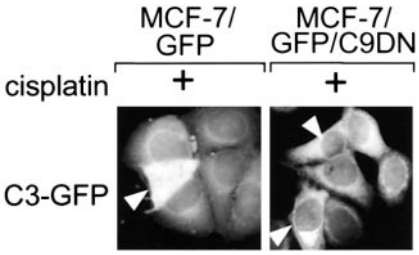
Caspase-mediated Disruption of the Nuclear–Cytoplasmic Barrier

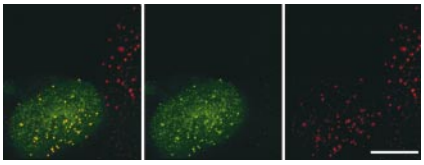
Faleiro et al. (page 951) report that caspases can disrupt the nuclear-cytoplasmic barrier by increasing the diffusion limit of nuclear pores, promoting the disassembly of the cell, and allowing caspases to enter the nucleus during apoptosis. The results help explain how caspases, which are ordinarily located in the cytoplasm, can reach molecular targets within the nucleus to carry out apoptosis.



Although the caspase-mediated signaling pathways that carry out apoptosis have been studied extensively, the mechanisms governing transport of caspases into the nucleus have remained poorly understood. By examining the localization of GFP-tagged caspase-3, the authors determined that the protein enters the nucleus during apoptosis, and that the activity of caspase-9 is required for this translocation. Caspase-9 activity also allows other large proteins to enter the nucleus, apparently by increasing the diffusion limit of nuclear pores. The findings point to a mechanism in which caspase-9 activation during apoptosis leads to an increase in nuclear pore permeability, allowing cytoplasmic caspases to reach nuclear substrates and promoting cell destruction.

Chromatin Assembly during Centromere Specification

Shelby et al. (page 1113) present evidence that chromatin assembly and DNA replication at kinetochores are not coupled, providing strong evidence for one of the two major models for centromere specification. For most chromatin, new histone synthesis is tightly coupled to DNA replication, but the new work shows that in the kinetochore, replication and chromatin assembly are temporally separated.

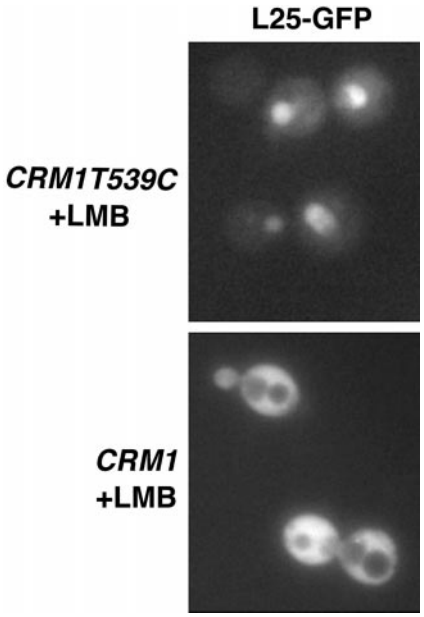


Although previous studies have shown that centromere specification is not determined solely by DNA sequences, the epigenetic mechanisms involved in this process have remained unclear. In the new work, the researchers used epitope-tagged CENP-A, a histone H3-like protein localized to the inner kinetochore plate of mammalian mitotic chromosomes, as a marker for inner kinetochore plate DNA. Immunoprecipitation and cytological analysis show that DNA in the prekinetochore domain replicates during S-phase, concurrent with the replication of bulk chromatin. However, consistent with previous findings, the team found that CENP-A synthesis is induced in G2-phase. The results appear to exclude the "last-to-replicate" model of centromere maintenance, which calls for replication of centromeric DNA at a unique time late in the cell cycle. Instead, the new data support a model in which centromeres are maintained by a distinct mechanism of chromatin assembly that is not coupled to DNA replication.

Nuclear Export of Ribosomal Subunits

In the first report to describe a nuclear export pathway for ribosomal subunits, Ho et al. (page 1057) demonstrate that the yeast protein Nmd3p directs the nuclear export of nascent 60S ribosomal subunits by a pathway involving the nuclear receptor Crm1p. Because Nmd3p is highly conserved throughout eukaryotes, the mechanism of 60S subunit export is likely to be similar in other systems.

As discussed by Aitchison and Rout on page F23, previous work showed that Nmd3p is required for a late step



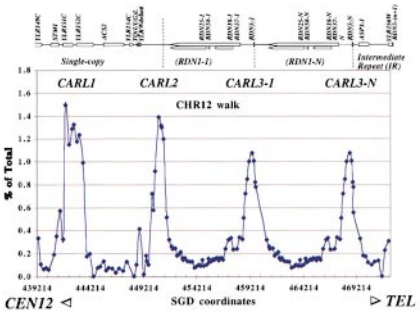
in 60S subunit biogenesis, but it remained unclear how the assembled subunits are exported from the nucleus. In the new study, the authors demonstrate that Nmd3p shuttles between the nucleus and cytoplasm, and that deletion of the nuclear export signal (NES) within the protein causes it to accumulate in the nucleus and also inhibits nuclear export of 60S subunits. The 60S subunits that accumulate in the nucleus under these conditions can be coimmunoprecipitated with the mutant Nmd3p. Specific inhibition of Crm1p also blocks Nmd3p shuttling and 60S subunit export, indicating that Crm1p is the receptor for Nmd3p export.

In addition to being conserved throughout eukaryotes, Nmd3p-like proteins are also found in archaebacteria, suggesting that the protein may have additional functions that predate the evolution of the nucleus.

Mapping Cohesin Binding Sites

By combining chromatin immunoprecipitation with high-resolution PCR-based chromosomal walking, Laloraya et al. (page 1047) have determined the chromosomal addresses of Mcd1p, a cohesin subunit. The work helps address several unresolved questions about the activity of cohesin, a conserved multiprotein complex that mediates sister chromatid

cohesion, and suggests that noncoding chromosomal DNA may serve an important structural function.

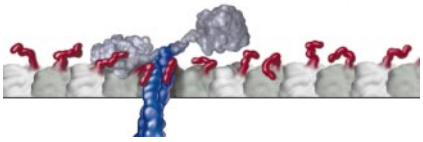


Recent work on budding yeast has shown that cohesins bind to specific regions of chromosomes to mediate cohesion, but the distribution of cohesin binding sites throughout the genome had not been determined, and the mechanisms of cohesin regulation also remained obscure. In the new study, the authors have constructed a detailed map of Mcd1p binding sites, called cohesin associated regions (*CAR*s). Averaging 0.8–1.0 kb in size, *CAR*s exhibit a conserved spacing of ~9 kb in single copy regions and in rDNA repeats, and are sequestered to intergenic regions and associated with AT-rich sequences. In contrast to previously proposed models, Laloraya et al. show that cohesins are not excluded from telomere proximal regions, and that cohesins are enriched at the centromere during mitosis by de novo loading rather than recruitment from arm regions.

The presence of *CAR*s in repetitive DNA and at the boundaries of silent chromatin suggests that cohesin binding may function in other forms of DNA metabolism, including mitotic recombination, gene splicing, and genomic packaging.

Modulating Kinesin Processivity

Taken together, two papers in this issue present a detailed view of the action of conventional kinesin, supporting a model in which a coiled-coil domain and a linker domain contribute important but distinct activities to drive the motor protein's highly processive movement along microtubules. Conventional kinesin drives the movement of membrane organelles, and while a basic model of its activity has emerged recently, key details have remained controversial. The implications of the new work are discussed in detail by Taylor and Borisy on page F27.



Tomishige et al. (page 1081) added disulfide bridges to kinesin to restrict the movement of either the neck linker domain or the neck coiled-coil domain to distinguish between two major theories of kinesin movement. One theory calls for an unzippering of the neck linker from the catalytic core to allow the dimeric protein to move along a microtubule, whereas the other involves unwinding of the neck coiled-coil. The new work tests both theories in functional motor assays, including assays at the single molecule level. Disulfide cross-linking of the neck coiled-coil had little effect on processive movement, but cross-linking the neck linker disabled movement, supporting neck linker unzippering as the mechanism for kinesin movement.

Although a major conformational change in the neck coiled-coil does not seem to be required for kinesin motor activity, Thorn et al. (page 1093) show that the coiled-coil domain still has a role in determining motor processivity. Increasing the positive charge in the neck coiled-coil domain increases the processivity of the motor protein, whereas mutations that make the domain more negatively charged decrease processivity. The results suggest that the neck coiled-coil domain enhances processivity by an electrostatic interaction with tubulin. Posttranslational modifications of tubulin that affect its charge might thus have a role in regulating conventional kinesin motor processivity in the cell.

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