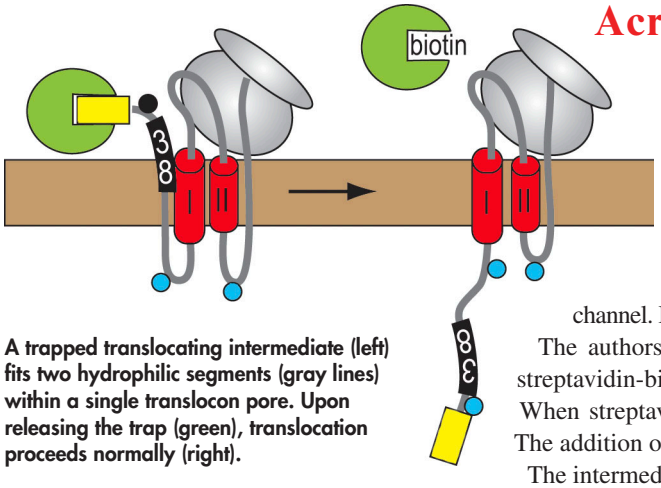
In This Issue



A trapped translocating intermediate (left) fits two hydrophilic segments (gray lines) within a single translocon pore. Upon releasing the trap (green), translocation proceeds normally (right).

Acrobatic flexibility of the translocon

The machinery that inserts membrane proteins into the ER is unexpectedly flexible, say Kida et al. Even with several segments of a multispanning membrane protein already looped around inside the pore, more can be inserted.

Proteins are inserted into the ER in eukaryotes and the plasma membrane in prokaryotes by the Sec61-based translocon. Data from several studies, including crystal structures, indicate that the translocon's pore consists of a single Sec61α subunit, thus creating a narrow channel. But the new studies suggest the pore is much larger than expected.

The authors trapped intermediates in the translocation process by adding a streptavidin-binding peptide tag to the NH_2-terminal end of the inserted protein. When streptavidin was added, translocation stalled, resulting in intermediates. The addition of biotin removed the streptavidin and revived translocation.

The intermediates revealed that stalled translocating peptides within the pore do not jam the translocon. Two distant hydrophilic segments of the same protein fit within the pore at the same time. As each of these lipid-averse segments needs Sec61α to protect it from the membranous environment, the structure suggests that the pore is not as narrow as previously thought. Another intermediate revealed that a stalled hydrophilic domain did not prevent the subsequent membrane insertion of as many as six successive hydrophobic segments.

To explain the flexibility, the authors suggest that perhaps two Sec61 complexes—one for each hydrophilic segment—combine for a translocation event. This model is supported by previous EM images of Sec61 oligomers. It is also possible that a single Sec61 complex undergoes drastic, unpredicted conformational changes or is somehow made larger by accessory proteins. JCB

Reference: Kida, Y., et al. 2007. *J. Cell Biol.* 179:1441–1452.

To bud where no bud has gone before

Like lightning, yeast bud sites never strike twice in the same place. Now, Tong et al. reveal that a zone of GTPase inhibition prevents a new bud site from overlapping with the previous site.

The process of budding leaves behind a scar in the cell wall. Each cell cycle creates a new yeast scar, as new division sites never fall on top of previous ones. Scientists wondered whether the scars might make the cell wall too rigid for a new bud site to form there. But the new findings show that the physical properties of the wall are not to blame.

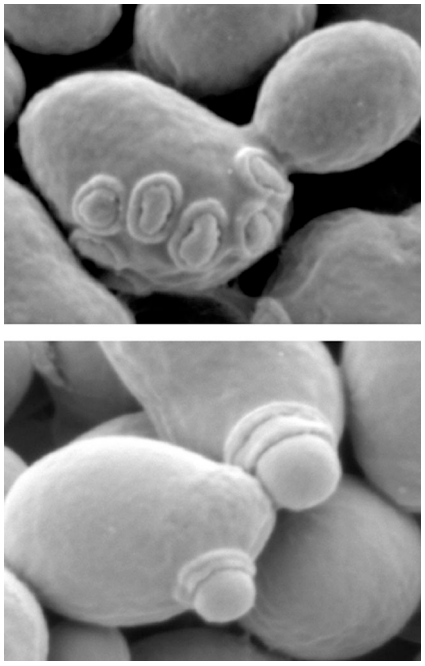
New bud sites repeatedly formed at the same site—on top of a scar—when a GTPase-activating protein (GAP) called Rga1 was deleted. This GAP's target is Cdc42, a polarity-inducing Rho GTPase. In its GTP-bound form, Cdc42 points the cytoskeleton and thus vesicular traffic toward the new bud site. But Rga1 inactivates Cdc42 by inducing it to hydrolyze its GTP to GDP.

During and after cytokinesis in wild-type cells, Rga1 was concentrated between two rings of septins, which help separate the cells. Its presence prevented active Cdc42 from accumulating there. Cdc42-GTP instead formed a patch just outside the Rga1 ring, creating a new bud next to, but not on top of, the previous site.

Although other GAPs that inactivate Cdc42 localized between the septin rings, they could not substitute for Rga1 to prevent budding on top of scars. This sort of specialization might help explain why humans have 68 GAPs for just 17 Rho GTPases.

The Rga1 mutant cells survived in culture despite their overlapping bud sites. In the wild, however, the mutation would be disadvantageous; in nature, buds often remain attached to their mother cell and would thus obstruct subsequent buds. Yeast cells probably senesce before their surface becomes completely covered with scars. JCB

Reference: Tong, Z., et al. 2007. *J. Cell Biol.* 179:1375–1384.



New buds usually form next to old bud sites (top) but can form on top of the old one in cells missing Rga1 (bottom).

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Translation turns on dendritic cells

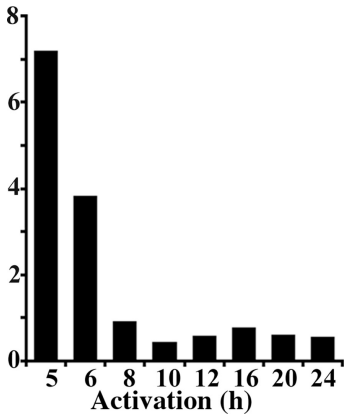
Peaks and valleys in protein translation prepare one set of immune cells for duty, according to findings from Lelouard et al. With the ebb and flow of translation, antigen-presenting dendritic cells (DCs) adjust the origin of their antigens.

DCs are professional immunity activators that alert T cells to the presence of invaders by displaying antigens on their surface. This display is ramped up by a maturation program initiated when a DC encounters inflammatory stimuli such as pathogenic components. Scientists have identified an abundance of transcriptional changes that take place during maturation. The new results show that a boost in translation is necessary to put the new transcripts into action.

Translation peaked in DCs about 4 hours after their activation. During this stretch, translation—and the PI3K/AKT/mTOR signaling pathway that activated it—was needed for maturation-associated changes in the DCs. These changes include producing T cell-activating cytokines and activating the antigen-presenting machinery.

After 4 to 8 hours, protein synthesis levels declined, due at least in part to proteasome-mediated cleavage of the eIF4GI translation initiation factor. At 16 hours, overall translation levels were even lower than they were before activation.

Some transcripts, however, escaped the translation shutdown. As also occurs during stress conditions, eIF4GI cleavage initiated an unusual translation pathway that bypasses the need for a 5′ cap on transcripts. Cap-independent translation, which favors the synthesis of anti-apoptotic proteins, made mature DCs resistant to apoptosis-inducing drugs. This pathway might thus help activated DCs survive the stress of their heavy new transcription and translation loads while they search for and then activate T cells.



Overall translation levels (black bars) peak several hours after DCs are activated and then decline.

As DCs switched translation pathways, they also changed the source of antigens presented on MHC class I molecules. During the early, translation-heavy stage, antigens were derived from pieces of newly synthesized proteins; when the authors blocked translation, antigen presentation was limited. But later on, translation inhibitors did not interfere with MHC class I presentation.

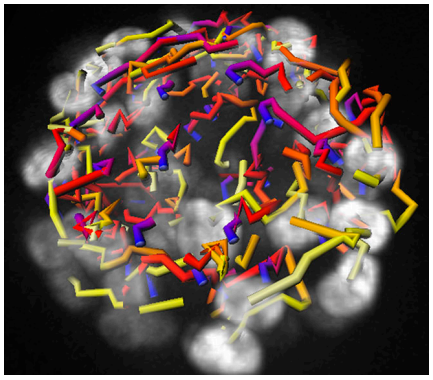
The authors speculate that once DCs have matured, their antigens are mainly derived from exogenous sources, such as pathogens. Although normally presented on MHC class II molecules, exogenous peptides can also crossover to the class I pathway. An alternative possibility is that late antigens come from a pool of stored, presynthesized self-peptides. JCB

Reference: Lelouard, H., et al. 2007. *J. Cell Biol.* 179:1427–1439.

Motility without invasion

Breast cancer cells dance around the subject before becoming invasive, if results from Pearson and Hunter are any indication. The findings show that mammary epithelial cells can become motile within their environment without being invasive. Their motility, however, places them one step closer to metastasis.

Many breast cancers are hallmarked by the unchecked activity of the ERK1/2 MAP kinase pathway. Pearson and Hunter investigated the mechanism by which these kinases, which are normally activated by extracellular growth factors, lead to tumorigenesis. They imaged breast epithelial cells in a 3D model that mimics their in vivo environment. In this model, the cells polarize on a basement membrane to form hollow spheres called acini.



The movements (colored lines) of breast epithelial cells within acini can occur without invasion.

Within mature acini, the provoked action of ERK1/2 encouraged cells to leave their appointed locations. They glided along the basement membrane (underneath other cells) or within the lumen of the acini (on top of the other cells). Motility required the activation of a myosin motor by a kinase that is a known target of ERK1/2.

The movements resembled those that occur in developmental contexts, such as in the forming kidney or salivary gland. These programs are shut down when cells differentiate but might be wrongly reactivated in cancers.

Dangerously invasive cells are characterized by their ability to break through the basement membrane, but this escape was not seen in the ERK-activated acini. The cells did not display the usual set of molecular changes, including increases in N-cadherin and vimentin, that accompany the epithelial–mesenchymal transition. Nonetheless, the movements disrupted the architecture of the acini, as the wandering cells squeezed between more well-behaved stationary cells.

Although not yet invasive, motile breast cells might suggest that a more aggressive form of cancer is brewing. With the ability to flee already in place, these cells would require fewer mutations to become fully invasive. The authors hope to identify molecular markers of this motility that will help physicians diagnose those patients who are at a higher risk for metastases. Motile but noninvasive cells might also permeate other epithelial cancers, including lung and bladder cancers. JCB

Reference: Pearson, G.W., and T. Hunter. 2007. *J. Cell Biol.* 179:1555–1567.