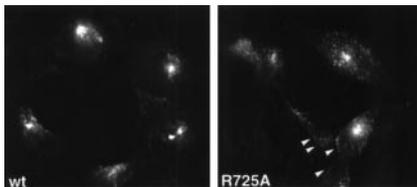


Dynamin, the Regular GTPase

Dynamin has provided cell biology with one of its most appealing models for protein action. Dynamin has been implicated in the formation of endocytic vesicles, it can self-assemble *in vitro* into spirals, and (at least with certain acidic lipids) it can act alone to pinch off individual vesicles. A theory therefore arose suggesting that the GTPase activity of dynamin was driving a constriction event by which the spiral pinched off new vesicles.

This model suggests that the hydrolysis of GTP is what drives dynamin action. But, in a 1999 *Nature* paper, and on page 1137 of this issue, Sever et al. present an alternative hypothesis. As with other GTPases, it now appears that dynamin is active when it is in its GTP-bound (not GTP-hydrolyzing) form.

The authors use two dynamin mutants that have normal basal GTPase activity, but are defective in the catalysis (R725A) or self-assembly (K694A) needed for assembly-stimulated GTPase activity. Both mutants were shown in the *Nature* paper to accelerate the earliest steps in receptor-mediated endocytosis, and it was assumed that both were driving faster vesicle formation. In the current paper, Sever et al. investigate the morphological basis for this counter-intuitive finding, and discover that the two mutants have, in fact, different effects on clathrin-coated vesicle formation.



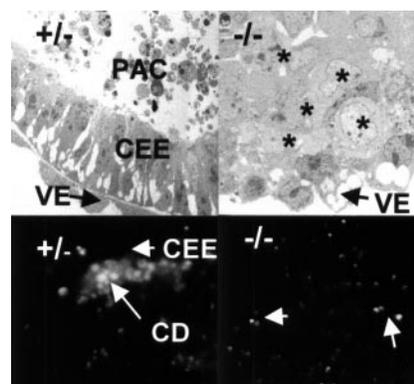
At the center of the experiments are biochemically-defined structures, termed constricted coated pits, which contain receptors that are accessible to small, but not large, extracellular probes. In normal cells, the constricted coated pits are almost instantly converted to free vesicles, but in cells expressing the catalysis-defective R725A mutant, the constricted

coated pits linger. This suggests that stimulated GTPase activity may be needed for disassembly of dynamin spirals and thus vesicle release.

As reported in the *Nature* paper, the formation of constricted coated pits is faster than normal in cells expressing either mutant dynamin. Thus, self-assembly may not be necessary for vesicle formation, and dynamin's GTP-bound form (more prevalent thanks to the mutations) may drive vesicle formation. Sever et al. propose that the GTP-bound form of dynamin recruits other proteins that are the true effectors of endocytosis.

Basement Membrane Required for Cavitation

Just before it forms its first cavity, the mammalian embryo consists of a basement membrane (BM), sandwiched between visceral endoderm (VE; on the outside) and epiblast (on the inside). Epiblast cells contacting the BM then polarize to form the columnar epiblast epithelium (CEE); the remaining epiblast cells subsequently detach from the CEE and die of apoptosis, leaving a cavity. On page 1215, Murray and Edgar use a laminin-1 knockout to show that both polarization and apoptosis of epiblast cells is dependent on the presence of a BM.

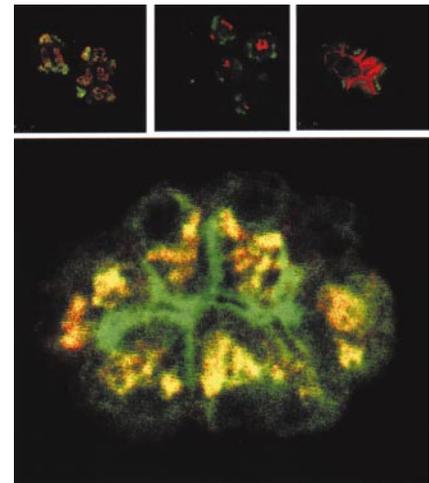


The mutant embryos lack a BM, but show normal differentiation of both VE and epiblast cells. The failure to then undergo polarization (for the outermost ring of epiblast cells) or apoptosis (for all other epiblast cells)

can be rescued by adding exogenous laminin, which forms a visible BM. Early addition of exogenous laminin to a heterozygote can even accelerate the normal formation of a BM and the processes of polarization and apoptosis. Thus, the BM may directly promote the survival of the CEE cells, while indirectly (by inducing CEE polarization) promoting the death of other cells.

Earlier evidence suggested that an apoptosis-inducing signal was generated by endodermal cells. Murray and Edgar establish that BM is an additional requirement for apoptosis, and their ability to accelerate apoptosis with exogenous laminin suggests that any endodermal signal is either present (but insufficient by itself) before the BM is usually formed, or rapidly induced by the BM.

Polarizing Calcium Waves

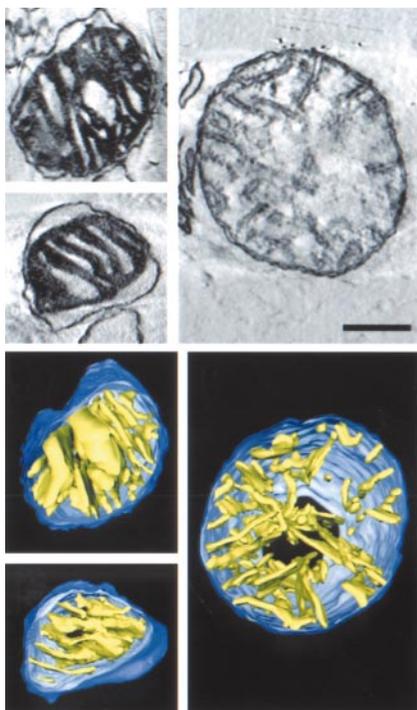


Calcium waves in secretory cells initiate at the apical pole before propagating along the cell periphery to the basal pole. On page 1101, Shin et al. report that the Sec6/8 complex, previously implicated in polarized secretion, is required for the correct functioning and localization of calcium signaling proteins.

In pancreatic acinar cells, Sec6/8 is present in a subapical domain that is similar to, but distinct from, the location of the Golgi apparatus. It colocalizes with a calcium-releasing receptor

for inositol-(1,4,5)-triphosphate (IP₃), and Sec8 coimmunoprecipitates with multiple calcium-signaling proteins. This linkage appears to be indirect, through actin, as the signaling proteins are lost from the Sec8 immunoprecipitate after addition of gelsolin, which cleaves actin filaments. An antibody to Sec8 abolishes calcium waves induced by carbachol, but not calcium waves induced by a nonmetabolizable IP₃. Before the response to carbachol addition is measured, the antibody is allowed to equilibrate for some time, so the relevant inhibition of Sec6/8 may be at the level of protein transport and localization, rather than a direct effect on calcium signaling activity.

Limited Damage to Apoptotic Mitochondria



On page 1027, von Ahsen et al. report that mitochondria that have released cytochrome *c* in response to apoptotic stimuli can nevertheless maintain active protein transport across their inner membrane. This

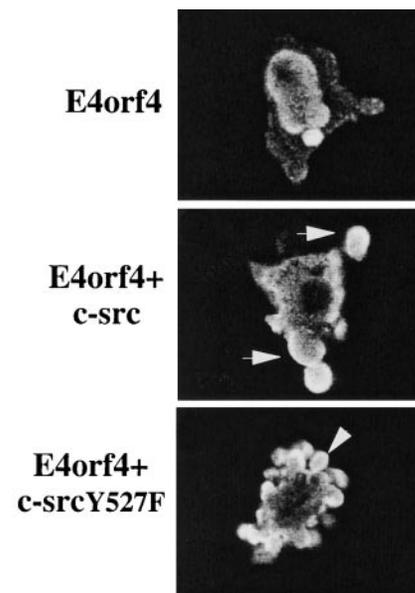
suggests that cyt *c* release is caused by an event specific to the outer mitochondrial membrane, whereas earlier models proposed that cyt *c* release started with the opening of the inner membrane's permeability transition (PT) pore, leading to matrix swelling and thus, indirectly, to mechanical rupture of the outer membrane.

Cyt *c* is lost, but protein transport is maintained under three conditions: in frog or human mitochondria incubated in buffer supplemented with the proapoptotic Bid or Bax; in HeLa mitochondria after cell permeabilization and addition of Bid; and in UV-irradiated or staurosporine-treated HeLa cells. But, in this last case, caspases must be inhibited to preserve the inner-membrane integrity. Normally, any released cyt *c* pairs with Apaf-1 to activate caspases and thus ensure the final destruction of the mitochondria, but recently discovered apoptosis-inhibitory proteins may short-circuit this process, perhaps even allowing a recovery of mitochondrial integrity in some situations.

Cytoplasmic Apoptosis

The adenovirus E4orf4 protein induces p53- and caspase-independent apoptosis. On page 1037, Lavoie et al. report that E4orf4 works by modulating Src tyrosine kinase activity. The resultant changes at cell adhesion and cortical sites turn on a cytoplasmic execution program that is independent of nuclear or mitochondrial events.

Soon after E4orf4 is first expressed, and long before loss of cell viability, cells round up and bleb. Lavoie et al. show that these cells still adhere to fibronectin, but no longer spread. Src coimmunoprecipitates with E4orf4, and activated Src promotes E4orf4-induced blebbing, whereas kinase-dead Src or an inhibitor of Src kinase activity inhibits the blebbing. This is surprising, given that Src kinases are better known for transducing an anti-apoptotic signal.



The effects of E4orf4 on Src activity are not simple. There is no change in Src tyrosine kinase activity *in vitro*. Perhaps a change in Src's binding partners is behind the E4orf4-induced decrease in phosphorylation of some Src substrates (such as FAK) and increase in phosphorylation of other substrates (such as cortactin). Underphosphorylated FAK may fail to transduce a cell-survival signal from focal adhesions, leading to the disorganization of these cell contacts. Cortactin, meanwhile, redistributes from the cytoplasm to the cortex after E4orf4 expression, and may participate in reorganizing actin into the contractile fibers that round up the cell. Disrupting actin with cytochalasin D inhibits E4orf4-induced blebbing and greatly decreases the formation of apoptotic nuclei. It will be important to determine how the peripheral events are transduced to cause the final execution phase of apoptosis. E4orf4 may help in this task and in determining the normal apoptogenic function (if any) of Src.

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