In Brief

Building, Maintaining, and Using the Golgi

Using the ER to Build the Golgi

On page 69, Rossanese et al. suggest that the Golgi is an outgrowth of the endoplasmic reticulum (ER), and that this property can explain the divergent organization of the Golgi in two yeast species.

The context for the paper involves two competing theories of Golgi organization. Both implicate transport vesicles in the stable compartments model, the vesicles carry out anterograde transport of secretory cargo between Golgi cisternae of constant composition; in the cisternal maturation model, the vesicles recycle Golgi components to ever earlier cisternae as the cisternae mature and move through the stack. The latter model involves bulk transport of components by maturation of the Golgi stacks.

If the cisternae are progressing and maturing, new cisternae must be created. Rossanese et al. propose that the cisternae are created near transitional ER (tER) sites, where vesicles bud from the ER. In *Saccharomyces cerevisiae*, a species with a dispersed Golgi, they find that proteins defining the tER are distributed throughout the cell, as are Golgi cisternae. In the yeast *Pichia pastoris*, however, there is a small number of organized Golgi stacks, which are closely apposed to discrete tER sites. Mammalian cells also contain discrete tER sites, but they may lose the tER–Golgi association because of microtubule-dependent transport of the Golgi elements.

“We’ve shown a correlation,” says senior author Benjamin Glick. “What we need to do now is to show a causation by identifying a *Pichia* gene that controls transitional ER formation, and then knocking out its function.”

Tethering the Golgi

Many organelles are positioned by the microtubule cytoskeleton. On page 83, Infante et al. report that GMAP-210 has the characteristics of a linker between the cis-Golgi and microtubules. GMAP-210, previously identified as an autoantigen, is now known to interact with the cis-Golgi network to determine its localization. In the presence of excess GMAP-210, the Golgi is enlarged, and microtubules nucleate from the Golgi. It is not clear if the microtubules are driving an unfolding of Golgi membranes or actively increasing the amount of membrane in the organelle.

When expressed in vivo, the amino terminus localizes to the cis-Golgi, whereas the carboxyl terminus localizes to the centrosome, and induces the dispersion of the pericentriolar material. GMAP-210 may link the cis-Golgi to the minus ends of microtubules that have been released from, but remain close to, the centrosome.

Sorting Rafts

The sorting determinants for apical transmembrane proteins have remained elusive; the only obvious feature for some of these proteins is the involvement of glycolipid- and cholesterol-enriched membranes (GEMs; also known as rafts). One protein that could potentially associate with rafts thanks to its intrinsic properties is MAL, a protein that is so hydrophobic it acts more like a lipid. On page 141, Puertollano et al. report that MAL may act as an adapter that may help shepherd some apical proteins into rafts. They also identify MAL as the first protein known to be necessary for apical transport of the influenza HA protein in rafts.

MAL overexpression causes a massive de novo formation of vesicles. In the current study, Puertollano et al. reduce the level of MAL with antisense oligonucleotides. HA incorporation in rafts is reduced, and a significant percentage of the HA is missorted to the basolateral side of the cell.

Some HA enters rafts even in cells that express no MAL, so it seems that HA’s access to rafts is not entirely MAL-dependent. As MAL is present only in rafts, and is associated with HA (by coimmunoprecipitation) even in conditions in which most or all rafts have dispersed, in addition to its proposed role in raft vesiculization, MAL may be stabilizing HA’s association with rafts and acting as a sorting receptor for apical transport.

Mechanotransduction in Cartilage

Articular cartilage protects the ends of long bones from compression forces, but it is also maintained by those forces. Chondrocytes in the cartilage respond to mechanical pressure by producing more proteoglycan and less metalloproteinase. Donald Salter’s group has characterized a number of components in this pathway, and on page 183 they add interleukin 4 (IL-4) to the list. This discovery splits what had been presumed to be a linear pathway into at least two signal transduction cascades.

Millward-Sadler et al. stimulate chondrocytes in vitro with a cyclical substrate-induced strain. Although they use changes in gas pressure above the culture dish as the direct stimulus, it is the bending of the plastic culture dish that provokes a response (a glass dish does not work). This causes the cells to either depolarize (after stimulation at 0.104 Hz) or hyperpolarize (at 0.33 Hz). Walking, which normally strengthens cartilage, stimulates at ~0.9 Hz, and presumably causes changes similar to 0.33-Hz stimulation.

Conditioned media from hyperpolarized cells can hyperpolarize naïve chondrocytes. This can be mimicked by IL-4 and blocked by IL-4 antibodies. Chondrocytes from IL-4 knockout mice do not hyperpolarize in response to 0.33-Hz stimulation.
Initially, the chondrocytes may sense mechanical stimulation through integrins. This signal is somehow transferred through a tyrosine kinase, and leads rapidly to release of pre-formed IL-4. In turn, IL-4 switches on phospholipase C and protein kinase C, and opens small conductance potassium channels.

Millward-Sadler et al. use the hyperpolarization as a read-out for pathway activation, but they are uncertain if hyperpolarization participates in the strengthening of cartilage. “We’re not quite sure what this potassium efflux means,” says Salter. “We already have two pathways activated that are known to affect gene transcription.” The cartilage defects seen in osteoarthritis could be caused by changes in the components of these pathways, especially the documented alterations in integrin and integrin ligand expression.

**Linking the Kinetochore to the Spindle**

The budding yeast centromere consists of a compact 125 bp of DNA, with four important protein components (the members of the CBF3 complex) binding to the 25-bp CDEIII unit. However, CBF3 is not sufficient for the attachment of chromosomes to microtubules in vitro. Hyland et al. (page 15) report that Ctf19p and probably some associated proteins are important in binding CBF3 and centromere DNA to spindle components.

There are many chromosome transmission fidelity (ctf) mutants, and they all lose chromosomes. Hyland et al. select two ctf19 mutants for special attention because the mutants die when a CBF3 component is overexpressed; these mutants also interact genetically with all the other CBF3 components.

Ctf19p can be chemically cross-linked to centromere DNA (probably indirectly), and extracts from ctf19 cells show defects in a minichromosome–microtubule binding assay. But cells deleted for CTF19 are alive. Residual microtubule-binding activity may come from proteins that normally form a complex with Ctf19p, as Ctf19p sediments at 20S with other proteins. At least some of these proteins are not CBF3 components, as shown by Johannes Lechner and colleagues in a paper in press at *Genes and Development*.

Ctf19p localizes to the spindle pole body region. This result is not altogether surprising, as budding yeast centromeres cluster at the spindle pole in interphase cells. During mitosis, however, centromeres move towards the middle of the spindle. A centromeric subset of Ctf19p may be undetectable by fluorescence (there have been problems detecting CBF3 components with similar protocols), or Ctf19p may have a specialized function at the spindle pole. For example, after chromosomes move to the poles in anaphase A, Ctf19p could help tether chromosomes to the spindle pole during the pole separation of anaphase B.