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.