Transcription factory proteomes

Eukaryotic nuclei contain three RNA polymerases (I, II and III), which are responsible for transcribing different groups of genes. These polymerases form larger complexes, which in turn are organized into putative ‘transcription factories’ that contain high concentrations of transcript production machinery. However, the question of whether transcription factories actually exist has been controversial, owing to the fact that they have not previously been isolated. In this issue of Nature Methods, Cook and colleagues describe an approach to purify active transcription factories containing RNA polymerases I, II or III, and then use mass spectrometry to analyze their protein content. They found that although the different transcription factories contained some common components, each had unique proteomes. The method could help address the burning question of whether transcription occurs within these factories.

Article p963

Enriching nuclease-modified cells

Zinc-finger and TALE nucleases can be used to make tailored modifications at specific sites in mammalian cells, but this process is often inefficient, and isolating the desired clones is laborious. Kim, Kim and colleagues report a simple episomal fluorescence–based reporter that can be used to enrich for cells modified by the nuclease of interest at an endogenous genomic site. Cells in which the nuclease is active are identified and enriched by flow cytometry based on their fluorescence profile. The authors used the reporter to enrich modified mouse or human cells, to test both zinc-finger and TALE nucleases, and to isolate biallelically modified cells.

Brief Communication p941

Gene expression in complex samples

Changes in the state of a tissue, such as those that occur during disease, are typically accompanied by changes in gene-expression patterns. However, the analysis of such expression changes is usually confounded by the fact that diseased tissue may also have an altered distribution of cell types. Kuhn, Luthi-Carter and colleagues describe a computational approach, dubbed population-specific expression analysis (PSEA), to disentangle these effects. Using linear regression modeling to cell type–specific references, PSEA is used to measure gene-expression changes in complex samples in spite of changes in cell populations. The researchers applied PSEA to analyze gene-expression changes in brain samples from humans with Huntington’s disease.

Brief Communication p945

0-glycoproteomics with SimpleCells

O-glycosylation is the most abundant and complex form of protein glycosylation, with many crucial biological functions. However, O-glycosylation is poorly understood because of technical difficulties in studying this post-translational modification: there is no known consensus motif that can be used for prediction, and the heterogeneity of O-glycosylation makes it difficult to study experimentally. Clausen, Levery and colleagues now report an approach to identify O-glycan attachment sites to proteins. To facilitate the isolation of O-glycoproteomes, the researchers used zinc-finger nuclease gene-targeting technology to glycoengineer cell lines, dubbed ‘SimpleCells’, displaying greatly simplified O-glycans. The O-glycoproteins and the O-glycan attachment sites are then readily amenable to analysis by lectin chromatography and mass spectrometry.

Article p977

RNA dynamics by NMR spectroscopy

Researchers are increasingly realizing that noncoding RNAs do not exist in a single conformation but undergo substantial dynamic motions to carry out their biological functions. Perhaps the best technology to study these dynamic motions is solution–state NMR spectroscopy, which offers atomic resolution and is sensitive to motions ranging from the picosecond to second timescales. Al-Hashimi and colleagues review the state of the field, describing the various NMR techniques that facilitate characterization of RNA dynamics at different timescales and presenting interesting applications. They also discuss specific areas in which technical developments are needed and describe how the technology can play a greater role in linking RNA dynamic motions to RNA function.

Review p919