Queen bees and DNA methylation

Genetically identical honeybee larvae develop into either worker bees or queen bees depending on exposure to royal jelly. Now Ryszard Maleszka and colleagues report that disruption of epigenetic programming can alter the larval developmental trajectory, mimicking the effect of royal jelly on development (*Science* 319, 1827–1830; 2008). The authors used RNAi to silence the *de novo* DNA methyltransferase Dnmt3 in larvae. This led to a large increase in the number of queens that developed from the larvae (72%) compared to the number of queens that developed from control RNAi–injected larvae (23%). Genomic methylation patterns in honeybee are not yet well defined, but methylation patterns in a number of genes are known. The authors investigated the effect of Dnmt3 RNAi on *dynactin p62*, a gene known to be differentially methylated during development. The authors determined that *dynactin p62* has about 10% less overall DNA methylation in queen larvae versus worker larvae, and that Dnmt3 RNAi similarly results in a 10% decrease in overall *dynactin p62* methylation. Although it is not clear whether differential methylation of this gene contributes to the phenotypic differences, this study identifies an intriguing connection linking epigenetic regulation, nutritional exposure and developmental transitions. ***EN***

Single-molecule sequencing

Timothy Harris and colleagues report first-generation single-molecule DNA sequencing using True Single Molecule Sequencing (tSMS) technology (Helicos BioSciences), and an application to resequence the M13 phage genome (*Science* **320**, 106–109; 2008). By allowing for direct sequencing of an individual DNA strand, this sequencing-by-synthesis approach avoids the amplification step of other sequencing methods and is intended to reduce errors inherent to amplification by PCR or cloning. The M13 phage genome, at a length of ~7 kb, provided a test case for the technology. The authors sequenced for 224 cycles (2 passes at 112 cycles each), resulting in an average read length of ~23 bases. The forward and reverse genome coverage were 96× and 105×, respectively. They found reduced accuracy in regions of base repeats, with homopolymers of >2 bases in length showing reduced incorporation and accuracy. In order to test how well mutations might be identified, the authors aligned sequence to ten M13 reference genomes, each of which had 50 introduced single nucleotide changes. They found that they were able to detect these mutations, at set thresholds, with a discovery rate of over 98% and with zero false positives. *OB*

Foxh1 and forebrain development

Identifying a significant number of transcription factor targets in early embryos is difficult, given the limited amount of material available and the complexity of the signaling pathways involved. Cristoforo Silvestri and colleagues report a successful attempt to define a key network downstream of Foxh1 in the developing mouse forebrain (*Dev. Cell* **14**, 411–423; 2008). In cooperation with Smad proteins, Foxh1 mediates TGFβ-dependent gene expression. The authors first established the consensus Smad-Foxh1 composite enhancer in a mammalian cell line, and searched for these composite regulatory elements in the human, mouse and rat genomes. They found 169 targets common to human and mouse, 21 of which were positioned in a similar manner relative to the nearest gene, including 16 newly identified Foxh1 targets. Of 12 target genes whose expression was successfully detected, 8 showed changes in expression in response to activin or in the absence of Foxh1 in embryoid bodies or early embryos. Three of the targets were the retinal dehydrogenases, which catalyze the rate-limiting step in the generation of retinoic acid. In Foxh1-null embryos, expression of these enzymes was abolished specifically in the developing forebrain, suggesting that TGFβ signaling, via Foxh1, initiates retinoic acid signaling in the forebrain. *AP*

Written by Orli Bahcall, Emily Niemitz and Alan Packer

Genetic variants and response to warfarin

Common variants in *CYP2C9* and *VKORC1* are associated with dose requirement for warfarin, an anticoagulant drug. The optimal dose of warfarin, defined by a standardized measure of clotting tendency, the international normalized ratio (INR), varies widely among individuals and is determined by trial and error. Now Ute Schwarz and colleagues report the results of a prospective study on the effects of variants of *CYP2C9* and *VKORC1* on variability in INR during initial warfarin therapy (*N. Engl. J. Med.* **358**, 999–1008; 2008). The authors determined that the common A haplotype of *VKORC1* had a significant effect on early response to warfarin, with a decreased time to the first INR within the therapeutic range and to the first INR above 4. In contrast, variants in *CYP2C9* did not affect the time to the first INR within the therapeutic range, but some *CYP2C9* variants did decrease the time to the first INR above 4. As most of our understanding of the effects of variants in *CYP2C9* and *VKORC1* on warfarin dosage is based on stably anticoagulated individuals, this identification of specific effects of *VKORC1* variants during initiation of therapy adds useful information for pharmacogenetic testing for warfarin treatment. *EN*

Schizophrenia and structural variants

Jonathan Sebat and colleagues report an analysis of structural variants in individuals with schizophrenia (*Science*, published online 27 March 2008; 10.1126/science.1155174). The authors carried out a genome-wide scan for microdeletions and microduplications over 100 kb in 150 schizophrenia or schizoaffective disorder cases and 268 control individuals, using ROMA 85K probe microarrays for event discovery and Illumina 550K and NimbleGen 2.1M HD2 arrays to validate events and refine breakpoints. Overall, they identified and validated 53 previously unreported microdeletions and microduplications, ranging in size from 100 kb to 15 MB. Although they found a similar frequency for 115 common variants (with frequency >1%) between cases and controls, previously unreported structural variants were found in 15% of cases, compared to only 5% of controls. Cases were also more likely than controls to have a rare structural variant that influenced a gene. They further examined frequencies of structural variants in an independent cohort of youth with childhood onset schizophrenia (COS), carrying out a genome-wide scan for structural variants in 83 COS cases using the Affymetrix 500K platform and available parents as controls, and report 27 newly identified deletions and duplications in 23 COS cases. They found that the genes disrupted by rare structural variants in cases in these studies were overrepresented in pathways relevant to brain development. ***OB***