Review Article

The Actin Cytoskeleton in Yeast and Animal Cells

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Abstract
The cell is the smallest functional unit of all known living organisms and is the building block of life. All cells are derived from pre-existing cells and irrespective of whether cells reproduce sexually or asexually, cells need to establish polarity, segregate the chromosomes and undergo cytokineses to generate the daughter cells. Cells also need to maintain correct shape and interact with their environment and many cells also have the ability to change shape and migrate from one place to another. These spatial and mechanical functions depend on a system of filaments collectively called as cytoskeleton. The entire focus of this review would be on the proteins associated with the actin network in yeast and mammalian cells.

Keywords: Actin cytoskeleton, Arp2/3, Wiskott Aldrich Syndrome Protein, Las17p, WIP, Vrp1p

1. Introduction
1.1 Actin filaments
Actin is a highly conserved 42 kDa protein expressed in all eukaryotic cells from fungi to metazoans and constitutes one of the most abundant cellular proteins. Diploid yeast cells have two copies of the gene encoding Act1p, the yeast actin. In humans, there are two copies each of the genes encoding the different actin isoforms, α-actin, β-actin and γ-actin. α-actin is expressed in muscle cells and is associated with muscle contraction, β-actin and γ-actin are expressed in non-muscle cells where β-actin is associated with actin polymerization in the cell leading edge and γ-actin is associated with stress fibers. In any cell, the richest area of actin is found in the narrow region that lies underneath the plasma membrane, called cell cortex. In the cell cortex, the actin filaments are arranged in a network that excludes most organelles from the cortical cytoplasm. Actin exists in two forms, a globular monomer called G-actin and as a filamentous polymer F-actin which is a linear chain of G-actin subunits. Actin filaments consist of two F-actin filaments which are interwoven with each other in a helical pattern. Each G-actin molecule has a binding site for a nucleotide (ATP or ADP) and a divalent cation (Mg2+). F-actin filaments are polarized structures with a fast growing barbed end and a slow growing pointed end. The barbed end of the filament is favored for polymerization and ATP-G-actin monomers are preferentially added to this end. Once the G-actin subunit has been incorporated into the filament at the barbed end, the bound nucleotide undergoes hydrolysis to ADP, thus reducing the affinity of G-actin with the neighboring subunits and in turn leads to the depolymerization of the filament at the pointed end. This process of active addition and dissociation of G-actin to and from the F-actin filament is called treadmilling (Fig. 1).

Figure 1: Treadmilling of actin filaments.

Treadmilling is caused by the hydrolysis of the ATP associated with G-actin. Filaments depolymerize at the pointed end and a steady concentration of ATP-G-actin is maintained which enables polymerization at the barbed end to balance depolymerization.

2. Arp2/3 complex
The two major classes of proteins which mediate the nucleation of actin filaments are the Formins and the Arp2/3 complex. Formins are associated with the nucleation and elongation of unbranched actin filaments and they participate in cytokineses and formation of actin filament bundles. The Arp2/3 complex is active at the leading edge of motile cells and it produces new actin filaments from the sides of the existing filaments. Among the two families of actin nucleators, the Arp2/3 complex has been the best characterized and it consists of seven subunits (Arp2, Arp3, ARPC1, ARPC2, ARPC3, ARPC4 and ARPC5). The complex was first discovered in Acanthamoeba castellanii and has subsequently been isolated from several other organisms including vertebrates and yeast. The subunit composition of the Arp2/3 complex is well conserved in all these organisms. In vitro studies have shown that purified Arp2/3 complex has minimum activity for the nucleation of actin filaments. However, the ability of the Arp2/3 complex to promote actin polymerization can be magnified many fold by the activation of the Arp2/3 complex by nucleation promoting factors (NPF). The main feature that is conserved in NPFs is the presence of an Arp2/3-binding sequence called the CA domain. The CA domain consists of a short stretch of basic and acidic amino acids located at the C and A region respectively. It has been shown that though the CA region in the NPFs is sufficient for binding with the Arp2/3 complex, it is not capable of activating the complex in vitro. In order to activate the Arp2/3 complex, the NPFs must possess adjacent binding sites for either G-actin or F-actin. The NPFs have thus been classified into two categories based on the presence of binding sites for G-actin or F-actin. NPFs which possess
one or two G-actin binding WH2/V domains are called Class I NPFs and include WASP, WAVE and Myosin I. Class II NPFs which include cortactin and Abp1p, do not have any G-actin binding WH2/V domain but instead contain F-actin binding domains (FAB)10.

2.1 Activation of the Arp2/3 complex

Class I NPFs activate the Arp2/3 complex by first forming a ternary complex with Arp2/3 complex and G-actin (Fig. 2A). In this ternary complex, the interaction between the Arp2/3 complex and the CA domain of the NPF may cause a conformational change in the complex, resulting in its activation. Nucleation could then be initiated by the presentation of G-actin by the WH2 domain of the NPF11. Class II NPFs activate the Arp2/3 complex by a slightly different mechanism (Fig. 2B). It is hypothesized that the binding of the CA domain of the Class II NPFs to the Arp2/3 complex may induce a conformational change that enables the complex to bind to the side of the existing actin filament. The binding of the Arp2/3 complex to the side of the mother filament might induce further conformational changes and subsequently leads to the activation of the complex and nucleation 9.

Figure 2: Activation of the Arp2/3 complex by Class I and Class II NPFs12.

A. Class I NPFs bind to inactive Arp2/3 complex and form a ternary complex consisting of the NPF, Arp2/3 complex and G-actin. The formation of the ternary complex induces a conformational change in the Arp2/3 complex resulting in its activation.

B. Class II NPFs bind with inactive Arp2/3 complex to form a binary complex. The binary complex then binds to the side of the mother filament which results in the activation of the Arp2/3 complex.

2.3 Wiskott Aldrich Syndrome (WAS)

The Wiskott Aldrich Syndrome protein (WASP) is one of the best characterized members of Class I family of NPFs. Mutations in the gene encoding WASP located on the X-chromosome, cause the genetic disease Wiskott Aldrich Syndrome (WAS) predominantly in males13. Patients suffering from WAS exhibit a variety of symptoms ranging from thrombocytopenia, eczema and bleeding disorders. However, only 27% of the patients suffering form WAS having all the above mentioned manifestation of WAS (Classic WAS). Almost 90% of the patients only have manifestation of eczema and thrombocytopenia at the onset of the disease. WAS patients also suffering from immune disorders characterized by recurrent bacterial and viral infections. The immune system dysfunction is probably due to reduced proliferation of T and B lymphocytes in response to antigens. Studies show that the patients also have a predisposition towards lymph node cancer (reviewed in Millard et al., 2004). The disease occurs at a frequency of 4/1,000,000 live male births and it can be treated by bone-marrow transplantation. Symptomatic treatment like splenectomy might also help by artificially raising the platelet count.

2.4 Wiskott Aldrich Syndrome protein (WASP)

WASP is a 502-residue proline rich protein that is mainly expressed in hematopoietic cells. WASP belongs to a large family of proteins called the WASP family of proteins (Fig. 3) which also includes N-WASP, WAVE1, WAVE2 and WAVE3, all of which are implicated with cytoskeletal regulation14. Yeast cells express only a single member of the WASP family, called Las17p in S. cerevisiae and Wsp1p in S. pombe.

Figure 3: Domain structure of the members of the WASP family of proteins14.

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<th>Protein</th>
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WH1: WASP homology domain 1, SH: Scar homology domain, BR: Basic region, GBD: GTPase binding domain, PRO: Proline rich region, V: Verprolin homology domain, C: Cofilin homology domain or connecting region, A: Acidic region.
WASP consists of a pleckstrin-homology (PH) domain at the N-terminal region which partially overlaps with the WASP homology (WH1) domain, followed by the GTPase binding domain (GBD), which is succeeded by a proline rich sequence and the verprolin homology-cofilin homology-acidic (VCA) region. The PH domain has been shown to interact with phospholipids like Phosphatidylinositol (4,5)-biphosphate. The WH1 domain has been identified in many proteins which regulate cytoskeletal organization and has been shown to bind with proline rich proteins like WIP and WIRE. The GBD has been shown to interact with Cdc42, a member of Rho family of GTPases which regulate actin cytoskeleton. The proline rich region has been shown to interact with SH3 domain containing proteins like Nck and Fyn. The VCA region has been shown to interact with G-actin and also bind and activate the Arp2/3 complex. The presence of many domains which interact with signaling proteins suggests an important role for WASP in the signaling cascades which regulate important physiological activities. Fifty percent of the mutations causing WASP are point mutations in the WH1 domain.

3. Regulation of WASP

WASP has been shown to adopt an inactive conformation in vitro through the binding of the VCA region with the GTPase binding domain (GBD), thereby masking the VCA region (Fig. 4A). The masked VCA region is unable to activate the Arp2/3 complex. Under such an inactive state, the protein cannot promote actin polymerization. It has been shown that GTPases like Cdc42 and phospholipids like Phosphatidylinositol (4,5)-biphosphate can cooperatively stimulate WASP to promote actin polymerization. The binding of Cdc42 and phospholipids with WASP disrupts the inactive conformation of WASP and exposes the VCA region (Fig. 4B). The exposed VCA region is therefore free to bind and activate the Arp2/3 complex. The activated Arp2/3 complex serves a very important role as an actin nucleating factor and promotes the formation of F-actin. Recently, a slightly different model for the activation of N-WASP has been suggested. According to this model, WASP/N-WASP exists as an inactive complex with WIP. The activation of WASP/N-WASP by Cdc42 requires the presence of an adaptor protein Toca-1 which is capable of binding simultaneously with both WASP and Cdc42. The exact role played by Toca-1 in the activation of WASP/N-WASP needs further investigation.

Figure 4: The putative mechanism of activation of WASP.

4. WASP interacting protein (WIP)

It has been reported that most of the WASP in mammalian cells exists as a complex with WIP. WIP was discovered in a yeast two-hybrid screen using WASP as bait and it has been shown that the C-terminal region of WIP (consisting of the WASP binding domain) interacts with the WH1 domain of WASP (Fig. 5). WIP is a proline rich protein belonging to the Verprolin family which also includes WIRE/WICH and...
CR16 \(^{(29)}\) and the yeast protein Vrp1p \(^{(20)}\). Among the mammalian members of the Verprolin family, WIP and WIRE are expressed in a ubiquitous manner but the expression of CR16 is restricted to the brain, heart, lungs and colon. WIP is a 503 residue protein that has the actin-binding verprolin homology (V) domain which contains the KLKK motif and shows significant similarity to the N-terminal region of the yeast protein Verprolin (Vip1p). Apart from these two domains, WIP also has a number of proline-rich regions containing putative SH3-binding motifs that have the consensus sequence PPPX(P).

In mammalian cells, over expression of WIP in transfected mammalian cells induces actin polymerization and formation of membrane projections \(^{(24)}\), suggesting a role for WIP in cytoskeletal organization. A WASP-WIP complex is also readily detected in resting cells. Some of the mutations causing Wiskott Aldrich Syndrome have been shown to affect WASP-WIP binding suggesting that such interaction might be crucial for WASP’s function. Studies done using yeast have shown that mutations in Wasp affect binding with WIP which leads to instability of the protein \(^{(22–24)}\). The contribution of WIP to WASP’s activity needs further investigation.

### 4.1 Actin cytoskeleton in Saccharomyces cerevisiae

In *S. cerevisiae*, the filamentous actin (F-actin) is organized into cortical actin patches, actin cables and the actomyosin ring \(^{(5)}\). Actin patches are complex structures composed of around 30 proteins. Actin cables are cross-linked bundles of actin filaments. Among these actin structures, the cortical actin patches take up most of the filamentous actin in the cell. Actin patches are concentrated at sites of polarized growth and play a crucial role in the endocytic machinery of the cell. Actin patches might also play a role in exocytosis as mutating some of the patch components results in the accumulation of post-Golgi vesicles. The primary role of actin cables is in the transport of cargo from the mother cell to the bud in a budding cell. During the early stages of cell cycle in *S. cerevisiae*, polarity factors accumulate at the location of the nascent bud site. At the bud emergence, the assembly of the actin cytoskeleton is initiated from this nascent bud site and the reorientation of the cables takes place in order to enable the growth and secretion to the future bud tip. These cables then serve as tracks for the delivery of cargo to the nascent bud, using the motor activity of the myosin proteins. One of the components of the cortical actin patches in yeast cells is the Arp2/3 complex. The *S. cerevisiae* Arp2/3 complex is regulated by Class I NPFs Las17p, Myo3p, Myo5p and Class II NPFs Pap1p and Abp1p.

### 4.2 Actin cytoskeleton and endocytosis in yeast

The earliest indication that actin is involved in endocytosis in yeast was provided by the observation that temperature-sensitive actin mutants were unable to carry out fluid phase endocytosis \(^{(21)}\). The endocytic process can be divided into the following steps, coated pit formation, membrane invagination and sequestration, detachment of the newly formed vesicle and the movement of the vesicle away from the plasma membrane and into the cytoplasm of the cell. Each of these steps could involve the motor activity of myosins using the actin cytoskeleton as tracks \(^{(7)}\). The specific role played by actin in endocytosis is not clear. It is suggested that the actin cytoskeleton could invaginate the plasma membrane by inducing a curvature on the cytosolic side of the membrane which would in turn enable the nascent vesicle to pinch off from the plasma membrane. It is also possible that the cortical cytoskeleton needs to be removed from the plasma membrane in order to make the plasma membrane less rigid and thus assist in endocytosis. It was also shown recently that FM4-64 labeled endosomes in *S. cerevisiae* co-localize with actin patches labeled with Abp1p-GFP both during the assembly of the actin patches at the bud cortex and also during the movement of the patch away from the bud and towards the mother cell \(^{(25)}\). Therefore, cortical actin patches could be putative sites of internalization during endocytosis in yeast cells.

### 4.3 The Saccharomyces cerevisiae WASP homologue, Las17p

Las17p, the *S. cerevisiae* homologue of WASP is one of the first proteins to be recruited to the cortical actin patches in yeast \(^{(20)}\). The domain organization of Las17p is similar to that of mammalian WASP. Las17p exhibits a modular structure with a WASP Homology 1 domain (WH1) at the amino terminal, the WH2 (WASP Homology 2 domain) and an acidic domain (A) at the carboxy terminal part of the protein (Fig. 3). A key difference between WASP and Las17p is the lack of a GTPase binding domain (GBD) in Las17p. Cdc42p regulates the localization of Las17p to regions of polarized cell growth, although unlike WASP, Las17p does not bind directly to Cdc42p \(^{(26)}\). The WH1 domain of Las17p interacts with the C-terminal region of the yeast WIP, Vip1p in a manner analogous with the interaction between WASP and the WASP Interacting protein (WIP) \(^{(27)}\). The proline rich regions of Las17p interact with the SH3 domains of Bzz1p, Hof1p, Myo3p, Myo5p and Rvs167p. The VCA domain of Las17p interacts with G-actin and also binds and activates the Arp2/3 complex \(^{(28)}\).

A purified fragment encompassing only the VCA domain of Las17p is able to activate the *S. cerevisiae* Arp2/3 complex albeit to a lesser degree compared to the full-length Las17p \(^{(29)}\). Moreover, genetic studies showed that complete deletion of LAS17 causes severe defects than the deletion of only the VCA domain of Las17p. These results suggest that the activity of full length Las17p in activating the Arp2/3 complex is not only due to its VCA domain but also due to additional regions of Las17p. It is possible that the N-terminal region of Las17p contributes to the activation of the Arp2/3 complex through the activity of other cytoskeletal proteins (Vip1p, Myo3p, Myo5p) that bind to the N-terminal region of Las17p \(^{(29)}\). The mobility of vacuoles or endosomes during endocytosis also requires actin polymerization induced by Las17p. The involvement of Las17p in endocytosis was further substantiated by the finding that the fusion of vacuoles was inhibited in presence of antibodies raised against Las17p \(^{(27)}\). The lack of a GBD domain in Las17p and the observation that purified full-length Las17p is able to promote actin polymerization *in vitro* even in the absence of Cdc42p \(^{(26)}\), suggests that Las17p is constitutively active.

### 4.4 Vrp1p

Vrp1p is a proline rich protein related to mammalian WIP \(^{(21)}\). Vrp1p colocalizes with actin patches and plays a similar role as Las17p in the distribution of cortical actin patches. Additionally, Vrp1p is also involved in regulating endocytosis in *S. cerevisiae* \(^{(30)}\). Deletion or mutation in the gene encoding Vip1p leads to depolarization of the cortical actin patches and loss of endocytosis and also abolishes cell growth at 37°C \(^{(31)}\). Vrp1p is also required for proper vacuolar morphology and is also involved in the fusion of vacuoles \(^{(23)}\). Vrp1p has a domain organization similar to that of mammalian WIP. Vrp1p interacts with G-actin through the V domain located at the N-terminal region of the protein and also through two additional actin binding domains located at the regions 270-364 and 460-490 in the protein (Fig. 6) \(^{(30)}\). The N-terminal region of Vrp1p also has the Hof1p trap domain (HOT) which interacts with SH3 domain containing protein Hof1p. The C-terminal region of Vrp1p interacts with Las17p through the Las17p Binding Domain (LBD) \(^{(32)}\).

Both the N-terminal and the C-terminal halves of Vip1p show some redundancy in activity. Expression of either the N-terminal or the C-terminal fragment is able to rescue the growth defects of the *vpl1A* mutant cells. However, only the C-terminal fragment of Vip1p is capable of...
restoring the polarization of the actin patches in the \(vrp1\Delta\) cells. Both the N-terminal and the C-terminal fragments of Vrp1p are capable of independently binding with type I myosins.\(^{14}\) It has been shown that Vrp1p is essential for the polarized distribution of Las17p to the cortical patches\(^{15}\), suggesting that Vrp1p plays an important role in Las17p-mediated activation of the Arp2/3 complex. Studies have also shown that Las17p and Vrp1p function both as a complex and also independently of each other\(^{14,15}\).

\[\text{Figure 6} \quad \text{Domain organization of Vrp1p.} \]

Vrp1p has the G-actin binding V domain and the Hof1p binding HOT domain (H) at the N-terminus. Two additional actin binding domains (VH2N and VH2C) are also located one each at the N-terminus and the C-terminus respectively. The C-terminal fragment of Vrp1p also has the Las17p binding domain (LBD).

4.5 Myosins in \(S. \text{cerevisiae}\)

The myosins are a super-family of motor proteins that generate directed movement along actin filaments in an ATP-dependent manner. \(S. \text{cerevisiae}\) has 3 classes of myosins: type I myosins (Myo3p, Myo5p), type II myosins (Myo1p) and type V myosins (Myo2p, Myo4p). The type I myosins localize to the cortical actin patches and are involved in the activation of the Arp2/3 complex and regulate endocytosis.\(^{31}\) The type II myosins localize to the bud neck and play a role in cytokinesis similar to type II myosins in fission yeast\(^{16}\). Lastly, the type V myosins accumulate at the bud tip and are involved in cargo transport along actin cables.\(^{6}\)

\[\text{Figure 7} \quad \text{Domain organization of Myo3p/Myo5p (The numbers indicate location of domains in Myo5p).} \]

The N-terminus of type I myosins regulates the motor activity through the motor head domain. At the C-terminus, the TH1 and TH2 domain bind with cellular membrane and F-actin respectively. The SH3 domain binds with proline rich proteins like Las17p and Vrp1p. The CA domain binds and activates the Arp2/3 complex.

The type I myosins contain a motor head domain at the N-terminus whereas the C-terminal region contains the neck region (N) followed by the TH1 domain, TH2 domain, SH3 domain and the acidic domain (CA) (Fig. 7). It has been shown that the motor head domain of myosins interacts with actin and is capable of molecular motion through the hydrolysis of ATP.\(^{30}\) The motor domain of the type I myosins also contains a phosphorylation site (TEDS) which regulates the motor activity of the protein as the phosphorylation of the TEDS site leads to the activation of the ATPase activity of the motor domain.\(^{31}\) The neck region (N) is made up of \(\alpha\)-helical structures and binds with Calmodulin.\(^{31}\) The motor activity of myosins can be inhibited by Ca\(^2+\)-mediated release of Calmodulin from the neck region (N). The TH1 domain of type I myosins has been shown to mediate the binding of the protein to cellular membranes. The TH2 domain of type I myosins has been shown to bind with F-actin while the SH3 domain has been shown to bind directly with proline rich proteins like Las17p and Vrp1p. The C-terminal region of Myo5p (encompassing the CA domain) is able to promote actin polymerization in the presence of yeast cytosol and the deletion of the CA domain prevents Myo5p induced Arp2/3 dependent actin polymerization in vitro.\(^{8}\)

References

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