Review TRENDS in Cell Biology Vol.17 No.11

WASP-interacting protein (WIP): working in polymerisation and much more

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The migration of cells and the movement of some intracellular pathogens, such as *Shigella* and *Vaccinia*, are dependent on the actin-based cytoskeleton. Many proteins are involved in regulating the dynamics of the actin-based microfilaments within cells and, among them, WASP and N-WASP have a significant role in the regulation of actin polymerisation. The activity and stability of WASP is regulated by its cellular partner WASP-interacting protein (WIP) during the formation of actin-rich structures, including the immune synapse, filopodia, lamellipodia, stress fibres and podosomes. Here, we review the role of WIP in regulating WASP function by stabilising WASP and shuttling WASP to areas of actin assembly in addition to reviewing the WASP-independent functions of WIP.

Introduction

The architecture of eukaryotic cells is maintained through structures composed of intermediate filaments, tubulin microtubules or actin filaments. In response to internal signals or external cues, the cytoskeleton must reorganize to fulfil numerous cellular requirements, including proliferation, division, transcription, shape change and migration. Approximately 15 years ago, the paradigm was developed that small GTPases of the Rho family are crucial for regulating the formation of actin-containing structures: Cdc42 was thought essential for the formation of filopodia, Rac1 was important for lamellipodia protrusion and Rho was crucial for stress-fibre formation and cell contractility [1]. However, the protein(s) downstream of small GTPases that couple cellular signalling to actin cytoskeleton remodelling remained somewhat of a mystery. In a cluster of publications around the same time, three groups reported that WASP (Wiskott-Aldrich syndrome protein), the protein deficient in Wiskott-Aldrich syndrome (WAS) [2] bound to active Cdc42 [3–5] (Box 1). WASP is encoded on the X chromosome of mammals and is expressed exclusively in cells of haematopoietic origin. Mutations in the *WASP* gene result in an X-linked primary immunodeficiency characterised by eczema, thrombocytopenia and recurrent infections [2]. The identification of WASP was followed by publications showing that WASP and its ubiquitously expressed homolog neural WASP (N-WASP) [6] bind to and activate the Arp2/3 complex [7,8], which is a multimer of seven evolutionarily conserved proteins that together function as one of the major nucleating centres for the assembly of filamentous (F) actin at the cell cortex. The WASP family of proteins has since expanded to include a second subfamily termed suppressor of cAMP receptor–WASP-family verprolin homology (SCAR/WAVE) proteins, which also affect actin dynamics [9,10]. Early and important questions to be addressed were how WASP regulates the activation of Arp2/3-mediated actin polymerisation and which of the several WASP-associated proteins regulate WASP activation of Arp2/3. WASP-interacting protein (WIP) was first identified as a partner of WASP and a regulator of WASP-dependent and -independent actin polymerisation. In the past 18 months, nearly 20 articles on the biological functions of WIP have been published. These recent studies suggest that WIP contributes to multiple activities in diverse cell types and in different organisms. In this review, we attempt to integrate recent results with previous data and propose a model for WIP function as a chaperone for WASP and as a protein shuttle to direct WASP to areas of active cytoskeletal remodelling.

Structure of WIP

WIP, a proline-rich protein, is 503 amino acids (a.a.) long and binds to WASP and N-WASP at its C-terminal region (Figure 1). The WASP-binding region of WIP was mapped initially to a.a. 377–503 [11] and was later limited to a.a. 416–488 [12]. NMR studies have shown subsequently that the a.a. 461–485 sequence is sufficient to bind to N-WASP [13]. This region is thought to wrap around the WH1 domain of WASP (a.a. 26–147) like thread around the spool-shaped WH1 domain, such that WIP makes contact with WASP at two areas. WIP a.a. 461–468 adopt a polyproline type II helical structure (a left-handed helix with three residues per turn) that docks to a region on one face of the WH1 domain spool that contains aromatic residues with a central-conserved tryptophan (Trp54). The highly basic residues spanning a.a. 476–485 of WIP make contact with the opposite face of the spool through an electrostatic

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Available online 18 October 2007.

 0962-8924/$ – see front matter doi:10.1016/j.tcb.2007.08.005

Box 1. WASP

The WASP subfamily has a WASP homology 1 (WH1) domain at the N-terminal region that binds to WIP, followed by a GTPase-binding domain (GBD) that binds activated Cdc42. At the C-terminal end is the VCA domain consisting of the V (verprolin homology or WH2), C (cofilin homology) and A (acidic) domains. The region between the GBD and the VCA domain is rich in proline and binds to several SH3 domain-containing molecules, including Nck, Grb2 and cortactin. The domains of WASP are shown in Figure I. The SCAR–WAVE proteins (numbering three in humans and other mammals) have a SCAR–WAVE homology domain at the N-terminal end and do not have a defined GBD domain. The proline-rich region and C-terminal VCA domain is conserved between the WASP and the SCAR–WAVE subfamilies of proteins.

The WASP- and N-WASP-mediated actin-nucleating events so crucial for cytoskeletal remodelling within cells occur at the C-terminal portion of these molecules (Figure I). Two groups reported near-simultaneously that N-WASP and SCAR bind actin monomers and the actin-nucleating protein complex Arp2/3 [7,61,62], thus acting as a scaffold for actin-filament formation in what is often referred to as the dendritic-nucleation model of filament assembly [63]. By activating the Arp2/3 complex, WASP serves as a key integrator of cellular signals that culminate in actin-cytoskeleton remodelling. Biochemical and structural evidence suggests that WASP and N-WASP are in a closed inactive auto-inhibited conformation in resting cells owing to intramolecular interactions between the VCA domain on the one hand and the GBD domain and the basic region upstream of the GBD on the other hand [64,65]. WASP and N-WASP are activated *in vitro* following binding of active Cdc42. Several SH3 domain-containing proteins that include Nck, Grb2 and cortactin also activate WASP [66–68]. In addition, phosphorylation of WASP at Y291 by kinases that include Btk, Fyn, Ick and Itk also activates the actin-assembly function of WASP [69–71]. By contrast, WIP contributes to maintain the auto-inhibited conformation of WASP and, on cellular activation, facilitates WASP translocation to areas of active actin assembly where inhibition is released.

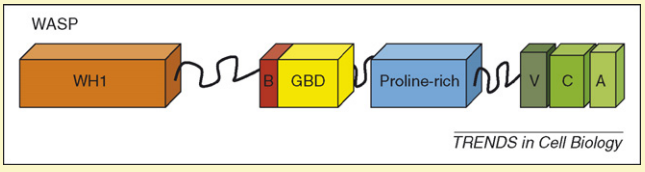

Figure I. Domain organisation of Wiskott-Aldrich syndrome protein (WASP). Abbreviations: A, acidic domain; B, basic region; C, cofilin homology; GBD, GTPase-binding domain; WH1, WASP homology 1 domain; V, verprolin homology.

interaction with a complementary network of acidic residues of WASP that are anchored into the correct alignment by Arg76. Recent studies show that a third region of WIP, spanning a.a. 451–461, enhances the overall affinity of WIP binding to WASP with a.a. 454–459 being in contact with WASP [14].

WIP isoforms and related proteins

The *WIP* gene is encoded by seven exons. Several cDNAs encoding shorter forms of WIP have been deposited in the Genbank. It is hard to determine if all are true isoforms or if some are partial cDNAs and nonproductive splice forms. One of the first WIP isoforms to be identified is *Prpl2* (GenBank Accession number X86019). The Prpl2 protein differs from WIP only at the C-terminal end (Figure 1). Prpl2 is encoded by exons 1–6 of WIP and by an additional exon, we term exon 6A, which is located between exons 6 and 7 of the *WIP* gene. *Prpl2* cDNA arises by alternate splicing to exon 6A rather than to exon7. Because *Prpl2* mRNA is not detected readily by RT PCR, splicing to exon 6A should be a relatively infrequent event. Another isoform of WIP was identified recently [15]. This unique short form of WIP arises by transcription continuing into intron 4, which results in a truncated 403 a.a.-long protein, 377 a.a. encoded by the first four exons of WIP followed by a novel stretch of 26 a.a. (Figure 1). This truncated form of WIP, termed mini-WIP, does not have the WASP-binding sequences of full-length WIP. The mini-WIP isoform is expressed in peripheral blood cells but not in fibroblasts. Because mini-WIP contains all the motifs present in WIP, except for the WASP-binding region, mini-WIP could act to regulate WIP function by competing with WIP for binding to various partners.

Since the discovery of WIP, two other proteins, CR16 and WIRE (*WIP-re*lated)/WICH (*WIP-C*R16 *h*omologous), have been found to belong to the same family (Figure 1). All three proteins are proline-rich and have docking sites for several SH3 domain-containing adaptor proteins and have the profilin-binding actin-based motility homology-2 (ABM-2) motif. CR16 was first discovered as glucocorticoid-regulated brain protein [16] and, although N-WASP–CR16 complexes have been detected in the brain [17], the biological function of CR16 is at present unknown. Recently, male-specific sterility was reported in mice with a deletion of CR16 [18]. WIRE/WICH protein was discovered simultaneously by two different groups [19,20]. WIRE/WICH protein is 440 a.a. long and shows 30% amino acid identity to WIP. CR16 and WIRE/WICH proteins lack the poly-glycine region found in the N-terminal region of WIP. Coexpression of WASP or N-WASP and WIRE/WICH in Cos-7 cells and affinity precipitation of WIRE/WICH showed that WIRE/WICH binds to WASP and to N-WASP but not SCAR–WAVE proteins [19]. Other studies report that WIRE/WICH binds to N-WASP but poorly to WASP [20]. The relative affinity of WIRE/WICH to WASP and N-WASP proteins needs to be established. WIRE/WICH might cooperate with WASP in actin-assembly functions; indeed, in cells expressing both WIRE/WICH and WASP ectopically, PDGF (platelet-derived growth factor) treatment translocated both WIRE/WICH and WASP to sites of actin assembly in the cell periphery, suggesting that WIRE/WICH and WASP are involved in actin polymerisation. In addition, WIRE/WICH cooperates with N-WASP in the formation of actin-containing microspikes and cross-links actin filaments [20,21].

Functions of WIP

Studies to date show that WIP has four major cellular functions in regulating actin dynamics: (i) WIP is essential for WASP stability [22–24]; (ii) WIP binds to N-WASP and WASP and regulates their activation by Cdc42 [25,26]; (iii) WIP participates in conveying WASP to areas of active actin assembly following antigen-receptor and chemokine-receptor signalling [27–30]; and (iv) WIP participates in the reorganisation of the actin-based cytoskeleton and stabilizes actin filaments in a WASP-independent manner (reviewed in [31]).

WIP is essential for WASP stability

Co-precipitation experiments demonstrate that, in cells, approximately 80% of WIP and WASP exist as a mutual