In Brief

Nuclear Organization

Subnuclear Chromosome Location

By tracing the localization of human chromosomes 18 and 19, Croft et al. (page 1119) have found that these chromosomes adopt distinct addresses within the nucleus early in cell division, apparently remaining in the same subnuclear locations through the remainder of the cell cycle. The work is the first demonstration that whole chromosomes may occupy distinct compartments within the nucleus, and suggests that genome mapping efforts will need to account for nuclear locations for a complete understanding of gene activity.

Chromosomes 18 and 19 are similarly sized, but differ significantly in their gene content, CpG island density, and hyperacetylated histone H4 association. Tracing chromosome localization in two or three dimensions with fluorescence in situ hybridization, the researchers found that chromosome 18, which has a lower density of genes, localizes to the periphery of the nucleus, whereas chromosome 19 occupies a more central position. In cells with a reciprocal translocation between chromosomes 18 and 19, chromosome 18 material on the translocated chromosome is still located peripherally, and the translocated portion of chromosome 19 is oriented towards the center of the nucleus, indicating that relatively small segments of DNA can determine chromosome location. "I am very much of the opinion that subnuclear localization will become integral to our understanding of mechanisms of gene expression and repression," says senior author Wendy Bickmore. Another recent paper (Brown, K.E., J. Baxter, D. Graf, M. Merkenschlager, and A.G. Fisher. 1999. *Mol. Cell.* 3:207–217) suggests that the subnuclear localization of some individual genes may change during the cell cycle.

Nuclear Oscillation and Recombination

On page 1233, Yamamoto et al. report that nuclear oscillation, a phenomenon associated with meiosis in the fission yeast *Schizosaccharomyces pombe*, is driven by the dynein microtubule motor protein and appears to be required for efficient meiotic recombination. During meiotic prophase, when homologous chromosomes are being paired, the nucleus of the yeast cell oscillates between the cell poles while telomeres cluster at the spindle pole body (SPB) at the leading edge of the moving nucleus. These events are believed to be involved in chromosome alignment, but their roles remain unclear.

Reasoning that cytoplasmic dynein, which is required for microtubule-mediated nuclear movement in other fungi, might be involved in *S. pombe* meiotic nuclear oscillation, the researchers disrupted the gene encoding the dynein heavy chain. The nuclei of cells lacking functional dynein do not oscillate, but the telomeres in these cells cluster normally, indicating that the two phenomena are not linked. The colocalization of homologous regions on a pair of homologous chromosomes is inhibited in the mutant cells, as is the overall frequency of meiotic recombination, but the cells are nonetheless able to complete meiosis to form viable spores. Nuclear oscillation may cause chromosomes to be dragged by their telomeres, facilitating alignment. First author Ayumu Yamamoto adds that the findings may be applicable to other systems: "We believe that other eukaryotes have similar mechanisms for aligning homologous chromosomes, since clustering of telomeres near a centrosome, an equivalent of the SPB, has been observed in many organisms, and nuclear movement has also been observed in some organisms during meiotic prophase."

Import and Localization of Splicing Factors

The SR proteins, a family of essential splicing factors, contain arginine/serine-rich (RS) domains that function as nuclear localization signals (NLS), but the nuclear import pathway for these proteins has remained unknown. Using a yeast two-hybrid screen, Kataoka et al. (page 1145) have identified a novel import receptor, which binds to RS domains and mediates the nuclear import of several of the SR proteins.

After determining that nuclear import of SR proteins is mediated by a saturatable factor in an in vitro import assay, the researchers used the yeast two-hybrid system to identify a gene product from a HeLa cell cDNA library, which binds specifically to an RS domain. The protein, dubbed transportin-SR, shows significant sequence similarity to the importin β-transportin family members involved in previously described nuclear import pathways. Biochemical experiments showed that transportin-SR binds specifically to intact SR proteins and mediates their nuclear import in the in vitro system.

Senior author Gideon Dreyfuss suggests that multiple nuclear import pathways provide the cell with additional levels of regulation of the compartmentalization of proteins. Dreyfuss adds that more remains to be discovered: "There are some proteins that get imported into the nucleus and for which neither the specific signal nor the receptor have so far been identified, so one can anticipate that there will be yet additional nuclear transport pathways."

Once RNA splicing factors are imported into the nucleus, they associate with sites of active transcription, but the mechanism of this association is poorly understood. Beginning on page 1133, Jolly et al. report that an endogenous gene that lacks introns nonetheless becomes associated with splicing factors when it is transcriptionally active.

First author Caroline Jolly emphasizes that there is still considerable controversy over the role of splicing factors in transcription, and "we may have to wait to collect data from ~200 or 300 endogenous genes to start understanding what the real situation is."

Reasoning that a comparison of two similarly regulated endogenous genes would be an informative start, the team

studied the mammalian heat-shock genes hsp90a and hsp70. Both are strongly induced when cells are subjected to stresses such as heat or heavy metals, but hsp90a contains 10 introns, while hsp70 is intronless. Using fluorescence in situ hybridization and immunofluorescence, the researchers monitored the locations of hsp90a and hsp70 transcription relative to splicing factor–containing speckles in the nucleus in stressed and unstressed cells. Under normal conditions, hsp90a displayed a low level of background transcription and some association with speckles, while hsp70 appeared fully repressed and did not associate with speckles. When induced by stress, both genes were highly transcribed and strongly associated with speckles.

There are two nonexclusive interpretations of these data. The induced gene may be recruited (perhaps by proteins not directly involved in splicing) to existing speckles, which already have intron-containing genes with associated splicing factors. Alternatively, the induced gene may be recruiting splicing factors, suggesting that introns are not required for transcription site localization of splicing factors. If the latter explanation is correct, the splicing factors may be present so they can scan the gene for introns.

Ubiquitin in Mitochondrial Inheritance

By searching for suppressors of a mutation that causes aberrant mitochondrial inheritance in yeast, Fisk and Yaffe have discovered that proper mitochondrial segregation requires protein ubiquitination. Previous work had identified several mitochondrial and cytoplasmic proteins involved in mitochondrial inheritance, but the underlying mechanisms of the process remained unclear.

Mutations in *MDM1*, a gene that encodes an intermediate filament-like protein, prevent mitochondria from being properly distributed during cell division. In a report beginning on page 1199, the researchers describe the isolation of two second-site suppressors of an *mdm1* mutation. The suppressors map to RSP5, a gene encoding a ubiquitin–protein ligase, and BUL1, the product of which binds to the Rsp5 protein and facilitates its activity. A mutant form of the Rsp5 protein that lacks ubiquitin ligase activity fails to complement the *mdm1* mutation, demonstrating that ubiquitination is required for mitochondrial segregation.

The finding adds another activity to ubiquitin, which is already known to direct proteins to a number of possible fates, including proteosomal degradation. Senior author Michael Yaffe says he is now trying to biochemically purify and identify other components in the mitochondrial segregation pathway. “In preliminary studies we have detected three or four ubiquitinated species that appear to be located on the mitochondrial surface,” he says. “None of these appear to be among the abundant proteins of the mitochondrial outer membrane.”

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