Single Cell Cytochrome c Release

Using single-cell analysis, Waterhouse et al. (page 319) tracked changes in the mitochondrial transmembrane potential after apoptotic cytochrome c release and found that, in the absence of caspase activity, permeabilized mitochondria can regenerate their membrane potential sufficiently to generate ATP. The findings help resolve an apparent paradox in apoptosis: ATP is required for programmed cell death, but the release of cytochrome c through the mitochondrial outer membrane could potentially interfere with ATP generation via oxidative phosphorylation.

In the new work, cells were triggered to undergo apoptosis in the presence of caspase inhibitors, causing the release of cytochrome c from mitochondria but preventing downstream apoptotic events. In this system, the mitochondrial transmembrane potential rapidly depolarizes, but recovers to its original levels within 60 min, after which it is maintained at levels sufficient to generate ATP using cytoplasmic cytochrome c. The findings conflict with observations from bulk-cell analysis, which have not shown a transient loss of the mitochondrial transmembrane potential. However, since mitochondrial membrane permeabilization is not synchronized in a population of cells, the loss and rapid recovery of membrane potential in individual cells would appear as an overall maintenance of membrane potential in the population.

Second Checkpoint in ERK Signaling

Aplin et al. (page 273) describe an additional integrin-regulated checkpoint in the activation of extracellular signal-regulated kinases (ERK) by growth factors. Previous work has shown that integrin-mediated anchorage stimulates growth-factor-mediated activation of ERK and increases in cyclin D1 levels, but activating ERK directly in the absence of integrin engagement gives conflicting results. In some cell types, forced activation of ERK induces cyclin D1 expression in the absence of adhesion, while in other cell types ERK activation alone is not sufficient to induce cyclin D1 expression.

In the new work, the authors expressed active forms of Raf and MEK, components of the ERK cascade, in the absence of cell adhesion. Though this activates the ERK pathway, ERK fails to translocate to the nucleus in the nonadherent cells, preventing the phosphorylation of downstream ERK targets in the nucleus. In adherent cells, treatment with cytochalasin D, but not colchicine, inhibits nuclear translocation and downstream activity of ERK, suggesting that integrin–actin interactions, but not intact microtubules, are important for ERK translocation. The findings imply two integrin-regulated checkpoints in the ERK cascade: the regulation of growth factor activation of ERK and the accumulation of active ERK in the nucleus. Cell type–specific differences in the stringency of the second checkpoint could explain the contradictory results in earlier studies of ERK signaling.

Selective Vacuole Targeting

Kim et al. (page 381) describe the identification of two homologous genes in the yeasts Saccharomyces cerevisiae and Pichia pastoris involved in targeting cytoplasmic proteins and organelles to the vacuole for degradation. Though there are three overlapping pathways by which cytoplasmic components are transported to the vacuole in yeast, the newly discovered gene products appear to be involved only in the selective Cvt and pexophagy pathways, not in nonspecific macroautophagy. Most of the vacuole transport machinery identified to date is involved in both selective and nonspecific pathways, so the new findings help illuminate the poorly understood mechanisms that confer specificity on vacuole targeting.

In S. cerevisiae, the Cvt9 gene is a required component for the uptake of the prAPI protein into vacuoles, while the P. pastoris gene Gsa9 was identified by its role in the turnover of peroxisomal enzymes. The two genes are structurally and functionally homologous. Biochemical and morphological studies demonstrate that the selective uptake of peroxisomes into the vacuole for degradation requires Cvt9 or Gsa9, but nonspecific macroautophagy induced by nitrogen starvation does not require either protein. Cvt9 and Gsa9 are localized in a punctate perivacuolar structure. The results suggest that a protein complex containing Cvt9 might identify a membrane compartment re-
quired for selective vacuole targeting, and that Cvt9 and Gsa9 may selectively sequester cytoplasmic proteins and organelles for degradation.

**Myopalladin and Muscle Sarcomere Structure**

Bang et al. (page 413) have discovered a novel protein that links two regions (Z-lines and I-bands) critical to muscle sarcomere structure by linking a structural protein to a protein involved in gene expression. Z-lines, which define the borders of individual sarcomeres in vertebrate striated muscle, contain the COOH-terminal ends of nebulin or nebulette filaments, but the molecular mechanism anchoring these filaments inside Z-lines remains unknown.

The new protein, myopalladin, is highly homologous to palladin, a ubiquitously expressed protein that colocalizes with α-actinin in a variety of cellular structures and helps organize the actin cytoskeleton and focal adhesions. The high degree of homology suggests that sarcomeres and nonmuscle stress fibers may be assembled by similar mechanisms.

The authors also discovered that the NH₂-terminal region of myopalladin interacts within the I-band with cardiac ankyrin repeat protein (CARP), which is believed to play a role in controlling muscle gene expression. Overexpressing a truncated CARP-binding form of myopalladin disrupts myofilaments, suggesting that the interaction of myopalladin with CARP is critical for the assembly or maintenance of sarcomeric structures in cardiac myocytes.

**Pushing the Nucleus to the Center**

In an effort to identify the mechanisms responsible for nuclear positioning, Tran et al. (page 397) used green fluorescent protein (GFP) fusion proteins to analyze the dynamic interactions of microtubules and nuclei in living Schizosaccharomyces pombe cells. The data point to a new mechanism for nuclear positioning, in which the opposing pushing forces of microtubules, rather than pulling forces or molecular motors, are primarily responsible for determining the position of the nucleus.

Analysis of GFP-tubulin in interphase S. pombe cells shows a microtubule cytoskeleton composed of three to four microtubule bundles extending the length of the cell, with the plus ends facing the cell tips and the nucleus attached near the overlapping minus ends, in the middle of the cell. Shortly after growing to the cell tip, a microtubule exhibits catastrophe and shrinks back to the center of the cell. The nucleus moves away from the cell tip only when a microtubule contacts the cell tip and continues to grow, demonstrating that the microtubules primarily exert pushing forces.

Based on their results, the authors propose a model in which regulated microtubule dynamics provides opposing, transient pushing forces that constantly sense the geometric center of a cell and maintain the nucleus in that position. In this model, an off-center nucleus would be pushed by microtubules more frequently and more forcefully from the side closest to a cell tip, driving the nucleus back to the center. A computer model of this process shows that this mechanism can largely account for observed nuclear positioning movements.