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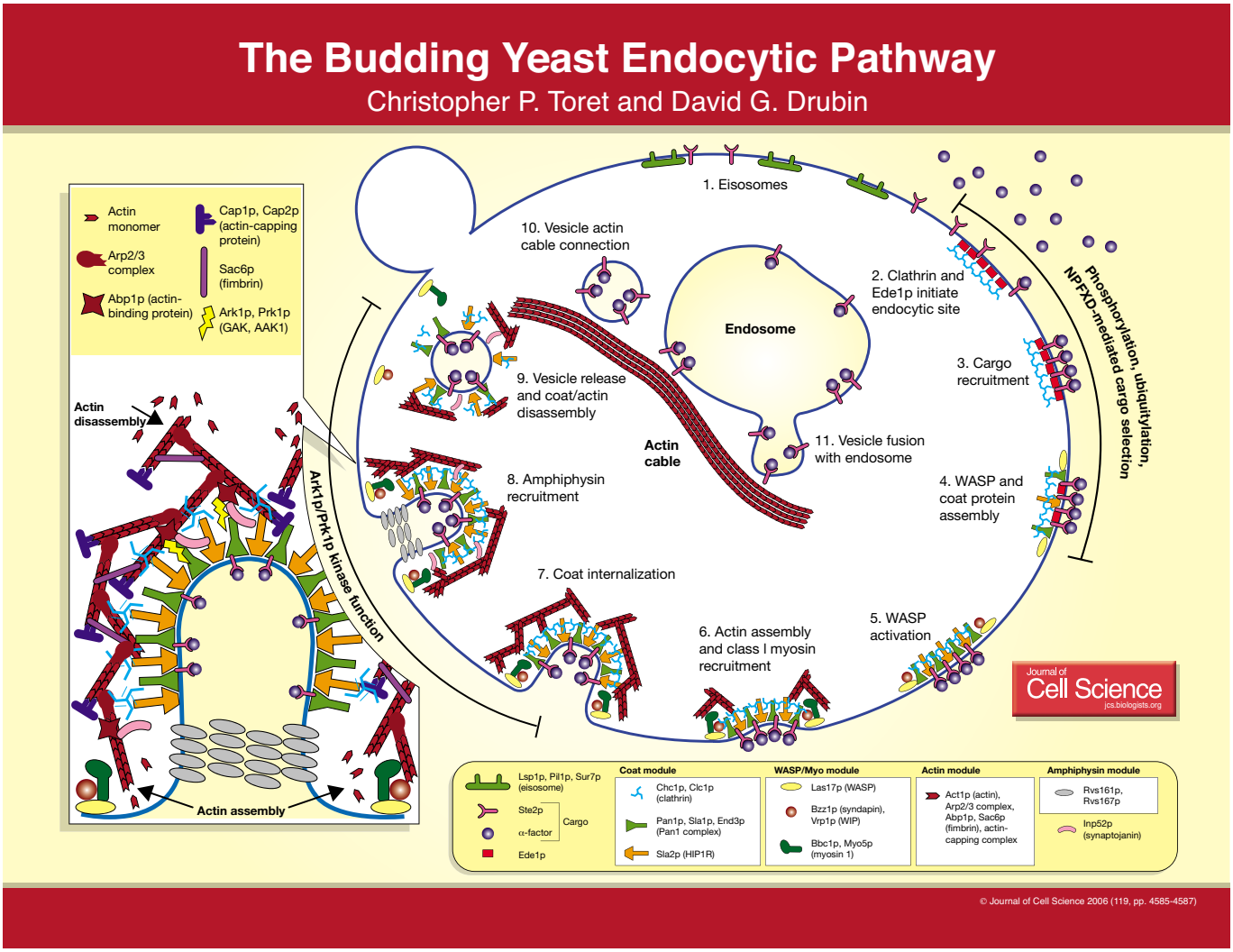
We apologise for an error in the poster accompanying this article. The WASP/Myo module was omitted in Step 7 of the budding yeast endocytic pathway.

Below, Step 7 is shown correctly.



The online version shows the corrected poster.

The budding yeast endocytic pathway

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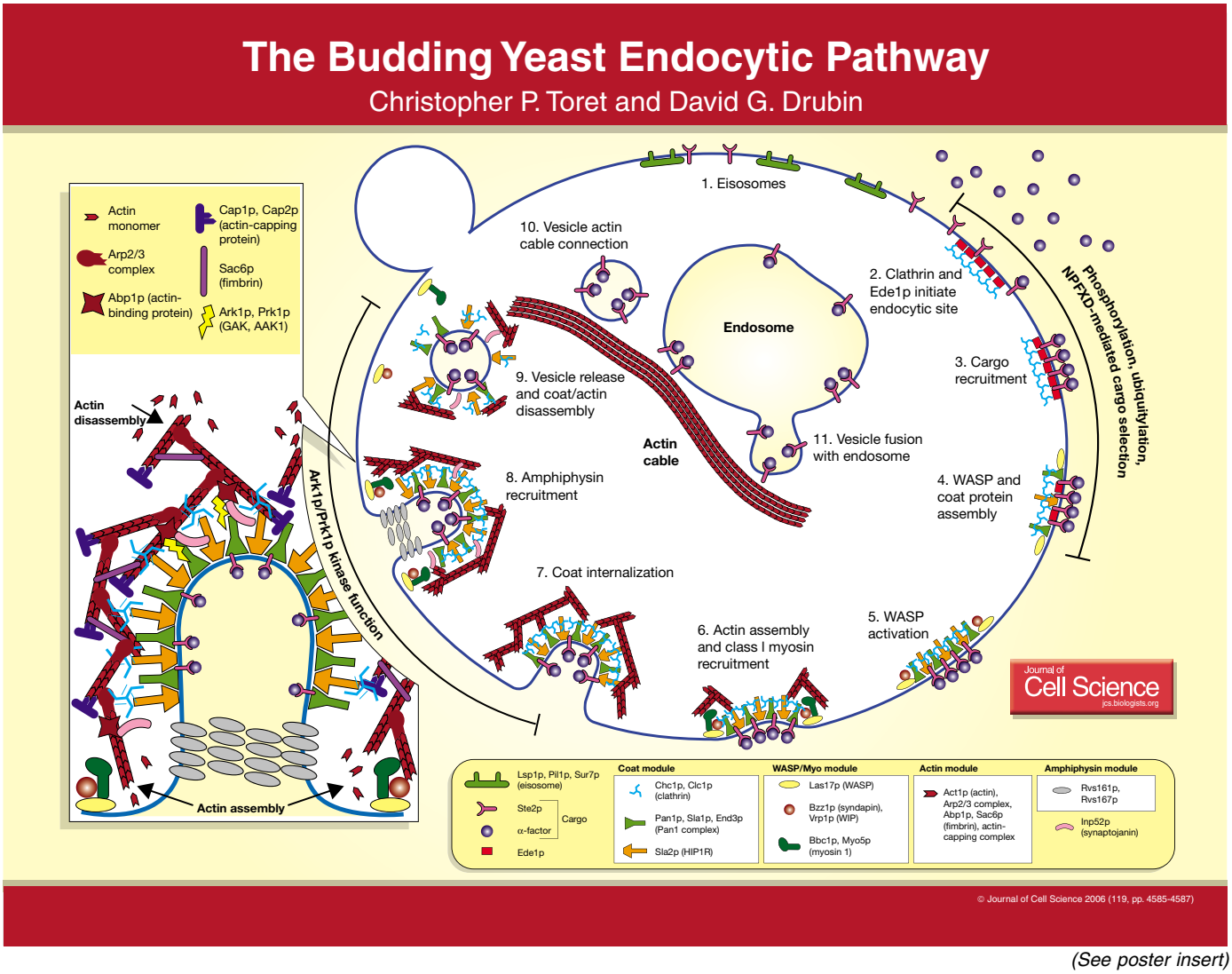
Recent live-cell imaging studies, coupled with powerful genetic, biochemical and pharmacological tests of function, have expanded our understanding of the molecular events that underlie clathrin/actin mediated-endocytosis in budding yeast. Many features of this pathway are evolutionarily conserved (Engqvist-Goldstein and Drubin, 2003; Kaksonen et al., 2006). Therefore, insights into the intricate molecular choreography of endocytic events in budding yeast will provide a basis for elucidating such mechanisms in more complex organisms. This poster depicts our current understanding of the dynamics of endocytosis in budding yeast.

Step 1. Stable plasma membrane protein structures called eisosomes have been suggested to function as organizing sites for endocytosis, although the nature of this proposed linkage to endocytic sites needs to be further explored (Walther et al., 2006). Two components of eisosomes, Pil1p and Lsp1p, are proposed targets of the two sphingolipid-regulated kinases Pkh1p and Pkh2p (Zhang et al., 2004). Sur7p, another eisosome protein, has been shown to localize with both the arginine permease (Can1p) and the uracil permease (Fur4p) (Malinska et al., 2004).

Step 2. Clathrin (which forms the characteristic cage around the budding vesicle) and Ede1p, an Eps15 homology (EH)-domain- and ubiquitin associated (UBA)-domain-containing protein, are the earliest proteins known to arrive at endocytic sites (Newpher et al., 2005; Toshima et al., 2006). Clathrin is not essential for endocytosis in budding yeast but is required for proper recruitment of later endocytic proteins (Kaksonen et al., 2005). Ede1p may function as an adaptor and plays a role in proper initation of endocytic sites (Kaksonen et al., 2005). Ent1p, Ent2p, Yap1801p, Yap1802p, and AP-2 all have both clathrin- and PtIns(4,5)P_2-binding domains and may function to recruit clathrin to endocytic sites (Newpher et al., 2005). However, their precise



(See poster insert)

dynamics remain to be established in budding yeast. PtIns(4,5)P_2 has an important yet undefined role at endocytic sites. It may serve to recruit or regulate these endocytic proteins.

Step 3. The yeast mating pheromone α-factor and its receptor, Ste2p, comprise one cargo known to cluster at endocytic sites after the arrival of early endocytic proteins at the endocytic site (Toshima et al., 2006). Binding of α-factor to Ste2p stimulates its internalization. Ste2p phosphorylation, ubiquitylation, and an NPFXD motif, function as internalization signals (Engqvist-Goldstein and Drubin, 2003; Hicke et al., 1998; Howard et al., 2002). The precise order and timing of the receptor modifications remains to be resolved. Furthermore, whether other endocytic cargo molecules show similar modifications and dynamics is not known.

Step 4. One to two minutes after clathrin appears at endocytic sites, Las17p (the yeast ortholog of the human Wiskott-Aldrich syndrome protein, WASP), which activates the Arp2/3 complex to promote actin assembly, begins to be recruited (Kaksonen et al., 2003). The actin assembly machinery probably encircles the coat that will be internalized because the WASP/Myo module proteins remain at the plasma membrane when the coat is internalized (steps 7-8). Concurrently with recruitment of Las17p to endocytic sites, Ede1p begins to disappear from the endocytic sites as additional coat proteins (Sla2p and the Pan1 complex) begin to accumulate (Kaksonen et al., 2003). The Pan1 complex contains End3p, an EH-domain-containing protein, and Sla1p, which is both a negative regulator of Las17p and the cargo adaptor for NPFXD-mediated endocytosis (Howard et al., 2002).

Step 5. Vrp1p (a Las17p-interacting protein orthologous to human WIP) and Bzz1p (a Las17p-interacting protein orthologous to human syndapin) are next recruited to the site. Bzz1p may relieve Sla1p inhibition of Las17p (Sun et al., 2006).

Step 6. Actin assembly is initiated. Vrp1p and the assembling actin recruit Myo5p, a type I myosin motor protein. Vrp1p stimulates the capacity of Myo5p to activate the Arp2/3 complex, and it promotes Arp2/3-mediated actin assembly for internalization (Sun et al., 2006). Bbc1p (an SH3-domain-containing protein) is recruited concurrently with Myo5p and appears to negatively regulate both Myo5p and Las17p (Kaksonen et al., 2005; Sun et al., 2006).

Step 7. The actin cytoskeleton is possibly linked to the coat module through Sla2p (Kaksonen et al., 2003; Newpher et al., 2006). Myosin motor activity and the assembling actin at the plasma membrane drive the coat module and underlying plasma membrane inward (Sun et al., 2006).

Step 8. As the coat module internalizes, the amphiphysins Rvs161p and Rvs167p are recruited to the endocytic site (Kaksonen et al., 2005). These proteins contribute to the release of the forming vesicle, although the scission mechanism remains to be established. Actin assembly forces may also contribute to vesicle scission. Several other proteins associated with the assembling actin meshwork provide important activities. Actin-binding protein (Abp1p), the actin-filament-crosslinking protein fimbrin (Sac6p) and the actin-capping protein heterodimer complex (Cap1p and Cap2p) contribute to productive actin meshwork dynamics (Kaksonen et al., 2005; Sun et al., 2006).

Step 9. Abp1p also plays a role in recruiting coat-disassembly factors, and inhibits the nucleating activity of the Arp2/3 complex (D'Agostino and Goode, 2005; Sun et al., 2006). After the coat has moved inwards approximately 200 nm, the coat and WASP/myosin module are disassembled. The protein kinases Ark1p and Prk1p are major regulators of endocytic site disassembly (Sekiya-Kawasaki et al., 2003; Zeng et al., 2001). Although their precise dynamics need to be characterized, recruitment of the two proteins is dependent on Abp1p (Cope et al., 1999). The Pan1 complex is disrupted by phosphorylation by these kinases (Zeng et al., 2001). The PtIns(4,5)P_2 phosphatase activity of Inp52p may contribute to endocytic coat disassembly. Inp52p arrives at endocytic sites late in the pathway and is also recruited by Abp1p (Stefan et al., 2005). The failure of the early components to disassemble from endocytic patches in the absence of actin assembly supports a role for the actin cytoskeleton as the trigger for a negative feedback loop to disassemble the endocytic machinery after internalization. In budding yeast endocytosis, no clathrin disassembly role has yet been identified for Swa2p, which is the yeast ortholog of the clathrin-uncoating protein auxillin.

Step 10. During its formation, the endocytic vesicle becomes associated with actin cables (Huckaba et al., 2004).

Step 11. The endocytic vesicles fuse with early endosomes in a process that is made more efficient by the association of the vesicles and early endosomes with actin cables (Toshima et al., 2006). The proteins that mediate association of endocytic vesicles and early endosomes with actin cables, and the underlying mechanisms for facilitating efficient docking and fusion, remain to be identified.

Although we know much about the budding yeast endoyctic pathway, much remains to be learned about the mechanisms that orchestrate this complex molecular choreography, about the ultrastructure of the endocytic complexes, and about the force-producing mechanisms.

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