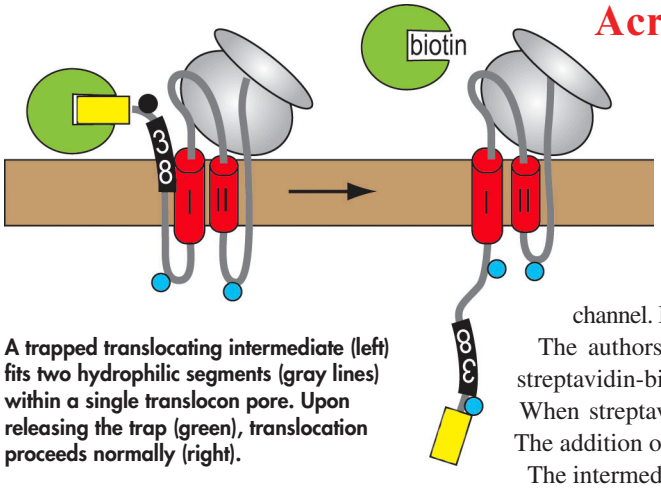
In This Issue



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.

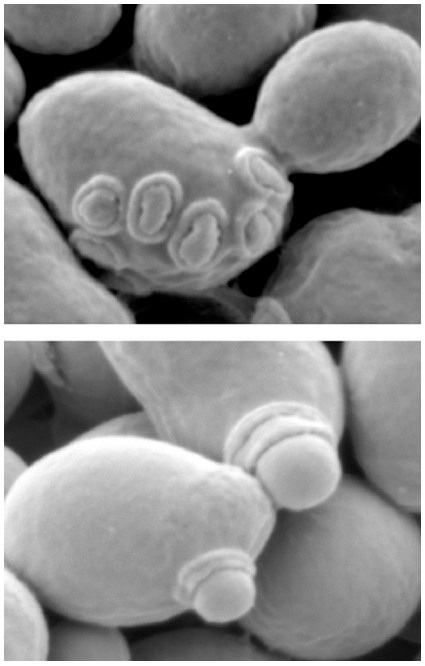
New bud sites repeatedly formed at the same site—on top of a scar—when a GTPase-activating protein (GAP) called Rga1 was deleted. This GAP's target is Cdc42, a polarity-inducing Rho GTPase. In its GTP-bound form, Cdc42 points the cytoskeleton and thus vesicular traffic toward the new bud site. But Rga1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