Dissecting cryptic variation

Organisms tend to be robust and invariant, harboring 'cryptic' genetic variation that has no apparent effect on phenotypes. The nature of this variation, and the evolution of robustness, has only begun to be explored. Josselin Milloz and colleagues now report a quantitative assessment of variation in a developmental signaling network that underlies vulva cell fate patterning in *Caenorhabditis elegans* (*Genes Dev.* 22, 3064–3075; 2008). The vulva develops from a stereotypical pattern of cell-fate decisions, and is known to be regulated by an EGF receptor–dependent pathway through Ras and MAPK, as well as Notch and Wnt signals. The authors analyzed seven independent wild isolates of *C. elegans*, introgressing mutations in the Ras, Notch and Wnt pathways, and scoring the number of induced vulval cells. They found that variation in Ras signaling has markedly different effects on the phenotype, suggesting the presence of cryptic variation in this pathway. Transgenic reporter constructs that quantitatively measure Ras output reveal that lines with higher background levels of Ras signaling have a higher vulval-cell inductive index in the Ras-mutation introgression assay. The effect of the Ras pathway mutations was largely uncorrelated across tissues, suggesting that robustness in this context has a degree of tissue-specificity. ***AP***

Shaping limb morphology in bats

Although all vertebrate limbs share the same basic morphology, evolutionary innovations within particular vertebrate lineages have led to the emergence of highly specialized variations that are adapted for specific functions. A notable example is the adaptation of bat forelimbs for flight, enabled in part by the dramatic elongation of digits 2 to 5 and retention of interdigital webbing. Nicola Illing and colleagues (*Proc. Natl. Acad. Sci. USA* **105**, 16982–16987; 2008) now show that these unique features in bats coincide with the induction of a second wave of expression of a key patterning molecule, *Shh*, during limb development. In all vertebrates, including bats, *Shh* expression in limbs first appears at the distal posterior margin, where it acts to maintain limb outgrowth and establish normal patterning along the anteroposterior axis. Illing and colleagues examined two species of bats and found, unexpectedly, that *Shh* expression is reinitiated at a later stage of limb development in the interdigital mesenchyme, where it likely acts, in concert with *Fgf8*, to promote ongoing proliferation of nascent digital elements and survival of interdigital cells, contributing to the unique morphological features of the bat wing. *KV*

Zip it

The SNF2 family of proteins is mainly comprised of ATP-driven DNA helicases, although how they work as mechanical enzymes is poorly understood. Mutation affecting one such uncharacterized protein, HARP (HepA-related protein), is linked to the etiology of Schimke immuno-osseous dysplasia (SIOD), which combines abnormalities of the immune and skeletal systems. Now, Timur Yusufzai and James Kadonaga report that HARP is not a canonical ATP-driven DNA helicase that generates single-stranded DNA (ssDNA) regions (*Science* **322**, 748–750; 2008). Rather, it is an ATP-driven molecular zipper shown to possess distinct properties that allow it to anneal complementary stably bound ssDNA strands. To gain insight into the molecular basis of SIOD, they generated mutations affecting the conserved ATPase region of HARP associated with SIOD: the R764Q substitution causes a severe form of SIOD, and the R586W substitution causes a mild form of SIOD. Both mutants preferentially bound fork DNA, but had little (R586W) to no (R764Q) detectable ATPase and annealing helicase activities. These results show that the mutants reduce the ATPase and annealing helicase activities of HARP in a manner that correlates with the severity of SIOD. Hence, HARP is critical in promoting the rewinding of stably unwound DNA and acts to oppose helicase activity throughout the genome to ensure proper transcription, replication and repair. *LK*

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Environmental and genetic buffering

Phenotypic robustness, also known as buffering, is used to explain why organisms may show limited phenotypic changes even when exposed to varying environmental conditions. Mark Siegal and colleagues now consider the mechanisms of how organisms may limit physical variation while both genetic and environmental variations are present (*PLoS Biol.* **6**, e264; 2008). They begin by identifying the key genetic players in yeast. To assess phenotypic variation, they used quantitative single-cell morphological phenotyping of 4,718 haploid *S. cerevisiae* single-gene knockout strains. They then identified roughly 300 candidate capacitor genes that showed higher phenotypic variance than expected under environmental variation, simulated by stochastic fluctuations in a constant microenvironment. For validation of these capacitors, they repeated phenotyping in 50 strains of the highest variance and 50 control strains. The 300 capacitors showed enrichment in Gene Ontology categories for critical cellular processes, and were also more likely to be network hubs, showing high degrees of both genetic and protein–protein interactions. Most capacitors also showed reduced growth rate when knocked out, which could allow persistence of these mutations. Capacitors with a duplicate gene pair showed different network properties from singleton capacitors, suggesting different mechanisms of buffering environmental variation. *OB*

Noncoding RNA and silencing

Large noncoding RNAs are known to have a role in epigenetic gene silencing in *cis*, but the mechanism is not well understood. Now, Peter Fraser and colleagues report that the *Air* noncoding RNA recruits the histone-modifying enzyme G9a to the chromatin of a nearby gene and that this interaction is involved in transcriptional silencing (*Science* advance online publication 6 November 2008; doi:10.1126/science.1163802). *Air* RNA is expressed from an imprinted locus; *Air* is expressed from the paternal allele and is required for imprinting of nearby genes *Igf2r*, *Slc22a2* and *Slc22a3*, which are expressed from the maternal allele in the mouse placenta. Fraser and colleagues found that *Air* RNA is localized to the promoter of the paternal, silenced *Slc22a3* allele where there is enrichment of H3K9me3 and that *Air* interacts with the H3K9 methyltransferase G9a. In mice expressing a truncated version of *Air* that does not accumulate at the promoter, there is reduced G9a enrichment at the promoter and loss of silencing of the *Slc22a3* paternal allele. The authors also showed that G9a-null placentas have loss of silencing of the *Slc22a3* paternal allele. These results suggest that noncoding RNAs can recruit histone modifiers to target loci for silencing. ***EN***