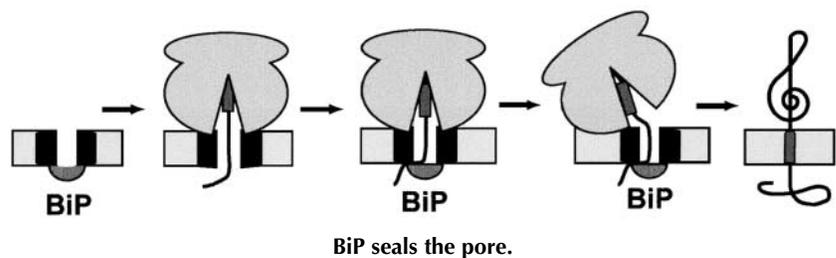


It's a chaperone . . . it's a gatekeeper . . . it's BiP

Imagine inserting a piece of string into an inflated balloon without letting any of the air out. An analogous problem confronts cells in the production of integral membrane-spanning proteins, which must be inserted partway into the ER without letting the ER calcium stores leak into the cytoplasm. On page 261, Haigh and Johnson show that the ER luminal chaperone protein BiP controls one end of a double door system at the translocon, the pore through which nascent proteins enter the ER, and propose a model to explain how the cell accomplishes the daunting task of regulating this double door.

On the cytoplasmic side of the ER membrane, the ribosome binds to the translocon to seal it while translocating a nascent polypeptide into the ER lumen. But the ribosome must break this seal to allow a cytoplasmic domain to extend into the cytoplasm. Some mechanism must exist to seal the luminal side of the translocon when this happens.

Using a clever fluorescence quenching assay in isolated microsomes, the authors found that BiP is required to seal the



luminal side of the translocon pore at certain stages during the integration of a transmembrane protein, and that BiP can do this even in the absence of other luminal proteins. This activity of BiP requires ATP hydrolysis, suggesting that the protein may use similar mechanisms in its diverse duties as a chaperone and pore sealer.

The authors propose that translation of a transmembrane sequence causes a BiP-mediated closure of the luminal side of a translocon, allowing the ribosomal seal to be opened on the cytoplasmic side without breaching the ER membrane. Future studies will focus on identifying the domains of BiP responsible for sealing the translocon, and determining how BiP and the ribosome coordinate their actions across the ER membrane. ■

Sorting out secretion

Proteins that are destined to be secreted from a cell undergo a multistep sorting and transport process, but until recently a critical portion of the secretory pathway—the transport of cargo from the Golgi apparatus—has been difficult to dissect. On page 271, Harsay and Schekman show that one population

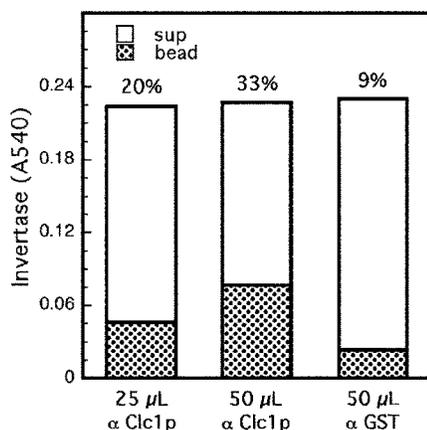
of yeast secretory proteins apparently takes a detour through an endosomal compartment on its way from the Golgi apparatus to the cell surface. Although some types of mammalian cells appear to use a secretory pathway involving endosomes, the new work is the first demonstration of such a system in yeast, a model system that should help define additional steps in this poorly understood process.

Previous work demonstrated that yeast *sec6* mutants exhibit a post-Golgi secretion defect that causes the accumulation of two populations of secretory vesicles, distinguished by their differing buoyant densities and cargos. In this genetic background, Harsay and Schekman found that mutations in VPS genes, affecting transport to an endosomal compartment, also block protein sorting to the high-density secretory vesicles. In these double mutants, proteins normally targeted to the high-density vesicles are instead sorted into the

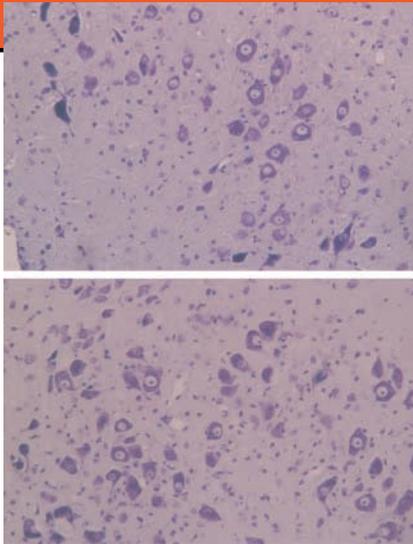
light-density vesicles.

This is the first time newly synthesized soluble exocytic proteins have been shown to move through an endosomal compartment on the way to being secreted. While it is still unclear why the cell would have two separate secretion pathways, one possibility is that the less abundant high-density vesicles, which are enriched in enzymes for particular metabolic processes, may allow rapid responses to environmental changes without causing membrane expansion.

The cell's ability to reroute secretory proteins from one pathway to another may also explain why it has been difficult to isolate mutants defective in the post-Golgi portion of the secretion process: both pathways would have to be shut down simultaneously to block secretion. Using mutations that block the high-density vesicle pathway, the authors are now trying to identify genes involved in the light-density vesicle pathway. ■



Going in or out? Isolated clathrin vesicles contain outward-bound invertase.



Motoneurons are lost in injured mice lacking Stat-3 (top) relative to wild type (bottom).

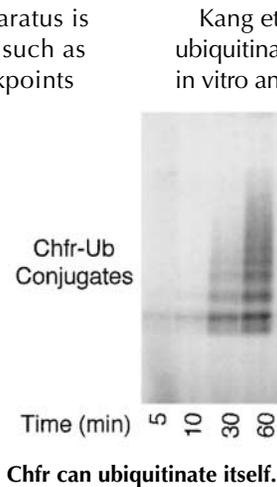
Survival gets more complicated

CNTF and related proteins have been shown to promote the survival of motoneurons in two different settings: during embryonic development and after nerve injury in adults. Because the signal and the response are apparently the same in the two situations, a simple explanation is that embryonic and adult neurons use the same signaling pathways to promote survival. But on page 287, Schweizer et al. show that the simplest hypothesis is not always the correct one.

Earlier work had shown that CNTF activates the LIF receptor to promote neuronal survival, and that Stat-3 is an important downstream effector of the LIF receptor. The authors used Cre-mediated recombination in a transgenic mouse system to delete the Stat-3 gene in neurons of the facial nucleus and spinal cord. Surprisingly, the mice develop normally, with no neuronal defects. When the facial nerves of adult mice are damaged, however, they show a dramatic loss of motoneurons compared with wild-type controls. Therefore, Stat-3-mediated signaling appears to be essential for motoneuron survival after injury, but dispensable during embryonic development, suggesting that neurons use different pathways at different times to transduce the same signal. ■

Finding degradation at the checkpoint

When the mitotic apparatus is disturbed by agents such as nocodazole, mitotic checkpoints delay mitosis to avert disaster. Defects in the anaphase-onset checkpoint are rarely seen in cancers, but defects in the G₂/M (prophase) checkpoint are present in multiple tumor cell lines. Now, on page 249, Kang et al. report that the recently identified G₂/M checkpoint protein Chfr is a ubiquitin ligase that promotes the degradation of a regulatory protein. In addition to describing a novel cell cycle checkpoint mechanism, the authors have developed an experimental system that should be useful in future studies on Chfr.



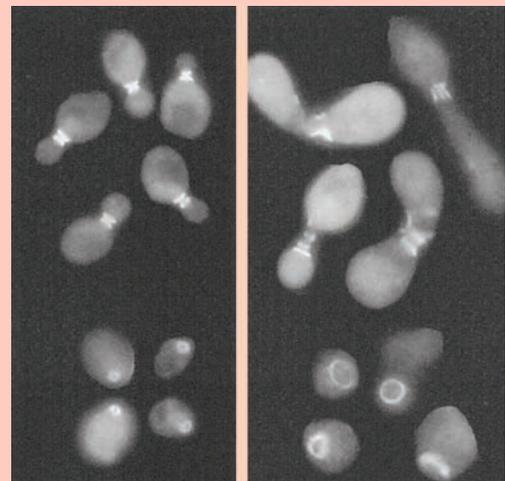
Kang et al. found that Chfr can ubiquitinate itself and other proteins in vitro and in vivo. In a *Xenopus* egg extract system, recombinant Chfr delays the activation of the kinase Cdc2 during the G₂/M transition. Using this system, the authors determined that Chfr ubiquitinates polo-like kinase 1 (Plk1), leading to its degradation. Plk1 ordinarily triggers a cascade that leads to Cdc2 dephosphorylation and mitosis, so its degradation leaves Cdc2 phosphorylated and halts progression into mitosis. Future work on the cell-free system should help uncover the mechanisms that link Chfr activity to mitotic stresses, such as microtubule defects. ■

A GTPase that goes both ways

Cellular GTPases are generally placed into one of two categories: signaling switches such as the oncogene ras or assembly factors such as the translation elongation factor EF-Tu (EF-1a in eukaryotes). But on page 315, Gladfelter et al. show that yeast Cdc42p, a well-characterized ras-like switch, can also behave as an EF-Tu-like assembly factor. The work is the first description of a GTPase changing hats in this way, and it suggests that GTPases may defy strict categorization.

Like all ras-like GTPases, Cdc42p activates downstream effectors when bound to GTP, and stops signaling after it hydrolyzes the GTP to GDP.

In an effort to study Cdc42p activity during the assembly of the septin ring, a structure important in yeast budding, the authors characterized two *cdc42* mutants with defects in septin ring assembly. Surprisingly, both of the recessive mutants exhibited reduced GTP hydrolysis, and overexpression of a Cdc42p GTPase activating protein could overcome their septin ring defects. If Cdc42p acted only as a ras-like GTPase, mutations that reduce GTP hydrolysis should lead to constitutive signaling and be dominant. The authors propose that, in addition to its ras-like signaling capabilities, Cdc42p can function as an EF-Tu-like factor that uses GTP hydrolysis to drive the assembly of a structure. It remains to be seen whether other signaling GTPases are similarly multitasking. ■



Normal septin ring assembly (left) is disturbed in a Cdc42p mutant that hydrolyzes GTP slowly (right).