RESEARCH HIGHLIGHTS

METHODS IN BRIEF

GENETICS

Targeted structural variations

Genomic structural variations are implicated in both normal phenotypic variation and in disease. Engineered nucleases, such as zinc-finger nucleases, can be designed to generate double-strand breaks at specific genomic locations and thus should be able to stimulate chromosomal rearrangements. Kim and colleagues report that zinc-finger nuclease pairs targeted to two endogenous locations in the genome of human cells can result in cells harboring not only deletions, as previously reported, but also genomic inversions and duplications, at an experimentally tractable frequency. The resulting cells can be screened for otherwise isogenic cells that differ only in the induced genomic change. The researchers use this approach to invert a 140-kilobase chromosomal segment carrying the gene encoding a blood coagulation factor that is implicated in severe hemophilia.

Lee, H.J. et al. Genome Res. advance online publication (19 December 2011).

GENE EXPRESSION

In vivo detection of protein binding in the transcriptome

Immunoprecipitation of RNA-binding proteins cross-linked to RNA and sequencing of bound fragments is a commonly used method to determine binding sites in the transcriptome. To achieve nucleotide resolution, photoactivatable ribonucleosides can be used to enhance cross-linking and reduce background. Jungkamp and colleagues now optimize the technique for use in the transparent nematode *Caenorhabditis elegans* (*in vivo* photoactivatable ribonucleoside cross-linking and immunoprecipitation or iPAR-CLIP). The group tested parameters for efficient and uniform labeling of RNA and demonstrated tissue-specific results. They performed iPAR-CLIP on epitope-tagged GLD-1, a protein required for normal oogenesis, and recovered 439 binding sites including all known sites with high reproducibility. They extensively validated the sites, which included using a proteomic approach to confirm the effect of *gld-1* knockdown on the amounts of protein targets. Jungkamp, A.-C. *et al. Mol. Cell* 44, 828–840 (2011).

IMAGING

Clearing the way to spinal cord regeneration

Several chemical agents have been used to make tissues transparent and allow imaging deeper into living matter. Combining tissue clearance with light sheet-based illumination, for example, one can observe macroscopic specimens such as whole brains with microscopic resolution. In recent work, Ertürk and colleagues screened for the optimal clearing solution to visualize adult rodent tissue containing a large amount of lipids and a sturdy extracellular matrix such as the spinal cord. They settled on tetrahydrofuran, which rendered the tissue transparent by dehydration and lipid extraction without affecting the fluorescence signal of various fluorescent proteins and tracers. The group used the method to follow the trajectories of regenerating sensory axons in the unsectioned spinal cord, but the procedure can also be applied to other tissues.

Ertürk, A. et al. Nat. Med. 18, 166–171 (2012).

GENE EXPRESSION

Ribosome profiling in bacteria

Ribosome profiling, a method in which ribosome-protected fragments of RNA are deep-sequenced, is helping to answer what happens as nascent polypeptides leave the ribosome in eukaryotic cells. Oh and colleagues now capture bacterial ribosomes in the act of translation by chloramphenicol treatment or fast filtration of culture. Polysome purification and micrococcal nuclease digestion produces monosomes, and then the researchers reverse-transcribe and sequence the ribosome-protected footprints. The researchers profile nascent-chain interactions with the chaperone trigger factor by chemically cross-linking to stabilize transient interactions and selectively purifying the ribosomal fraction associated with the protein. They show that trigger factor associates with nascent peptide 100 amino acids beyond the ribosomal exit site, allowing other proteins to access the newest parts of the polypeptide. This is in contrast to *in vitro* studies that indicated immediate surveillance by trigger factor.

Oh, E. et al. Cell 147, 1295-1308 (2011).