Meeting report

A cornucopia of delights for the mouse fancier
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A report on the Mouse Molecular Genetics meeting, Hinxton, UK, 5-9 September 2007.

This annual gathering of mouse enthusiasts covers many aspects of mouse molecular genetics, epigenetics, development and disease, and also provides a forum for showcasing cutting-edge technologies and resources. This year's meeting was held at a new European location, the Wellcome Trust campus in Hinxton, outside Cambridge.

A night at the movies
The first night of the meeting was movie night. Microscopy is a mainstay of biology and it is becoming increasingly clear that *in vivo* imaging will be essential for revealing the dynamics underlying gene regulation, lineage specification and embryonic morphogenesis. This point was beautifully highlighted in presentations from Sigolene Meilhac (Institut Pasteur, Paris, France) and Berenika Plusa (Manchester University, Manchester, UK), who tackled the problem of lineage specification (epiblast versus primitive endoderm) within the inner cell mass (ICM) of the blastocyst stage embryo. By imaging a nuclear-localized human histone H2B-green fluorescent protein (GFP) fusion protein in developing embryos, Meilhac visualized the dynamics of all cells within the ICM. Using the same fluorescent fusion protein, Plusa specifically followed the primitive endoderm cells as they become specified. The take-home message from these talks was that live imaging at cellular resolution will have a lot to reveal when applied beyond the analysis of preimplantation embryos to more complex postimplantation or adult situations.

Imaging and neurobiology
Taking imaging into a more complex anatomical setting, Anamaria Sudarov (Sloan-Kettering Institute, New York, USA)

discussed her work on the morphogenesis of the cerebellum, an initially smooth-surfaced structure that becomes foliated during late embryogenesis and the first two weeks after birth. Her dynamic analysis of the development and organization of cerebellar cytoarchitecture in the mouse revealed key events underlying initiation of the infolding of the surface to form fissures. She compared the cell behaviors observed in emerging fissures in wild-type embryos with those in *Engrailed2* mutants, which exhibit a specific foliation defect, and found that this well characterized defect is a result of aberrant cell behavior at the base of fissures, thus supporting a central role for these basal cells in cerebellar fissure formation.

Pushing the envelope of technology, Liqun Luo (Stanford University, Stanford, USA) discussed recent improvements in the combination of genetics and imaging, including the use of the new generation of red fluorescent protein (RFP) variant reporters, and novel applications of mosaic analysis with double markers (MADM) in mice. MADM exploits a site-specific recombinase to catalyze an interchromosomal recombination between two interrupted fluorescent protein cassettes. Upon Cre-mediated recombination, fluorescent protein expression is restored, creating sparsely labeled cells that can also be made homozygous mutant for a gene of interest. Luo presented data exploiting the MADM system for both lineage tracing, to investigate the modes of cell division underlying cerebellum development, and single-cell knockouts, by inactivating $p27^{Kip1}$ to model sporadic loss of heterozygosity (LOH). He also presented a double-fluorescent Cre reporter mouse. This strain expressed an RFP before Cre excision and GFP after excision. Given the popularity of Cre reporters within the mouse community, Luo's 'stoplight' mouse will certainly be of interest to many laboratories.

Organ development and disease models
Autosomal dominant polycystic kidney disease is the most common autosomal dominant disorder in humans and is

characterized by the growth of numerous cysts that replace much of the mass of the kidneys, leading to their failure. Matthias Treier (EMBL, Heidelberg, Germany) discussed the function of *Glis2* and *Glis3* and their relation to this disease. These are genes for zinc finger transcription factors closely related to the mammalian Gli and Zic families. Having generated mouse mutants for each of these genes, Treier could model the human disease phenotypes. He showed that polycystic kidney disease can be caused by mutations in *Glis3*, whereas mutations in *Glis2* cause nephronophthisis, a disease characterized by progressive destruction of the kidney tubules and glomeruli. He also showed that Glis proteins localize to both nuclei and primary cilia, supporting a direct role in cystic kidney 'ciliopathies'. Licia Selleri (Weill Medical College of Cornell University, New York, USA) discussed the ontogeny of the spleen, and presented work from her laboratory piecing together a cellular, genetic and molecular framework regulating the formation of this organ, about which very little is currently known. Analyzing various mouse mutants that do not develop a spleen, Selleri and colleagues have pieced together a pathway directing spleen development in which the homeobox transcription factor Pbx1 plays a pivotal role, near the top of a complex hierarchy.

Oliver Smithies (University of North Carolina, Chapel Hill, USA), who this year was awarded the Nobel Prize for Physiology or Medicine with Mario Capecchi and Martin Evans for pioneering work in developing the technologies for knock-out mice, charmed the audience as usual with a presentation of his studies on hypertension using mouse models and computer simulations.

Rosa Beddington is a prominent figure in the history of this meeting. She made many legendary contributions to the science, both in the auditorium and around the posters during the day, and at the bar late into the night. It was therefore fitting that the Rosa Beddington memorial lecture was given by Richard Harvey (Victor Change Cardiac Research Center, Sydney, Australia), who was introduced to the mouse by Rosa. Harvey presented new insights on cardiac progenitor specification and proliferation, demonstrating a major role of the homeodomain protein Nkx2-5 in these processes.

Nkx2-5 mutations in the mouse recapitulate human congenital heart disease, and the underlying defects are therefore of profound interest. Harvey's studies of *Nkx2-5* mutants reveal a negative feedback loop that normally represses *Nkx2-5* via the Bmp2-Smad1/5/8 pathway, its immediate upstream activator, in the secondary heart field (SHF) thereby regulating SHF proliferation and outflow tract (OFT) morphology. In hypomorphic *Nkx2-5* mice, cardiac progenitor cells are overspecified and hence cell proliferation in the SHF is reduced, resulting in a truncated OFT. The Bmp2-Smad1/5/8 pathway is overactive in *Nkx2-5*

mutants, leading to the overspecification of the SHF, and Harvey reported that this effect is probably mediated through direct binding of Nkx2-5 to the enhancer of the *Bmp2* gene in cardiac cells. Deletion of a *Smad1* allele in these mutants could rescue the OFT phenotype. These results suggest that Nkx2-5 is essential for control of SHF cell proliferation through repression of the Bmp2-Smad1/5/8 pathway, and is a key regulator controlling the balance between cell proliferation and cell differentiation in the cardiac fields.

MicroRNAs as key regulators of cardiac gene expression

Continuing the subject of cardiac development, Eric Olson (University of Texas Southwestern, Dallas, USA) presented evidence for microRNAs (miRNAs) as key players in cardiac physiology. Heart-muscle contractility is regulated by two genes, *αMHC* and *βMHC*, which encode a fast and a slow ATPase, respectively. Subtle changes in the expression of these genes can influence heart function, showing that their normal expression is finely tuned. The switch from predominant expression of *βMHC* in the embryonic heart to predominant *αMHC* expression in the adult heart is mediated by thyroid hormone, which stimulates *αMHC* and inhibits *βMHC* expression. Cardiac stress influences the balance of *αMHC* and *βMHC* by inducing predominantly *βMHC* expression.

Olson and co-workers have implicated miRNA-208 (miR-208) in this response, as its expression in the adult mouse heart is reduced in response to stress. Amazingly, miR-208 is encoded within intron 27 of *αMHC* and is almost exclusively expressed in the heart. Gene-expression profiling of miR-208 mutants compared with wild-type hearts revealed an increase in expression of fast muscle fiber genes, a hallmark of cardiac response to a hyperthyroid state. This suggested that miR-208 might act by repressing a common component of the stress response and the thyroid hormone signaling pathways. Olson provided evidence that this repression is mediated through THRAp1, a co-regulator of the thyroid hormone receptor, which in turn has been shown to repress *βMHC* expression. Taken together, these results suggest a complex network underlying the cardiac stress response, where understanding the role of miRNAs in cardiac physiology may open doors to create new therapies for congenital and acquired heart disease.

Epigenetics and imprinting

Wolf Reik (Babraham Institute, Cambridge, UK) opened the imprinting session with a discussion of the evolution of genomic imprinting in mammals. Imprinting is argued to have evolved owing to the role of maternal nutrition in embryogenesis, suggesting that mammalian imprinting appeared coincident with the placenta at the monotreme-marsupial boundary. He presented data showing the

identification of a marsupial *H19* gene (encoding a non-coding RNA) and demonstrated that it conserves both parental imprinting and differential methylation in placental mammals such as the mouse. Indeed, Reik and his colleagues believe *H19* to be the most ancient imprinted non-coding RNA. Work now in progress will determine whether it is also an imprinted gene in monotremes.

It seems almost axiomatic that genomic imprinting must be maintained through the requirement for specific dosage at imprinted loci, but this has not been systematically investigated outside the context of imprinting control. Simao Teixeira da Rocha (University of Cambridge, UK) has tackled this problem, investigating the effect of dosage at the imprinted *Dlk1* locus (which encodes Delta-like 1, a protein similar to Delta, the Notch receptor). Using BAC transgenesis, da Rocha was able to compare the effect of one, two or three copies of *Dlk1*. Whereas three copies proved lethal, two copies proved viable, although both growth and metabolism were abnormal; these phenotypes are significant if nutrition is the driving force behind the evolution of imprinting, as many believe.

The session ended with a fascinating talk from Anton Wutz (Research Institute of Molecular Pathology, Vienna, Austria), who, returning to non-coding RNAs, described the imaging of *Xist* RNA (the RNA that initiates and maintains X-chromosome inactivation in female mammals) in living cells. Wutz presented results showing that *Xist* RNA was clustered during interphase, indicating its association with the inactivated X chromosome, whereas during mitosis it appeared to be displaced from chromosomes. Fluorescence recovery after photobleaching (FRAP) was used to monitor the turnover of *Xist* RNA and to visualize the dynamics of its spreading along the X chromosome. Interestingly, despite its rapid dynamics, the *Xist* RNA remains on the chromosome from which it was transcribed.

Mouse genetics and genome resources

On the final two days the speakers found themselves competing with planes flying into the nearby Duxford airshow. Eddy Rubin (Joint Genome Institute, Berkeley, USA) spoke about his team's work using evolutionary sequence conservation to identify enhancer and repressor function (http://enhancer.lbl.gov). To investigate how enhancers work in concert *in vivo*, Rubin has analyzed the actions of combinations of enhancers driving a single reporter gene, finding examples of both independently acting and interacting enhancers. To date 47% of the highly conserved elements tested have proved to have enhancer function *in vivo*.

Allan Bradley (Sanger Institute, Cambridge, UK) set the scene for the genomics session where he discussed his "quest for homozygosity", whereby he envisioned embryonic stem cells (ES cells) as both the vehicle for genetic modifications as well as the platform for phenotypic analyses. His wide-ranging talk discussed the efficiency of transposon-based mutagenesis as well as the analysis of strain-based variations and their relevance for mouse genetics. Many of the strategies exploited ES cells deficient in the Bloom's syndrome protein, which display an increased mitotic recombination frequency that results in high rates of LOH; they thus provide an efficient tool for recovering homozygous mutant clones.

Tian Xu (Yale University, New Haven, USA) moved transposon-based mutagenesis from ES cells into the mouse in his discussion of the mutagenesis project he is spearheading that exploits a germline-mobilized PiggyBac transposon. In a massive purpose-built mouse facility at Fudan University in China, and making use of current communication technology, he and his co-workers are now isolating gene-trap mutations through phenotype-driven screens. Indeed, the role of good phenotyping in mutant analysis was underlined by Helmut Fuchs (National Research Center for Environment and Health, Munich, Germany) in his talk on the work of the German Mouse Clinic (GMC; http://www.mouseclinic.de). Using a substantial battery of tests, the GMC has identified novel phenotypes in over 90% of mutant lines examined, many of which were previously unreported. Indeed it seems that our much-missed colleague, the late Anne McLaren, was right when she proclaimed "a mouse with no phenotype does not exist!"