How to Make Two Different Adherens Junctions

Epithelial cells have an apical zonula adherens (ZA) to delineate the border between apical and basolateral membranes. The ZA forms by a poorly characterized process of cadherin and catenin clustering. On page 391, Hunter and Wieschaus find that a protein called Nullo is necessary for formation of a second set of adherence junctions, which appear transiently during fly embryo cellularization at the basal corners of the forming cells. Surprisingly, Nullo appears to act to prevent the clustering of junctional components.

Without Nullo, which is normally located at basal junctions, some cleavage furrows fail. In other furrows the apical ZA forms as usual, but basal Armadillo (Drosophila β-catenin) spreads apically. In contrast, excess Nullo has no effect on basal catenin localization, but selectively disrupts apical junctions: the catenin in lateral membranes stays fixed in place and no longer clusters to form an apical ZA.

Hunter and Wieschaus believe that formation of basal adherens junctions does not require clustering. Delivery of junctional components to the lateral membrane, which at the start of cellularization is only a small patch, assures initial localization. As long as the components do not drift as the lateral membrane extends downwards, a junction of sorts will be maintained and can differentiate lateral from apical membrane. Indeed, clustering of the components early in the process would be destructive. Components in opposite faces of a transient membrane infolding would be pulled together, and converted from an opposing to a parallel configuration. Nullo may be the protein that fixes junctional components in place and prevents this aberrant clustering. Nullo is degraded at mid-cellularization, thus allowing the subsequent clustering of lateral junctional components into an apical ZA.

Cytoskeleton Interactions

Microtubule and actomyosin cytoskeletons are often studied in isolation, but on page 361 Waterman-Storer et al. demonstrate that the two systems interact and remodel each other in Xenopus extracts, with possible implications for motility and cytokinesis. Waterman-Storer et al. use dual-wavelength and fluorescent speckle microscopy to examine the interactions of microtubules and actin. A jerking actin movement is seen only in the presence of microtubules, is correlated with microtubule movements, and is eliminated by inhibition of cytoplasmic dynein. The jerking movement probably results from a static link between actin and translocating microtubules. A slower gliding movement of actin along microtubules probably represents a motor linkage, and Waterman-Storer et al. identify myosin-V on microtubules as one possible effector. These interactions must be confirmed in living cells, but one possible role is in the initiation of cytokinesis. Waterman-Storer et al. hypothesize that microtubules translocating out from asters may sweep actin towards the center of the spindle, where the actin could coalesce into bundles that contract during cytokinesis.

Rev Rounds Up Microtubules

The Rev protein from HIV is a small, basic RNA-binding protein that helps shuttle unspliced or partially spliced viral RNAs out of the nucleus. Watts et al. find that Rev has another activity: the depolymerization of microtubules to form stable bilayered rings (page 349). Rev polymerizes in vitro into long tubes. Watts et al. found that the tubes could be depolymerized with decaglutamate, and wondered if COOH-terminal polyglutamate tracts on the exterior of microtubules might have the same effect. Addition of microtubules led to the formation of toroidal complexes visible by electron microscopy. Based on their mass and symmetry, the complexes appear to consist of two rings of 28, 30, or 32 tubulin subunits each, with a matched number of Rev units inside the tubulin rings. The outside of the rings correspond to the inside of microtubules, so dimers of Rev are probably helping to peel away two protofilaments of a microtubule to form the rings.

Ring formation is largely insensitive to changes in salt concentration, pH and temperature, suggesting that the driving force is not a simple charge interaction. Furthermore, part of Rev is similar to the tubulin-binding region of some kinesins, including the Kin I kinesins that are known to destabilize the ends of microtubules. If microtubule destabilization by Rev
can be demonstrated in cells, the search will be on for an effect on replication of the virus, which may benefit from disruption of either mitotic timing or cellular communication.

**Rad51 Recruited to Damage Sites**

On page 283, Tashiro et al. report that the recombinational repair protein Rad51 is recruited to sites of DNA damage. A similar recruitment was previously reported for the end-joining repair protein Mre11 after localized doses of soft x rays, but these workers reported that there was no Rad51 recruitment in this system.

Tashiro et al. introduce DNA breaks by labeling fibroblasts with BrdU and irradiating a small nuclear spot with ultraviolet radiation. Between 10 and 20 min later, Rad51 is recruited to a single spot that contains single-stranded DNA. Irradiating two spots close together results in two Rad51 accumulations close together; two irradiation spots far apart yield two Rad51 accumulations far apart. In both cases the many Rad51 foci that are normally scattered around replicating nuclei are now diminished in intensity.

Tashiro et al. use sequential incorporation of two different DNA labels to deduce that Rad51 is preferentially associated with replicated chromatin. This makes sense given the fact that Rad51 needs two copies of the DNA to carry out repair. Before replication, the two homologous chromosomes are generally separated in the nucleus, but after replication Rad51 can access two neighboring sister chromatids.

**Depolarization Activates PARP**

Poly (ADP-ribose) polymerase (PARP) is an abundant, conserved nuclear protein that modifies nuclear proteins responsible for DNA transcription, replication or repair. PARP is activated by DNA breaks, and its induction during apoptosis is associated with increased DNA repair. Homburg et al. report on page 293 that PARP is also induced in rat cortical neurons during neuronal activity. They suggest that PARP may be involved in helping active neurons to survive.

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PA RP and its substrates, including topoisomerase I, are poly ADP-ribosylated following trains of action potentials. Membrane depolarization does not turn on PARP by causing DNA breaks. Rather the PARP is directly activated by calcium, which is released from inositol-1,4,5-triphosphate (IP$_3$)-gated perinuclear stores. The steps from activated PARP to changes in neuron survival or function are yet to be determined.