In Brief

Glycans Not GPIs

Glycosyl-phosphatidylinositol (GPI)-anchored proteins are found predominantly at the apical surface of polarized epithelial cells. As the GPI anchor has a natural affinity for sphingolipid–cholesterol rafts, it has been widely assumed that this association represents the relevant sorting event responsible for transport to the apical domain.

Benting et al. (page 313) contradict this assumption and report that the sorting is mediated by N-glycans. They use rat growth hormone (rGH) as a test protein, and find that 60% of a GPI-anchored version is sorted to the basolateral side. Association with rafts does not necessarily result in apical sorting, as the protein is raft associated on both apical and basolateral sides. With the addition of one or two sites for N-glycosylation to the GPI-anchored rGH, the distribution of the protein shifts to 60% apical.

The new rule for protein sorting may be that there are no absolute rules. "Many of the sorting events we are looking at are much more plastic than we thought," says senior author Kai Simons. "It's not all or none." Simons does not, however, think that the idea of raft involvement in apical sorting is dead. He is now searching for lectins that may link glycosylated proteins to rafts.

Cytoskeletal Regulation by Ral and JNK

Activation of Ras by receptor tyrosine kinases leads to the activation of Raf kinases, phosphoinositide 3-kinase, and Ral-specific guanine nucleotide exchange factors (Ral-GEFs). On page 361, Sawamoto et al. show that Ral regulates cytoskeletal changes through the JNK (Jun kinase) pathway and thus may be involved in many cell shape changes after receptor activation.

Sawamoto et al. isolate a *Drosophila* Ral (*DRal*) gene using degenerate PCR, and express a dominant-negative version of the protein in the fly wing. Cells that normally produce a single hair now produce multiple hairs, many of them short, forked, curved, or twisted. The aberrant hairs emanate from multiple actin bundles of irregular morphology. Expression of mutant DRal in bristle cells leads to disruption of actin structures and loss of bristles.

Mutation of JNK suppresses the bristle phenotype, suggesting that wild-type DRal functions by negatively regulating the JNK pathway. Consistent with this, dorsal closure in the embryo is disrupted either by loss of JNK or expression of a constitutive DRal protein. In tissue culture cells, constitutive DRal reduces the level of activating phosphorylations on JNK. The pathway from JNK to the cytoskeleton can now be studied by screening for fly mutants that enhance or suppress the hair or bristle phenotypes.

Stabilizing Kinetochore Microtubules

Microtubules that reach from the spindle poles to the kinetochore are unusually stable. From attachment and detachment experiments, that stability appears to be conferred by the kinetochore. Zeng et al. (page 415) isolate Slk19p, a *Saccharomyces cerevisiae* kinetochore protein that may have the necessary stabilizing activity.

SLK19 was isolated based on synthetic lethality with a deletion in *KAR3*, which encodes a motor protein. Kar3p resides at the spindle poles and destabilizes microtubules, so in a simple world the inactivation of a stabilizing protein (Slk19p) would suppress not exacerbate a Kar3p defect.

Whatever the explanation for the lethality, it allows Zeng et al. to study the regulated loss of viability using a *kar3^{ts}/slk19Δ* strain. If a spindle has already been constructed, shifting this strain to the non-permissive temperature induces a rapid collapse of the spindle to yield a monopolar microtubule array, indicating that Kar3p and Slk19p work cooperatively to stabilize the bipolar spindle.

Zeng et al. localize Slk19p to the centromere first by immunofluorescence and then by cross-linking and immunoprecipitation. During anaphase some Slk19p is left in the midzone of the spindle suggesting that Slk19p may help stabilize the ends of midzone microtubules. These microtubules must lengthen extensively during anaphase B in budding yeast. Any proposed microtubule-stabilizing role must now be confirmed by testing whether Slk19p interacts with or influences the stability of microtubules in vitro.

Membrane Proliferation

As pancreatic cells or hepatocytes develop, or as B cells mature, a huge proliferation of membranes devoted to secretion is induced. A similar proliferation is seen in yeast upon the expression of p180, a protein that binds ribosomes to the endoplasmic reticulum (ER). On page 273, Becker et al. show that the induced membrane is capable of functional secretion, and that p180 turns on what appears to be the entire secretory system.

The p180 protein can be divided into three domains. The NH_2-terminal membrane anchor is all that is needed for membrane induction. Addition of the COOH-terminal coiled-coil domain adds a characteristic spacing between membrane bilayers, thus distinguishing the membranes from karmellae. (Karmellae are membranes induced by the overexpression of a number of ER proteins, but they have not been seen under normal conditions.) The central ribosome-binding domain of p180 converts the smooth membrane to a rough ER, and induces the production of secretory pathway proteins that function in the ER and Golgi, and in fusion at the plasma membrane.

Secretion compromised by either mutation or the overwhelming production of a foreign protein can be restored by expression of p180. Thus, the yeast system is valid for studying how increased secretory capacity is induced. This process is entirely mysterious. It is not clear, for example, if p180 is induced in pancreatic or B cells, or if this putative induction is necessary for increased secretion. The p180 pathway can now be studied by isolating yeast mutants

that cannot turn on secretory genes in response to p180 expression.

Cadherins in Adhesion and Motility

On page 501, Dufour et al. suggest that cross talk between cell–matrix and cell–cell adhesion systems explains at least some of the differences in how cells expressing different cadherins respond to the same extracellular environment.

In vitro both type I cadherins (such as N-cadherin) and type II cadherins (such as cadherin-7) mediate cell–cell adhesion. But in vivo type I cadherins are often associated with cohesive tissues and type II cadherins with motile cells. Neural crest cells (NCCs), for example, express N-cadherin until they migrate out of the neural tube as cadherin-7–expressing cells, only to resume N-cadherin expression when they reach their targets.

Dufour et al. test transfected cells on fibronectin, one of the substrates present when NCCs are migrating. Cells expressing cadherin-7 disperse over a greater area and move faster than cells expressing N-cadherin. The cadherin-7 cells have fewer cell–cell contacts, although the contacts are restored if cell adhesion to fibronectin is inhibited. Thus, the cell–matrix interaction can inhibit the cell–cell interaction. In contrast, if cells expressing N-cadherin are first allowed to cluster, they subsequently show little FAK phosphorylation when plated on fibronectin. This suggests that the cadherin system can dampen the response to cell–matrix interaction.

In vivo the consequences are clear. Cells expressing N-cadherin remain clustered after injection near the neural tube. Cells expressing cadherin-7 slowly disaggregate and migrate, although they do not then cluster at the normal NCC target sites.

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