Two ways to trap a gene in mice

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The sequencing of the human and mouse genomes has been heralded as one of the most important recent achievements in biological science. Attention has now turned to the functional annotation of the 25,000 or so genes encoded by the mammalian genome. Although there are many experimental approaches that address gene function, the most relevant approach for extrapolation to human development, physiology, and disease is to analyze the phenotype of mutations for each gene in a whole mammalian model, the mouse. Many phenotypes relevant to congenital traits and abnormal pathologies in humans have emerged from gene knockout studies in the mouse, providing a strong justification for expanding the collection of mouse mutants to include all genes. Recent discussions between funding agencies and the mouse genetics community (1, 2) have garnered support for an international, concerted effort to generate a resource of mutations in every gene in the mouse genome. This effort will use a combination of gene targeting and gene trapping in ES cells, two well-established technologies that were developed in parallel in the late 1980s and gradually perfected over the years (3, 4).

In this issue of PNAS, Friedel et al. (5) ingeniously combine gene targeting and gene trapping to mutate genes expressed in ES cells at a high efficiency. It may surprise many to learn that this hybrid method of “targeted trapping” is applicable to a majority of genes in the mouse.

Gene targeting has been widely used over the past 15 years to engineer precise modifications in the mouse genome. The collective efforts of many laboratories have thus far produced targeted mutations in ~3,600 genes, or just <15% of the genome (a list of targeted gene mutations is maintained by The Jackson Laboratory, www.informatics.jax.org). Gene targeting relies on rare homologous recombination events between an exogenous DNA construct introduced into cells and its cognate genomic locus, to engineer precise modifications in the genome. Typically, targeting constructs contain several kilobases of genomic DNA flanking a drug selection marker driven by a heterologous promoter. Improvements in molecular cloning methods, specifically recombinase-mediated bacterial artificial chromosomes (6–8), have reduced the effort and increased the precision for building targeting vectors. Despite these advances, gene targeting remains a labor-intensive undertaking that is not easily scalable because of the effort required to screen ES cell colonies for a small fraction of correctly targeted events (9).

Gene trapping, by contrast, relies on random integration of a promoterless reporter gene, such as βgeo, equipped with splice acceptor (SA) and polyadenylation (pA) signals. The reporter is activated after insertions into introns of expressed genes to generate a fusion mRNA that can be characterized by 5′ RACE. (B) Targeted trapping relies on homologous recombination to introduce a promoterless gene-trap cassette into predefined loci. The trapping cassette is flanked by genomic sequences of the target locus that do not contain the promoter of the target gene.

Gene trapping versus targeted trapping. (A) Gene trapping depends on random insertions of a promoterless reporter gene, such as βgeo, equipped with splice acceptor (SA) and polyadenylation (pA) signals. The reporter is activated after insertions into introns of expressed genes to generate a fusion mRNA that can be characterized by 5′ RACE. (B) Targeted trapping relies on homologous recombination to introduce a promoterless gene-trap cassette into predefined loci. The trapping cassette is flanked by genomic sequences of the target locus that do not contain the promoter of the target gene.

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cell surface proteins that may play a role in axon guidance. For this purpose, an astonishingly high frequency of correctly targeted events was observed for 16 of the 24 loci, averaging greater than 50%. Importantly, Friedel et al. establish the threshold of expression above which targeted trapping is effective. Semiquantitative RT-PCR was used to measure steady-state mRNA levels of selected target genes relative to transferrin receptor, a gene expressed at low levels in ES cells. Efficient targeting was observed for most genes expressed above 1% the level of transferrin receptor. This result agrees well with the fraction of genes that are thought to be accessible to random trapping, estimated to be approximately 60% of all mouse genes (14). Therefore, most of the genes accessible to targeted trapping are already represented in the existing gene-trap libraries, and a list of trapped genes in public and private resources (available from the International Gene Trap Consortium, www.genetrap.org) serves as a useful guide for identifying genes for targeted trapping. The work of Friedel et al. (5) comes at a propitious time and has important strategic implications for international efforts aimed at generating a complete collection of reporter-tagged null mutations in mice. The public gene-trap resources contain mutations in 40% of genes; however, the efficiency of trapping new genes has dropped to 10% (one new gene is trapped for every 10 colonies isolated) and will continue to diminish well before saturation is achieved. The efficiency of targeted trapping exceeds the current efficiency of random gene trapping; therefore, targeted trapping could be put to good use to systematically target the remaining 20% of trappable genes that are not currently represented in the public resource. Furthermore, a significant fraction of targeted genes are represented by single events and thus the generation of additional mutant cell lines for these loci will be beneficial. Although most gene-trap mutations studied in mice effectively mutate the endogenous gene at the site of insertion, the generation of null alleles is not always guaranteed. Hypomorphic mutations (16) or insertions that disrupt only a subset of mRNA isoforms (22) are possible. The rapid generation of additional null alleles by targeted trapping will be valuable in these cases. Finally, the mouse genetics community has expressed a strong desire for reporter-tagged, conditional null alleles to enable temporal or tissue-specific ablation of gene function (1, 2). Targeted trapping can be readily adapted for conditional mutagenesis by, for example, incorporating conditional gene-trap cassettes (23).

Gene targeting in ES cells will continue to be the workhorse for the functional analysis of genes in mice for many years to come. The detailed analysis of genes will require all manner of alleles beyond the generation of null mutations. Targeted trapping is a welcome addition to the arsenal of molecular tools with which to address gene function in the mouse.

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