indicating that SP7 interacts with ERF19 to suppress its activity.

An intriguing part of the study was that when plants were inoculated with a transgenic form of the hemibiotrophic fungal pathogen *Magnaporthe oryzae* that expressed SP7, the detrimental effects of the pathogen were less than in plants infected with the wild-type fungus. The authors suggest that SP7-mediated suppression of defense pathways in the plant allows the fungus to remain in a biotrophic state. This, in turn, allows the fungus to keep the plant alive for longer. It does not seem very intuitive that a protein that suppresses plant defense could be beneficial for the plant. However, it could allow a fungus like *M. oryzae* to simply remain undetected inside the host for a longer period until the plant has grown larger and more resources could be obtained from the host. Additionally, defense suppression could open up the possibility for other fungi to attack the plant. Such results also raise the question of whether a plant is more or less susceptible to fungal pathogens when colonized by an AMF that is expressing SP7 and, thus, suppressing part of the plant's ability to defend itself. Indeed, reports about whether AMF increase or decrease the ability of plants to protect themselves against fungal pathogens are rather contradictory. Nevertheless, the finding of an AMF effector protein that switches off parts of the plant defense mechanism is indeed a major milestone in understanding the AM symbiosis.

Given the global importance of the mycorrhizal symbiosis, coupled with the difficulty of working with the fungal partners, these two studies identifying the role of fungal effector proteins that allow mycorrhiza formation and allow the fungus to switch off the plant's defense responses truly further our knowledge of these plant–fungal associations.

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DOI: 10.1016/j.cub.2011.06.022

Cell Biology: Actin Keeps Endocytosis on a Short Leash

High-resolution structural analysis of branched actin networks at the sites of clathrin-mediated endocytosis sheds light on the role of actin in endocytosis and mechanisms controlling actin assembly.

Vladimir Sirotkin

The dynamic actin cytoskeleton plays many important roles in fundamental cellular processes, including cell motility, cell division, control of cell shape and endocytosis. Studies in organisms ranging from yeast to man have revealed a burst of actin assembly at the endocytic site or patch, peaking at the internalization of an endocytic vesicle [1–3]. Actin plays an obligatory role in endocytosis in yeast, while the role of actin in endocytosis in animal cells remains a subject of debate. Given that force generated by actin assembly is sufficient to deform cell membranes and to move particles within dense cytoplasm, actin was proposed to participate at multiple stages of endocytosis, including membrane invagination, scission and propulsion of the endocytic vesicle. In this issue of *Current Biology*, Collins *et al.* [4] provide remarkable electron microscopy images of the actin network around clathrin-coated structures in animal cells. The organization of the network helps to define the role of actin in endocytosis and provides important clues about the mechanisms controlling actin assembly at the endocytic sites.

The exact mechanism for the role of actin in endocytosis depends on the arrangement of filaments around the endocytic sites (Figure 1A). Several models have been proposed [1–3,5]. Work in budding yeast favors actin filament organization with fast-growing, barbed ends facing the plasma membrane and slow-growing, pointed ends anchored at the endocytic vesicle coat. In a second model, filament barbed ends are oriented towards the tip of endocytic invagination, so that growing filaments force elongation of the neck of the budding vesicle and propel the vesicle after the scission from plasma membrane. The images in the present study [4] support a third model, in which actin filaments form a collar-like structure around the neck of the endocytic vesicle. Growing filaments are oriented towards the vesicle neck, providing force for neck elongation, vesicle scission and propulsion.

Collins *et al.* [4] used platinum replica electron microscopy and electron tomography to produce striking images of the actin network at clathrin-coated structures in cultured mouse cells that were either 'de-roofed' by sonication or extracted with detergent. In the de-roofed preparations, many of the clathrin-coated structures are at the early stages of invagination and are surrounded by a branched actin network. Electron tomography has revealed that the actin network is located at the neck of the endocytic

invaginations and is specifically excluded from the clathrin coat at the tip of invagination. By decorating filaments with an S1 myosin-II fragment or by examining the orientation of the characteristic 70° angle of Arp2/3-complex-branched filaments, the growing ends of the filaments in the actin collar are found to be oriented towards the neck of the endocytic invagination. In this orientation, force generated by growth of the filaments in the actin collar can directly squeeze the vesicle neck. Expansion of the actin network at the neck can also result in elongation of endocytic invagination, away from plasma membrane. The actin collars are often asymmetric, which can help promote neck elongation and accounts for the lateral movement of clathrin structures [6].

Deeply invaginated clathrin structures show reorganization of actin into comet-tail-like structures [4]. In these structures, the growing filament ends are oriented towards the clathrin coat and can further elongate the neck of the budding vesicle or propel the vesicle in the cytoplasm after internalization. Notably, only comet tail and ruffle-like structures are observed in detergent-extracted cells. It will be interesting to determine whether the earlier structures are not preserved after detergent extraction or whether organization of actin patches varies with location within the cell, for example in ventral versus dorsal cell surfaces.

The observation of branched actin filament networks associated with clathrin structures provides direct confirmation that the Arp2/3 complex assembles actin filaments at sites of endocytosis. Targeting actin filament ends towards the vesicle neck places certain constraints on the mechanism controlling actin assembly. The Arp2/3 complex is intrinsically inactive and needs to be activated by activator proteins or nucleation promoting factors, such as members of the Wiskott-Aldrich Syndrome protein (WASp) family, cortactin and others [7]. Collins *et al.* [4] have postulated that the Arp2/3 complex activators are specifically localized or activated at the neck of endocytic invagination (Figure 1B). Specific localization of WASp to the neck of endocytic invaginations capped by clathrin at the tip has been observed in yeast by immunoelectron microscopy [8]. Mathematical modeling of actin patch assembly in fission yeast also shows that concentrating actin filament nucleation at the surface of endocytic invaginations is required to account for the rapid 10-second time course of actin assembly [9,10]. Localized actin assembly at the neck of the budding vesicle also accounts for the observation that actin depolymerization by latrunculin B treatment results in wider and shorter necks in wild-type and dynamin-depleted mouse cells [11].

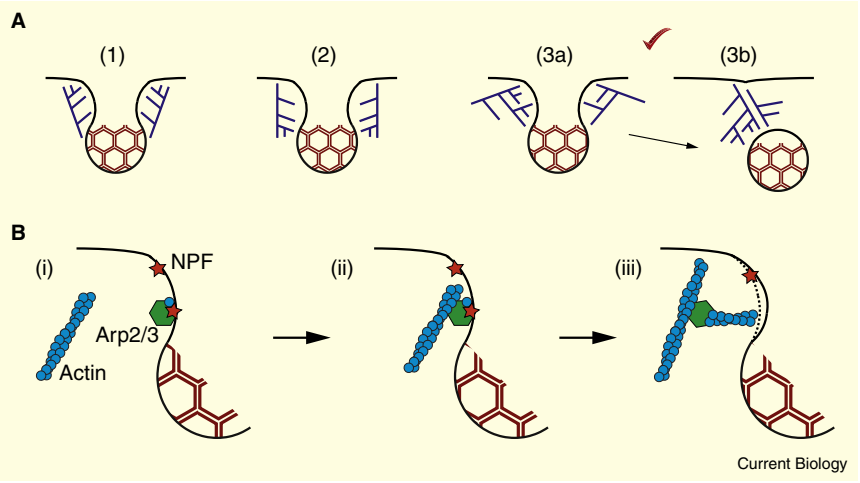

Figure 1. Actin networks at sites of clathrin-mediated endocytosis.
(A) Models of actin organization. Growing filament ends face: (1) plasma membrane, (2) clathrin coat, or, as observed by Collins *et al.* [4], (3a) neck of endocytic invagination and (3b) internalized vesicle. (B) Actin assembly at endocytic sites. Arp2/3 complex (i) binds to a nucleation promoting factor (NPF) at the neck of endocytic invagination, (ii) binds to a mother filament, and (iii) nucleates a branch that deforms the membrane.

Just how the stimulation of Arp2/3 complex by its activators is restricted to the neck of the budding endocytic vesicle is an important question for future research. One attractive possibility is that BAR-domain-containing proteins [12], which can sense and promote membrane curvature, can directly recruit Arp2/3 complex activators to the neck of the endocytic vesicle in a positive-feedback loop [13]. In this scenario, actin assembly promotes membrane curvature, which is recognized by the BAR proteins that bind to the emerging neck and recruit additional Arp2/3 complex activators that further stimulate localized actin assembly. Conversely, BAR proteins can suppress Arp2/3 complex activators at specific times or locations around endocytic invagination. Indeed, specific BAR proteins localize to the tubular invaginations in dynamin-deleted mouse cells [11] and in an *in vitro* reconstitution system [14], and several BAR domain proteins regulate the activity of Arp2/3 complex activators [13]. An additional mechanism that may focus filaments to the neck of endocytic invagination may involve the activity of myosin-I, which localizes to endocytic sites in yeast [2,10] and animal cells [15].

The work by Collins *et al.* [4] also sheds light on another mystery in the field — the source of the initial so-called mother filament required to trigger actin assembly at endocytic sites. The ternary complex comprising the Arp2/3 complex, activator, and actin monomer must bind to the side of pre-existing actin filament to complete activation and nucleate a daughter filament (Figure 1B). Thereafter, actin assembly proceeds in an explosive, auto-catalytic fashion. Collins *et al.* [4] now show that actin collars at sites of endocytosis are often associated with filaments in nearby actin structures. This suggests that actin assembly at endocytic sites may be triggered from fortuitously adjacent actin filaments and may explain why endocytic actin collars are asymmetric in shape.

Exclusion of actin filaments from the clathrin-coated tip of endocytic invaginations raises the possibility that, in addition to its role in promoting membrane invagination, clathrin may play a role in protecting the initial endocytic invagination from the force of actin assembly. Similar organization of endocytic sites, with actin at the neck and clathrin at the tip of endocytic

invaginations, has been observed in yeast [8] and in dynamin-deleted animal cells [11], although in both systems endocytic invaginations are tubular rather than spherical. Physical parameters, such as membrane tension [16], size of endocytosed material [17] and scission activity of dynamins [11], likely contribute to the shape of endocytic invaginations. An interesting question for future research is whether the actin organization around tubular invaginations is similar to the actin structures around spherical invaginations observed by Collins *et al.* [4].

The role of actin in endocytosis in animal cells has been questioned on the basis of the relatively mild effects of actin-disrupting drugs on endocytosis [1,2]. Collins *et al.* [4] now show that treatment of their cells with concentrations of actin-disrupting drugs sufficient to eliminate most actin structures fails to completely eliminate actin collars around clathrin-coated structures. This observation, together with the observed association of actin with at least 43% of clathrin structures, provides further evidence that the role of actin in endocytosis in animal cells might have been under-appreciated.

By providing high-resolution images of actin networks at the sites of clathrin-mediated endocytosis, Collins *et al.* [4] clarify the role of actin in promoting invagination, scission and propulsion of endocytic vesicles. Future research will undoubtedly focus on the mechanisms that target actin assembly to the neck of the budding endocytic vesicle and possible feedback mechanisms linking actin assembly with the progress of membrane invagination.

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DOI: 10.1016/j.cub.2011.06.029

Plant Genomics: Homoplasy Heaven in a Lycophyte Genome

The recent genomic sequencing of *Selaginella*, a member of the lycophyte lineage of vascular plants, opens up all kinds of new opportunities to examine the patterns of evolutionary innovation and the creation of the basic bauplan of plants.

William E. Friedman[1,2]

Steven Gould famously argued in *Wonderful Life* [1] that, if the tape of life (beginning with the Burgess Shale fauna) could be replayed, it would always and inevitably turn out differently. Historical contingency is a powerful and often stochastic determinate of the course of evolutionary innovation. Certainly, Gould was correct in many fundamental ways; his arguments opposed the deterministic, progressive, and highly teleological views of evolutionary history as one long slog leading ultimately and inevitably to the origin of humans. It is difficult to believe that certain rare evolutionary historical events have not been critical in setting the subsequent course of evolution.

Imagine if the one-time transition from splitting H_2S to H_2O as a source of electrons in an early cyanobacterium had not occurred some two to three billion years ago. It would be hard to argue that Earth without molecular oxygen would be quite the same place as we find it now. Life would have continued to evolve, but, in an atmosphere entirely devoid of oxygen, it is reasonable to posit that the course would not have led "inevitably" to the point we now occupy, some 4.5 billion years after the planet was born. The same could be said for the endosymbiotic event that led to the capture of a purple bacterium that ultimately established itself in the nucleated cells of eukaryotes and subsequently served as the mitochondrion. Indeed, myriad events