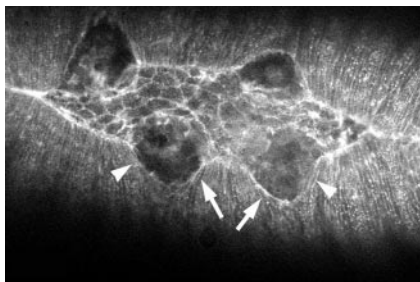


Cell Sheet Morphogenesis in *Drosophila*

Kiehart and colleagues (page 471) used time-lapse and real-time imaging of a GFP-tagged fusion protein, along with a series of elegant biomechanical analyses, to determine the major types of forces acting on cell sheets during dorsal closure in *Drosophila*. In addition to illuminating this important aspect of fly biology, the experiments demonstrate a powerful new system for studying morphogenesis and wound healing.

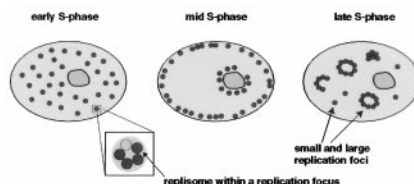
Using a transgene encoding GFP fused to an actin-binding fragment from the fly moesin protein, the authors followed cell shape changes and tissue movements during dorsal closure. The team then used a laser to destroy small groups of cells in precise locations, observing the resulting changes in cell sheet morphology to determine the forces acting on the sheet. The results suggest that both purse string-like forces in the leading edge of the lateral epidermis and contractility in the amnioserosa contribute to dorsal closure, while tension in the anisotropic lateral epidermis opposes dorsal closure.



The experiments also show that the epidermis of *Drosophila* embryos heals rapidly and reproducibly from repeated mechanical or laser wounds. Combined with the ability to observe cell movements and the well-established genetics of the fly, this finding should make the system broadly applicable for studies in wound healing, including genetic screens to identify loci involved in this poorly understood process.

Observing DNA Replication Factories in Living Cells

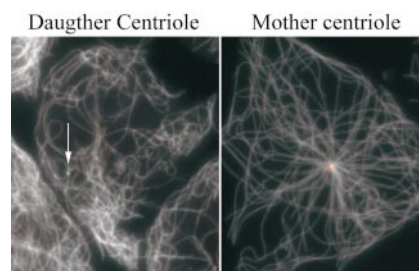
Using time-lapse imaging and a GFP-tagged component of the DNA replication machinery, Leonhardt and colleagues (page 271) examined the dynamics of DNA replication factories in living cells. The results suggest that in contrast with nuclear speckles and coiled bodies, replication factories are stably anchored in the nucleus in a pattern that changes by asynchronous assembly and disassembly of the foci. The approach should also be useful for future studies on nuclear organization.



A growing number of proteins have been shown to associate with subnuclear replication foci in a cell cycle-dependent manner, but the molecular mechanism of this association remains poorly understood. In this new work, the authors created stable cell lines expressing translational fusions of GFP and proliferating cell nuclear antigen (PCNA), a central DNA replication factor. In time-lapse imaging of the live cells, replication factories appear to be immobilized in the nucleus, shifting position by assembling and disassembling asynchronously throughout S-phase. At higher resolution, the larger factories appear as collections of several independent smaller-sized foci. The results are consistent with a model in which replication factories, anchored to the DNA and an underlying nuclear skeleton, remain stationary while replicating DNA is processed through them. The asynchronous assembly of the factories also suggests that early, middle, and late replication may be arbitrarily defined points in a continuous process.

Differing Behavior of Mother and Daughter Centrioles

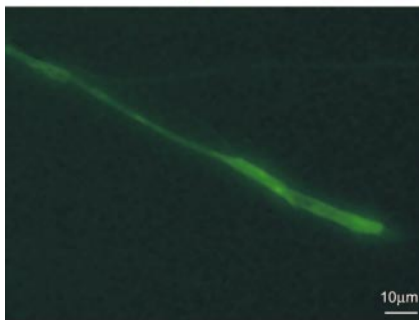
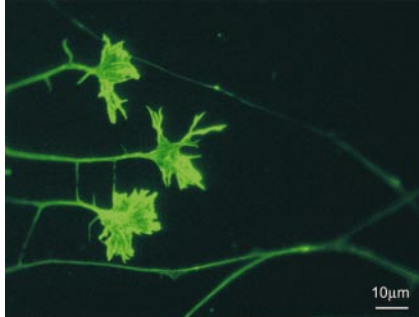
Applying GFP tagging and time-lapse imaging to studies on centrioles, Piel and colleagues (page 317) discovered unexpected complexities in the movements of mother and daughter centrioles and the role of cytoskeletal components in centriole behavior. The data, which show differences in both the motility and microtubule organizing activity of the two centrioles, suggest that centrioles may play a significant role in controlling microtubule arrays during cell locomotion and division.



After generating stable cell lines expressing a GFP-centrin fusion protein, the team followed the localization of the fluorescent protein, which is incorporated into mother and daughter centrioles. The mother-daughter centriole pair appears to split during or shortly after telophase. After mitosis, the mother centriole remains near the center of the cell, but the daughter moves around the cytoplasm. The centrioles replicate at the G1/S transition, and the movements of the daughter gradually diminish until its behavior is identical to that of the mother. Microtubule or actin inhibitors stop the movement of the daughter when administered together, but not when administered separately. While both centrioles can nucleate microtubules, only the mother anchors them.

Since most previous studies of the centrosome have employed fixed cells or isolated organelles, this new work provides important insights into cen-

troosome behavior. The authors propose that microtubules are nucleated near centrioles, then released to associate either with the mother centriole or with other anchoring sites, and that centriole splitting could be an important mechanism for controlling the cellular microtubule array.



Control of Growth Cone Collapse

Reports by Fournier and colleagues (page 411) and Wahl and colleagues (page 263) focus on the collapse of the growth cone, a structure that mediates axonal pathfinding during neuronal development. The results suggest that growth cone collapse is carried out by a combination of cytoskeletal reorganization and endocytosis, and that at least one growth cone collapse signal is transduced by the activation of Rho and Rho kinase. Repulsive environmental cues can

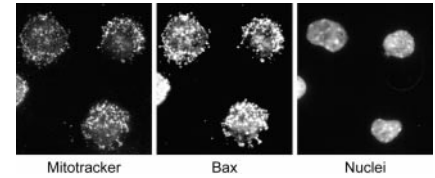
cause the complete collapse of the growth cone, arresting neurite outgrowth, or partial collapse, which causes asymmetry and steers the growth cone. Though growth cone collapse and steering are crucial to neuronal development, the molecular details of these phenomena remain unclear.

Fournier and colleagues studied the collapse response to Sema3A, a member of the class 3 semaphorin family that repulses the axons of sympathetic and sensory neurons. After Sema3A treatment, F-actin, NP-1, Plexin, and rac1 are redistributed to new membrane ridges and vacuoles. Sema3A also stimulates endocytosis during growth cone collapse. In dorsal root ganglion and retinal ganglion cell (RGC) systems, inhibitors of axon extension stimulate endocytosis. The authors suggest that the stimulation of endocytosis and actin filament rearrangement may be general mechanisms of growth cone collapse. Focusing on the signal transduction pathways involved in inducing growth cone collapse, Wahl and colleagues found that the repulsive guidance molecule ephrin-A5 induces the activation of the small GTPase Rho and its downstream effector Rho kinase. When RGC cultures are treated with ephrin-A5, Rho is activated while Rac is downregulated, and inhibitors of Rho GTPase and Rho kinase reduce the collapse rate of growth cones in this system.

Integrin-mediated Signals and Bax-mediated Apoptosis

In an effort to elucidate the mechanisms linking extracellular matrix (ECM) adhesion to the intracellular apoptotic machinery, Gilmore and

colleagues (page 431) studied the localization of the apoptotic protein Bax in mammary epithelial cells. Their results support a model in which integrin-mediated survival signals control the conformation and localization of Bax, suggesting new avenues for basic research on apoptosis as well as novel molecular targets for cancer therapy.



The loss of adhesion to the ECM induces apoptosis in most normal cells, a process apparently initiated by the loss of survival-promoting signals from integrins on the cell surface. In this new work, the authors show that a loss of ECM attachment in mammary epithelial cells causes Bax, a Bcl-2 family protein, to relocalize rapidly from the cytosol to mitochondria. At the same time, the BH3 domain of Bax becomes exposed. The conformational change in Bax and subsequent apoptosis require signaling by the integrin-associated kinase pp125FAK, and occur before the irreversible commitment to apoptosis. Based on these results, the authors present a model in which pp125FAK acts through PI3-kinase and pp60src to maintain Bax in a conformation that prevents its localization to the mitochondria; when ECM attachment and survival-promoting signaling are lost, Bax relocalizes to the mitochondria, leading to apoptosis.

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