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METHODS IN BRIEF

SEQUENCING

Single-molecule protein interaction detection

Gu et al. bring the power of single-molecule sequencing technology to profile protein interactions with a new approach called single-molecule-interaction sequencing, or SMI-seq. The approach is based on tagging proteins with DNA barcodes that can be read out by sequencing. First, proteins are barcoded with DNA tags by ribosome display or enzymatic conjugation; next, the barcoded proteins are randomly immobilized in a gel matrix; then, the DNA barcodes are amplified into in situ polymerase colonies ('polonies'). Barcoded proteins in a complex mixture can thus be identified and quantified by DNA sequencing, and protein-protein interactions can be detected by sequencing colocalized polonies. Gu et al. demonstrated the utility of this powerful platform for profiling the binding repertoire of an antibody and also for screening for small molecules that mediate G protein-coupled receptor activation.

Gu, L. et al. Nature doi:10.1038/nature13761 (21 September 2014).

IMAGING

Super-resolution imaging in live animals

Technical issues make it difficult to track single molecules in a living organism despite the development of super-resolution imaging techniques that improve light microscope resolution to a few nanometers. Zhan $et\ al.$ now show that complementation-activated light microscopy (CALM) can be used to follow individual voltage-dependent calcium channels in the muscle cells of living adult roundworms. CALM relies on fusing a protein of interest to a nonfluorescing GFP that lacks a single β -strand, which can be added back at low levels for complementation and sparse molecular labeling; it does not need photobleaching or low protein levels. The authors used dystrophin-mutant worms as models for human Duchenne muscular dystrophy and discovered that dystrophin modulates calcium-channel positional organization in muscle cell membranes and neuromuscular synapses by transducing changes in muscle tension. Zhan, H. $et\ al.\ Nat.\ Commun.\ 5$, 4974 (2014).

PROTEOMICS

Phosphohistidine proteomics

Mass spectrometry–based proteomic methods for detecting phosphorylation of serine, threonine and tyrosine are becoming well established, but this important regulatory post-translational modification (PTM) is also known to occur on other amino acid residues such as histidine. Oslund *et al.* describe a method for global proteomic profiling of phosphohistidine proteins, which they applied to study differences in *Escherichia coli* cells grown on glycerol versus on mannitol. First, to enrich for peptides containing this PTM, they utilized a panphosphohistidine antibody. Next, they found that collision-induced dissociation fragmentation of phosphohistidine-containing peptides in the mass spectrometer produced characteristic neutral-loss species. This observation allowed them to develop a software tool to identify phosphohistidine-containing peptides in mass spectrometry data by their characteristic fragmentation pattern.

Oslund, R.C. et al. J. Am. Chem. Soc. 136, 12899-12911 (2014).

MOLECULAR BIOLOGY

Large-scale transcription factor binding prediction

Of the approximately 170,000 eukaryotic transcription factors (TFs) believed to exist, only about 1% have characterized binding sequences. Weirauch *et al.* address the knowledge gap by using two protein-binding microarrays to determine the binding motifs of 1,032 cloned TFs, which come from over 130 species and represent 54 of 80 known DNA binding-domain classes. On the basis of sequence similarity, the researchers inferred motifs for another 58,000 TFs, with 89% accuracy according to cross-validation. Motifs are enriched in promoters, chromatin-immunoprecipitation sequence fragments and *Arabidopsis thaliana* expression quantitative trait loci. Weirauch *et al.* also develop an algorithm that generates ranked lists of human TFs whose binding can be altered by disease-associated genetic variants. The binding motif information is available in the catalog of inferred sequence preferences of DNA-binding proteins (CIS-BP) (http://cisbp.ccbr.utoronto.ca/).

Weirauch, M.T. et al. Cell 158, 1431-1443 (2014).