Meeting report **Mouse genomics gets the royal treatment** F William Buaas

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A report on the Genetics Society autumn meeting 'The mouse: genetics and genome', The Royal Society, London, UK, 14 November 2003.

Functional annotation of the mouse genome was the theme of the meeting and this is clearly a lofty goal. The mouse is a unique model organism for understanding mammalian biology owing to the availability of classical genetic approaches as well as sequence-based manipulation methods. The impressive group of mouse experimentalists gathered for the day exemplified many of the clever tactics being used in the mouse for functional dissection of a variety of biological systems. Highlights are covered here.

Tagging and tinkering with the mouse genome

Allan Bradley (The Wellcome Trust Sanger Institute, Hinxton, UK) estimated that only 15% of the mouse genome has been functionally assessed via gene-targeting strategies, and he presented approaches to increase that percentage using sequence-driven methods. He highlighted the usefulness of embryonic stem (ES) cell libraries generated by genome-wide gene-trapping strategies. The availability of the mouse genome sequence means that determining the exact genomic position of the insertions in these libraries is extremely rapid. The average distance between insertions is 25 kilobases, and 13,000 of the insertion lines are predicted to be null mutations. Notably, the targeting vector used to make the insertions includes a site-specific recombination site, LoxP, which facilitates systematic chromosome-engineering experiments. The dizzying collection of engineered chromosome segments generated by Bradley includes balancers, duplications and deletions, suggesting that it will soon be as easy to perform mutant phenotype screens in mice as it is in flies.

Another exciting reagent in Bradley's quiver is the Bloom syndrome mutant cell line (*Blm*^{-/-}): a *Blm*^{-/-} ES cell line has a

high level of mitotic recombination, which results in a 20-fold increase in the rate of loss of heterozygosity (LOH). Unlike other cell lines with increased rates of LOH, such as p53 mutant cells, the $Blm^{-/-}$ strain is genetically stable (except for the aforementioned phenotype). By introducing gene-trapping vectors into the $Blm^{-/-}$ cell line, Bradley's group has been able to identify recessive mutations in DNA mismatch repair genes: the gene-trap event induces a mutation, and the high rate of LOH generates the 'second hit' required to create homozygous mutant cells. This approach could be applied to a variety of cell-based phenotypes, and the gene-trap ES cell lines can be used for the production of mutant mice for whole-animal studies.

Neal Copeland (National Cancer Institute, Maryland, USA) addressed the ambitious task of generating and navigating the mouse cancer genome map. He articulated some of the difficulties encountered in this endeavor: the sheer number of loci of both major and minor effects, the difficulty of recognizing a cancer gene from its sequence, and the potential for cooperative mutations in multiple genes. The predominant strategy for identifying cancer genes that is being used in his and Nancy Jenkins' laboratory (at the National Cancer Institute, Maryland, USA) is to overexpress or inactivate genes using retroviral insertion vectors. If a cancer gene is mutated, a clonally derived tumor will be propagated. The mouse genome sequence facilitates the rapid genomic positioning of the insertion sites and provides a preliminary cancer genome map which, when combined with other groups' data, contains more than 235 candidate cancer genes. Copeland then reviewed a recent gene-therapy trial in France that used an IL2RG-containing retrovirus and succeeded in treating patients with X-linked severe combined immunodeficiency (SCID), but two of the ten individuals treated developed acute T-cell leukemia. Both tumors contained retrovirus insertions in the 5' end of the LMO2 gene, a recognized human T-cell proto-oncogene. Both the IL2RG and LMO2 loci were hit in Copeland and Jenkins' studies, leading them to suggest that *IL2RG* is a proto-oncogene and

the prevalence of leukemia in this particular SCID trial is due to the synergy between *IL2RG* and *LMO2*: the overexpression of *IL2RG* from the retrovirus provides the first hit and the second hit is the insertion of the retrovirus in a cooperating gene such as *LMO2*. The cancer map generated from their mouse experiments above will help predict such interactions in the future, thereby reducing the risks associated with gene-therapy trials.

The forward progress of forward genetics

The power and feasibility of phenotype-driven screens using the 'supermutagen' N-ethyl-N-nitrosourea (ENU) were showcased by Steve Brown (MRC Mammalian Genetics Unit and UK Mouse Genome Centre, Harwell, UK) and David Beier (Brigham and Women's Hospital, Harvard Medical School, Boston, USA). Brown outlined the ongoing, substantial mutagenesis program at Harwell, part of which has been published. He and his colleagues have screened 35,000 mice for dominant mutations, with an emphasis on sensory and neurological defects, and his review of their current figures is quite encouraging. Of the 1,500 abnormal phenotypes identified by the screen, 376 mutants were subjected to inheritance testing, 85 have been genetically mapped and 20 loci have been cloned. Brown concluded that at least half of the mutants display novel phenotypes and thus provide novel functional information. Furthermore, some mutants have assigned new phenotypes to previously mutated genes, emphasizing the unexpected information that can be obtained by examining multiple alleles. Many mutants identified by Brown's group affect auditory and vestibular functions, and hold promise as models for human deafness disorders. In particular, he detailed two mutants, Jeff and Junbo, which have middle ear defects and model the human inflammatory disease otitis media with effusion. The cloning of Jeff revealed a gene with no recognizable sequence motifs, which is expressed in the middle ear epithelium. In contrast, the Junbo mutation is a missense allele of Evi1, a gene that had previously been annotated as a proto-oncogene and shown by gene targeting to be required for embryonic development.

A more humble, but no less productive, ENU mutagenesis screen was described by Beier. Previously, his lab undertook a three-generation recessive screen to identify organogenesis mutants. The breeding scheme and phenotyping strategy for this screen facilitated the simultaneous production, identification and mapping of mutants with robust phenotypes, which were the subject of his talk. A broad spectrum of phenotypes was uncovered and their primary characterization suggests they represent several new mouse models for human congenital diseases. One mutant strain with cleftpalate defects contains a mutation in *Prdm16*, a zinc-fingerencoding gene related to *Evii* but with no previous functional data attributed to it. Another mutant, *little lung*, exhibits phenotypes similar to a congenital diaphragmatic

defect in humans; the molecular lesion is a novel splicingdefect allele of the Fog2 gene. Previous studies of Fog2 in the mouse revealed an essential role for this gene during heart development, which precluded the study of late developmental stages. The analysis of this hypomorphic Fog2allele resulted in a similar take-home message to the *Junbo* story - that such phenotype-driven forward genetic screens can reveal phenotypes and novel alleles that complement sequence-based approaches.

Building big pictures from small effects

The subject of complex quantitative traits was broached by Jonathan Flint (Wellcome Trust Centre for Human Genetics, Oxford, UK). His talk reminded us that most complex traits are affected by many 'loci of small effects' (each accounting for 5% or less of the phenotype). Flint pointed to the utility of the mouse for identifying chromosomal regions containing loci of small effects, but noted that gene identification has so far been successful only for loci of large effects. The critical reagent in his studies is the outbred HS mouse stock, which was founded from eight different inbred mouse strains, and has a high density of linkage disequilibrium and thus a variety of haplotypes. Flint and co-workers used this strain in earlier work to detect and finely map several behavioral traits that model anxiety. Even though the genomic region defined for one of their anxiety loci was 'small' by positional cloning standards, the number of potential causative polymorphisms numbered in the thousands. Flint discussed a comparative method involving the HS parental strains which excluded a large fraction of the putative candidates. Calculations of the statistical power of these methods concluded his talk and indicated that 2,000 HS mice would be sufficient to detect all quantitative trait loci (QTLs) that have a minimum of 5% phenotypic effect. The group's current goal is to phenotype and genotype 1,000 HS mice by the end of this year.

Joe Nadeau (Case Western Reserve University, Cleveland, USA) also takes advantage of natural variation in mouse strains, but his goal is to integrate complex systems with the study of health and disease. Nadeau's earlier work compared two inbred strains, A/J and C57BL/6J (B6), across a variety of physiological parameters with an emphasis on cardiovascular properties. Even though both strains have a comparable blood-flow output, there were numerous cardiovascular phenotypes that differed between the two and must therefore have different genetic causes. With the help of AXB and BXA recombinant inbred strains, which are derived from A/J and B6 strains, his group was able to separate many cardiovascular properties genetically, to define relations between the cardiovascular traits computationally, and to model a functional network. This network was used to account for the phenotypic changes in mice exhibiting pathological cardiovascular phenotypes due to single gene mutations. Nadeau then turned to studies focused on folate-homocysteine metabolism, noting that folic acid supplementation during pregnancy reduces the incidence of neural tube, craniofacial and cardiovascular defects. The A/J and B6 inbred strains were fed a folate-deficient diet followed by a replenishment phase. A temporal analysis of folate and homocysteine levels together with gene-expression profiling revealed that the two inbred strains respond quite differently to this type of environmental stress: unlike A/J mice, B6 inbred mice fail to reestablish their original folate and homocysteine levels, and their gene-expression status. Nadeau suggested that, because of its environmental history, the B6 strain might now be much more susceptible to a future disruption in the folate-homocysteine metabolic pathway. The small, yet significant, genetic variants in these two 'wild-type' strains are assuredly similar to the gene-environment phenomena characteristic of complex human diseases and should, therefore, provide useful further insights.

Traditional techniques

What about the traditional method of studying a few mutants at a time and focusing on them? Such approaches are still vital and thriving, as demonstrated by two speakers at the meeting. Rudi Balling (German Research Center for Biotechnology, Braunschweig, Germany) broadened the discussion to include the interaction between the genomes of the host and its pathogens, and touched on some points validating the mouse for the study of infectious diseases. His group is subjecting a small collection of the immunological mutants identified in the large German ENU mutagenesis program to a battery of secondary screens for susceptibility to different bacterial infections. Robin Lovell-Badge (National Institute for Medical Research, London, UK) compared and contrasted embryonic and adult stem cell populations and the unique environments they encounter. His group has been studying a handful of developmentally regulated Sox genes (Sox1, Sox2 and Sox3), which encode HMGbox transcription factors. Previous analysis of the Sox2-targeted mutant defined a function for the gene in the stem-cell populations of the pre-implantation embryo. He presented a variety of data using neurospheres - an in vitro model system for studying neural stem cell development and cell transplantations, which together with previous data indicate that Sox2 functions in both embryonic and adult neural stem cells. The Sox2 gene, therefore, provides a useful foothold for studying neural stem cell biology. Lovell-Badge also emphasized that a stem cell's 'history' influences its fate and potential more than its environment does.

Although most speakers emphasized methods of the postgenomic era, the meeting included a delightful talk from Mary Lyon who described some personal reminiscences with respect to her more than 50 years in the mouse genetics community. Lyon is probably most recognized for her X-chromosome inactivation hypothesis, and at the conclusion of her talk she was awarded the Mendel Medal for her major contributions to the field of mouse genetics. While we are now far removed from the tedious days of Lyon's early years when mapping was done with linkage testing stocks, it was apparent from this meeting that much of the rich history of mouse genetic resources, and enthusiasm continues in this post-genomic age of mouse genetics.