mal GASP as a protein that determines the degradative fate of some GPCRs.

Another recent study disclosed that a protein called sorting nexin–1 (SNX-1) is important for specifying the preferential targeting of PAR-1 to lysosomes (7). SNX-1 is a membrane-associated protein that is already known to promote lysosomal sorting and degradation of the epidermal growth factor receptor (8). Interestingly, Wang et al. (7) report that SNX-1 has a low affinity for GPCRs that prefer the recycling pathway. Thus, SNX-1 may be another candidate sorting protein involved in targeting GPCRs to the degradative pathway.

Some GPCRs can be modified by the covalent attachment of ubiquitin molecules (ubiquitination), which determines their sorting fate. Ubiquitination usually tags cellular proteins for degradation within intracellular organelles called proteasomes.

However, recent work has demonstrated that ubiquitination of some GPCRs targets them for degradation in lysosomes instead of proteasomes (9, 10).

It appears, then, that the sorting fate of individual GPCRs is determined by a complex array of protein interactions that direct the receptors to opposing pathways (recycling versus degradation). Although recent studies have identified several interacting proteins, including GASP, SNX-1, and NSF-1, that target internalized GPCRs for recycling or degradation, there are probably plenty more interacting proteins and sorting motifs waiting to be discovered. Moreover, a number of important questions persist: Given that even recycled GPCRs are eventually degraded, how are these receptors ultimately targeted to the degradative pathway? How do GASP and SNX-1 interact with the sorting machinery to direct GPCRs to lysosomes?

Are the mechanisms involving GASP and SNX-1 specific for some lysosomally sorted GPCRs, but not for others? How do the processes that regulate GPCR recycling and degradation modulate GPCR activity in vivo? The answer to this last question is likely to have tremendous implications for understanding the actions of drugs that target GPCRs and for designing new medications with fewer side effects and greater efficacy.

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PERSPECTIVES: CELL BIOLOGY

Formins Set the Record Straight

Fred Chang and Matthias Peter

Cell polarity, cell migration, cytokinesis, vesicle transport, and the formation of membrane protrusions are just some of the cellular processes that depend on actin filaments. Actin filaments are assembled by polymerization of monomers, and can be either branched or straight. For example, at the leading edge of motile cells the entire network of actin filaments is branched, whereas in microvilli, stereocilia, stress fibers, and contractile rings, actin is organized into bundles of linear filaments. Yeast contain linear structures called actin cables that enable directional transport of vesicles. A protein complex called Arp2/3 is the molecular machine that nucleates and drives actin monomers to polymerize into branched filaments (1). Now, two recent studies, including one on page 612 of this issue (2), identify the formins as a new class of actin nucleator that directs assembly of straight filaments (2, 3).
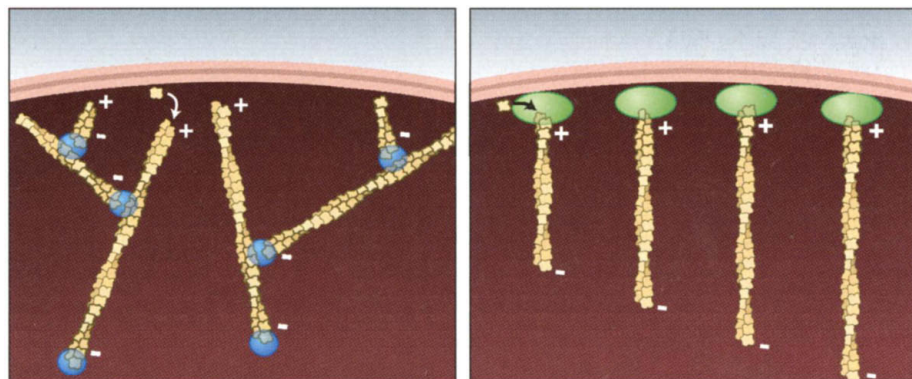
Nucleation of actin (the formation of a short filament by two or three actin monomers) is the critical first step in actin filament assembly. Actin monomers are poor initiators of new filament assembly, and thus actin nucleation is the rate-limiting step. Actin filaments have structurally distinct ends: the barbed end and the pointed end (see the figure). The barbed end is the faster growing end where most of new filament assembly takes place.

Much recent research has centered on the Arp2/3 complex, composed of at least seven proteins, which binds to the pointed ends and to the sides of existing actin filaments. Arp2/3 prefers to nucleate new filaments at a 70° angle to existing filaments, resulting in formation of a branched network (see the figure). In vivo, the Arp2/3 complex is required for cellular processes that use branched actin filaments, for example, the extension of membrane protru-

sions in animal cells, and the intracellular motility of the bacterium Listeria monocytogenes. Because accessory proteins such as tropomyosin promote Arp2/3 to form actin filaments with fewer branches (4), many researchers have presumed that Arp2/3 is the primary and perhaps sole actin nucleator in the cell.

Recent studies, however, suggest that there is another actin nucleator at work, one that may specifically direct formation of straight actin filaments. Formins are large multidomain proteins that are required for cytokinesis and maintenance of cell polarity (5). These cytoskeleton-organizing proteins direct assembly of actin structures, such as the contractile ring, actin cables, and stress fibers, and also regulate microtubule stability in eukaryotic cells. Formins become activated when they bind to Rho guanosine triphosphatases, such as Cdc42, and are known transducers of the Rho signaling

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Straight arrows and branched networks. The Arp2/3 complex and formins organize different actin structures. (**Left**) The Arp2/3 complex (blue) tends to nucleate new actin filaments at the sides of existing actin filaments, resulting in a branching filament network. (**Right**) In contrast, formins (green) nucleate the assembly of straight filaments. The Arp2/3 complex binds to the minus (pointed) end of the actin filament, whereas formins bind to the growing plus (barbed) end of the actin filament.

pathway. In budding yeast, the formins Bni1 and Bnr1 are important for assembly of actin cables. Loss of formin activity leads to rapid loss of actin cables, whereas overexpression of certain segments of formin proteins leads to extra actin cables (6, 7). The Arp2/3 complex does not seem to be required for actin cable assembly (6, 8), implying that these structures may be nucleated without the help of this complex.

In the new work, Pruyne et al. (2) and Sagot et al. (3) show that the highly conserved FH2 domain of the yeast formin Bni1 nucleates the assembly of actin filaments in vitro. Biochemical and electron microscopy experiments revealed that the FH2 domain induces actin nucleation and promotes assembly of long unbranched actin filaments from their barbed ends. In contrast to Arp2/3, which binds to the pointed ends, the formin fragment appears to attach to actively growing barbed ends. Consistent with this barbed-end association of formins, Bni1 is localized in the cortex at the tip of the yeast bud, where the barbed ends are thought to reside (see the figure). With Bni1 and the barbed ends of filaments fixed into the cortex of the yeast bud, the pointed ends of actin filaments are pushed into the cell interior as monomers are added to the barbed ends. Together these exciting findings identify formins as a new class of proteins that nucleate actin.

Formins are thought to bind to multiple proteins in large complexes. In particular, they have a proline-rich domain that binds to profilin, a small protein that binds to actin monomers and stimulates their polymerization. Genetic analyses suggest that formins and profilins cooperate during actin assembly in vivo. Indeed, formin-dependent actin nucleation is stimulated by profilin in vitro, most likely through recruitment of actin monomers (3). Formins also bind to other actin-organizing proteins such as Bud6 and Spa2. Thus, although a small portion of a formin can stimulate actin assembly itself, their true involvement is likely to be as participants in a large actin assembly molecular machine.

These studies begin to reveal how different actin structures are formed by different nucleators. Many cells have multiple formins that carry out either specific or overlapping tasks. For instance, each of the three formins in fission yeast promotes the assembly of a specific actin structure, such as the contractile ring required for cytokinesis. A recent study in fission yeast reveals that actin nucleation in the contractile ring requires both the Arp2/3 complex and the formin cdc12p (9). Thus, in vivo it is possible that these two types of nucleator work together. In addition, other nucleators may exist. For instance, human zyxin may generate new actin structures in an Arp2/3-independent manner (10). Future studies will define which actin nucleators are responsible for directing assembly of which actin structures.

Although the new results identify formins as direct actin nucleators, many important questions remain unanswered. What is the biochemical mechanism of actin nucleation by the formin FH2 domain? How do other domains of formin proteins and other formin-associated proteins contribute to or modify FH2 domain activity? Do different formins direct the organization of different actin structures? How do formins coordinate actin assembly with other activities such as microtubule dynamics? How is actin organization regulated by the spatial and temporal regulation of formins? Clearly, the formins are an important focal point for understanding the molecular processes that direct the cytoskeletal organization of cells. Given the current pace of progress, there is little doubt that these and other questions will be straightened out in the near future.

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PERSPECTIVES: ASTRONOMY

Of Clusters and Galaxies

Christine M. Clement

Globular clusters (see the first figure) are compact assemblies of stars that are the oldest stellar systems in the Milky Way Galaxy (1). They play a crucial role in the study of the formation and evolution of our galaxy. But as Yoon and Lee show on page 578 of this issue (2), some long-held assumptions about globular clusters need to be revised.

Forty years ago, Eggen et al. (3) proposed that the galaxy formed from a homogeneous, rotating, gaseous body that collapsed into a disk (see the second figure). According to this monolithic collapse model, most globular clusters formed from condensations of gas in the spherical halo before the remaining gas collapsed. As a result, globular clusters are located primarily in the halo. Their stars have lower metal abundances than Sun-like

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stars because they formed at an earlier epoch when the interstellar medium was less enriched in heavy elements (4).

But evidence is mounting that the formation of our galaxy was less straightforward than this model suggests. Capture from other galaxies is increasingly seen as a viable source of clusters. Yoon and Lee (2) propose that this mechanism may explain the unusual properties of certain metal-poor globular clusters.

In a slow monolithic collapse model, the age and metal abundance of a halo cluster should depend on its distance from the galactic center—the more distant clusters should be older and have a lower metal abundance. However, Searle and Zinn (5) found no radial abundance gradient in the clusters of the outer halo. They argued that some clus-

The globular cluster Omega Centauri. With about a million stars, it is the largest and brightest cluster in the Milky Way. A typical cluster contains ~100,000 stars. About 150 clusters have been identified in our galaxy.

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