Research Roundup

Sudden bacterial death syndrome

Just before they lyse, bacteria infected with lambda phage have no idea what is about to hit them, according to Angelika Gründling, Mike Manson, and Ry Young of Texas A&M University (College Station, TX). Gründling watched bugs spin around their single tethered flagella as an indicator that their membranes were still energized. The spinning stopped abruptly a few seconds before the cells lysed, thus contradicting the assumptions of one model for lysis timing.

That model was born from the observation that membrane energy poisons induce lysis prematurely. Energy poisons, some suggested, were merely accelerating a normal process of gradual proton leakage. In this model, phage-produced holin proteins would cause the leak, until a critical reduction in proton motive force (pmf) across the membrane triggered any remaining holins to form into pores and finish off the bug.

Gründling’s new observations suggest that there may indeed be a critical level of pmf, but that there is no significant leakage early on that would gradually lead the cell to this critical level. Instead, holins increase in concentration until a pore spontaneously precipitates in the membrane. The catastrophic proton leak that results initiates the formation of many other pores, leading to the bug’s rapid demise.

This triggering mechanism leads not only to rapid and complete lysis, but may also reduce competition from other phage. As new phage attach to a cell, they cause a transient drop in pmf. This drop may trigger holin aggregation, thus killing the cell before the invader has time to start competing with the resident phage.

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Bicoid mRNA is an anterior determinant for fly development that is produced in nurse cells before moving into the neighboring oocyte. In the simplest models, the mRNA was thought to use polarized microtubules to move to the anterior of the fly oocyte.

Now William Theurkauf and colleagues (University of Massachusetts Medical School, Worcester, MA) show that such a simple model will not work, because the mesh of oocyte microtubules is largely unpolarized. Instead, bicoid mRNA picks up transport factors in the nurse cells, before entering the oocyte and using those factors to mediate anterior localization, possibly on a polarized subset of microtubules. Without the factors, the mRNA can move on microtubules but its movement is undirected.

Byeong Cha in the Theurkauf lab began his experiments by injecting in vitro transcribed fluorescent bicoid mRNA into the center of oocytes. He found that the mRNA moved to whatever region of the oocyte cortex that was closest, including anterior and lateral areas. Bicoid mRNA enters oocytes through large ring canals in the anterior, so Theurkauf says “our initial bias was that it was just being trapped” as it entered.

But further experiments showed that fly oocytes do a more complete job than simple trapping. Cha found that injection of the fluorescent mRNA into nurse cells, followed by recovery of that injected mRNA and reinjection into the center of oocytes, resulted in full anterior localization. Theurkauf and Cha suggest that factors picked up by bicoid in the nurse cell allow the resultant complex to move along a subset of polarized microtubules that are hidden within the bulk of the non-polarized microtubule array.

Based on mutant analysis, one protein that must be transferred from nurse cells is Exuperantia. Theurkauf is now searching for other such proteins. One approach, pursued in collaboration with Paul Macdonald’s group (University of Texas at Austin), involves defining the cis elements that bicoid mRNA needs to pick up its factors in the nurse cell, and then using these sequences to fish out the binding proteins.

Formin a link to microtubules

A protein more often linked to effects on the actin cytoskeleton has been implicated in the polar stabilization of microtubules. Gregg Gundersen (Columbia University, New York, NY) and colleagues show that mDia1 and mDia2, two forms related to fly Diaphanous and budding yeast Bni1, can induce the formation of stable detyrosinated microtubules that may help polarize cells. Gundersen had earlier found that the one effector whose Rho interactions correlated perfectly with stable microtubule induction was mDia.

Expression of mDia2 induced formation of stable capped microtubules, and mDia2 co-sedimented with taxol-stabilized microtubules. Gundersen suspects that Dia is not itself capping microtubules, but is a scaffold for other capping proteins. Once capped, stable microtubules are gradually detyrosinated, and this modification, says Gundersen, may bias transport to the leading edge of the cell.


Transcription gets a licence

Ubiquitination of some transcriptional activators may be necessary both to make them functional activators and to signal their destruction, according to a recent study from William Tansey (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and colleagues. Tansey suggests that ubiquitination is a temporary licence for transcription that preprograms destruction into the very activation process, thus keeping activators under tight control.

Tansey has been trying to unite the proteolysis and transcription fields ever since he noticed that the determinants of ubiquitination and transcription activation were often overlapping or even inseparable. Selling this idea took some time. “It’s counterintuitive,” he says. “You have proteins that are destroyed not when they are no longer needed but when they are at their most active. People have some trouble with this idea.”

In the new experiments, Tansey found that a specific ubiquitin ligase was necessary for the VP16 transcriptional activation domain to activate transcription, and that this requirement could be circumvented by fusing a single ubiquitin (which does not signal destruction) to the activator.

One thing that ubiquitin may be doing, not necessarily related to actual destruction, is recruiting the proteasome. Stephen Johnson and colleagues (University of Texas Southwestern Medical Center, Dallas, TX) recently reported that the 19S regulatory particle of the proteasome is required for efficient transcription elongation, perhaps in some kind of chaperone role.

As for destruction, Tansey does not know how tightly it is coupled with transcription. “Are transcription factors truly a disposable thing—a one-shot deal?,” he asks. Further studies are needed to answer this question and to determine just how many different transcription factors are licensed by this mechanism.


Touching cells transform

The cytoskeleton of an epithelial cell is converted to a fibroblast-like morphology upon contact with a fibroblast. So say Edward Bonder (Rutgers University, Newark, NJ) and colleagues, who present what they hope will be the beginning of a lengthy study of the interactions between heterotypic cell types.

The main conclusion so far, says Bonder, is that “even though you have nontypical contacts there is still recognition,” leading to a structural transformation. Such heterotypic contacts occur frequently during development, such as when fibroblast-like neural crest cells migrate over the epithelial layer of the neural tube, or when fibroblasts directly contact an overlying epithelium before laying down an intervening barrier of basal lamina.

The initial cytoskeletal organization is very different in fibroblasts and epithelial cells. Fibroblasts have actin-filament bundles pointed out to the cell’s leading edge and protruding into lamellae, whereas epithelial cells have an arc of actin running parallel to the leading edge. Upon contact of the two cell types, little change is seen in the fibroblast, but the epithelial cell converts to a more fibroblast-like organization.

Despite expressing different cadherins, the two cell types appear to form adhesion complexes that contain β-catenin. The transient association is terminated when the fibroblast wheels on its axis and marches off in another direction. Although such interactions may serve primarily to keep two tissue layers separate, they may also be essential to cement that separation by inducing the formation of more permanent barriers such as the basal lamina.