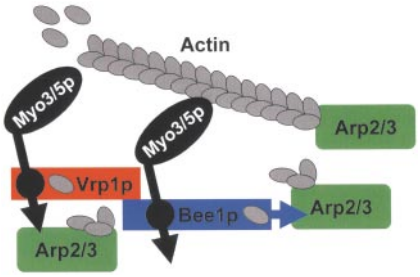
Role for Myosin in Actin Polymerization

Using different approaches, two groups have found evidence that myosin is involved in actin assembly. The dynamic nature of actin assembly implied an association with actin-based molecular motors, but the details of this association have remained obscure. In the new work, the two teams determined that type I myosins in yeast associate with WASP-like adapter proteins and components of the Arp2/3 actin nucleation complex, suggesting that myosin I proteins are involved in the assembly of cortical actin filaments.

Evangelista and colleagues (page 353) used two-hybrid and coimmunoprecipitation experiments to identify binding partners for the carboxy-terminal SH3 and acidic domains of Myo3p and Myo5p, functionally redundant type I myosin heavy chain proteins in *Saccharomyces cerevisiae*. They found that Bee1p (Las17p) and Vrp1p, homologues of the human WASP and WIP adapter proteins, interact with the myosin I SH3 domain. Arc40p and Arc19p, components of the Arp2/3 complex that regulate actin nucleation and assembly, associate with the myosin I acidic domain.



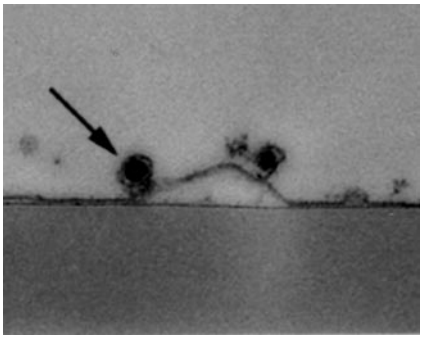
By affinity chromatography and mass spectroscopic analysis, Lechler et al. (page 363) approached the problem from the other direction, isolating and identifying proteins that interact with Bee1p. After discovering that Myo3p and Myo5p interact with Bee1p, the researchers found that the motor activity of these myosins is required for cortical actin polymerization in an in vitro reconstitution assay. The team also demonstrated a direct physical interaction between the Arp2/3 complex and the acidic tail of myosins using purified proteins. Polymerization requires phosphorylation of the myosin motor downstream of the small GTPase Cdc42p, placing myosin in a direct role in Cdc42-dependent actin polymerization. A comment by Laura Machesky on the two papers begins on page 219.

Characterizing Transport Systems In Vitro

An In Vitro Exocytosis System

In work that should facilitate future studies on the molecular mechanisms of exocytosis, Avery and colleagues (page 317) describe a cell-free system for regulated exocytosis in the PC12 neuroendocrine cell line. Though a variety of defined in vitro assays have been developed to study intracellular fusion events, studies on regulated exocytosis have generally been limited to biochemically complex permeabilized cell preparations.

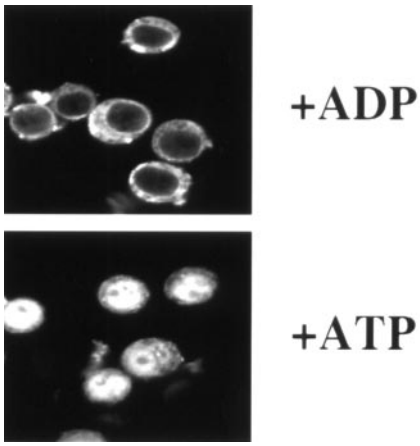
After growing PC12 cells on coated coverslips, the researchers incubated the cells with acridine orange, a fluorescent dye that accumulates in acidic compartments. Sonicating these cells produces flat plasma membrane patches with attached, dye-containing vesicles. Exocytosis causes the visible disappearance of the labeled vesicles, and exocytic membrane fusion can be monitored in single vesicles with atomic force microscopy.



Exocytosis in the in vitro system requires ATP, Ca^{2+}, and cytosol, and is sensitive to tetanus toxin, suggesting that the cell-free assay is similar to the in vivo process. In addition to potential applications in basic studies of exocytosis, the assay should also be adaptable to other labeling techniques, allowing the tracking of tagged proteins targeted to secretory granules.

Nuclear Import of Spliceosome Proteins

Hetzer and Mattaj (page 293) studied the nuclear import of U1A and U2B″ in vitro, and present evidence that the two spliceosome proteins are imported into the nucleus by a mechanism different from previously characterized nuclear import pathways. The mechanism requires hydrolyzable ATP, but does not depend on hydrolyzable GTP or Ran, a small GTPase required for almost all known types of protein nuclear import.

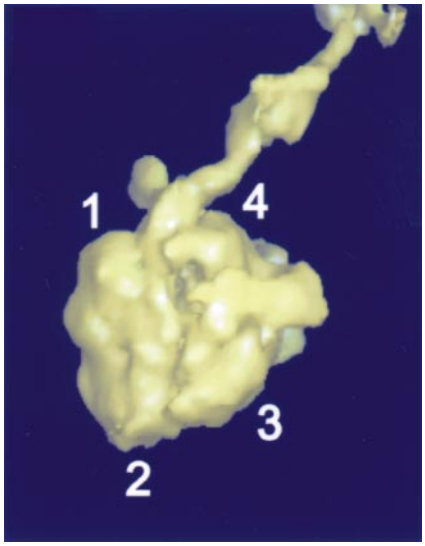


The unusually long nuclear localization sequences (NLSs) of U1A and U2B″ do not contain identifiable recognition signals for any previously described nuclear import pathways. Hetzer and Mattaj analyzed the import of U1A and U2B″ in permeabilized HeLa cells and determined that the process is active and saturable. Saturation of several known import pathways fails to inhibit the import of U1A and U2B″ NLSs, and the process also appears to be independent of Ran-mediated GTP hydrolysis. In contrast to all known import mechanisms, U1A/U2B″ import requires hydrolyzable ATP and factors found in

a nuclear fraction, and does not require cytosolic factors in the in vitro system. The authors were unable to purify the required activity from nuclear fractions by affinity chromatography, but suggest that U1A/U2B″ might bind directly to a nucleoporin rather than a soluble nuclear factor.

Pre-mRNPs Bind Specific Fibers in the Nucleoplasm

Studying the transport of a pre-mRNP particle in the salivary gland cells of the midge *Chironomus tentans*, Miralles and colleagues (page 271) have found that a population of the particle associates with specific fibers in the nucleoplasm, and have cloned and sequenced the cDNA of one component of the fibers. The

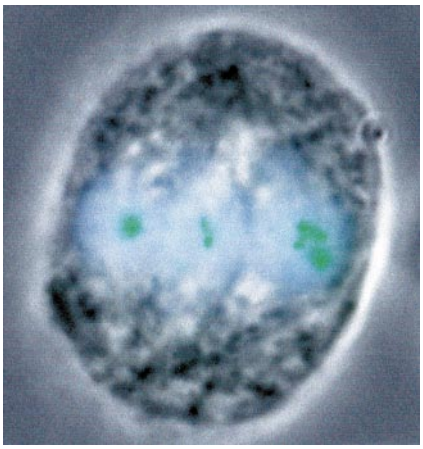


mechanisms of pre-mRNP transport from the gene to the nuclear envelope have been difficult to elucidate, with some models favoring free diffusion of the particles, while others favor physical attachment of pre-mRNPs to nuclear structures.

The team used electron tomography to visualize the structure of the Balbiani ring (BR) particle, a particularly large pre-mRNP, in the nuclei of midge salivary gland cells. The BR particles are present in three populations in the nucleus: one population bound to fibers, another associated with large complexes, and a population of apparently free particles. Since nearly all BR particles are eventually exported to the cytoplasm, the three populations may represent separate steps in a sequential export process. A previously described but unsequenced protein, hrp65, is a component of the fibers, and the researchers cloned and sequenced the hrp65 cDNA. The sequence shares some homology with known RNA-binding proteins. Immuno-EM shows that it localizes to the nucleus, and specifically to the portion of the fibers closest to the BR pre-mRNP particles. The results support a model in which mRNPs are transported through a combination of free diffusion and specific interactions.

Mitotic Silencing of rDNA Transcription and Pre-rRNA Processing

Beginning on page 259, Sirri and colleagues demonstrate that the mitotic silencing of rDNA transcription and the concurrent repression of pre-rRNA processing can be uncoupled, providing a novel system for studying the relationship between these two processes during mitosis. The transcription of rDNA is silenced and processing of pre-rRNA is arrested during mitosis, and previous work has suggested that the cdc2–cyclin B kinase pathway may be involved in regulating this process, but the regulatory details have remained obscure.



The researchers found that inhibiting the cdc2–cyclin B kinase pathway in mitotically synchronized HeLa cells can reverse the mitotic silencing of rDNA transcription. The resumption of rDNA transcription depends only on cdc2–cyclin B kinase, and is not caused by the exit of the cells from mitosis. Interestingly, 47S pre-rRNA accumulates in the cells after rDNA transcription resumes, indicating that pre-rRNA processing remains inhibited. The ability to uncouple the two processes suggests that they are regulated by distinct mechanisms, and the specificity of cdc2–cyclin B kinase inhibition should facilitate future studies on mitotic silencing.

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