Gene expression in early development

Katsuhiko Shirahige, Miho Ohsugi and colleagues report the largest transcriptome analysis of mouse preimplantation cells to date (*Genes Dev.* 27, 2736–2748, 2013). The authors synchronized over 140,000 *in vitro*–fertilized mouse embryos and parthenotes at specific stages—from fertilization to the two-cell stage—and compared their expression changes both to each other and to unfertilized oocytes using RNA sequencing. They identified 5,364 genes expressed in oocytes and early embryos whose expression was not detected in earlier microarray studies. Consistent with previous reports, the authors found extensive changes in gene expression between the one- and two-cell stages but unexpectedly discovered that these changes occurred for noncoding RNAs as well as for mRNAs. Among the new insights uncovered in their analysis is the discovery of fertilization-regulated transcription factors, including five whose expression was not detected in oocytes or parthenotes: *Foxd1*, *Nkx2-5*, *Sox18*, *Myod1* and *Runx1*. The authors suggest that these transcription factors are required for developmental progression, as parthenotes fail to develop past the four-cell stage. The freely available database is likely to be an important resource for future developmental research. ***BL***

Dynamic enhancer usage during development

To characterize the *in vivo* dynamics of enhancer usage during development, Axel Visel and colleagues generated genome-wide maps of the enhancers active in mouse forebrain, heart and liver (*Cell* **155**, 1521–1531, 2013). The authors profiled acetylation at histone H3 lysine 27 (H3K27ac), a histone modification associated with active enhancers and transcription start sites (TSSs), via chromatin immunoprecipitation and sequencing (ChIP-seq) of mouse tissue collected at seven different stages of pre- and postnatal development. Their analyses identified 105,394 H3K27ac-enriched regions, comprising 16,225 TSS-proximal regions and 89,169 TSS-distal regions that represent candidate enhancers, and characterized patterns of dynamic enhancer activity spatially and temporally across development. Almost 50% of candidate enhancers were predicted to be active in only one of the three tissues, and just 3% of candidate enhancers were marked by H3K27ac enrichment in all tissues and at all stages. To experimentally validate enhancer predictions, the authors used a transgenic mouse enhancer reporter assay to test candidate forebrain enhancers, finding that 23 of 32 (72%) candidate enhancers tested in transgenic mice drove expression patterns *in vivo* as predicted by H3K27ac marks. *EN*

GADL1 variants and lithium response

Lithium is a common treatment for bipolar disorder, but only some individuals exhibit a favorable response. To identify genetic factors influencing lithium response, the Taiwan Bipolar Consortium performed a genome-wide association study of individuals treated for bipolar I disorder (*N. Engl. J. Med.* **370**, 119–128, 2014). In the discovery phase, the authors studied 294 individuals who had shown good adherence to lithium treatment for bipolar disorder over at least a 2-year period. By testing common variants for association with treatment response using a standardized cutoff, they identified a cluster of SNPs at 3p24.1 that reached genome-wide significance. They subsequently replicated association of the two lead SNPs at 3p24.1 with lithium response in two additional cohorts. In a combined analysis of 394 subjects, the response allele at rs17026688 yielded an odds ratio of 82.2 and a positive predictive value of 83%. Through further resequencing of the region, the authors identified a 1-bp deletion in intron 8 of *GADL1* that is in complete linkage disequilibrium with rs17026688 and that may influence *GADL1* splicing. If confirmed in larger cohorts, these markers will be useful for identifying the individuals most likely to benefit from lithium treatment. *KV*

Written by Brooke LaFlamme, Emily Niemitz & Kyle Vogan

A CRISPR method for genome-wide screening

CRISPR (clustered regularly interspaced short palindromic repeats), together with the Cas9 nuclease, can be used to make targeted changes to genomic DNA in mammalian cells. Now, the inventors of CRISPR-Cas9, Feng Zhang and colleagues, report the use of their method in a genome-wide knockout screen of human cells (*Science* **343**, 84–87, 2014). They modified the method to allow for the efficient delivery of CRISPR guide RNAs and Cas9 in a single lentivirus vector. The lentiCRISPR method avoids the most common pitfall of RNA interference (RNAi) screening, incomplete RNA degradation. Using lentiCRISPR, they delivered a library of 64,751 unique guide RNAs targeting 18,080 genes to embryonic stem cells and melanoma cells and used RNA sequencing to identify guide RNAs that were depleted 14 d after transduction. The authors show that depleted RNAs correspond to essential genes. They also identified six genes expressed in melanoma cells that are involved in resistance to a chemotherapeutic agent, vemurafenib: two previously identified (*NF1* and *MED12*) and four novel (*NF2*, *CUL3*, *TADA2B* and *TADA1*) candidates. The authors note that the method can be expanded to carry out many other types of genomic screens. *BL*

Synthetic modeling of developmental enhancers

To quantitatively model the developmental functions of *cis*-regulatory modules (CRMs), Eileen Furlong and colleagues used synthetic engineering to systematically interrogate the individual and combinatorial contributions of transcription factor binding site motifs to enhancer activity in *Drosophila melanogaster* development (*PLoS Genet.* 10, e1004060, 2014). The authors engineered 63 synthetic *cis*-regulatory elements from 10 binding site motifs recognized by transcription factors of the Wingless and Dpp pathways and stably integrated them into the *Drosophila* genome. Each synthetic CRM was composed of six motifs and neutral spacer sequences, and enhancer potential was assessed by reporter expression during embryonic development. They determined that, for some motifs, homotypic clusters were able to drive specific spatiotemporal expression patterns that recapitulate *in vivo* expression of the corresponding transcription factor. To interrogate the importance of motif organization, the authors generated heterotypic CRMs composed of motifs for transcription factors known to interact and found that altering the spacing and organization of some motifs can affect enhancer activity in a tissue-specific manner. The authors quantified variability in CRM-driven expression and used fractional site occupancy modeling to determine that transcription factor cooperativity has an important role in CRM activity in some tissues. ***EN***