Verprolin: A cool set of actin-binding sites and some very HOT prolines

Verprolin: a cool set of actin-binding sites and some very HOT prolines

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Abstract

Spatiotemporal organisation of eukaryotic cells is established and maintained by the cytoskeleton, a highly dynamic and complex network of structural and signalling proteins. Many components of the cytoskeleton are functionally and structurally conserved between humans and yeast. Among these are verprolin (Vrp1p) in yeast and its human ortholog Wiskott-Aldrich Syndrome Protein (WASP)-Interacting Protein (WIP). Much of our understanding of the function of these proteins has come from genetic analysis in yeast. Verprolin-deficient yeast cells exhibit defects in cytokinesis, endocytosis, and actin cytoskeleton polarisation. Verprolin binds actin, the yeast ortholog of human WASP (Las17p or Bee1p), and the yeast ortholog of human PSTPIP1 (Hof1p or Cyk2p). We propose that verprolin acts as a chaperone that by transient bimolecular interactions maintains the proper function of its partners. Verprolin-related proteins and partners are implicated in cancer, immunodeficiency, and neurodegeneration. Therefore, elucidating how verprolin functions will have major impacts in cell biology and medicine.

Introduction

Verprolin (Vrp1p) is a budding yeast (*Saccharomyces cerevisiae*) protein, however related proteins are found in vertebrates, e.g. Wiskott-Aldrich Syndrome Protein (WASP)-Interacting Protein (WIP). Vertebrate verprolin-related proteins are the topic of an excellent review [1]. The focus here will be on budding yeast verprolin (Fig. 1). Knockout of the *VRP1* gene that encodes verprolin yields viable verprolin-deficient (*vrp1Δ*) yeast cells. The *vrp1Δ* cells are temperature-sensitive, i.e. they are viable at 24^{o}C, but not at elevated temperature (e.g. 37^{o}C). In contrast, wild type (*VRP1*) yeast cells are viable at both temperatures [2; 3; 4]. The majority of *vrp1Δ* cells remain viable for a few hours and continue to grow in size at 37^{o}C but are unable to divide [5].

S. cerevisiae cells are encased in a rigid wall. They divide by outgrowth of a daughter cell (bud) from a site on the mother cell surface (Fig. 2*a*). When the bud approaches the size of the mother cell it is released by cytokinesis (division of the cytoplasm) (Fig. 2*b*). In wild type cells newly synthesised cell wall material is deposited centripetally at the neck that separates mother cell and bud (bud neck) to form a septum. After cytokinesis the septum is split down the middle allowing the cells to separate [6]. Cytokinesis is delayed in *vrp1Δ* cells even at 24^{o}C. This is due to inadequate spatiotemporal control of cell wall deposition as the septum is aberrantly thick (Fig. 2*d*). When *vrp1Δ* cells are shifted from 24^{o}C to 37^{o}C cytokinesis fails due to an inability to make a septum [7] (Fig. 2*e,f*).

A structure important for cytokinesis and conserved between yeast and humans is the contractile actomyosin ring [8]. The actomyosin ring comprises linear actin filaments nucleated by two proteins related to human formin, Bni1p and Bnr1p [9]. In many *vrp1Δ* cells at 24^{o}C the actomyosin ring is a C-shaped structure rather than a closed ring [7]. After prolonged shift to 37^{o}C a topologically closed but unconstricted actomyosin ring is visible in the majority of arrested cells that seems to contain less F-actin [7]. It is possible that the actomyosin ring defects observed in *vrp1Δ* cells reflect reduced Bni1p and Bnr1p activity.

There are two redundant pathways by which cytokinesis can occur in *S. cerevisiae*. Cells carrying mutations that block each individual pathway are viable but those carrying mutations that block both pathways fail in cytokinesis and are inviable [7; 10]. One pathway is dependent on the actomyosin ring [8]. The alternative pathway is dependent on the yeast ortholog of human Proline-Serine-Threonine-Phosphatase-Interacting Protein 1 (PSTPIP1) (Hof1p or Cyk2p) [10; 11; 12] [13]. Hof1p localises initially to two rings, one on each side of the bud neck. During cytokinesis these condense into a single ring coincident with the actomyosin ring. The Hof1p and actomyosin rings are distinct [7; 10; 11; 12]. In *vrp1Δ* cells at 24^{o}C Hof1p localises to two rings and these condense into a single ring as in wild type cells. However, after shift to 37^{o}C Hof1p localisation is abolished and Hof1p adopts a diffuse distribution throughout the cytoplasm [5; 7; 14]. *vrp1Δ* is lethal when combined with mutations affecting the actomyosin ring [2; 7] or Hof1p ring [7] consistent with verprolin functioning in both pathways of cytokinesis.

Hof1p interacts with verprolin and this is important for Hof1p localisation and function. Like human PSTPIP1, Hof1p has a C-terminal Src Homology 3 (SH3) domain. SH3 domains are protein interaction modules that bind proline-rich motifs (PRMs). The Hof1p SH3 domain binds to an N-terminal site in verprolin containing three PRMs: PAVPSIPSS (residues 144-152), PIPDIPSS (residues 156-163), PIPIVPSS (residues 167-174). All three verprolin PRMs are essential for interaction with the Hof1p SH3 and are collectively referred to as the Hof One Trap (HOT) domain [5]. Verprolin does not extensively co-localise with Hof1p, but a small pool of verprolin may transiently relocate to the Hof1p ring during cytokinesis (see Myo5p-mRFP/Vrp1p-GFP movie in [15]).

Endocytosis is a vesicle-mediated membrane traffic pathway that delivers plasma membrane and extracellular material to endosomes [16]. Yeast cells internalise extracellular fluid by fluid-phase endocytosis (Fig. 3, *vrp1Δ* + *VRP1*, *top*). They also express cell surface receptors and internalise their ligands by receptor-mediated endocytosis [17]. One mutant isolated in a screen for endocytosis-defective mutants was *end5* (*end*ocytosis-defective *5*). *end5* mutant cells are unable to perform fluid-phase or receptor-mediated endocytosis at either 24^{o}C or 37^{o}C (Fig. 3, *vrp1Δ* + vector, *top*) [18]. The *end5* mutation is a frame-shift after codon 604 of *VRP1* (renamed *vrp1-E5*) [3; 18]. Interestingly, *vrp1-E5* retains potential function as modest over-expression of *vrp1-E5* rescues *vrp1Δ* viability at 37^{o}C (our unpublished data).

Cortical actin patches are small (~0.1 μm diameter) spots comprising filamentous actin (F-actin) at the cell cortex [19]. In wild type cells actin patches have a polarised distribution and this varies through the cell cycle. Actin patches cluster at the site on the

mother cell surface where the next bud will emerge (nascent bud site). When the bud emerges actin patches cluster in the bud (Fig. 4, *vrp1Δ* + VRP1). During nuclear division actin patch distribution becomes random but actin patches later repolarise to either side of the bud neck in preparation for cytokinesis [19]. Cortical actin patches are short-lived (~11 s) and their polarised distribution is achieved by polarisation of the sites at which they form [20; 21].

The F-actin in actin patches is in the form of a dendritic network comprising branched actin filaments [22]. The rate-limiting step in F-actin assembly is the nucleation of short filaments from actin monomers. The Arp2/3 complex, a seven-subunit complex highly conserved from yeast to humans, nucleates F-actin assembly by creating branches on the sides of existing actin filaments. For strong activity Arp2/3 requires stimulation by Nucleation Promoting Factors (NPFs) [15]. Verprolin associates with several NPFs including Las17p (or Bee1p) (yeast WASP) [21] and type I myosins [7; 15; 23; 24; 25; 26]. The verprolin-Las17p and verprolin-type I myosin complexes function in assembly of the branched F-actin that comprises actin patches and co-localise with actin patches [3; 4; 26; 27].

In *vrp1Δ* cells cortical actin patches form but are less polarised than in wild type cells (Fig. 4, *vrp1Δ* + vector) [2; 3; 4; 18; 20]. Cell polarity in yeast (as in humans) is under the control of the Rho-family GTPase Cdc42p. Las17p, type I myosins, and verprolin do not physically interact with Cdc42p so polarisation signals from Cdc42p must be transmitted via other proteins to the Las17p-verprolin and type I myosin-verprolin

complexes. The formin-related proteins Bni1p and Bnr1p function redundantly to transmit cell polarity signals from Cdc42p to the Las17p-verprolin complex in yeast [21].

Consistent with a role in actin patch assembly, verprolin binds actin monomers. Verprolin has a WASP Homology 2 (WH2) domain in the N-terminal region [4]. Despite an initial report to the contrary [4], the WH2 domain is non-essential [14]. A second actin-binding site is located between verprolin residues 270-364 and may function redundantly with the WH2 [14]. A third actin-binding site (specific for actin monomers) is located between residues 465 and 493 in the C-terminal half of verprolin (Verprolin Homology 2 C-terminal or VH2-C domain). The VH2-C domain shows strong homology to a sequence within residues 270-364, so the second N-terminal actin-binding site may also be a VH2 domain (tentatively designated VH2-N) [28].

Structure-function analysis of verprolin

When an N-terminal fragment of verprolin (residues 1-364, N-Vrp1p) (Fig. 1) is mildly over-expressed in *vrp1Δ* cells it partially rescues viability and cytokinesis at 37^{o}C. Importantly, N-Vrp1p lacks sequences required to localise to cortical patches (see below) and does not restore actin patch polarisation. Therefore, the functions of verprolin in cell viability and cytokinesis do not require localisation to cortical patches. Artificially anchoring N-Vrp1p to membranes with a lipid anchor or strong over-expression of N-Vrp1p both confer even more efficient rescue, but still do not restore a fully polarised actin patch distribution [14]. Interestingly, over-expression of Las17p in *vrp1* mutant cells also

rescues viability at 37^{o}C (and fluid-phase endocytosis) without restoring actin patch polarisation [3].

Further truncations of N-Vrp1p identified the minimal fragment sufficient to rescue *vrp1Δ* viability at 37^{o}C as the HOT domain and either the WH2 or VH2-N domain (Fig. 1). Intriguingly, the requirement for the HOT (but not WH2 or VH2-N) domain is efficiently bypassed by deleting the Hof1p SH3. In *vrp1Δ* cells also lacking the Hof1p SH3 (*hof1SH3Δ*) expression of the WH2 domain is sufficient for viability at 37^{o}C. Hence, N-Vrp1p supplies two distinct functions: binding of the Hof1p SH3 (dispensable in cells lacking the Hof1p SH3) and binding of actin monomers [5; 14].

When expressed at slightly elevated levels in *vrp1Δ* cells, a C-terminal fragment of verprolin (C-Vrp1p, residues 364-817) (Fig. 1) restores viability, cytokinesis, fluid-phase endocytosis, and also actin patch polarisation at 37^{o}C [14; 18]. C-Vrp1p also localises to cortical actin patches like full-length verprolin [14; 28]. Localisation of C-Vrp1p to cortical patches is strictly dependent on both its C-terminal Las17p-Binding Domain (LBD) and on Las17p [14; 28] (Fig. 1). C-Vrp1p does not interact with the Hof1p SH3 [7]. The ability of C-Vrp1p to restore cortical actin patch polarisation to *vrp1Δ* cells is dependent on both VH2-C and LBD domains [28]. In contrast, for viability and fluid-phase endocytosis at 37^{o}C the LBD is sufficient [28]. This suggests that fluid-phase endocytosis is important for viability at 37^{o}C, but a fully polarised actin patch distribution is not.

Another important role of Las17p binding is to anchor verprolin to membranes. Deletion of the LBD results in loss of C-Vrp1p from cortical patches and is accompanied by loss of all function. To determine whether it is patch localisation, cortical localisation, or Las17p interaction that is most important for C-Vrp1p function a test was performed to see whether anchoring this truncated form of C-Vrp1p to membranes using a lipid anchor would restore function. The lipid-anchored form of truncated C-Vrp1p evenly decorated membranes but did not form patches. Remarkably, this lipid-anchored form of truncated C-Vrp1p regained function in viability at 37^{O}C and fluid-phase endocytosis [28]. Association of verprolin with membranes is important for viability at 37^{O}C and fluid-phase endocytosis, but patch localisation and interaction with Las17p are not.

Human WIP partially substitutes for verprolin in yeast

Human WIP has a similar domain structure to budding yeast verprolin, containing an N-terminal WH2 domain and a C-terminal WASP-binding domain. High-level expression of human WIP in *vrp1-1* (Leu425 to Pro) mutant yeast cells partially rescues viability at 37^{O}C, fluid-phase endocytosis, and actin patch polarisation. However, the *vrp1-1* allele only reduces and does not abolish verprolin function as modest over-expression of the *vrp1-1* mutant gene in *vrp1Δ* cells restores viability at 37^{O}C (our unpublished data). Human WIP lacks the ability to bind yeast Las17p (our unpublished data), which may explain why high-level expression of human WIP in *vrp1Δ* cells (devoid of endogenous verprolin) only rescues viability at 37^{O}C and not actin patch polarisation [29] (our

unpublished data). The ability of human WIP to rescue viability at 37^{o}C is dependent on both the WH2 and the PRM APPPPP (residues 427-432) [29]. Dependence on the WH2 may be because WIP lacks VH2 domains.

A model for verprolin function: from multivalent adaptor to multivalent chaperone

Verprolin acts via physical association with other proteins. Some associations are likely stable (e.g. with Las17p) [21] while others may be transient (e.g. with Hof1p) [7]. Verprolin has the capacity to associate simultaneously with multiple partner proteins and has been described as a multivalent adaptor that holds these proteins together in a multi-protein complex [24]. It was a surprise to find that verprolin fragments function without multivalency. Interaction with verprolin alone confers an important physiological change in the partner protein. Multivalency, however, may facilitate better coordination of these various verprolin-dependent processes.

Our model is depicted in Fig. 5. In this model one major verprolin function is to prevent the Hof1p SH3 from inhibiting cytokinesis. One possibility is that verprolin stably associates with the Hof1p SH3 and thereby physically prevents it from interfering with cytokinesis. An alternative possibility is that verprolin only transiently associates with the Hof1p SH3 but this results in a lasting change in the SH3 and this prevents it from interfering with cytokinesis. We favour the second possibility because of the lack of extensive co-localisation of verprolin with Hof1p. According to our model, the Hof1p SH3 interacts excessively or improperly with other proteins and inhibits their function in cytokinesis in the absence of the verprolin HOT domain. The Hof1p SH3 has been shown

to interact with Bni1p and Bnr1p [12]. Bni1p and Bnr1p function in both cell polarity signalling and assembly of linear actin filaments. Therefore, inhibition of Bni1p and Bnr1p by the Hof1p SH3 may explain the defects in the actomyosin ring and actin patch polarisation in *vrp1Δ* cells.

Our model for verprolin function (Fig. 5) may also explain why over-expression of Las17p restores viability at 37^{O}C and fluid-phase endocytosis but not actin patch polarisation to *vrp1-E5* cells [3]. Las17p has a PRM with the sequence PIPEIPST (residues 275-282) that resembles the verprolin HOT domain PRMs and interacts with the Hof1p SH3 (our unpublished data). The existence of a potential Hof1p SH3-binding site in Las17p suggests that Las17p may be another protein targeted by the Hof1p SH3 in cells lacking the verprolin HOT domain. Las17p is essential at 37^{O}C for fluid-phase endocytosis [3] and Hof1p localisation to the bud neck [14]. In the absence of the verprolin HOT domain these Las17p functions may be inhibited.

In our model, verprolin association with Las17p via its LBD induces conformational changes in Las17p that increase its NPF activity on Arp2/3 and may protect Las17p from degradation (Fig. 5). This explains why the verprolin LBD alone satisfies the verprolin-requirement for viability at 37^{O}C and fluid-phase endocytosis [28]. Stimulation of Las17p NPF activity or increased Las17p protein levels resulting from expression of C-Vrp1p in *vrp1Δ* cells would be predicted to counterbalance the inhibitory effects of the Hof1p SH3. The verprolin-Las17p interaction also physically attaches verprolin to membranes, which appears to be important for verprolin function.

Originally, it was proposed that verprolin provides actin monomers to Arp2/3 for incorporation into filaments via a physical interaction [15; 21; 24]. However, the verprolin WH2 domain remains functional *in vivo* even after the domains that mediate interaction with NPFs are deleted [5]. In our model it is the conformation (or covalent modification) of actin monomers following transient interaction with verprolin WH2 or VH2 domains that is important for Arp2/3- and formin-dependent F-actin assembly (Fig. 5). This may reduce non-specific interactions that might otherwise result in aggregation of actin monomers. In support of this, *vrp1Δ* cells contain aberrant cytoplasmic bar-shaped structures that stain with anti-actin antibodies but not F-actin specific reagents and may be non-specific aggregates [20].

Our model may also apply to human WASP and WIP. The APPPPP PRM in human WIP essential for rescue of *vrp1* [29] overlaps extensively with the PRM PPPPPPST, which resembles the verprolin HOT domain PRMs. Indeed, human WIP interacts with the Hof1p SH3 (our unpublished data). Human WIP function in yeast is therefore analogous to N-Vrp1p function as both interact with actin monomers and the Hof1p SH3, but not with Las17p and restore viability at 37^{o}C but not a polarised actin patch distribution. WIP- and WASP-related proteins are implicated in an ever-expanding list of human diseases including immune-deficiency [30], cancer [31], and neurodegeneration [32]. Insights into the molecular basis of verprolin function may enable improved therapies for these diseases.

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Figure legends

Figure 1 *Domain structure of verprolin*

Schematic depicting the domain structure of full-length verprolin (*above*) and the N-Vrp1p and C-Vrp1p fragments (*below*). Numbers refer to amino acid residues.

Figure 2 *Cytokinesis defects in verprolin-deficient cells*

Transmission electron micrograph of wild type (*WT, a,b*) and *vrp1Δ* (*c-f*) cells during cytokinesis at 24^{o}C (*a-d*) and after shift to 37^{o}C for 3-4 h (*e,f*). Note the disorganised septum in *vrp1Δ* cells at 24^{o}C (*d, black arrow*) compared to wild type cells (*b, black arrow*). Note the maintenance of cytoplasmic continuity between mother cell and large bud in *vrp1Δ* cells after shift to 37^{o}C (indicates failure in cytokinesis) (*e,f*). Note the aberrantly thick cell wall enclosing the mother cell (*e,f, double black arrowhead*) compared to wild type cells (*a, double black arrowhead*). *Single black arrowhead* in *b-f*, bud neck. Bars, 1 μm.

Figure 3 *Loss of fluid-phase endocytosis in verprolin-deficient cells*

Shown are *vrp1Δ* cells carrying the wild type *VRP1* gene on a plasmid (*left*) and *vrp1Δ* cells carrying empty vector (*right*) grown at 24^{o}C and incubated for 1 h at 24^{o}C with a

fluorescent endocytic dye (Lucifer Yellow). The upper and lower panels show the same field of cells with fluorescence optics to visualise Lucifer Yellow and differential interference contrast optics to visualise vacuoles (indentations), respectively. The wild type cells internalise the dye but *vrp1Δ* cells do not. Bar, 5 μm.

Figure 4 *Depolarisation of cortical actin patches in verprolin-deficient cells*

Shown are *vrp1Δ* cells carrying the wild type *VRP1* gene on a plasmid (*left*) and *vrp1Δ* cells carrying empty vector (*right*) grown at 24^{o}C stained with fluorophore-conjugated phalloidin (stains F-actin). In wild type cells cortical actin patches polarise to small buds but in *vrp1Δ* cells they are more depolarised. Bar, 5 μm.

Figure 5 *Model for verprolin function*

Verprolin is depicted as a multivalent chaperone. Misfolded partner proteins (irregular shapes) can transiently bind to appropriate binding sites on verprolin and undergo long-lasting conformational change to an active form. Actin monomers are depicted in red, the Hof1p SH3 domain in yellow, and Las17p in green. The two barrel-shaped structures are the Arp2/3 complex (*left*) and formins (*right*). The correctly folded actin is shown emerging from verprolin and passing to the Arp2/3 complex and formins for use in assembly of branched or linear actin filaments, respectively. The misfolded form of the Hof1p SH3 domain is shown here binding Las17p and formins which is interpreted here as being inhibitory to Las17p and formin function. Some cellular structures and processes that depend on branched and linear actin filaments are listed below each type of filament.

Fig. 1

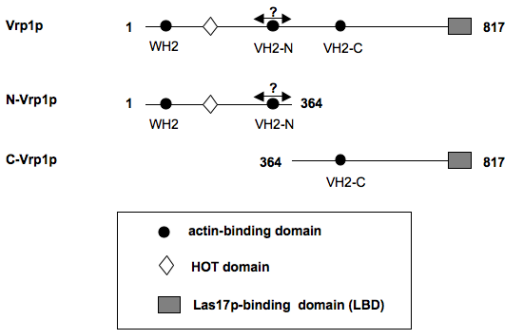

Figure 1 Domain structure of verprolin

Fig. 2

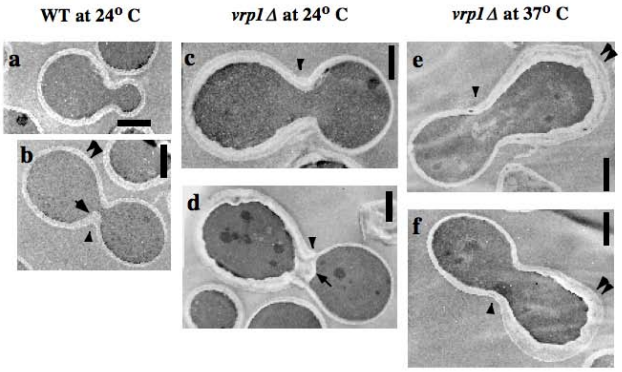

Figure 2 Cytokinesis defects in verprolin-deficient cells

Fig. 3

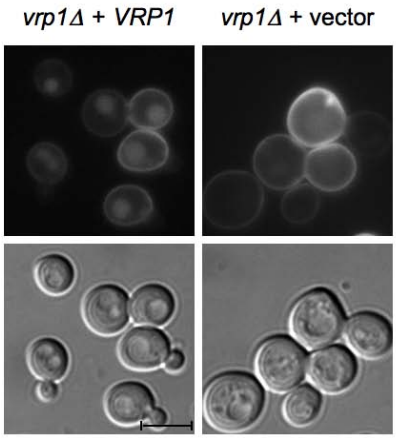

Figure 3 Loss of fluid-phase endocytosis in verprolin-deficient cells

Fig. 4

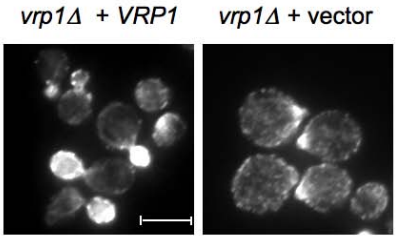

Figure 4 Depolarisation of cortical actin patches in verprolin-deficient cells

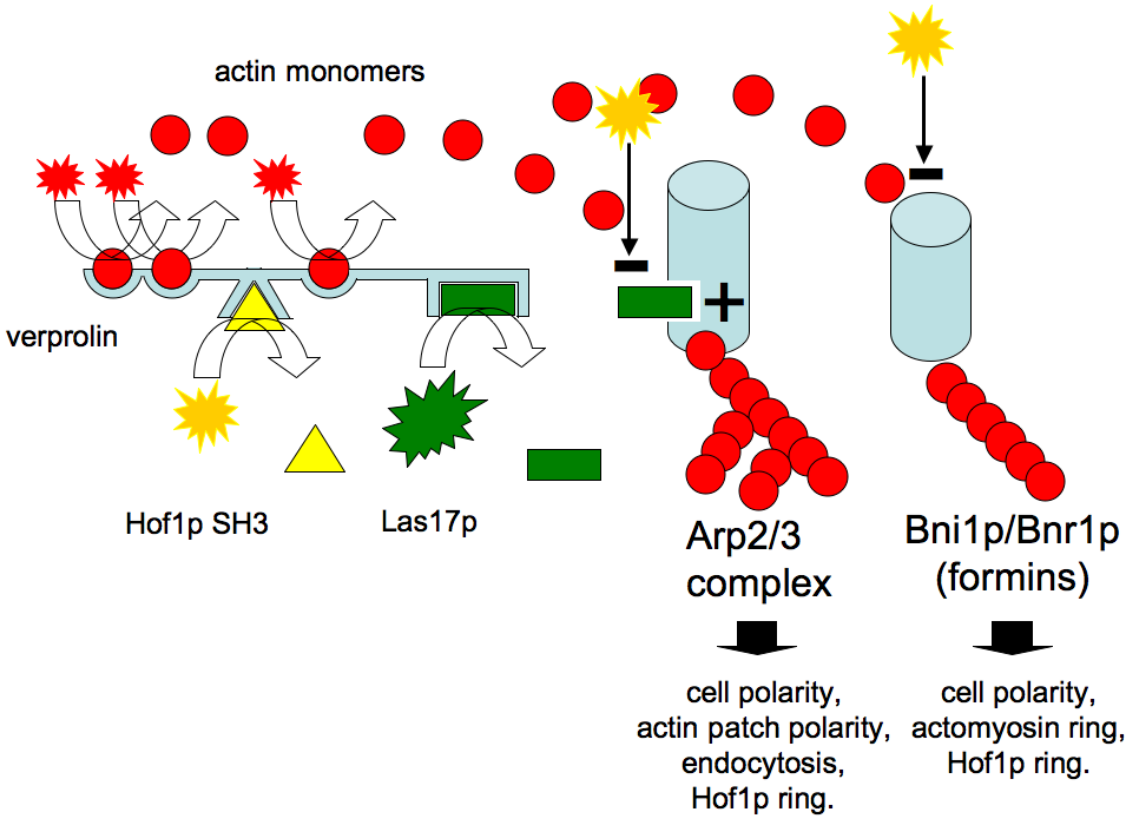

Figure 5 Model for verprolin function