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New genetic resources for mammalian developmental biologists

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Abstract

The utilization of homologous recombination in embryonic stem cells as a means to generate mice carrying pre-determined modifications of genomic sequences has revolutionized the study of developmental biology. Recognizing the potential efficiencies that can be obtained by high-throughput production at centralized technology centers, a number of large-scale efforts for generating mice with targeted mutations have been funded. These programs are reaching fruition, and a variety of libraries of embryonic stem cells with defined mutations are now available.

Introduction and context

There can be little doubt that the ability to use homologous recombination in embryonic stem (ES) cells as a method to generate mice with defined genomic modifications has made them the premier model system for the study of mammalian development and organogenesis. This has been further enhanced by recombinase-based methods that allow mutations to be induced in a tissue- or temporal-specific fashion. However, the task of generating mice with genome modifications remains time- and resource-intensive, and is particularly daunting for investigators without experience in this domain. This problem has been recently addressed by the initiation of large-scale targeted and gene-trap mutagenesis efforts, many of which have progressed to maturity, providing a vast resource for investigators wishing to use the mouse as a model system. The following summarizes the present status of several of these programs. For additional information, a thorough review of the history and methodologies of these and other programs has recently been published [1].

Major recent advances

In 2004, a proposal was made to pursue a large-scale project to generate a library of ES cells carrying mutations of all known or presumptive genes [2]. This effort has made remarkable progress, and very large numbers of targeted ES cells are now available to investigators at modest cost. The project is being undertaken by a consortium whose diverse responsibilities include the generation of targeting vectors, the selection and characterization of mutant ES cells, the distribution of reagents, and the presentation of data.

The largest components of this effort are represented in the KOMP (Knock-Out Mouse Project) and EUCOMM (European Conditional Mouse Mutagenesis) programs. KOMP was initiated in 2006 and is itself represented in two complementary efforts [3]. CSD is a collaborative team from the Children's Hospital Oakland Research Institute (CHORI), the Wellcome Trust Sanger Institute, and the University of California at Davis School of Veterinary Medicine that has a goal of generating 5000 targeted ES cells. The CSD strategy uses a remarkable high-throughput method of recombineering developed at the Sanger Institute to generate targeting vectors that will generate conditional mutant alleles. This is paired with an automated ES cell colony-picking technology and allele characterization by long-range polymerase chain reaction (PCR). The other major component of KOMP is an effort by investigators at Regeneron to generate 3500 deletion alleles. This employs a method of bacterial artificial chromosome (BAC)-mediated homologous recombination and replacement that they have

developed and adapted for high throughput [4]. Successful targeting is assayed by using quantitative PCR to discriminate between wild-type and hemizygous cell lines for each mutant allele. The KOMP has been highly productive; as of May 2010 they have generated a total of 8092 targeting vectors, mutated ES cell lines for 5643 loci, and generated over 300 mutant mouse lines.

EUCOMM is a parallel effort with a goal of generating 8000 targeted conditional mutant alleles [5]. This program employs the same gene targeting strategy. The project was developed at the Wellcome Trust Sanger Institute, the Helmholtz Zentrum München German Research Center, the Medical Research Council (MRC) Mammalian Genetics Unit, Harwell, UK, the Institut Clinique de la Souris, Strasbourg, France, the Consiglio Nazionale delle Ricerche, Monterotondo, Italy, the University of Frankfurt, Germany, the Center for Cardiovascular Research at the Charité, Berlin, Germany, the University of Technology, Dresden, Germany, and the European Molecular Biology Laboratory (EMBL), Monterotondo, Italy. EUCOMM has produced 5980 vectors, 3473 mutated ES cells, and over 400 mutant mouse lines.

A third major project involving gene targeting by homologous recombination is being pursued as part of the NorCOMM (North American Conditional Mouse Mutagenesis) project [6]. The goal in this effort is up to 500 targeted loci, and the targeting protocol maximizes the utility of the modified locus for further manipulation (e.g., replacement with specific mutation ['knock-in'], different reporters, recombinases, and so on).

These three projects (and the Texas A&M Institute for Genomic Medicine, see below) are associated in the International Knockout Mouse Consortium (IKMC). The IKMC data coordination center provides unified access to information about vector design and status of ES cell line and mutant mouse generation [7]. A genome-view perspective of alleles that have targeted mutations can be obtained using the 'IKMC genes' ribbon on the University of California Santa Cruz Genome Browser [8] or the 'KO alleles' DAS (distributed annotation system) track on the ENSEMBL Genome Browser. A crucial aspect of any large-scale biological resource is facilitating distribution; this is done by the KOMP Repository at the University of California at Davis [9] and by the European Mouse Mutant Cell Repository (EuMMCR) for targeted ES cells or from the European Mutant Mouse Archive (EMMA) for mutant mouse strains. The KOMP repository also provides blastocyst injection under a fee-for-service proviso. Note that all vectors can be obtained for investigators still inclined to 'do-it-yourself' gene-targeting.

A unifying aspect of these projects is the use of C57BL/6-derived ES cells for targeting. This has been an area of ongoing discussion, in which the efficiency of germ-line passage obtained using 129-derived lines was balanced against the desirability of having targeted mutations isogenic on a C57BL/6 background. The latter was chosen as a goal for both KOMP and EUCOMM. Interestingly, this requirement did not specify a sub-strain, and it has turned out that C57BL/6N has been more robust for ES cell line development than C57BL/6J. The feeder-independent JM8 cell line, which is the primary line used for targeting by the EUCOMM and KOMP projects, is C57BL/6N-derived [10].

These targeted libraries complement several large gene-trap resources, such as that generated as part of the BayGenomics project, the German Gene Trap Consortium, the Toronto Centre for Modelling Human Disease, and other efforts. Data regarding the insertion sites, targeting vectors, and transcript characterization for all publicly available gene-trap cell lines are now curated and accessible via a central database maintained by the International Gene-Trap Consortium [11]. A recent addition is a library originally generated at Lexicon Genetics and now distributed by the Texas A&M Institute for Genomic Medicine. This library contains over 350,000 cell lines representing more than 10,000 unique genes, and is notable for being in C57BL/6N-derived ES cells [12]. However, the cost of mice generated from this resource is substantially more than that for mice obtained from most public-domain programs.

Future directions

While we are potentially on the cusp of a new era in the utilization of genetically modified mice for developmental analysis, a number of unknowns exist. First and foremost is the robustness of the newly developed resource with respect to the generation of germline-competent mutant mice. Many variables affect this, including genetic background, genomic stability, culture conditions, and technical expertise. Both the Sanger Institute and Regeneron have excellent success in generating germline-competent mice in their own facilities. For example, targeted JM8 clones successfully colonized the germline in over 65% of experiments [10]. As multiple cell lines have been derived for each targeted locus, the likelihood of obtaining a germline-competent mouse for any specific gene of interest is high. This assumes such success can be obtained elsewhere.

Another issue is the fact that the resource has been created in a C57BL/6N-derived cell line. Although the J and N substrains are very closely related (only 102 of 139,561 genotyped single-nucleotide polymorphisms are discordant [10]), several phenotypic differences have been noted [13,14] and molecular differences, such as a deletion of the *Nnt* gene in C57BL/6J, have been found [15,16].

Finally, there is a spectrum of opinions regarding how such resources can best be utilized. The European Community and the Wellcome Trust Sanger Institute have developed a vigorous effort to support systematized and centralized phenotyping in a large-scale fashion [17]. This model has been less consistently embraced by the research community in the USA. While there are clear efficiencies and opportunities for discovery inherent by phenotypic screening in an unbiased fashion, there is also a case to be made for facilitating analysis by individual investigators who are invested in specific gene families, pathways, or phenotypes. As an example, relatively modest but targeted ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis programs have been arguably as productive, with respect to mutation discovery and functional characterization, as much larger, broadly focused efforts [18-21].

In summary, an extraordinary resource of gene deletions, disruptions, and conditional mutant alleles is now available at relatively low cost to investigators worldwide. The substantial investment required to create these libraries is likely to yield substantial insight into the role of specific genes in mammalian biology.

Abbreviations

CHORI, Children's Hospital Oakland Research Institute; CSD, CHORI/Sanger/UC Davis; ES, embryonic stem; EUCOMM, European Conditional Mouse Mutagenesis; IKMC, International Knockout Mouse Consortium; KOMP, Knock-Out Mouse Project; PCR, polymerase chain reaction.

Competing interests

DRB is a member of the scientific advisory boards for the KOMP and EUCOMM programs.

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