Rafting across polarized cells

For raft proteins, direct delivery is not the travel route of choice, according to Roman Polishchuk, Jennifer Lippincott-Schwartz (NIH, Bethesda, MD), and colleagues. They find that, contrary to earlier evidence, glycosyl phosphatidylinositol–anchored raft proteins make a detour to the basolateral membrane of polarized MDCK cells before crossing the cell to their final destination in the apical membrane.

Direct delivery had been plausible, as sorting of GPI-anchored proteins into rafts, and thus away from basolateral proteins, occurs in the trans-Golgi network (TGN) before either departs for plasma membranes. Few or no apical proteins were ever seen in the basolateral membrane. And finally, transport across the cell seemed unlikely: the main method of departure, clathrin-mediated endocytosis, did not transport rafts.

Lippincott-Schwartz recently found that rafts are continually endocytosed by a nonclathrin pathway, overcoming the clathrin objection. And at the basolateral surface, she says, “all of these biochemical experiments would have potentially missed a transient appearance.”

The NIH team found that GPI-YFP and basolateral proteins were segregated as they left the TGN, but nevertheless shared the same tube-shaped carriers. (Others have claimed one cargo type per carrier, but did not prebleach to reduce background.) Those carriers must be paying a visit to the basolateral membrane, as both proteins got stuck intracellularly when fusion to the basolateral membrane was prevented with tannic acid, a cell-impermeable fixative. When tannic acid was applied to the apical side, only the apical cargo was intracellular, presumably after making its transient basolateral stop.

Evidence for this trip across the cell came from a tracer and antibody, which were applied to the basolateral side but travelled with GPI-YFP apically.

A nonraft apical protein went straight to the apical side of the cell. Why should the cell bother with the more circuitous route taken by GPI-YFP?

Myc conquers all

More Myc makes you stronger, according to Eduardo Moreno and Konrad Basler (Universität Zürich, Switzerland) and Claire de la Cova, Laura Johnston (Columbia University, New York, NY), and colleagues. They find that cell clones producing more dMyc overproliferate and outcompete their neighbors, with the neighbors dying off by apoptosis.

The concept of cell competition is not a new one. Fly cells mutant for various ribosomal proteins are known to suffer from competition-based elimination by wild-type cells. But now the two groups show that competition occurs in response to varying levels of the growth promoter dMyc, even when the “weaker” of the two cell groups are expressing wild-type levels of dMyc.

The Swiss group found that weaker cells could be rescued by either stimulating their rate of endocytosis (with an activated Rab5) or turning on genes downstream of the survival factor Dpp: outcompeted cells also had reduced expression of Dpp targets. Basler suggests weaker cells lose out because their lower metabolism is not driving a sufficiently robust endocytic cycle, leaving them with insufficient endocytosed survival factors.

But when it comes to Dpp, says Johnston, “we haven’t been able to find any evidence.” This colors her thinking of what the more competitive cells are doing. “We think they are not just sopping up nutritional and growth factors,” she says. “We think there’s a signal being sent” between cells of different metabolic capabilities.

The genesis and identity of such a signal remain unknown, but Johnston thinks it will connect competition to regulation of organ size. Her team found that fly wing discs repressed for apoptosis showed much greater variability in size than normal. They are currently testing whether this effect is based on competition or some other apoptosis-related phenomenon.

Basler remains skeptical of a connection to organ size. “We look at [competition] only as an artificial phenotype—at best it is an elimination plan for weak cells,” he says. “It’s more like a policeman—present but normally not needed.” Where it might be important, he says, is in a stem cell niche where every cell must be a high performer. Incipient cancers may take advantage of this biology by expanding their domain at the expense of outcompeted normal cells.

References: de la Cova, C., et al. 2004. *Cell.* 117:107–116.

Moreno, E., and K. Basler. 2004. *Cell.* 117:117–129.
Telomeres together

An enzyme dissolves a unique pairing connection between telomeres, according to Jasmin Dynek and Susan Smith (New York University, New York, NY).

Overexpression of the enzyme, called tankyrase 1, is known to extend telomere length—it makes TRF1 fall off telomeres so that telomerase can gain access. So presumably too little tankyrase 1 will have the opposite effect, and simply shorten telomeres.

Or not. Dynek and Smith now find that cells lacking tankyrase 1 have a completely unexpected phenotype. They arrest in mitosis when their telomeres, though not covalently linked, nevertheless fail to separate. Tankyrase 1 is a poly(ADP-ribose) polymerase that dissociates TRF1 from telomeres based on the excess negative charge of all those ADP-ribose groups on TRF1. Whether it is TRF1 or another protein that is the relevant target for dissolving telomere pairing is unknown.

Telomere pairing is known to help chromosomes pair during meiosis, and may help damaged telomeres to repair each other by homologous recombination. It appears that the tankyrase mechanism is used especially for regulating telomere pairing, either instead of or in addition to the separase–cohesin system used on chromosome arms. Smith is now interested in how this pairing is regulated during the cell cycle.

Reference: Dynek, J., and S. Smith. 2004. Science. 304:97–100.

Being intolerant of tumors

Using the immune system to attack cancers is a good idea, but it may only work if Toll-like receptors (TLRs) are activated to break tolerance, says Yiping Yang (Duke University, Durham, NC), Drew Pardoll (Johns Hopkins, Baltimore, MD), and colleagues.

The favored method for delivering cancer immunotherapy has been dendritic cells: they are the central antigen-presenting cells for generating T cell responses; they can be grown in vitro in large quantities; and their use in mice with cancer led to some spectacular results. “That led to a whole wave of excitement,” says Pardoll. “But it hasn’t really panned out.”

Perhaps the biggest problem is tolerance. Most cancer immunotherapy focuses not on tumor-specific antigens, which vary too much because each tumor has different mutations, but on self-antigens that are overexpressed in the tumor. Although some collateral damage of normal self-tissue is alright for many tissues, the real problem is getting the immune response off the ground when it is challenged with a self-antigen.

Now Yang et al. show that viral but not dendritic cell vectors can do the job. Only the viral vectors can suppress tolerance to a cancer self-antigen in a mouse model, thus leading to increased survival. Ligands that activate TLRs also do the trick, and may be more promising in humans, as humans generally mount antibody responses that neutralize viral vectors.

Reference: Yang, Y., et al. 2004. Nat. Immunol. 10.1038/ni1059.

Get out! And don’t come back!

An active mechanism keeps vesicles that are departing the ER from turning round and fusing back to it, say Faustin Kamena and Anne Spang (Max Planck, Tübingen, Germany).

Directionality is a challenge for vesicle traffic. Although vesicles traveling from ER to Golgi and from Golgi to ER have very different job descriptions, recycling of transport proteins ensures that the compositions of the two vesicle types are similar if not identical. The two vesicle types start off with different coats (COPII for ER to Golgi and COPI for Golgi to ER) but shed them soon after departure.

Kamena and Spang figured that vesicles departing from the ER might be prevented from doing a U-turn by the same ER machinery that welcomes fusion of Golgi-derived vesicles. Sure enough, a mutant of the ER protein Tip20p allowed COPII vesicles, recently departed from the ER, to fuse back to the ER.

The researchers were lucky that they tested two different Tip20p mutants. Only one of those mutants (tip20–8, and not tip20–5 or any of the other ER fusion mutants) showed the U-turn phenotype. Now, the team can look for suppressor mutations that rescue tip20–8 but not tip20–5, and isolate proteins that bind only one of the two mutant proteins. Either approach may give clues about what, exactly, is sensed as different about a departing versus an arriving vesicle.

Reference: Kamena, F., and A. Spang. 2004. Science. 304:286–289.