Technology Watch

NUCLEOSOME TURNOVER

Nucleosome disassembly and reassembly have been difficult to study. Previous approaches have included the mapping of histone variant H3.3, which is deposited independently of DNA replication. Using a new approach, Deal *et al.* assay the kinetics of native histones by pulsing *Drosophila melanogaster* S2 cells with the methionine surrogate azidohomoalanine (Aha), which is incorporated into histones that are being translated. The authors then couple biotin to Aha, break up the chromatin into single nucleosomes and use streptavidin beads to affinity purify nucleosomes containing newly synthesized histones. They subsequently hybridize the nucleosomal DNA to a high-density tiling microarray to create a map of nucleosomes that have turned over during the pulse. The landscapes created using this CATCH-IT (covalent attachment of tags to capture histones and identify turnover) technology show good correlation with H3.3 maps but with better defined chromatin features. Varying the length of the Aha pulse also enables turnover kinetics to be measured. For example, Deal *et al.* show that nucleosomes in active genes and at epigenetic regulatory elements, such as binding sites for polycomb or trithorax group proteins, are replaced several times during a cell cycle of ~20 hours. These findings have important implications for understanding the maintenance and transmittance of epigenetic information.

ORIGINAL RESEARCH PAPER Deal, R. B. *et al.* Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. *Science* **328**, 1161–1164 (2010)

N-GLYCOSYLATION MAPPED

N-linked glycosylation sites have been difficult to map owing to the complexity of attached sugar molecules and the low expression levels of many *N*-glycoproteins. The authors took advantage of a filter-aided sampled preparation (FASP)-based method, which has been shown to allow highly efficient capture of membrane proteins (many of which are *N*-glycosylated) because it achieves complete protein solubilization. Combining FASP with an *N*-glycosylation enrichment method by adding the affinity reagent — in this case a mixture of lectins — to the top of the filter after on-filter protein digestion, captured all three classes of *N*-glycosylated peptides. Deglycosylated peptides were then identified using on-line liquid chromatography electrospray mass spectrometry (LC-MS/MS) on a linear ion trap orbitrap instrument (the LTQ Orbitrap Velos), which has superior sequencing capabilities compared with ion trap fragmentation and shows no loss of sensitivity. Examination of four different mouse tissues and blood plasma identified 74% of known mouse *N*-glycosylation sites and an additional 5,753 sites on a wide range of proteins. Analysis of the *N*-glycoproteome shows that almost all modifications are attached to the canonical *N*-linked glycosylation motif, and 52% of the *N*-glycoproteome is located in the plasma membrane or oriented towards the extracellular space, whereas 20% is inside the lumen of subcellular organelles.

ORIGINAL RESEARCH PAPER Zielinska, D. F. *et al.* Precision mapping of an *in vivo* *N*-glycoproteome reveals rigid topological and sequence constraints. *Cell* **141**, 897–907 (2010)

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