cooper DN, Ferec C: **A systematic analysis of LINE-1 endonuclease-dependent retrotranspositional events causing human genetic disease.** *Hum Genet* 2005, **117**: 411-427.
- Gilbert N, Doucet AJ, Bucheton A: **Genomic instability associated with human LINE-1 retrotransposition [in French].** *J Soc Biol* 2004, **198**:419-424.
- Han JS, Boeke JD: **LINE-1 retrotransposons: modulators of quantity and quality of mammalian gene expression?** *Bioessays* 2005, **27**:775-784.
- Kazazian HH Jr: **Mobile elements: drivers of genome evolution.** *Science* 2004, **303**:1626-1632.
- Ostertag EM, Kazazian HH Jr: **Biology of mammalian L1 retrotransposons.** *Annu Rev Genet* 2001, **35**:501-538.
- Craigie R: **Hotspots and warm spots: integration specificity of retroelements.** *Trends Genet* 1992, **8**:187-190.
- Cost GJ, Boeke JD: **Targeting of human retrotransposon integration is directed by the specificity of the L1 endonuclease for regions of unusual DNA structure.** *Biochemistry* 1998, **37**: 18081-18093.
- Feng Q, Moran JV, Kazazian HH Jr, Boeke JD: **Human L1 retrotransposon encodes a conserved endonuclease required for retrotransposition.** *Cell* 1996, **87**:905-916.
- Jurka J: **Sequence patterns indicate an enzymatic involvement in integration of mammalian retrotransposons.** *Proc Natl Acad Sci USA* 1997, **94**:1872-1877.
- Myers JS, Vincent BJ, Udall H, Watkins WS, Morrish TA, Kilroy GE, Swergold GD, Henke J, Henke L, Moran JV, et al.: **A comprehensive analysis of recently integrated human Ta L1 elements.** *Am J Hum Genet* 2002, **71**:312-326.
- An W, Han JS, Wheelan SJ, Davis ES, Coombes CE, Ye P, Triplett C, Boeke JD: **Active retrotransposition by a synthetic L1 element in mice.** *Proc Natl Acad Sci USA* 2006, **103**:18662-18667.
- Babushok DV, Ostertag EM, Courtney CE, Choi JM, Kazazian HH Jr: **L1 integration in a transgenic mouse model.** *Genome Res* 2006, **16**:240-250.
- Ivics Z, Hackett PB, Plasterk RH, Izsvak Z: **Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells.** *Cell* 1997, **91**:501-510.
- Ding S, Wu X, Li G, Han M, Zhuang Y, Xu T: **Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice.** *Cell* 2005, **122**:473-483.
- Carlson CM, Dupuy AJ, Fritz S, Roberg-Perez KJ, Fletcher CF, Largaespada DA: **Transposon mutagenesis of the mouse germline.** *Genetics* 2003, **165**:243-256.
- Dupuy AJ, Fritz S, Largaespada DA: **Transposition and gene disruption in the male germline of the mouse.** *Genesis* 2001, **30**: 82-88.
- Horie K, Yusa K, Yae K, Odajima J, Fischer SE, Keng VW, Hayakawa T, Mizuno S, Kondoh G, Ijiri T, et al.: **Characterization of Sleeping Beauty transposition and its application to genetic screening in mice.** *Mol Cell Biol* 2003, **23**:9189-9207.
- Keng VW, Yae K, Hayakawa T, Mizuno S, Uno Y, Yusa K, Kokubu C, Kinoshita T, Akagi K, Jenkins NA, et al.: **Region-specific saturation germline mutagenesis in mice using the Sleeping Beauty transposon system.** *Nat Methods* 2005, **2**:763-769.
- Bestor TH: **Transposons reanimated in mice.** *Cell* 2005, **122**: 322-325.
- Wei W, Gilbert N, Ooi SL, Lawler JF, Ostertag EM, Kazazian HH, Boeke JD, Moran JV: **Human L1 retrotransposition: cis preference versus trans complementation.** *Mol Cell Biol* 2001, **21**: 1429-1439.
- Collier LS, Carlson CM, Ravimohan S, Dupuy AJ, Largaespada DA: **Cancer gene discovery in solid tumours using transposon-based somatic mutagenesis in the mouse.** *Nature* 2005, **436**: 272-276.
- Dupuy AJ, Akagi K, Largaespada DA, Copeland NG, Jenkins NA: **Mammalian mutagenesis using a highly mobile somatic Sleeping Beauty transposon system.** *Nature* 2005, **436**:221-226.
- Izsvak Z, Stuwe EE, Fiedler D, Katzer A, Jeggo PA, Ivics Z: **Healing the wounds inflicted by sleeping beauty transposition by double-strand break repair in mammalian somatic cells.** *Mol Cell* 2004, **13**:279-290.
- Yant SR, Park J, Huang Y, Mikkelsen JG, Kay MA: **Mutational analysis of the N-terminal DNA-binding domain of sleeping beauty transposase: critical residues for DNA binding and hyperactivity in mammalian cells.** *Mol Cell Biol* 2004, **24**:9239-9247.

26. Gilbert N, Lutz-Prigge S, Moran JV: **Genomic deletions created upon LINE-I retrotransposition.** *Cell* 2002, **110**:315-325.
27. Symer DE, Connelly C, Szak ST, Caputo EM, Cost GJ, Parmigiani G, Boeke JD: **Human II retrotransposition is associated with genetic instability in vivo.** *Cell* 2002, **110**:327-338.
28. Abbott A: **Laboratory animals: the Renaissance rat.** *Nature* 2004, **428**:464-466.
29. Kazazian HH Jr, Wong C, Youssoufian H, Scott AF, Phillips DG, Antonarakis SE: **Haemophilia A resulting from de novo insertion of LI sequences represents a novel mechanism for mutation in man.** *Nature* 1988, **332**:164-166.
30. Ostertag EM, DeBerardinis RJ, Goodier JL, Zhang Y, Yang N, Gerton GL, Kazazian HH Jr: **A mouse model of human LI retrotransposition.** *Nat Genet* 2002, **32**:655-660.
31. Han JS, Boeke JD: **A highly active synthetic mammalian retrotransposon.** *Nature* 2004, **429**:314-318.
32. Miki Y, Nishisho I, Horii A, Miyoshi Y, Utsunomiya J, Kinzler KW, Vogelstein B, Nakamura Y: **Disruption of the APC gene by a retrotransposal insertion of LI sequence in a colon cancer.** *Cancer Res* 1992, **52**:643-645.
33. Nakamura Y, Nishisho I, Kinzler KW, Vogelstein B, Miyoshi Y, Miki Y, Ando H, Horii A, Nagase H: **Mutations of the adenomatous polyposis coli gene in familial polyposis coli patients and sporadic colorectal tumors.** *Princess Takamatsu Symp* 1991, **22**:285-292.
34. Morse B, Rotherg PG, South VJ, Spandorfer JM, Astrin SM: **Insertional mutagenesis of the myc locus by a LINE-I sequence in a human breast carcinoma.** *Nature* 1988, **333**:87-90.
35. Chalitchagorn K, Shuangshoti S, Hourpai N, Kongruttanachok N, Tangkijvanich P, Thong-ngam D, Voravud N, Sriuranpong V, Mutirangura A: **Distinctive pattern of LINE-I methylation level in normal tissues and the association with carcinogenesis.** *Oncogene* 2004, **23**:8841-8846.
36. Jurgens B, Schmitz-Drager BJ, Schulz WA: **Hypomethylation of LI LINE sequences prevailing in human urothelial carcinoma.** *Cancer Res* 1996, **56**:5698-5703.
37. Menendez L, Benigno BB, McDonald JF: **LI and HERV-W retrotransposons are hypomethylated in human ovarian carcinomas.** *Mol Cancer* 2004, **3**:12.
38. Roman-Gomez J, Jimenez-Velasco A, Agirre X, Cervantes F, Sanchez J, Garate L, Barrios M, Castillejo JA, Navarro G, Colomer D, et al.: **Promoter hypomethylation of the LINE-I retrotransposable elements activates sense/antisense transcription and marks the progression of chronic myeloid leukemia.** *Oncogene* 2005, **24**:7213-7223.
39. Suter CM, Martin DI, Ward RL: **Hypomethylation of LI retrotransposons in colorectal cancer and adjacent normal tissue.** *Int J Colorectal Dis* 2004, **19**:95-101.
40. Muotri AR, Chu VT, Marchetto MC, Deng W, Moran JV, Gage FH: **Somatic mosaicism in neuronal precursor cells mediated by LI retrotransposition.** *Nature* 2005, **435**:903-910.
41. Schwahn U, Lenzner S, Dong J, Feil S, Hinzmann B, van Duijnhoven G, Kirschner R, Hemberger M, Bergen AA, Rosenberg T, et al.: **Positional cloning of the gene for X-linked retinitis pigmentosa 2.** *Nat Genet* 1998, **19**:327-332.
42. Ostertag EM, Prak ET, DeBerardinis RJ, Moran JV, Kazazian HH Jr: **Determination of LI retrotransposition kinetics in cultured cells.** *Nucleic Acids Res* 2000, **28**:1418-1423.
43. Luo G, Santoro IM, McDaniel LD, Nishijima I, Mills M, Youssoufian H, Vogel H, Schultz RA, Bradley A: **Cancer predisposition caused by elevated mitotic recombination in Bloom mice.** *Nat Genet* 2000, **26**:424-429.
44. Consortium CKMP: **The Knockout Mouse Project.** *Nat Genet* 2004, **36**:921-924.
45. Consortium EMM: **The European dimension for the mouse mutagenesis program.** *Nat Genet* 2004, **36**:925-927.
46. Adkins RM, Gelke EL, Rowe D, Honeycutt RL: **Molecular phylogeny and divergence time estimates for major rodent groups: evidence from multiple genes.** *Mol Biol Evol* 2001, **18**:777-791.
47. Springer MS, Murphy WJ, Eizirik E, O'Brien SJ: **Placental mammal diversification and the Cretaceous-Tertiary boundary.** *Proc Natl Acad Sci USA* 2003, **100**:1056-1061.
48. Goodman M, Porter CA, Czelusniak J, Page SL, Schneider H, Shoshani J, Gunnell G, Groves CP: **Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence.** *Mol Phylogenet Evol* 1998, **9**:585-598.
49. Lindblad-Toh K: **Genome sequencing: three's company.** *Nature* 2004, **428**:475-476.
50. Cohen-Tannoudji M, Babinet C: **Beyond 'knock-out' mice: new perspectives for the programmed modification of the mammalian genome.** *Mol Hum Reprod* 1998, **4**:929-938.
51. Abbott A: **Laboratory animals: the Renaissance rat.** *Nature* 2004, **428**:464-466.
52. Zhou Q, Renard JP, Le Friec G, Brochard V, Beaujean N, Cherifi Y, Fraichard A, Cozzi J: **Generation of fertile cloned rats by regulating oocyte activation.** *Science* 2003, **302**:1179.
53. Justice MJ, Noveroske JK, Weber JS, Zheng B, Bradley A: **Mouse ENU mutagenesis.** *Hum Mol Genet* 1999, **8**:1955-1963.
54. Kitada K, Ishishita S, Tosaka K, Takahashi R, Ueda M, Keng VW, Horie K, Takeda J: **Transposon-tagged mutagenesis in the rat.** *Nat Methods* 2007, **4**:131-133.
55. Brouha B, Schustak J, Badge RM, Lutz-Prigge S, Farley AH, Moran JV, Kazazian HH Jr: **Hot LIs account for the bulk of retrotransposition in the human population.** *Proc Natl Acad Sci USA* 2003, **100**:5280-5285.
56. Farley AH, Luning Prak ET, Kazazian HH Jr: **More active human LI retrotransposons produce longer insertions.** *Nucleic Acids Res* 2004, **32**:502-510.
57. Prak ET, Dodson AW, Farkash EA, Kazazian HH Jr: **Tracking an embryonic LI retrotransposition event.** *Proc Natl Acad Sci USA* 2003, **100**:1832-1837.
58. DeBerardinis RJ, Goodier JL, Ostertag EM, Kazazian HH Jr: **Rapid amplification of a retrotransposon subfamily is evolving the mouse genome.** *Nat Genet* 1998, **20**:288-290.
59. Goodier JL, Ostertag EM, Du K, Kazazian HH Jr: **A novel active LI retrotransposon subfamily in the mouse.** *Genome Res* 2001, **11**:1677-1685.
60. Han JS, Szak ST, Boeke JD: **Transcriptional disruption by the LI retrotransposon and implications for mammalian transcripts.** *Nature* 2004, **429**:268-274.
61. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexander S, An P, et al.: **Initial sequencing and comparative analysis of the mouse genome.** *Nature* 2002, **420**:520-562.
62. Seto M, Jaeger U, Hockett RD, Graninger W, Bennett S, Goldman P, Korsmeyer SJ: **Alternative promoters and exons, somatic mutation and deregulation of the Bcl-2-Ig fusion gene in lymphoma.** *EMBO J* 1988, **7**:123-131.
63. Ishida Y, Leder P: **RET: a poly A-trap retrovirus vector for reversible disruption and expression monitoring of genes in living cells.** *Nucleic Acids Res* 1999, **27**:e35.
64. Xin HB, Deng KY, Shui B, Qu S, Sun Q, Lee J, Greene KS, Wilson J, Yu Y, Feldman M, et al.: **Gene trap and gene inversion methods for conditional gene inactivation in the mouse.** *Nucleic Acids Res* 2005, **33**:e14.
65. Swergold GD: **Identification, characterization, and cell specificity of a human LINE-I promoter.** *Mol Cell Biol* 1990, **10**:6718-6729.
66. Yang N, Zhang L, Zhang Y, Kazazian HH Jr: **An important role for RUNX3 in human LI transcription and retrotransposition.** *Nucleic Acids Res* 2003, **31**:4929-4940.
67. Hammer RE, Swift GH, Ornitz DM, Quaife CJ, Palmiter RD, Brinster RL, MacDonald RJ: **The rat elastase I regulatory element is an enhancer that directs correct cell specificity and developmental onset of expression in transgenic mice.** *Mol Cell Biol* 1987, **7**:2956-2967.
68. Kruse F, Rose SD, Swift GH, Hammer RE, MacDonald RJ: **Cooperation between elements of an organ-specific transcriptional enhancer in animals.** *Mol Cell Biol* 1995, **15**:4385-4394.
69. Li Q, Peterson KR, Fang X, Stamatoyannopoulos G: **Locus control regions.** *Blood* 2002, **100**:3077-3086.
70. Pinkert CA, Ornitz DM, Brinster RL, Palmiter RD: **An albumin enhancer located 10 kb upstream functions along with its promoter to direct efficient, liver-specific expression in transgenic mice.** *Genes Dev* 1987, **1**:268-276.
71. Hammer RE, Krumlauf R, Camper SA, Brinster RL, Tilghman SM: **Diversity of alpha-fetoprotein gene expression in mice is generated by a combination of separate enhancer elements.** *Science* 1987, **235**:53-58.
72. Swift GH, Hammer RE, MacDonald RJ, Brinster RL: **Tissue-specific expression of the rat pancreatic elastase I gene in transgenic mice.** *Cell* 1984, **38**:639-646.

73. Shmerling D, Danzer CP, Mao X, Boisclair J, Haffner M, Lemaistre M, Schuler V, Kaeslin E, Korn R, Burki K, et al.: **Strong and ubiquitous expression of transgenes targeted into the beta-actin locus by Cre/lox cassette replacement.** *Genesis* 2005, **42**:229-235.
74. Burgess-Beusse B, Farrell C, Gaszner M, Litt M, Mutskov V, Recillas-Targa F, Simpson M, West A, Felsenfeld G: **The insulation of genes from external enhancers and silencing chromatin.** *Proc Natl Acad Sci USA* 2002, **99**(suppl 4):16433-16437.
75. Chung JH, Bell AC, Felsenfeld G: **Characterization of the chicken beta-globin insulator.** *Proc Natl Acad Sci USA* 1997, **94**:575-580.
76. Pikaart MJ, Recillas-Targa F, Felsenfeld G: **Loss of transcriptional activity of a transgene is accompanied by DNA methylation and histone deacetylation and is prevented by insulators.** *Genes Dev* 1998, **12**:2852-2862.
77. Maksakova IA, Romanish MT, Gagnier L, Dunn CA, van de Lagemaat LN, Mager DL: **Retroviral elements and their hosts: insertional mutagenesis in the mouse germ line.** *PLoS Genet* 2006, **2**:e2.
78. Kingsmore SF, Giros B, Suh D, Bieniarz M, Caron MG, Seldin MF: **Glycine receptor beta-subunit gene mutation in spastic mouse associated with LINE-1 element insertion.** *Nat Genet* 1994, **7**:136-141.
79. Mulhardt C, Fischer M, Gass P, Simon-Chazottes D, Guenet JL, Kuhse J, Betz H, Becker CM: **The spastic mouse: aberrant splicing of glycine receptor beta subunit mRNA caused by intronic insertion of LI element.** *Neuron* 1994, **13**:1003-1015.
80. Takahara T, Ohsumi T, Kuromitsu J, Shibata K, Sasaki N, Okazaki Y, Shibata H, Sato S, Yoshiki A, Kusakabe M, et al.: **Dysfunction of the Orleans reeler gene arising from exon skipping due to transposition of a full-length copy of an active LI sequence into the skipped exon.** *Hum Mol Genet* 1996, **5**:989-993.
81. Yajima I, Sato S, Kimura T, Yasumoto K, Shibahara S, Goding CR, Yamamoto H: **An LI element intronic insertion in the black-eyed white (Mitf^[mi-bw]) gene: the loss of a single Mitf isoform responsible for the pigmentary defect and inner ear deafness.** *Hum Mol Genet* 1999, **8**:1431-1441.
82. Perou CM, Pryor RJ, Naas TP, Kaplan J: **The bg allele mutation is due to a LINE1 element retrotransposition.** *Genomics* 1997, **42**:366-368.
83. Kohrman DC, Harris JB, Meisler MH: **Mutation detection in the med and medJ alleles of the sodium channel Scn8a. Unusual splicing due to a minor class AT-AC intron.** *J Biol Chem* 1996, **271**:17576-17581.
84. Chen J, Rattner A, Nathans J: **Effects of LI retrotransposon insertion on transcript processing, localization and accumulation: lessons from the retinal degeneration 7 mouse and implications for the genomic ecology of LI elements.** *Hum Mol Genet* 2006, **15**:2146-2156.
85. Yoder JA, Walsh CP, Bestor TH: **Cytosine methylation and the ecology of intragenomic parasites.** *Trends Genet* 1997, **13**:335-340.
86. Naas TP, DeBerardinis RJ, Moran JV, Ostertag EM, Kingsmore SF, Seldin MF, Hayashizaki Y, Martin SL, Kazazian HH: **An actively retrotransposing, novel subfamily of mouse LI elements.** *EMBO J* 1998, **17**:590-597.
87. Bourc'his D, Bestor TH: **Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L.** *Nature* 2004, **431**:96-99.
88. De La Fuente R, Baumann C, Fan T, Schmidtman A, Dobrinski I, Muegge K: **Lsh is required for meiotic chromosome synapsis and retrotransposon silencing in female germ cells.** *Nat Cell Biol* 2006, **8**:1448-1454.
89. Huang J, Fan T, Yan Q, Zhu H, Fox S, Issaq HJ, Best L, Gangi L, Munroe D, Muegge K: **Lsh, an epigenetic guardian of repetitive elements.** *Nucleic Acids Res* 2004, **32**:5019-5028.
90. Chen H, Lilley CE, Yu Q, Lee DV, Chou J, Narvaiza I, Landau NR, Weitzman MD: **APOBEC3A is a potent inhibitor of adeno-associated virus and retrotransposons.** *Curr Biol* 2006, **16**:480-485.
91. Muckenfuss H, Hamdorf M, Held U, Perkovic M, Lower J, Cichutek K, Flory E, Schumann GG, Munk C: **APOBEC3 proteins inhibit human LINE-1 retrotransposition.** *J Biol Chem* 2006, **281**:22161-22172.
92. Stenglein MD, Harris RS: **APOBEC3B and APOBEC3F inhibit LI retrotransposition by a DNA deamination-independent mechanism.** *J Biol Chem* 2006, **281**:16837-16841.
93. Mikl MC, Watt IN, Lu M, Reik W, Davies SL, Neuberger MS, Rada C: **Mice deficient in APOBEC2 and APOBEC3.** *Mol Cell Biol* 2005, **25**:7270-7277.