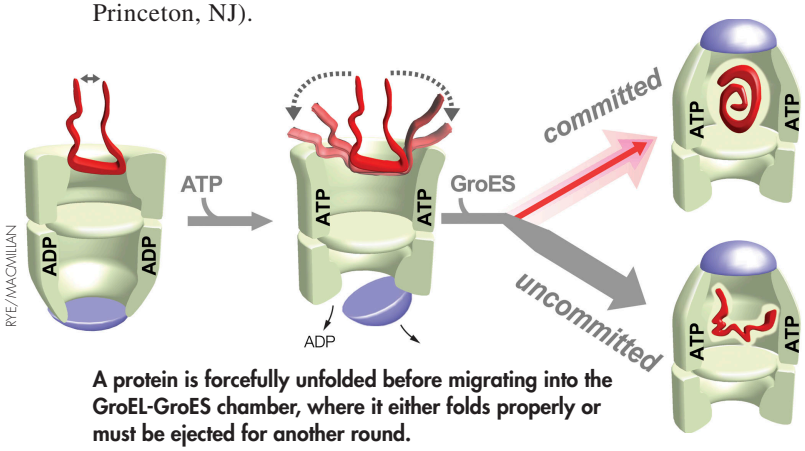
Research Roundup

To fold, first unfold

Proteins unfold at the mouth of a chaperone before being swallowed and refolded, according to Zong Lin, Damian Madan, and Hays Rye (Princeton University, Princeton, NJ).



A protein is forcefully unfolded before migrating into the GroEL-GroES chamber, where it either folds properly or must be ejected for another round.

The double-ringed GroEL chaperone complex is well-known for capturing partially folded proteins and confining them within its central cavity to facilitate folding. GroEL is especially important to proteins that have a complex folding pattern, such as Rubisco. A prominent model of GroEL function suggests that the chaperone first partially unfolds its substrate, disrupting misfolded and inhibitory conformations, thus giving them a chance to refold properly once inside the chaperone cavity. But the importance of this effect for successful refolding has been unclear.

To explore this question, the authors tagged the two loose ends of partially folded Rubisco for FRET analysis. They found that the two ends of Rubisco were close upon initial binding to the open end of GroEL. But when ATP bound to the complex and triggered a GroEL conformational change, the Rubisco ends separated. The same conformation change allowed the complex to bind GroES, GroEL's smaller partner, thereby causing Rubisco to enter the cavity.

But did this unfolding aid in proper folding? ATP-driven unfolding works too fast to answer that question, so the authors examined the slower, passive unfolding of Rubisco on a single-ringed form of GroEL called SR1. By incubating Rubisco with SR1 for varying lengths of time before adding ATP and GroES, they showed that more time with SR1, and therefore more time to unfold before entering the SR1 cavity, produced a higher proportion of successfully folded Rubisco molecules.

"We think this represents a really clean demonstration that unfolding can directly stimulate productive folding," Rye says. JCB

Lin, Z., et al. 2008. *Nat. Struct. Mol. Biol.* doi:10.1038/nsmb.1394.

GABA fine-tunes the glycine receptor

A pair of neurotransmitters deliver a one–two punch to the glycine receptor to fine-tune hearing, say Tao Lu, Laurence Trussell (Oregon Health and Science University, Portland, OR), and Maria Rubio (University of Connecticut, Storrs, CT).

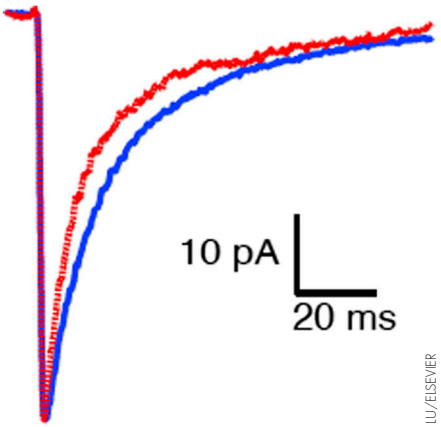
"In the past, regulation of synaptic responses was assumed to be largely due to postsynaptic variables, and the transmitter was a constant," Lu says. "We are saying that the transmitters may also vary significantly in composition."

A major inhibitory neurotransmitter of the auditory pathway is glycine. The binding of glycine to its receptors briefly prevents a synapse from being activated. This inhibitory effect is thought to help the brain interpret the relative timing and intensity of signals from either ear.

Another inhibitory transmitter, called GABA, is expressed in many of the same glycine-sensitive synapses and is packaged together and coreleased with glycine in several areas of the brain. GABA is a weak agonist of the glycine receptor, but it is too weak to activate the receptor by itself. GABA also has its own receptors, so the significance of the corelease has been controversial.

To address this question, the authors applied glycine with and without GABA to isolated membrane patches containing only glycine receptors. Glycine's inhibition of postsynaptic neuronal signaling was shortened in the presence of physiological levels of GABA. More GABA caused an even faster loss of inhibitory power.

In vivo, the two transmitters occurred together in over half the presynaptic terminals in the examined auditory region. When the authors blocked synaptic uptake of GABA precursors, the acceleration effect diminished. When they mimicked the action of the glycine receptor by applying blocking currents to an auditory neuron, they found that a small change in the decay rate of the inhibitory signal had a large effect on the neuron: a 1-ms reduction in the decay constant cut the neuron's inhibition window from 4 to 2 ms.



The inhibition of synaptic firing by glycine (blue line) is shortened by the addition of GABA (red line).

Questions remaining include how the ratio of the two transmitters is regulated and whether that ratio varies among synapses and over time. "We expect to see a wide variation in relative concentrations of the two," Trussell says, matched to the individual needs of the synapse. JCB

Lu, T., et al. 2008. *Neuron.* 57:524–535.

Text by Richard Robinson
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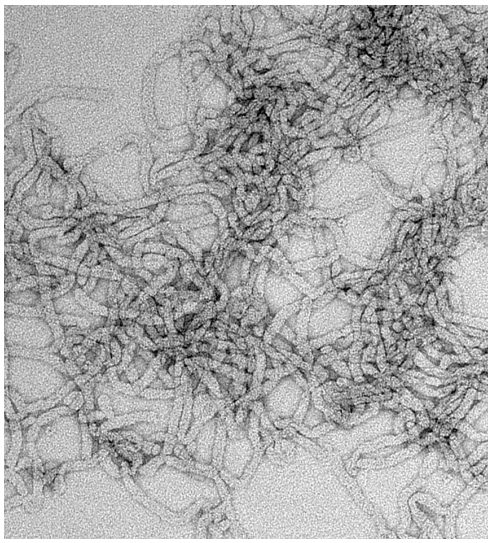


RAPOPORT

Tubules form from a mix of Yop1p and bacterial polar lipids.

How the ER gets its shape

Two proteins are sufficient to form tubules at the ER, say Junjie Hu, Tom Rapoport (Harvard Medical School, Boston, MA), and William Prinz (National Institutes of Health, Bethesda, MD).

The ER often appears as a tubular network. Previous work showed its tubular shaping involves a class of integral membrane proteins, comprising the reticulons and a protein family that includes DP1 in mammals and Yop1p in yeast. To investigate the tubule-forming abilities of these proteins, the authors purified Yop1p or a reticulon from yeast cells, mixed each with lipids, and reconstituted artificial membranes. The resulting proteoliposomes had the shape of tubules with a constant diameter (~15–17 nm). These tubules were narrower than normal ER tubules because they contain a higher concentration of tubule-inducing proteins.

"The reticulons and DP1/Yop1 were known to be required for ER tubule formation," Rapoport says. "This study shows they are sufficient." The structure of these proteins is not known yet, but they form hairpin bends in the membrane and have the propensity to form oligomers. These characteristics suggest that they may form tubules via two effects: external wedging to induce local curvature, and protein linking to form a scaffold that enforces a smooth bend. "The synthesis of the two is most efficient," says Rapoport. "We think this is a paradigm for how membranes are shaped in general." JCB

Hu, J., et al. 2008. *Science.* 319:1247–1250.

Lipid is new Star in nuclear regulation

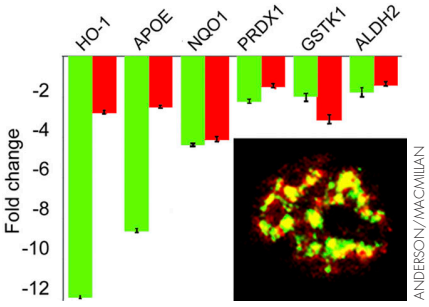
A lipid known for its cytoplasmic duties jacks up mRNA levels by activating a polyadenylating enzyme, say Richard Anderson and colleagues (University of Wisconsin, Madison, WI).

The PtdIns4,5P_2 phosphoinositide is generated by PIPK-α, which is activated by an as-yet uncharacterized stress–response pathway and which links to mRNA-processing complexes called nuclear speckles. In their new study, the authors used the enzyme's speckle-targeting region as bait to find PIPK-α–associated proteins, which they reasoned might be regulatory targets of PtdIns4,5P_2. This strategy led them to a new polyadenylating enzyme they named "speckle-targeted PIPK-α–regulated poly(A) polymerase," or Star-PAP. The close proximity of PIPK-α and Star-PAP might facilitate the lipid's ability to turn on the polymerase.

A knockdown of either Star-PAP or PIPK-α reduced the polyadenylation and subsequent expression levels of about 2,000 mRNAs, many of which are involved in response to oxidative stress. Treatment of cells with an oxidative stressor increased the association of Star-PAP with PIPK-α and the RNA polymerase machinery and thereby increased Star-PAP's activity. Together, the data show the Star-PAP assembly is positioned to extend poly(A) tails on transcripts that are needed for surviving oxidative stresses.

"This is a novel gene expression regulatory mechanism," says Anderson. "It's also the first poly(A) polymerase [found to be] regulated by a signaling pathway. This suggests that other PAPs may be regulated as well." JCB

Mellman, D.L., et al. 2008. *Nature.* 451:1013–1018.



Knockdown of Star-PAP (green) or PIPKI-α (red) reduces multiple stress response mRNAs.

New exit: caspase-1 for secretion

A caspase activates secretion of many inflammatory response proteins without signal sequences, say Martin Keller, Hans-Dietmar Beer, and colleagues (Swiss Federal Institute of Technology, Zürich, Switzerland), revealing a new pathway for secretion.

The cytokine interleukin-1α (IL-1α) has no secretion signal peptide but is nonetheless secreted as part of the inflammatory response. IL-1α is also not a substrate for caspase-1, but its secretion is reduced in macrophages that do not express the protease.

In the new work, the authors showed that caspase-1 inhibition reduced secretion of IL-1α and almost 80 other inflammatory response proteins, many of which lack secretion signal peptides, including FGF-2. Many of the transported proteins were not caspase-1 substrates, yet catalytic activity of the enzyme was required for their secretion, for reasons that are not yet clear. Both IL-1α and FGF-2 bound to caspase-1, suggesting that the enzyme may carry them directly.

"Unlike signal sequence–driven secretion, which is regulated at the level of transcription," says Beer, "unconventional secretion can rapidly release a wide variety of proteins." The proteins involved trigger detoxification, tissue repair, and cell survival, suggesting caspase-1 is helping to regulate the entire inflammatory response. JCB

Keller, M., et al. 2008. *Cell.* 132:818–831.