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Endocytosis: Actin in the Driving Seat

Dispatch

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Endocytosis is an essential eukaryotic process which has been reported to require a functional actin cytoskeleton. New data obtained using the model yeast *Saccharomyces cerevisiae* significantly advances our understanding of the interface between the endocytic machinery and actin.

Endocytosis has a number of important functions in eukaryotic cells: it is critical for controlling the protein–lipid composition of the plasma membrane and the uptake of nutrients as well as pathogens, and it also plays an important role in the regulation of cell signaling. While a number of pathways for endocytosis have been characterised, all of these require remodelling of the cell cortex. The importance of a dynamic actin cytoskeleton for facilitating endocytosis has been recognized for many years from work on the budding yeast *Saccharomyces cerevisiae*, and the generality of this role is increasingly supported by studies in mammalian cells [1].

In yeast, the first evidence for a link between endocytosis and the actin cytoskeleton came from genetic screens for endocytic mutants [2–4]. Many of these endocytic (*end*) mutants were subsequently found to have defects in the actin cytoskeleton or to be the result of mutations in genes encoding proteins known to associate with actin. However, the mechanistic details of how the actin cytoskeleton acts in endocytosis in yeast have been unclear. Indeed, following electron microscopy studies localising the Ste2p pheromone receptor to sites distinct from those containing actin [5], there was speculation as to whether the actin cytoskeleton played a direct role in endocytosis at all. These doubts, including other longstanding contradictions among published data, now appear to have been laid to rest by several recent studies. The new data, most notably those reported by Kaksonen *et al.* [6], give a clear idea of how actin is coupled to endocytosis in yeast, and reveal significant similarities to the role of actin during endocytosis in mammalian cells.

In their elegant study, Kaksonen *et al.* [6] used real-time fluorescence microscopy to localize a number of GFP-tagged proteins previously implicated in endocytosis or the organization of the actin cytoskeleton. The life cycle of an actin patch in yeast has been previously described [7], but the new work of Kaksonen *et al.* [6] has added an important functional dimension. Their data show that, rather than being generated *de novo*, the actin patches form at pre-existing sites containing endocytic machinery. A cortical actin patch appears to be generated in order to support the process of endocytosis. Actin polymerisation at this site is then proposed to drive the endocytic vesicles into the cell. In this respect, the process may be extremely similar to that in mammalian cells, where evanescent field microscopy revealed the recruitment of actin at late stages of vesicle formation and apparent movement of the vesicle into the cell coupled to polymerisation of actin [8].

Kaksonen *et al.* [6] measured the life-time of several GFP-tagged protein patches and recorded their kinetic behavior. The proteins studied were: Las17p, a homologue of the actin regulator WASP; Sla2p, homologous to Huntingtin interacting protein HIP1-R; Sla1p, an endocytic adaptor protein; Pan1p, an Eps15 homologue; Abp1p, an actin-binding protein with a mammalian homologue; and Arc15p, a component of the Arp2/3 complex which nucleates actin polymerisation. The authors observed changes in the composition of a cortical patch through its life-time and found that the behavior of the patch correlates with its specific protein composition. While patches containing Abp1p and Arc15p were seen to be short lived, they displayed distinctive motility: slow movement followed by rapid movement. Patches containing the proteins Sla1p, Pan1p and Sla2p had a longer life-time and showed restricted movement followed by slow movement. Las17p-containing patches were the longest lived, but these showed relatively little movement at any point in their life-time (Figure 1).

Warren *et al.* [9] had previously shown that Abp1p and Sla1p patches can exist separately, but also co-localize, and they postulated that this association is required for endocytosis. Kaksonen *et al.* [6] investigated localisation of these and several other pairs of tagged proteins and were able to show that the patches evolve in such a way as to explain many earlier findings. Their model proposes that Las17p is a component of early patches, which then recruit other proteins of the endocytic machinery: Sla1p, Sla2p and Pan1p. At this stage the patches are relatively immotile. Actin and its associated proteins — marked by Abp1p and Arc15p — are then recruited, and this is signified by an initial slow rate of patch movement while the endocytic proteins are present. It is thought that these proteins are then disassembled from the patch, and there is rapid movement of the endocytosed vesicle into the cell.

Interestingly, different Arp2/3-activating activities are present during the immotile, slow and rapid movement stages (Las17, Pan1p and Abp1p, respectively), a first indication these different activator proteins have distinct roles. In the model [6], Las17p is the earliest activator in the patch. Given that actin is not detected in the patch until a later stage, it seems likely that Las17p has additional roles at this early stage, which might include recruitment of other proteins such as Sla1p [10]. Within the later-stage patches, Las17p is thought to remain at the plasma membrane as the patch invaginates and moves inwards. This localisation might indicate that Las17p has a role in driving polymerisation of actin immediately adjacent to the membrane.

Pan1p is the second activator that localises to a developing actin patch, and this activator is found on

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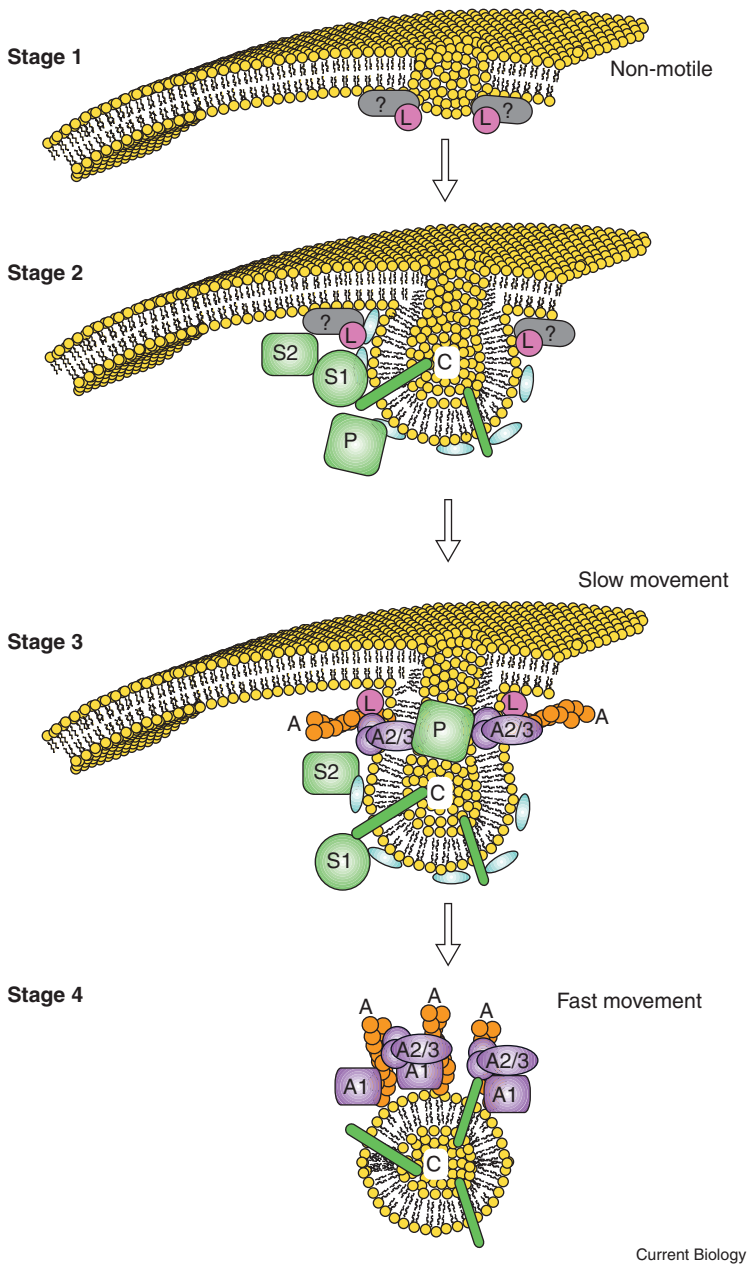

Figure 1. A schematic model of cortical patch development in budding yeast.
From their visualization of GFP-tagged proteins, Kaksonen *et al.* [6] propose distinct stages in the life cycle of a cortical patch. Las17p characterises stage one patches, which are relatively long lived (>30 sec) but almost static. Other proteins (designated **?**) that are likely to be localized at this stage include verprolin (Vrp1p), which is required for localisation of Las17p itself [7]. It is not yet clear whether the patches at this stage recognize any specific cargoes. Stage two patches exist for an intermediate amount of time (~30 sec); they are initially static but then show some limited slow movement. This stage of the patch is characterised by the presence of the endocytic proteins Sla1p, Sla2p and Pan1p. At this stage there are known interactions with cargoes, such as the pheromone receptor Ste2p [14]. The final stage of patch development is characterised by recruitment of actin and associated proteins. These patches at first move slowly, while the endocytic proteins are still present, but then they lose endocytic proteins and exhibit a short rapid burst of movement, as actin polymerisation drives internalisation of the endocytic vesicle into the cell. L, Las17p; S1, Sla1p; S2, Sla2p; P, Pan1p; C, cargo; A, actin; A1 Abp1p; A2/3 Arp2/3 complex (incorporating the tagged protein Arc15p).

patches as they move into the cell. Pan1p might therefore participate in driving this movement; alternatively it might be involved in membrane scission events, as *pan1* mutants accumulate long plasma membrane invaginations [11]. Lastly, Abp1p localises to patches at the late stage, when they are proposed to be moving into the cell and when the recruitment of actin to the patches is maximal. This late role, proposed to follow invagination and possibly scission, would explain why lack of Abp1p does not effect endocytosis *per se*. This notion of sequentially functioning Arp2/3 activators has also been suggested from work on the activators cortactin and WASP in mammalian cells [12].

One final aspect of the new study by Kaksonen *et al.* [6] came from analysing a strain lacking Sla2p, a component of the endocytosis machinery. As previously noted [13], in the absence of Sla2p, cortical patches appear elongated, giving the appearance of actin tails overlapping the fluorescence signal from the endocytic adaptors. At this arrested stage, a cargo protein (the yeast pheromone receptor Ste2p), adaptors and actin were seen to colocalise, the first definitive demonstration that these components do all come together. Strikingly, while these patches contain both actin and early endocytic patch markers, they do not move, indicating that they represent an intermediate step in the endocytic process.

The Sla2p-deficient strain was used to address the important question of whether the actin filaments recruited to endocytic sites are pre-formed or nucleated at the site. By applying the technique of fluorescence recovery after photobleaching (FRAP) to

the actin tails in Sla2p-deficient cells, it was clearly demonstrated that actin is nucleated at the endocytic sites. Furthermore, the rate of polymerisation was found to be comparable to that observed in highly motile cells, indicating that a capacity for high actin polymerisation rates is fundamental to eukaryotic cells.

Despite the considerable step forward reported by Kaksonen *et al.* [6], there are still important aspects of the endocytic process that remain unresolved, most strikingly the mechanistic contributions of the plethora of proteins that localize to cortical patches. Kaksonen *et al.* [6] discuss the localisation and behavior of six proteins within cortical patches. But more than 20 other proteins have been reported to have at least overlapping localisation with cortical actin. The molecular functions of these are largely unknown, though genetic studies point to redundancy among many of the patch components. The ease of genetic exploitation in yeast however, means that studies as performed by Kaksonen *et al.* [6] may soon reveal further insights into this essential eukaryotic process.

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