5.4 Writing and Reading the Histone Code

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The human genome is estimated to contain 30,000-40,000 unique genes; the DNA sequence and chromosomal locations of all these genes are becoming widely known. A central challenge facing the biomedical community is how to derive medically valuable knowledge about the function of these genes from the now-available DNA sequence data. Though every gene exists within every cell in the human body, only a small percentage of genes are activated in any given cell. To manage this genetic information efficiently, nature has evolved a sophisticated system that facilitates access to specific genes. This system relies on a DNA-histone protein complex called chromatin to efficiently package the genetic information that exists within each cell. This packaging system making certain genes more readily accessible to transcription factors and other machinery that must engage our genetic template. Chromatin modifications, and the regulation of the enzymes responsible for adding or subtracting them, are poised to take center stage in the study of cancer in the current post-genomic or epigenomic era. Moreover, the implications of chromatin and its modification are beginning to be appreciated in clinical oncology. The identification of altered DNA methylation and histone acetylase activity in a range of human cancers, coupled with the use of HDAC inhibitors in the treatment of leukemia make a compelling argument. It is clear that the regulatory signals provided by chromatin modifications will revolutionize our view of cancer as new models of epigenetic carcinogenesis are advanced. We favor the view that there exists an epigenetic indexing system for our genome, or a "histone code", that represents a fundamental regulatory mechanism that acts outside of the DNA itself. We predict that this "code" impacts on most, if not all, chromatin-templated processes with far-reaching consequences for cell fate decisions and for normal and pathological development (see Strahl and Allis, 2000; Cheung et al., 2000; Jenuwein and Allis, 2001). Most of our current research is centered around chromatin and its regulation through post-translational modification.


6.1 Proteomics of the Gsp1pGTPase System in S. cerevisiae

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The S. cerevisiae nuclear pore complex (NPC) is a macromolecular protein assembly that acts as the major conduit for passage of cargo between the cytoplasm and the nucleoplasm of cells. Gsp1p (called Ran in vertebrates) is a highly conserved protein (from yeast to humans) that plays a central role in nucleocytoplasmic transport. The interplay between karyopherins (the shuttling receptors responsible for the transport of cargo across the NPC) and the nuclear pore complex is governed in part by Gsp1p, where Gsp1p-GTP modulates the interaction of karyopherins with cargo and nucleoporins (the protein constituents of the NPC). The Gsp1p effectors, Rna1p and Prp20p are important for keeping Gsp1p in its GDP- and GTP-bound form, in the cytoplasm and nucleoplasm, respectively. Gsp1p has also been implicated in cell cycle progression and may play roles in mitotic spindle assembly and nuclear envelope formation.

We have used a proteomic strategy to identify proteins that interact with Gsp1p and its effectors. Bacterially expressed GST-fusions of these proteins were used as bait to capture interacting proteins from yeast extracts, which were identified using mass spectrometry. Using cell extracts prepared from yeast arrested at different stages of the cell cycle (G1, S and G2/M), we detected changes in the pattern of interacting proteins, and the differences were quantified using ¹²⁵I-Protein A Western blots. For example, the levels of karyopherins present in extracts did not change in a cell cycle dependent manner, the interactions of several karyopherins with Gsp1p-GTP did change. The results suggest that the proteomic approach used here is useful in detecting changes in protein interactions based on responses to different cellular states.

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6.2 Functional Proteomic Analysis of Mitotic Substructures Reveals Conserved Cell Division Components

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Attaining a molecular understanding of how an exact copy of the genome is transmitted to daughter cells during cell division is a key problem in biology, yet a systematic identification of cell division proteins has been lacking. To obtain an accessible and enriched source of cell division proteins, we isolated mammalian midbodies and identified 159 midbody proteins by tandem liquid chromatography and mass spectrometry (LC/ LC/MS/MS). The midbody is a protein-dense, microtubule-based structure within the persisting intercellular bridge of dividing animal cells. Functions of midbody proteins were systematically evaluated in C. elegans using RNA-mediated interference (RNAi) and in vivo time-lapse video microscopy. Approximately 90% of the midbody proteins were conserved between mammals and nematodes, 88% of these were required for cell division, and 68% had no previously known function in cell division. Surprisingly, the majority of midbody factors were vesicular trafficking and secretory proteins plus microtubule- and actin-binding proteins, many of which are known to function in neuronal trafficking events. Depletion of the C. elegans homologues disrupted cytokinesis and/or celluarization, chromosome segregation, and mitotic or meiotic events including spindle formation and alignment. Thus, a comparative functional proteomic analysis of a subcellular assembly provides a highly efficient method to identify novel factors participating in complex cellular processes.