EXPLORE THE ISSUE BEING INVESTIGATED

How Actin-binding Proteins Interact with the Cytoskeleton to Determine the Morphology of Yeasts

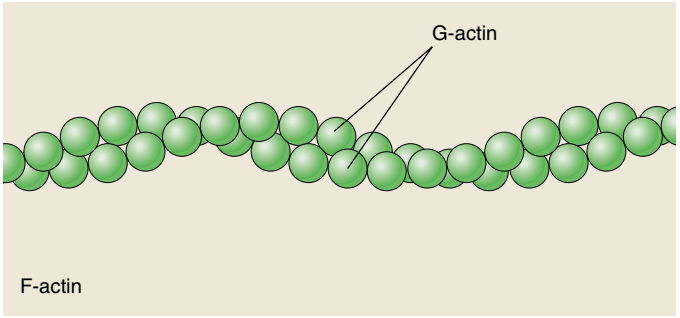
Eukaryotic cells have a complex cytoskeleton that localizes organelles, provides for complex plasma membrane functions like endocytosis, and determines cell shape and motility. The dynamic nature of the cytoskeleton is driven largely by the turnover of actin filaments.

Actin is one of the most abundant proteins in eukaryotic cells, comprising 10 to 20% of total cytoplasmic protein. It exists in two forms in the cell: single globular monomers called G-actin and monomers bound together in long filaments called F-actin. G-actin monomers are the building blocks of F-actin, and serve no function in the cell independently. F-actin directs the movement of cellular structures, the motility of the cell, and the establishment of the cytoskeleton. It is able to do this because F-actin filaments are not static structures in the cell. Their functions result from the dynamic assembly and disassembly of the actin filament. Actin filaments are constantly built and then broken down as the needs of the cell change.

The assembly and disassembly of actin filaments is not a random process. Monomers are added to one end of a filament and are removed from the opposite end, giving the filament directed growth. Many of the functions of actin are "directional" in nature: extending out the plasma membrane and cytoplasm in a pseudopodium to give a cell motility, for example, or dragging cellular structures from one location in the interior of the cell to another. The addition of monomers to one end with the loss of monomers from the other end creates a "treadmilling" action which allows the actin filament to crawl through the cell in a specific direction. The process of actin-powered movement is quite complex, with many components playing active parts. The process has been studied *in vitro* and has revealed the identity of many different proteins that help to stabilize the "growing" end of the filament, or destabilize the "shrinking" end. The more difficult experimental problem is in assessing which of the proteins play important roles *in vivo*.

One way to study a complex process in the cell is to knock out particular components and see what happens to the living cell. An interesting and little-understood component of the cytoskeleton is an actin-associated protein called cofilin that appears to bind to both G-actin and F-actin.

Every type of eukaryotic cell studied contains cofilin, which suggests that cofilin performs an important function in the cell. Supporting this, *null* mutations of cofilin (mutations that completely knock out the function of cofilin) are lethal in the cells of yeast, flies, and worms. *In vitro* studies show that cofilin stimulates the disassembly of actin filaments. Cofilin would seem to be a good candidate for study *in vivo*.



Actin. The protein, actin, is present in two forms in the cell, as F-actin (actin filaments) and as G-actin (globular actin monomers). G-actin subunits link together to form F-actin. F-actin assembles and disassembles in the cell. G-actin monomers are added to one end and they are removed from the other end. Researchers examined cofilin's role in the disassembly of the actin filament.

Pekka Lappalainen, now at the University of Helsinki, and David Drubin of the University of California, Berkeley examined the function of cofilin in yeast cells using site-directed mutations of the *COF1* gene (the gene for cofilin). Lappalainen and Drubin could not use a *null* mutation of *COF1* because, as stated above, *null* mutations of the cofilin gene are lethal. Instead, they used two temperature-sensitive alleles of cofilin. These are mutations that disrupt the function of cofilin at elevated temperatures.

To examine the effects of disrupting cofilin, the researchers treated wild type and mutant cells with the chemical latrunculin-A (Lat-A) which inhibits actin filament assembly. If cofilin is involved with actin disassembly *in vivo* as *in vitro*, then there should be a disruption of actin disassembly process in the mutant cells. Actin filaments should disassemble in the wild type cells but they should remain intact in the mutant cells.

To see actin in the cell, Lappalainen and Drubin used a rhodamine-phalloidin staining treatment which causes patches of actin filaments in the cells to become visible through the microscope. Using this experimental treatment, they were able to directly examine the role cofilin plays in the cell.