In This Issue

Text by Ben Short
bshort@rockefeller.edu

Stress management for mRNAs

An enzyme involved in mRNA degradation turns into a transcript stabilizer during stress, Yoon et al. report.

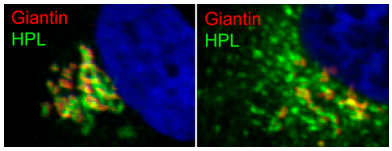
mRNA fates are determined by their association with regulatory proteins in ribonucleoprotein (mRNP) complexes. mRNPs can accumulate in large, cytosolic aggregates; P bodies, for example, contain translationally repressed mRNAs complexed with proteins that initiate mRNA destruction. One of these proteins is the enzyme Dcp2p, which removes the 5′ cap from mRNAs to spur their degradation. Yoon et al. found that budding yeast Dcp2p is phosphorylated during cell stress, when cells alter the fate of many of their mRNAs to aid their survival and recovery.

Dcp2p was phosphorylated by the stress-activated kinase Ste20p. Blocking this modification—either by mutating the phosphorylation site or deleting the kinase—prevented Dcp2p from accumulating in P bodies during stress and inhibited the formation of a second type of RNA–protein aggregate called stress granules. Yeast stress granules can depend on P bodies for their formation and contain repressed mRNAs that may be poised to re-begin translation. A Dcp2p mutant mimicking the phosphorylated form accumulated normally in P bodies and restored stress granules to yeast lacking Ste20p.

The phosphomimetic form of Dcp2p also stabilized a subset of yeast mRNAs, including a number of transcripts encoding ribosomal proteins. Senior author Roy Parker thinks that Dcp2p phosphorylation changes the fate of these transcripts by altering the decapping enzyme's interactions with other regulatory proteins, promoting the mRNAs' stable accumulation in stress granules instead of initiating their degradation. The mRNAs are thus poised to be translated once conditions improve, allowing the yeast to rapidly recover.

Yoon, J.-H., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200912019.

The story of *O*-glycosylation



A marker recognizing *O*-glycosylated proteins (green) labels the Golgi (red) in unstimulated cells (left), but stains the ER after EGF treatment (right).

The tyrosine kinase Src boosts protein glycosylation by stimulating the transport of specific enzymes from the Golgi to the ER, Gill et al. reveal.

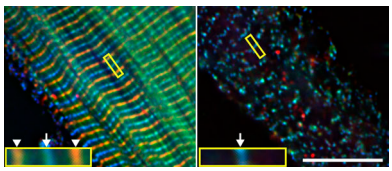
Src is activated downstream of growth factor receptors, but although a portion of the kinase localizes to the Golgi, little is known about its function at this organelle. Gill et al. discovered that growth factor stimulation induced a group of glycosylating enzymes called GalNac-Ts to relocate from the Golgi to the ER. This redistribution was blocked by inhibiting Src or by preventing the formation of COP-I transport vesicles. These vesicles only convey GalNac-Ts in response to Src activation: other glycosylation enzymes stayed put in the Golgi.

GalNac-Ts add *N*-acetylgalactosamine sugars to serine and threonine residues of secretory proteins—the initial step in the *O*-glycosylation pathway. Src activation and enzyme redistribution increased *O*-glycosylation levels, perhaps because ER-localized GalNac-Ts have access to their protein substrates for longer, or because they face less competition from other glycosylation enzymes that remain in the Golgi.

Senior author Frederic Bard now wants to understand how GalNac-Ts are specifically recruited into COP-I vesicles upon Src activation—he suspects that the kinase phosphorylates an adaptor protein that links the enzymes to the COP-I machinery. Another question is how increased *O*-glycosylation affects cell behavior. One possibility is that changing the glycosylation pattern of cell surface proteins will alter their interactions with neighboring cells or the extracellular matrix, suggesting a potential new way for growth factors and Src to influence cell adhesion.

Gill, D.J., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201003055.

Nebulin doesn't measure up



Nebulin (red) stabilizes actin filaments (green) to protect them from latrunculin A. In the protein's absence (right), the depolymerizing drug causes filament disassembly.

Pappas et al. demonstrate that the giant disease-related protein nebulin stabilizes actin filaments to control their length in skeletal muscle.

Nebulin is big enough to stretch the entire length of muscle thin filaments, binding individual actin subunits via a series of repeated domains. Alternative splicing produces nebulin molecules in various sizes that match the filament lengths of different muscle tissues, suggesting that nebulin acts as a "molecular ruler," setting filament length by binding a defined number of actin monomers. The protein's size has hampered efforts to test its function directly, so Pappas et al. synthesized a truncated "mini-nebulin" to replace the longer version.

Muscle cells expressing mini-nebulin contained filaments that were longer than mini-nebulin itself, indicating that the protein doesn't measure out filament length directly. Instead, nebulin regulates thin filament size by stabilizing them: both full-length and mini-nebulin protected filaments from the depolymerizing drug latrunculin A. Filaments never depolymerized to lengths shorter than mini-nebulin, suggesting that the protein binds the filaments to set their minimum size. But photobleaching experiments revealed that mini-nebulin also stabilized filament ends not directly bound by the protein, allowing the filaments to grow longer still.

Senior author Carol Gregorio now wants to investigate how mini-nebulin stabilizes parts of the actin filament it has no contact with. The shortened protein will also enable studies of nebulin mutations that cause nemaline myopathy, a human disease characterized by protein aggregates and muscle weakness, and sometimes short, thin filaments.

Pappas, C.T., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201001043.