In Brief

Maintenance of Mitochondrial DNA

Beginning on page 401, Aiken Hobbs et al. describe the localization of the yeast mitochondrial protein Mmm1p, and propose that the protein is part of a complex that controls mitochondrial structure and DNA segregation. The results are consistent with a model in which a “mitoskeleton” might play a role in regulating mitochondrial activity and morphology.

Mmm1p was identified previously as a protein involved in mitochondrial structure, since the mitochondria of mmm1 mutants form large, spherical structures instead of the tubular reticular structures found in the periphery of wild-type yeast cells. In the new work, Aiken Hobbs et al. analyzed the localization of a Mmm1p-GFP fusion protein in vivo. The protein localizes to small, punctate structures on the outer membranes of mitochondria, adjacent to a subset of mitochondrial DNA (mtDNA) nucleoids. Temperature-sensitive Mmm1p mutants have a defect in mtDNA transmission to daughter cells, as well as a loss of normal mitochondrial nucleoid structure, at the nonpermissive temperature. Deletion of mmm1 causes a dramatic disorganization of the mitochondrial inner membrane structure.

Based on their findings, the authors suggest that a protein complex containing Mmm1p forms a connection between the outer and inner mitochondrial membranes and anchors mtDNA nucleoids to the cytoskeletal matrix. Future studies in this system should help to reveal the mechanisms governing both mitochondrial structure and mtDNA maintenance.

Timing of DNA Replication

Using fluorescence in situ hybridization and time-lapse microscopy of GFP-tagged chromosomal domains, Heun et al. (page 385; also see the Comment by Gilbert on page F11) examined the subnuclear localization of early- and late-firing DNA replication origins. Their results suggest that late-firing origins are transiently enriched at the nuclear periphery in G1 phase, where a modified chromatin structure is established, determining the timing of origin firing.

Although previous studies have found a correlation between decreased transcripational activity and delayed origin firing, the molecular mechanisms that control origin firing have remained unclear. Heun et al. analyzed the localization of early- and late-firing origins in fixed and live yeast cells, and found that late-firing origins are significantly enriched in a zone immediately adjacent to the nuclear envelope in G1 phase. The local chromatin structure of an origin appears to be dominant over its nuclear positioning in controlling origin timing in S phase, but the establishment of a late replication state correlates with G1-phase localization to the nuclear periphery. In contrast, early-firing origins are distributed randomly in the nucleus throughout the cell cycle.

Heun et al. propose that late firing requires the presence of specific DNA elements flanking the origin, as well as contact with the nuclear periphery during G1 phase, whereas early firing is the default condition for replication origins. Once the late-firing state has been established in G1 phase, subnuclear location seems to be less important, as late-firing origins are randomly localized and mobile within the nucleus.

Conserved Mitotic Spindle Components Identified

In results that suggest that mitotic machinery is conserved across eukaryotes, Wigge and Kilmartin (page 349) have purified and analyzed a complex of spindle components from Saccharomyces cerevisiae, and have also characterized homologues of some components of the complex in Schizosaccharomyces pombe and in human cells. The new work builds on previous studies from the same lab, in which MALDI mass spectrometry was used to identify components in an enriched preparation of spindle poles.

In the new study, Wigge and Kilmartin purified a centromere-associated complex containing the novel spindle components Ndc80p, Spc25p, Spc24p, and the previously described protein Nuf2p, and found that temperature-sensitive mutations in the NDC80, SPC25, and SPC24 genes cause defects in chromosome segregation. The fission yeast S. pombe has homologues to Ndc80p, Nuf2p, and
Spc24p. There is a human homologue of Ndc80p (Chen, Y., D.J. Riley, P.L. Chen, and W.H. Lee. 1997. Mol. Cell. Biol. 17:6049–6056) and an EST database search revealed that Nuf2p also has a human homologue. All of the homologues localize to the centromeres in their respective species. The apparently high degree of conservation between yeast and human mitotic machinery suggests that yeast cells undergo a simplified version of vertebrate mitosis, and that further biochemical analysis of the Ndc80p complex in yeast will help elucidate the fundamental mechanisms of eukaryotic cell division.

**Arterial Injection of Muscle-derived Stem Cells**

Torrente et al. (page 335) have found that multipotent stem cells purified from the muscle tissues of normal newborn mice, when injected arterially into mdx mice, can participate in the regeneration of muscle fibers. The work should facilitate future studies on the behavior of transplanted stem cells in these mice, which are the principal animal model for the human disease Duchenne muscular dystrophy (DMD), and could also lead to improved therapies for the condition.

Using a muscle cell culture system, the authors isolated an enriched population of Sca-1+, CD34+ cells derived from normal newborn mouse muscle tissue. In vitro, these cells can undergo both myogenic and multilineage differentiation. When injected arterially into mdx mice, the multipotent cells attach to capillaries in muscles, migrate into muscle tissues, and partially restore normal dystrophin production. Interestingly, the efficiency of stem cell engraftment increases significantly when the muscle of the injected limb is injured after injection, and the transplanted cells participate in tissue regeneration.

**Voltage-dependent Anion Channel in Apoptosis**

Using antibodies against the voltage-dependent anion channel (VDAC) of mitochondria, Shimizu et al. (page 237) have demonstrated that the VDAC plays an essential role in Bax- and Bak-induced, but not Bid-induced, apoptosis in mammalian cells. Although previous work in the same laboratory has suggested that the VDAC is required for apoptogenic cytochrome c release from mitochondria, direct evidence for this model has been lacking, and several other explanations have been proposed.

To address the issue directly, the authors generated antibodies that inhibit VDAC activity. In isolated mitochondria, the antibodies block Bax-induced, but not Bid-induced, cytochrome c release, an event that triggers the apoptotic pathway. The antibodies also prevent Bax-induced cytochrome c release and apoptosis in whole cells, and significantly inhibit apoptosis induced by etoposide, paclitaxel, and staurosporine. These findings provide the first direct evidence that the VDAC is required for various forms of apoptosis, including Bax-induced apoptosis. The authors propose that Bax and Bak trigger the opening of the VDAC and subsequent cytochrome c release, leading to other mitochondrial changes associated with apoptosis, while Bid and related apoptotic proteins target a different molecule on the mitochondrial outer membrane.

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