Secretion for cytokinesis

The actomyosin contractile ring involved in separating a dividing cell in two only gets so far. The job is finished, according to work from Adam Gromley, Stephen Doxsey (University of Massachusetts Medical Center, Worcester, MA), and colleagues, by a burst of secretory vesicle fusion.

Doxsey’s group was looking for a function for a vertebrate centrosomal protein called centriolin. A defect in cytokinesis was not what they expected to find when they knocked down centriolin function, but there it was. “We saw a thin wisp of cytoplasm retained between [daughter] cells,” says Doxsey.

In wild-type cells, this normally transient wisp harbored a ring of centriolin, which then recruited several components of the secretory pathway, including the exocyst. Later, SNARE proteins followed.

Unlike the actomyosin ring, the centriolin/SNARE ring did not constrict during cytokinesis. Instead, the authors saw, secretory vesicles from one of the daughter cells moved to the ring, piling up on that side. After accumulating briefly, the vesicles apparently fused in a rapid burst. The daughter cells then split apart on the vesicle side of the ring, leaving the cell on the opposite side with an intact lingering ring, similar to the bud scar of yeast. As in centriolin mutants, the cells remained linked when vesicle fusion was impaired.

The triggers for vesicle transport and for fusion are unknown. “It’s clearly highly regulated,” says Doxsey. “But what the cell is monitoring—DNA, centrosomes, or something else—is still unclear.” The group is especially keen to determine how the asymmetric vesicle secretion is generated. They hypothesize that differences in the centrioles—one daughter gets the original and the other a copy—might be involved.

Reference: Gromley, A., et al. 2005. Cell. 123:75–87.

Polymerase with a protein template

The Rev1 DNA polymerase has forsaken Watson and Crick. Instead of a complementary base, this polymerase uses a protein template, according to Deepak Nair, Aneel Aggarwal (Mount Sinai School of Medicine, New York, NY), and colleagues.

The findings, says Aggarwal, “explain the two mysteries of this polymerase: why it works so well with template G, and why it only puts C opposite it.” The group determined the crystal structure of yeast Rev1 in complex with template DNA and its favorite incoming nucleotide, dCTP. They found several features that distinguish Rev1 from standard eukaryotic polymerases.

First, Rev1 is its own template. An arginine residue within Rev1 acts like a surrogate template G by forming hydrogen bonds with the incoming C. Any other base results in steric hindrance and unfavorable electrostatic interactions. “The paradigm is that coding is provided by the DNA sequence,” says Aggarwal. “Here, the protein dictates what comes in.”

In fact, the incoming C initially does not even contact the template G, which the group found is rotated out of the DNA helix by Rev1. The correct template is ensured, however, by hydrogen bonding between this twisted G and a part of Rev1 called the G-loop. Bases other than G would create steric hindrance, although an empty sugar backbone would not, which is consistent with the known ability of Rev1 to add a C opposite an abasic site.

The twist of the G also explains how Rev1 is able to polymerize through damaged DNA containing N2-adducted Gs, as the N2 group is turned away from Rev1. These adducts are created by common carcinogens, including those in cigarette smoke. Defects in human Rev1 might be associated with increased stalling of replication at these adducts, resulting in DNA breaks and eventually leading to cancers.

Reference: Nair, D.T., et al. 2005. Science. 309:2219–2222.
Wnt for adhesion

Wnt signaling keeps a group of migrating cells together, say Florian Ulrich, Michael Krieg, Carl-Philipp Heisenberg (Max Planck Institute, Dresden, Germany), and colleagues. Wnt achieves this by promoting the recycling of adhesion molecules.

Signaling via Wnt11 is needed during vertebrate gastrulation, when the group of cells that will form the axial mesendoderm migrate en masse to the animal pole. Heisenberg’s group finds that these cells are uncoordinated and migrate in various directions in the absence of Wnt11.

The authors found that Wnt11 mutants adhered less tightly, both to each other and to matrix proteins. "The ability to cohere," says Heisenberg, "allows cells to align their [migratory] processes better. Loose clusters can project processes in all sorts of directions. Coherent ones can only put processes where there aren’t any cells in the way."

Adhesion in these cells is mediated by E-cadherin, which the authors show is properly recycled only in the presence of Wnt. The activation of endocytosis corrected the migratory problems of Wnt11 mutants, whereas loss of endocytosis weakened cell adhesion strengths. Wnt might induce endocytosis via its known ability to activate actomyosin contraction.

Though more surface cadherin should result from a block in endocytosis, Heisenberg thinks the total amount is less important than its dynamicity. "For cells to form cohesive clusters," he says, "they need to undergo a lot of junctional remodeling, which might be dependent on E-cadherin recycling."

Presenting lipid antigens

Immune cells inspect not just protein but also lipid antigens, thanks to presentation by the MHC relative CD1. Now, results from Peter van den Elzen, Michael Brenner (Harvard Medical School, Boston MA), and colleagues show that immune cells co-opt normal pathways of fat metabolism to deliver these lipid antigens to CD1.

Lipids circulate as part of large particles containing apolipoproteins such as ApoE. Cells that need more lipids secrete ApoE, which grabs onto fats and is then recaptured via receptor-mediated endocytosis. Brenner noticed that dendritic cells were dumping out much more ApoE than is required for their metabolic needs for fats.

But much of this ApoE is probably used to search for foreign lipids, according to the findings. The authors show that ApoE binds directly to lipid antigens and brings them into dendritic cells much more efficiently than does macropinosis (which dendritic cells use to engulf foreign peptides). Endocytosis "deposits the lipids in endosomal compartments," says Brenner, "right where CD1 is waiting. Then they come to the surface, where they can activate T cells."

In the absence of ApoE, dendritic cells required hours rather than minutes in contact with lipid antigens to activate T cells. This lag is precious lost time during which a pathogen might be rapidly multiplying. Blocking ApoE-dependent endocytosis might be beneficial, however, in preventing lipid-filled macrophages from initiating autoimmune disorders such as atherosclerosis.

Marks of death on nuclei

Mammalian red blood cells get rid of their nuclei as they mature. Now, Hideyuki Yoshida, Shigekazu Nagata (Osaka University Medical School, Osaka, Japan), and colleagues show that the extruded nuclei cover themselves with the marks of dying cells. As a result, macrophages engulf and degrade the nuclei, as they do apoptotic cells.

The mark of an apoptotic cell that calls in macrophages is phosphatidylserine (PtdSer). Macrophage receptors such as MFG-E8 initiate phagocytosis when they sense this phospholipid in the dying cell’s plasma membrane. Yoshida et al. found that PtdSer also appears on the plasma membrane surrounding an extruded nucleus. Masking PtdSer prevented macrophages from engulfing the nuclei.

PtdSer is normally retained in the inner leaflet of the plasma membrane by an ATP-dependent mechanism. But once separated from their cell body, the extruded nuclei were unable to generate their own ATP. PtdSer thus rapidly appeared on the outer surface of the membrane. Since ATP levels remain high in apoptotic cells, lone nuclei and dying cells must generate the PtdSer marks via different means.

Autoimmune problems in mice expressing a mutant MFG-E8 have been attributed to persistent apoptotic cells. But since at least tenfold more red blood cells than dying cells are generated per day, the unscavenged nuclei are probably the bigger issue.