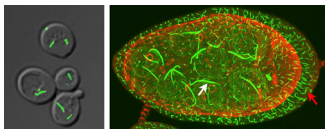
In This Issue

Text by Ben Short
bshort@rockefeller.edu

The cytoplasm gets some new threads



CTP synthase (green) forms filaments in both yeast (left) and *Drosophila* egg chambers (right).

Cytoskeletal proteins aren't the only filament-forming molecules in cells, say Noree et al., who identify several metabolic enzymes and translation factors that also assemble into cytoplasmic threads.

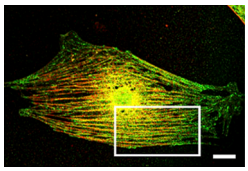
Many cytosolic proteins assemble into large, supramolecular complexes such as the P bodies that regulate mRNAs. In an effort to identify novel components of these complexes, or even new structures entirely, Noree et al. screened a yeast library for GFP-tagged proteins that form cytoplasmic aggregates big enough to see by fluorescence microscopy.

The screen uncovered nine proteins that formed cytoplasmic filaments. Several translation factors co-assembled into one type of filament, while the metabolic enzymes glutamate synthase, GDP-mannose pyrophosphorylase, and CTP synthase each formed their own unique cytoplasmic strands. CTP synthase also formed filaments in *Drosophila* tissues and mammalian neurons. CTP synthase fibers probably comprise the inactive form of the enzyme, because a mutation blocking feedback inhibition by CTP also prevented filament assembly, whereas boosting CTP levels increased the enzyme's aggregation.

Self-assembly may therefore control enzymatic activity depending on the cell's metabolic state, though senior author James Wilhelm thinks these structures might also be used for other purposes. CTP synthase filaments are specifically found in the axons of rat nerve cells, suggesting they have a specialized function there, perhaps as a metabolically regulated scaffold for other proteins. Wilhelm now wants to investigate possible functions of these new cytoplasmic filaments, as well as study the mechanism and regulation of their assembly.

Noree, C., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201003001.

Myosin II contracts out GTPase activation



The Dbl family GEF βPIX (green) colocalizes with myosin II (red) on actin stress fibers.

Lee et al. report that contractile myosin filaments bind guanine nucleotide exchange factors (GEFs) to prevent the activation of Rho family GTPases, potentially coordinating the dynamics of migrating cells.

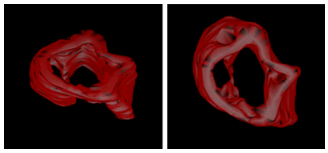
Cells move by sending out adhesive protrusions at their leading edge, a process controlled by actin-regulating GTPases like Rac and Cdc42. Myosin II motors then contract the actin cytoskeleton to pull the rest of the cell forward. How the alternating protrusions and contractions are synchronized is unknown, but Lee et al. found a hint when they discovered that myosin II directly binds members of the Dbl family of GEFs.

Dbl GEFs preferentially bound myosin II assembled into actomyosin fibers, and interacted via their catalytic domains. The GEFs were thus inactive when associated with myosin filaments. Inhibiting myosin's contractile ATPase activity triggered their release, allowing them to activate GTPases and induce membrane protrusions. The motility-stimulating growth factor PDGF also liberated GEFs from myosin fibers, and blocking this release reduced PDGF-induced Rac activation and cytoskeletal rearrangements.

The interaction between myosin II and Dbl family GEFs could therefore coordinate contraction and protrusion. Senior author Eung-Gook Kim now wants to investigate whether migrating cells employ this mechanism by using biosensors to monitor where and when GTPases are activated in response to changes in myosin II contractility.

Lee, C.-S., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201003057.

Migrating cells CLIC into gear



Electron tomography of newly internalized CLICs reveals a complex morphology that suggests a function in protein sorting.

A clathrin-independent pathway is the predominant endocytic mechanism in fibroblasts, say Howes et al., and it boosts cell migration by rapidly internalizing adhesion proteins.

Although clathrin-mediated endocytosis is the best-studied route for internalization, researchers have long known that other pathways also exist. One of these is the clathrin-independent carrier (CLIC) pathway, which also functions without the endocytic coat protein caveolin. CLIC endocytosis seems to specialize in internalizing glycosylphosphatidylinositol-linked membrane proteins, but a lack of definitive markers has hampered efforts to understand the pathway further.

Howes et al. used quantitative electron microscopy to follow the internalization of a non-specific surface marker in fibroblasts. To their surprise, they found that three times as much material was endocytosed through CLICs as through the clathrin-dependent pathway—enough to consume the entire plasma membrane in less than 12 minutes—indicating that the CLIC pathway can rapidly remodel the cell surface. Electron tomography revealed that internalized CLICs have a complex morphology with distinct domains that probably sort cargo to endosomes or back to the plasma membrane.

To find out how fibroblasts use this pathway, Howes et al. purified newly formed CLICs and analyzed their contents by mass spectrometry. The carriers contained many proteins involved in cell migration, which were specifically internalized by CLICs at the leading edge of moving cells. Blocking the pathway impaired fibroblasts' ability to migrate. Senior author Robert Parton now wants to investigate how CLIC endocytosis facilitates cell migration. The pathway may be coordinated with clathrin and caveolin-dependent endocytosis for efficient directional movement.

Howes, M.T., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201002119.