CMLS, Cell. Mol. Life Sci. 54 (1998) 1145–1153
1420-682X/98/101145-09 $ 1.50 + 0.20/0


CMLS Cellular and Molecular Life Sciences

# Review

# Understanding the molecular basis of Wiskott-Aldrich syndrome

**A. Abo**

Onyx Pharmaceuticals, 3031 Research Drive, Richmond (California 94806, USA),
Fax +1 510 222 9758, e-mail: arie@onyx-pharm.com

Received 17 April 1998; received after revision 2 June 1998; accepted 2 June 1998

**Abstract.** Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency disorder associated with lymphocytes and platelet abnormalities. The gene that encodes the Wiskott-Aldrich protein (WASP) was recently isolated, and shown to be defective in WAS patients. WASP contains multiple domains that interact with various signalling proteins, including the guanine triphosphatase (GTPase) Cdc42Hs and SH3 domain-containing proteins. Biochemical and genetic evidence strongly suggests that WASP is an important protein in the regulation of cell morphology. Recent progress in the identification of molecular partners for WASP suggests a molecular mechanism for the cellular abnormalities of WAS.

**Key words.** Wiskott-Aldrich syndrome protein; actin cytoskeleton; signal transduction; transmembrane signalling; T-cell activation; immunodeficiency.

## Introduction

Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder described as a clinical triad of immunodeficiency, eczema and thrombocytopenia. Boys affected with this syndrome typically suffer from rashes, pyrogenic and opportunistic infections, and bleeding problems [1–3]. The rapid destruction of abnormally shaped platelets is the primary cause for severe thrombocytopaenia in WAS patients [4]. The immunodeficiency in WAS is primarily associated with T- and B-lymphocyte dysfunction. Defective immune responses in WAS patients include defects in transmembrane signalling of T and B cells [5, 6], failure to respond to polysaccharide antigens [7], reduced levels of isohaemaglutinin [8], decreased function and number of T lymphocytes and increased incidence of lymphomas [7]. Severe immunodeficiency is observed in many WAS patients, which can be treated with bone marrow transplantation, indicating that the defect is intrinsic to the cells and not to the microenvironment [7].

The gene that encodes the Wiskott-Aldrich protein (WASP) was recently isolated by positional cloning. WASP expression was restricted to cells of the haematopoetic lineage [9]. More than 50 different WASP mutations were identified in WAS patients [10–16]. WASP is likely to be a key protein for lymphocytes and platelet functions. Delineation of the biochemical roles of WASP in leukocytes will contribute to our understanding of leukocyte function and will suggest a molecular pathogenesis for WAS.

## WAS cellular defects are associated with cytoskeletal abnormalities

The clinical features of WAS point to a link between WASP function and the actin cytoskeleton [1–3]. The cell cytoskeleton is a complex network that provides structural support by linking the plasma membrane with the cell's interior. The reorganization of the cytoskeleton is a dynamic process regulated by extracellular signals. During signal transduction, many proteins in the cytoskeleton become modified to integrate information received from the receptor, thereby producing the cytoskeletal rearrangement necessary for signal transduction.

The most common clinical manifestation of WAS is severe thrombocytopaenia. In addition, mutations in the WASP gene lead to abnormal cytoskeletal organization of platelets [4] and subsequently the destruction of platelets by the spleen. After splenectomy, circulating platelets are restored to normal function, size and numbers [4]. In addition, normal platelets transfused in WAS patients were not destroyed, whereas transfused autologous platelets were rapidly cleared from the blood. This finding suggests that the destruction of WAS platelets is due to abnormal platelets [4].

Patients with WAS often suffer from severe immunodeficiency involving B and T lymphocytes. Electron microscopy scans of T cells from WAS patients have shown that the microvilli are decreased in size and density compared with those of normal cells [17, 18]. WASP may play an important role in stabilizing microvilli projections and thereby facilitate the anchorage of surface molecules in cell protrusion.

A large body of evidence suggests a defect in various types of surface molecules from WAS leukocytes. Some patients exhibited a decrease in platelet glycoprotein glycosyl phosphatidylinositol (GPIb) and in the antiadhesive molecule CD43 (silophorin) in T cells [19, 20]. However, it is unclear whether the reduction in expression of these surface proteins reflects the abnormality of leukocyte function. Interestingly, selective expression of CD43 was defective in the microvilli of leukaemic rat cells and human T cells [1, 21]. It is possible that reduction of CD43 at the cell surface is a direct result of an abnormal cell surface overall as well as defective microvilli caused by a mutation in the gene that encodes for WASP.

The failure of WAS B and T cells to proliferate when treated with polysaccharide antigens [7] and with immobilized anti-CD3 monoclonal antibody, respectively, appears to be related to an underlying transmembrane signalling defect [5, 6]. Anti-CD3 monoclonal antibody initiates a normal $Ca^{2+}$ flux and induces normal tyrosine phosphorylation of CD3$\zeta$ in T cells from WAS patients [6] , indicating that CD3 signalling is partially intact. In contrast, the proliferative response was detected in WAS T cells challenged with allospecific Ag, phytohaemagglutinin (PHA) or phorbol 12-myristate 13-acetate (PMA) plus ionomycin [6]. This finding suggests that defects in WAS cells are membrane-proximal in T-cell signal transduction and are most likely due to the inability of the T-cell receptor to communicate the necessary signal to the cytoskeleton. Delivering such a signal from the receptor is probably required for cell polarization and receptor aggregation mediated by WASP-dependent actin cytoskeletal rearrangements. This hypothesis is supported by the observation that actin bundling is necessary for T-cell activation by anti-CD3 [22], and disruption of actin polymerization blocks T-cell proliferation [23, 24]. However, this defect could be related to a failure to release cytokines that are essential for T- and B-cell proliferation.

## WASP contains several domains that suggest potential functions

WASP is a 62–65-kDa protein whose expression is restricted to haematopoietic cells and was found to be mutated in cells of WAS patients [9]. WASP contains various domains found in proteins that are implicated in the control of cell morphology (fig. 1). WASP contains longer repeated proline-rich sequences, typically 4 to 5 consecutive residues with one stretch of 11 prolines [9]. The role of repetitive proline sequences is still uncertain. In several instances they have been shown to be involved in association of multiple protein complexes.

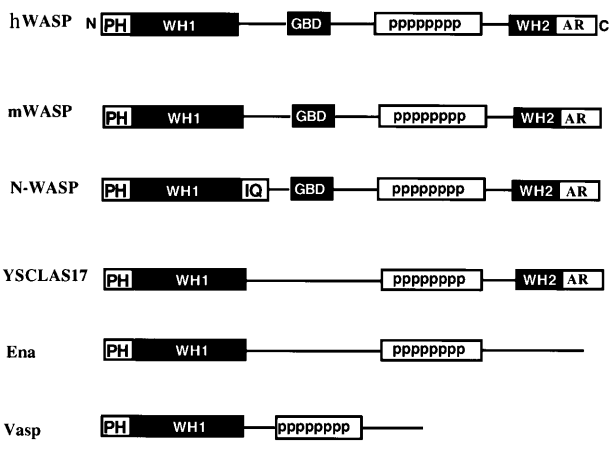


Figure 1. The molecular structure of WASP and WASP-related proteins. Schematic representation of domain structure found on WASP and WASP-related proteins: PH (plekstrin homology); WH1,2 (WASP homology); GBD (GTPase binding domain); PPPPPPP (proline-rich region); and AR (acidic residues). hWASP, human; mWASP, mouse; N-WASP, neuron; YSCLAS17, *S. cervisiae* WASP homologue.

Another protein containing proline repeats, vasodilator-stimulated protein (VASP), was recently shown to interact with the monomeric actin-binding protein, profilin [25]. VASP is implicated in the assembly of actin filaments which are essential for the motility of *Lesteria monocytogenes* in the cytoplasm of infected eukaryotic cells [25–27]. The proline-rich motifs of VASP typically appear as $Gly(Pro)_5$, and three of the four copies are in a tandem repeat configuration. Moreover, a peptide corresponding to the VASP $Gly(Pro)_5$ tandem repeat competes with VASP in binding to profilin, supporting the notion that this region is important in VASP-pro-filin binding [26]. Interestingly, human WASP also contains a $Gly(Pro)_5$ sequence [9], whereas the mouse protein contains two such motifs [28], suggesting that the effect of WASP on actin polymerization may be mediated through a direct interaction between WASP and profilin.

In addition to the long consecutive proline sequence, WASP contains several proline-rich sequences that were shown to interact with SH3 domains. Several proteins were shown to interact in vivo and in vitro with this region through their SH3 domain, including NCK [29], Fyn, Fgr [30], PLC$\gamma$, Src [30] and PSTPIP [31]. Thus, WASP may be implicated in bringing together various signalling molecules such as tyrosine kinases, phosphatases and adapter proteins to regulate and integrate cellular signalling.

WASP contains a putative GTPase-binding domain (GBD) that was found in several effector proteins for the GTPases Rac and Cdc42Hs [32–34]. The GBD [known also as a Cdc42Hs/Rac interactive binding region (CRIB [35])] was initially identified in p21 activated kinase (PAK) [36] and shown to be necessary and essential for direct interaction with Cdc42Hs and Rac [36, 37]. WASP contains the GBD consensus sequence PXXXXHXX-HVGXXXXXG and could bind preferentially to the activated form of Cdc42Hs and not to Rac [32–34].

At the N and C termini two novel putative domains were described for WASP – WH1 and WH2 (WASP homology) – that are conserved among several proline-rich proteins shown to be involved in the regulation of the actin cytoskeleton [32]: VASP (discussed above) was implicated in actin polymerization [26], and verprolin was shown to play a role in cytoskeletal organization and cellular growth in *Saccharomyces cerevisiae* [38]. In addition, Ena, the protein product of the *Drosophila* gene *enabled* is involved in axonal architecture [39], and recently the mouse homologue Mena was shown to induce the formation of abnormal F-actin-rich outgrowth [40], consistent with a role in regulation of actin cytoskeleton. The fact that WH1 and WH2 are conserved among polyproline-rich proteins that are implicated in cytoskeletal organization suggests that they represent novel functional domains associated with the cytoskeleton. WASP C-terminus contains a highly charged domain composed of mainly acidic residues (AR) EDEDDDEDFEDDDEWED [9]. WASP mutants lacking the C-terminus fail to colocalize with polymerized actin, suggesting that this domain may be implicated in connecting WASP to the actin cytoskeleton [32].

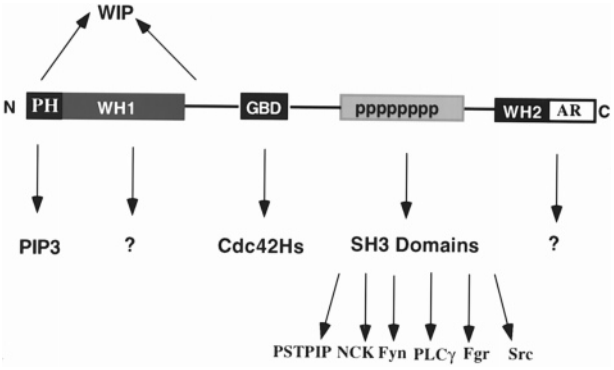


Figure 2. WASP interactive proteins. Multiple proteins interact with various domains on WASP. Several proteins containing SH3 domains interact with the WASP prolin region. The GTPase Cdc42Hs binds WASP through the GBD. WIP interacts with the N-terminus of WASP, including PH and WH1 domains. The phosphoinositide PIP3 interacts with the PH domain.

A yeast protein YSCLAS17 [32] (also known as Bee1 [41]) exhibits a similar sequence and domain structure to WASP. YSCLAS17 contains all of the domains found on WASP except the GBD. Interestingly, BEE1-disrupted cells were defective in the organization of actin, budding and cytokinesis [41].

Recently, a WASP-related protein N-WASP was identified in brain tissue. N-WASP contains the same domain organization as WASP [42]. In addition to the domains found on WASP, N-WASP contains an IQ motive that is known as a calmodulin-binding site (fig. 1). Miki et al. [42] identified a plekstrin homology (PH) domain on N-WASP and WASP at the N-terminus of WASP which was also shown to bind phosphatidylinositol 4,5-bis-phosphate (PIP2).

## Signalling proteins connecting WASP to the cytoskeleton

Substantial progress has been made in identifying proteins that interact with WASP. Various signalling molecules were identified and shown to interact with a specific domain on WASP (fig. 2).

WASP interacts with the Rho-like GTPase CDC42 Hs [32–34] through its GBD. WASP interacts with the active GTP-bound form of CDC42Hs but not with the Rho-like proteins Rac and Rho. Actin polymerization was induced by the overexpression of WASP (fig. 3)

and was regulated by CDC42Hs [32]. The dominant-negative mutant of CDC42Hs (CDC2Hs-N17) blocked the induction of actin polymerization by WASP, and subsequently WASP no longer colocalized with polymerized actin. The Rho-like GTPases play key roles in integrating extracellular signals from various receptors to initiate the reorganization of cell cytoskeleton and mitogenesis (for a review, see ref. 43). CDC42Hs plays a pivotal role in the regulation of two pathways, a kinase cascade leading to transcription activation and a second pathway regulating the organization of the cytoskeleton [43]. CDC42Hs activates the PAK kinases that are related to STE20 [36, 37] and is implicated in the activation of the JNK MAP kinase cascade [44–46].

The role of CDC42Hs in filopodia formation was previously demonstrated by microinjection experiments and was shown to be independent of Rho and Rac [47, 48]. Microvilli require an intact actin-based cytoskeleton, but the signal transduction pathways and molecular mechanisms that underlie the formation of filopodia are not known. Recent studies of leukocyte adhesion suggest a central role for microvillus receptor presentation [49]. Clustering of L-selectin on microvilli greatly increases contact with native ligand or monoclonal antibody, whereas exclusion from microvilli causes a dramatic decrease in the capacity of the receptor to initiate adhesion despite normal ligand binding [49]. This observation is reminiscent of studies performed on leukocytes from WAS patients which showed that cells lacking microvilli failed to signal from the receptor despite normal ligand binding [6]. It is conceivable that WASP and CDC42Hs play a key role in the formation of microvilli in leukocytes. Interestingly, N-WASP was recently shown to regulate the formation of filopodia in fibroblast [50]. In contrast, WASP did not interact with the CDC42Hs effector mutant CDC42L61(C40) which was previously shown to be important in the induction of filopodia [51]. These data suggest that WASP is not the effector responsible for the formation of filopodia; however, further work is required to understand the physiological role of CDC42Hs and WASP in the regulation of morphological changes.

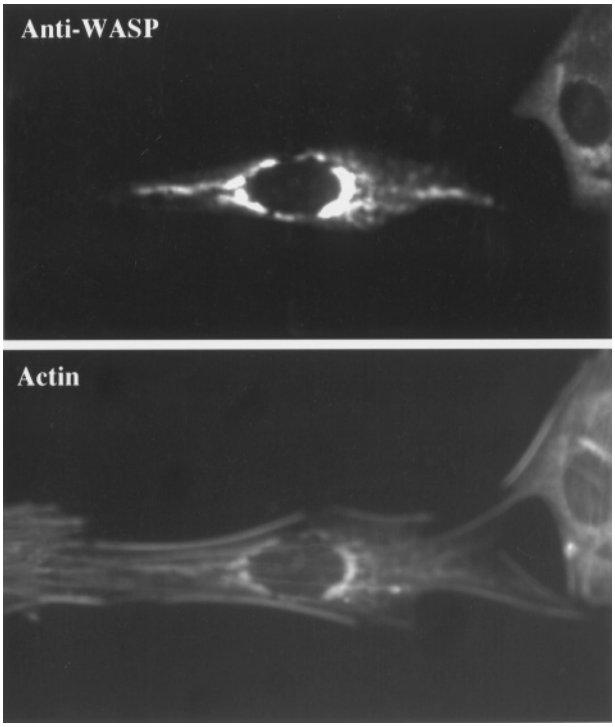


Figure 3. Microinjection of WASP into cells induces WASP clustering and actin polymerization. Expression vector encoding FLAG-tagged WASP was injected into the nucleus of porcine aortic endothelial cells (PAE). To visualize WASP expression, cells were labelled with anti-FLAG antibody and were costained with phalloidin to visualize polymerized actin. Note the clustering of WASP around the nucleus, which is colocalized with polymerized actin clusters.

Several groups isolated WASP with a panel of GST-SH3 proteins in an attempt to isolate the SH3-binding protein. WASP can bind the SH3 domain of adapter proteins such as NCK [29] and GRB2 [52]; protein tyrosine kinases, including Src, Fyn, Fgr and phospholipase PLC$\gamma$ [30]; and other proteins, including PSTPIP [31]. NCK and Fyn were also shown to coimmunoprecipitate with WASP in HL-60 and U-937 cells [29, 30]. Analysis with inhibitory peptides identified the exact sequences on WASP (aa 307–322, aa 375–388, aa 336–349) responsible for interaction with Src, Fgr and PLC$\gamma$ SH3 domains [53]. It is possible that WASP is regulated directly by tyrosine kinases such as as Fyn and Src, or alternatively WASP may be connected by the adapter protein NCK to a pathway that is regulated by a tyrosine kinase. Further work is required to understand the physiological significance of these interactions.

WASP has been shown to interact in the yeast two-hybrid system with the SH3 domain of PSTPIP [31], a protein that was previously described to be associated with the PEST family members of phosphatase HSCF [54]. When coexpressed with WASP, PSTPIP inhibits the formation of actin clusters [31]. Moreover, the affinity to WASP was reduced dramatically when the SH3 domain of PSTPIP was phosphorylated by Src kinase [31]. These data suggest a mechanism in the regulation of WASP-dependent actin polymerization. Further work in haematopoietic cells is required to understand the physiological relevance of WASP and PSTPIP interactions.

Using the yeast two-hybrid system, a novel WASP-interacting protein (WIP) was recently identified [55].

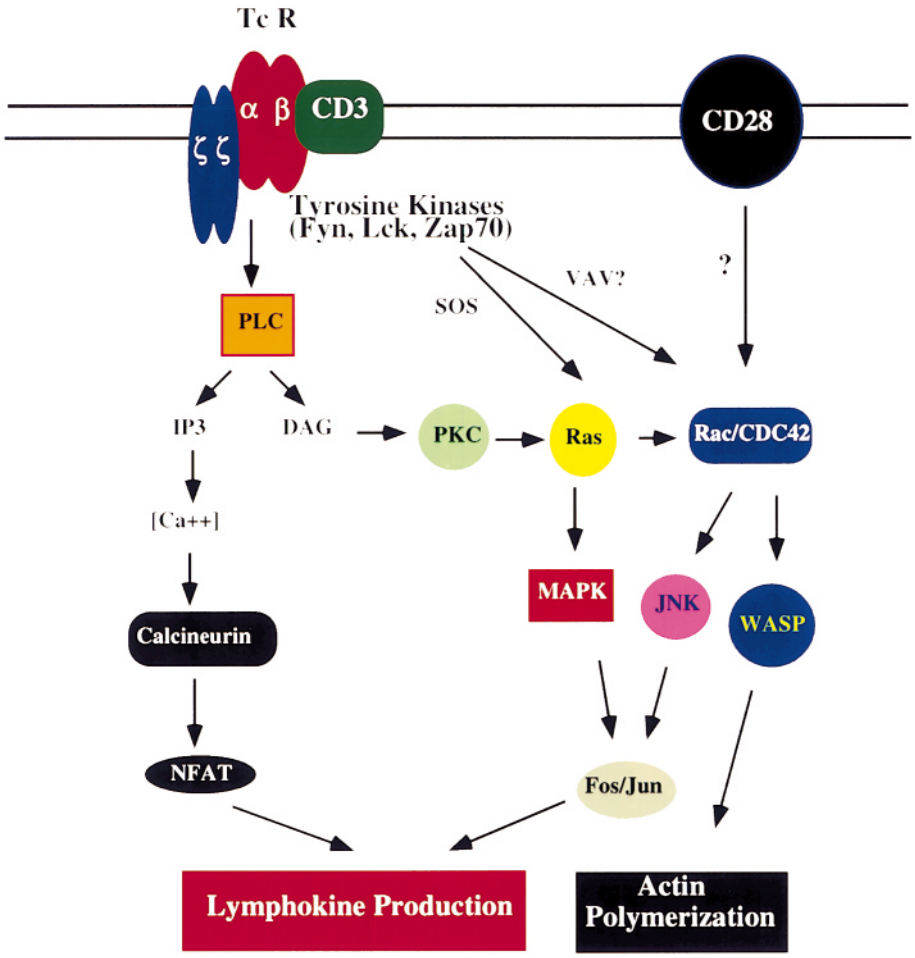


Figure 4. A potential role for WASP in T-cell signal transduction. A schematic diagram of the signal pathways mediated by T-cell receptor (TCR)/CD3, CD28 and the potential role for WASP. Ligation of TCR by the appropriate stimulus (i.e. monoclonal antibody C305) initiates a chain of events leading to transcriptional activation of early responsive genes. Phosphorylation of the $\zeta$ chain by tyrosine kinase Lck leads to the interaction of Zap70 with the $\zeta$ chain and subsequently to the activation of Zap70 kinase activity. Subsequently, PLC is phosphorylated and activated to elevate the $Ca^{++}$ level and to stimulate PKC. Elevation of $Ca^{++}$ causes stimulation of the $Ca^{++}$-dependent phosphatase calcineurin. The transcription factor NFAT is dephosphorylated and subsequently translocates to the nucleus to initiate lymphokine production. Activation of the Ras pathway is also essential for T proliferation and is implicated in the activation of the MAP kinase cascade, thereby activating the transcription factor AP1. Greater activation of AP1 is achieved by CD28 ligation to initiate the JNK MAP kinase pathway. CDC42 could possibly be activated by CD28 or TCR to regulate the WASP-dependent cytoskeletal rearrangements that are essential for transcriptional activation.

In contrast to the protein described above, WIP is a prolin-rich protein with some degree of homology to verprolin. Mapping the interaction between WIP and WASP identified the N-terminus of WASP (first $\sim$170 amino acids on WASP, including the PH and WH1 domains) for WIP binding. Expression of WIP in B cells resulted in increased actin polymerization; however, the effect on WASP localization was not reported [55].

The data described above clearly suggest a strong link between WASP and regulation of the actin cytoskeleton. Which specific events do WASP and its regulators control? How does WASP regulate the actin cytoskeleton? Why is this event crucial for cell signalling? To answer these questions we will have to investigate the role of WASP and its regulators in haematopoietic cells in particular in T and B cells, where mutation in WASP leads to signalling defects.

### T-cell receptor signalling transduction and the WASP link

Transmembrane signalling was shown to be defective in B and T cells from WAS patients. The T-cell receptor (TCR) is a multisubunit complex that comprises the polymorphic antigen-binding subunits: TCR$\alpha\beta$ or $\gamma\delta$ chains and the signal transduction subunits CD3$\gamma\delta\varepsilon$ and $\zeta$ chains [56–58] (fig. 4). The TCR$\zeta$ chain plays a key role in T-cell signal transduction. Stimulation of TCR induces tyrosine phosphorylation by Lck and possibly Fyn. Phosphorylation of the receptor allows recruitment of Zap70 and consequently phosphorylates a substrate(s), thereby initiating a chain of events leading to the transcriptional activation of various cytokines. The intracellular signals that regulate these transcription factors involve protein kinase C [59], activation of calcineurin, a calcium-dependent phosphate [48], the Ras pathway [60] and the MAP kinase cascade [61]. Recently, the Rho-like GTPase, Rac, was implicated in the control of JNK MAP kinase activation during TCR stimulation, yet the biochemical pathway linking Rac to JNK is not known. A potential candidate is Vav, a protein primarily expressed in haematopoietic cells [62] that contains a dbl homology (DH) domain found on guanine nucleotide exchange factors (GEF) for small GTPases [63]. Vav binds Zap70 and many other proteins [64], and null mutations in Vav produce an immunodeficiency very much like WAS [65]. It was recently demonstrated that Rac and JNK are part of the Vav signalling pathway [66], and phosphorylation of Vav by Lck activates Vav nucleotide exchange activity of Rac [67].

T cells of WAS patients fail to transmit the signal from the TCR to initiate a proliferative response, whereas a soluble stimulus such as PMA and ionomycine induces a normal signal. In contrast, early events such as tyrosine phsphorylation of the $\zeta$ chain appear to be normal [6]. These data place WASP downstream from TCR-induced tyrosine kinase phosphorylation and $Ca^{2+}$ entry and strongly suggest that WASP plays a key role in connecting the TCR and/or other surface molecules to the cytoskeleton and that this connection is essential for proper signalling. Understanding how WASP functions will lead to an understanding of a new signalling pathway that is independent of tyrosine phosphorylation and $Ca^{2+}$ and that connects the cell surface with the nucleus.

WASP may play an important role in T-cell costimulation (fig. 4). T lymphocytes require two stimuli for activation of early immune response genes essential for proliferation and communication with other cell types (for a review, see ref. 68). The primary stimulus is initiated by the TCR, whereas the second stimulus is initiated by activating the CD28 protein. Interactions with the TCR are responsible for activation of the $Ca^{2+}$/calcineurin pathway [69] and the Ras/MAP kinase pathway [70], and CD28 interactions result in activation of the JNK kinase cascade [71]. The JNK kinase cascade is activated by the GTPases Rac and CDC42Hs [44]. Ligation of CD28 may be involved in the stimulation of a GEF necessary for activation of CDC42Hs-dependent pathways (fig. 4). Thus, WASP-dependent events may be downstream of CD28. By controlling the cytoskeleton, WASP may play a central role in bringing together the CD3 complex and CD28, thereby allowing amplification and integration of the JNK and MAP kinase pathways.

Two models explain the accepted view of how ligands stimulate a receptor. According to the first model, the occupancy of the ligand induces a conformational

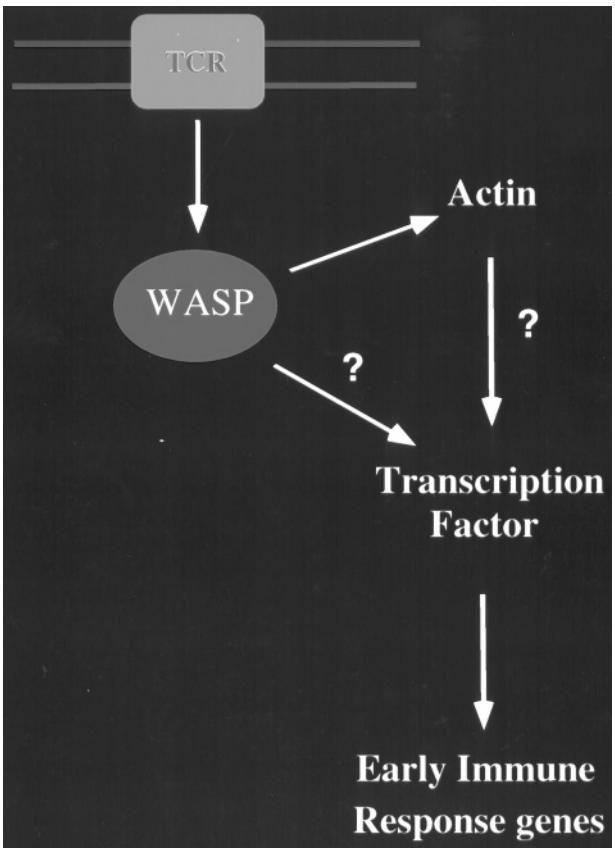


Figure 5. A potential role for WASP in the regulation of actin polymerization and transcriptional activation. T cells from WAS patients did not proliferate when stimulated through the T-cell receptor. A large body of evidence suggests that WASP plays a critical role in regulating the actin cytoskeleton. WASP could play a role in coordinating cytoskeletal reorganization with transcription activation.

change in the receptor sufficient to enable it to connect to the signal-transduction molecules [72, 73]. The second model suggests that the ligand induces receptor dimerization or aggregation to allow cross-activation of the cytoplasmic part of the receptor. The TCR-ligand interaction most likely fits the latter model, with the added caveat that oligmerization of the receptor is almost certainly required for activation as shown using multivalent synthetic ligands that do not induce conformational changes [73]. The TCR recognizes the major histocompatibility and antigen peptide complex on the antigen-presenting cell in order to initiate the activation of various tyrosine kinases including Fyn, ZAP70 and Syk. Receptor aggregation may require reorganization of the actin cytoskeleton. Several T-cell surface molecules including CD11a/CD18 and CD44 associate with the cytoskeleton upon receptor cross-linking. Recently, TCR ligation was shown to recruit the $\zeta$ chain to the cytoskeleton and is directly correlated with interleukin (IL)-2 production [23]. WASP could be an important candidate in connecting the TCR to the cytoskeleton to initiate receptor aggregation.

The data discussed in this review strongly suggest that WASP plays a critical role in regulating the actin cytoskeleton and gene transcription in lymphocytes. However, these two functions may be a direct consequence of each other or simply represent unrelated events both of which are regulated by WASP (fig. 5). For example, WASP may function in the organization of the cytoskeleton and be essential for transmembrane signalling, thereby affecting gene transcription. Understanding the molecular and cellular mechanisms of WASP signalling pathways will provide us with a better understanding of WAS and a basic understanding of leukocyte activation.


1 Remold-O'Donnell E., Rosen F. M. and Kenney D. (1996) Defects in Wiskott-Aldrich syndrome blood cells. Blood **87:** 2621–2631
2 Peacocke M. and Siminovitch K. A. (1993) The Wiskott Aldrich syndrome. Semin. Dermatol. **12:** 247–254
3 Kirchhausen T. and Rosen F. M. (1996) Disease mechanism: unravelling Wiskott-Aldrich syndrome. Curr. Biol. **6:** 676–678
4 Mullen C. A., Anderson K. D. and Blases R. M. (1993) Splenectomy and/or bone marrow transplantation in the managment of Wiskott-Aldrich syndrome: long-term follow up of 62 cases. Blood **82:** 2961–2964
5 Simon H. U., Mills G. B., Hashimoto S. and Siminovitch K. A. (1992) Evidence for defective transmembrane signaling in B cells from patients with Wiskott-Aldrich syndrome. J. Clin. Invest. **90:** 1396–1405
6 Molina I. J., Sancho J., Terhorst C., Rosen F. S. and Remold-O'Donnell E. (1993) T cells of patients with the Wiskott-Aldrich syndrome have a restricted defect in proliferative responses. J. Immunol. **8:** 4383–4390
7 Ochs H. D., Slichter S. J., Harker L. A., Von B. W., Clark R. A. and Wedgwood R. J. (1980) The Wiskott-Aldrich syndrome: studies of lymphocytes, granulocytes and platelets. Blood **55:** 243–252
8 Krivit W. and Good R. A. (1959) Aldrich's syndrome (thrombocytopenia, eczema and infection in infants). Am. J. Dis. Child. **97:** 137–145
9 Derry J. M. J., Ochs H. J. and Francke U. (1994) Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. Cell **78:** 635–644
10 Kwan S. P., Hagemann T. L., Radtke B. E., Blaese R. M. and Rosen F. S. (1995) Identification of mutations in the Wiskott-Aldrich syndrome gene and characterization of a polymorphic dinucleotide repeat at DXS6940 adjacent to the disease gene. Proc. Natl. Acad. Sci. USA **92:** 4706–4710
11 Kwan S. P., Hagemann T. L., Blaese R. M., Knutsen A. and Rosen F. S. (1995) Scanning of the Wiskott-Aldrich syndrome (WAS) gene: identification of 18 novel alterations including a possible mutation hot spot at Arg 86 resulting in thrombocytopenia, a mild WAS phenotype. Hum. Mol. Genet. **4:** 1995–1998
12 Kolluri R., Shehabeldin A., Peacock M., Lamhonwah A. M., Teichertkuliszewska K. and Siminovitch K. A. (1995) Identification of WASP mutations in patients with Wiskott-Aldrich syndrome and isolated thrombocytopenia reveals allelic hetrogeneity at the WAS locus. Hum. Mol. Genet. **4:** 1119–1126
13 Derry J. M. J., Kerns J. A., Weinberg K. I., Ochs H. D., Volpini V., Estivill X. et al. (1995) WASP gene mutations in Wiskott-Aldrich syndrome and X-linked thrombocytopenia. Hum. Mol. Genet. **4:** 1127–1135
14 Zhu Q., Zheng M., Blaese R. M., Derry J. M. J., Junker A., Francke. U. et al. (1995) The Wiskott-Aldrich syndrome and X linked congenital thrombocytopenia are caused by mutations in the same genes. Blood **86:** 3797–3804
15 Wengler G. S., Notarangelo L. D., Beradelli S., Pollonni G., Mella P., Fasth A. et al. (1995) High prevalence of nonsense, frame shift and splice-site mutations in 16 patients with full blown Wiskott-Aldrich syndrome. Blood **86:** 3654–3684
16 Schindelhauer D., Weiss M., Hellebrand H., Golla A., Hergersberg M., Seger R. et al. (1996) Wiskott-Aldrich syndrome: no strict genotype-phenotype correlation but clustering of missense mutations in the amino-terminal part of the WASP gene product. Hum. Genet. **96:** 68–76
17 Molina I. J., Kenney D. M., Rosen F. S. and Remold-O'Donnell E. (1992) T cell lines characterize events in the pathogenesis of the Wiskott-Aldrich syndrome. J. Exp. Med. **176:** 867–874
18 Kenney D., Cairns L., Remold-O'Donnell E., Peterson J., Rosen F. S. and Parkman R. (1986) Morphological abnormalities in the lymphocytes of patients with the Wiskott-Aldrich syndrome. Blood **68:** 1329–1332
19 Pidard D., Didry D., Le-Deist F., Griscelli C., Bellucci S. and Nurden A. T. (1988) Analysis of the membrane glycoproteins of platelets in the Wiskott-Aldrich syndrome. Br. J. Hematol. **69:** 529–535
20 Parkman R., Remold-O'Donnell E., Kenney D. M., Perrine S. and Rosen F. S. (1981) Surface protein abnormalities in lymphocytes and platelets from patients with Wiskott-Aldrich syndrome. Lancet **2:** 1387–1389
21 Yonemura S., Nagafuchi A., Sato N. and Tsukita S. (1993) Concentration of an integral membrane protein, CD43 (leukosialin, sialophorin), in the cleavage furrow through the interaction of its cytoplasmic domain with actin-based cytoskeletons. J. Cell. Biol. **120:** 437–449
22 Parsey M. V. and Lewis G. K. (1993) Actin polymerization and pseudopod reorganization accompany anti-CD3-induced growth arrest in Jurkat T cells. J. Immunol. **151:** 1881–1893
23 Rozdzial M. M., Malissen B. and Finkel T. H. (1995) Tyrosine-phosphorylated T cell receptor $\zeta$ chain associates with the actin cytoskeleton upon activation of mature T lymphocytes. Immunity **3:** 623–633
24 Caplan S., Zeliger S., Wang L. and Baniyash M. (1995) Cell surface-expressed T cell antigen-receptor $\zeta$ chain is associated with the cytoskeleton. Proc. Natl. Acad. Sci. USA **92:** 4768–4772
25 Reinhard M., Giehl K., Abel K., Haffner C., Jarchau T., Hoppe V. et al. (1995) The proline-rich focal adhesion and microfilamemt protein VASP is a ligand for profilins. EMBO J. **14:** 1583–1589

26 Haffner C., Jarchau T., Reinhard M., Hoppe J., Lohmann S. M. and Walter U. (1995) Molecular cloning, structural analysis and functional expression of the proline-rich focal adhesion and microfilament-associated protein VASP. EMBO J. **14:** 19–27

27 Theriot J. A., Rosenblatt J., Portnoy D. A., Goldschmidt-Clermont P. J. and Mitchison T. J. (1994) Involvement of profilin in the actin-based motility of L. monocytogenes in cells and in cell free extracts. Cell **76:** 505–517

28 Derry J. M. J., Wiedemann P., Blair P., Wang U., Kerns J. A., Lemahieu V. et al. (1995) The mouse homolog of the Wiskott-Aldrich syndrome protein (WASP) gene is highly conserved and maps near the *scurfy* (sf) mutation on the X chromosome. Genomics **29:** 471–477

29 Rivero-Lezcano O. M., Marcilla A., Sameshima J. H. and Robbins K. C. (1995) Wiskott-Aldrich syndrome protein physically associated with Nck through Src homology 3 domain. Mol. Cell. Biol. **15:** 5725–5731

30 Banin S., Truong O., Katz D. R., Waterfield M. D., Brickell P. M. and Gout I. (1996) Wiskott-Aldrich syndrome protein (WASp) is a binding partner for c-Src family protein-tyrosine kinases. Curr. Biol. **6:** 981–988

31 Wu Y., Spencer S. D. and Lasky L. A. (1998) Tyrosine phosphorylation regulates the SH3-mediated binding of the Wiskott-Aldrich syndrome protein to PSTPIP, a cytoskeletal-associated protein. J. Biol. Chem. **273:** 5765–5770

32 Symons M., Derry J. M. J., Karlak B., Jiang S., Lemahieu V., McCormick F., Francke U. and Abo A. (1996) Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. Cell **84:** 723–734

33 Aspenstrom P., Lindberg U. and Hall A. (1996) Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott-Aldrich syndrome. Curr. Biol. **6:** 70–75

34 Kolluri R., Tolias K. F., Carpenter C. L., Rosen F. S. and Kirchhausen T. (1996) Direct interaction of the Wiskott-Aldrich syndrome protein with the GTPase Cdc42. Proc. Natl. Acad. Sci. USA **93:** 5615–5618

35 Burbelo P. D., Drechsel D. and Hall A. (1995) A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases. J. Biol. Chem. **270:** 29071–29074

36 Manser E., Leung T., Salihuddin H., Zhao Z. S. and Lim L. (1994) A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature **367:** 40–46

37 Martin G. A., Bollag G., McCormick F. and Abo A. (1995) A novel serine kinase activated by Rac1/CDC42Hs-dependent autophosphorylation is related to PAK65 and STE20. EMBO J. **14:** 1970–1978

38 Donnelly S. F., Pocklington M. J., Pallotta D. and Orr E. (1993) A proline rich protein, verprolin, involved in cytoskeletal organization and cellular growth in the yeast *Saccharomyces cerevisiae*. Mol. Microbiol. **10:** 585–596

39 Gertler F. B., Comer A. R., Juang J., Ahern S. M., Clark M. J., Liebel E. C. et al. (1995) *Enabled*, a dosage-sensitive supressor of mutations in the *Drosophila* Abl tyrosine kinase, encodes an Abl substrate with SH3 domain-binding properties. Genes Dev. **9:** 521–533

40 Gertler F. B., Niebuhr K., Reinhard M., Wehland J. and Soriano P. (1996) Mena, a relative of VASP and Drosophila Enabled, is implicated in the control of microfilament dynamics. Cell **87:** 227–239

41 Li R. (1997) Bee1, a yeast protein with homology to Wiscott-Aldrich syndrome proteins, is critical for the assembly of cortical actin cytoskeleton. J. Cell Biol. **136:** 649–658

42 Miki H., Miura K. and Takenawa T. (1996) N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. EMBO J. **15:** 5326–5335

43 Hall A. (1998) Rho GTPases and the actin cytoskeleton. Science **279:** 509–513

44 Minden A., Lin A., Claret F. X., Abo A. and Karin M. (1995) Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell **81:** 1147–1157

45 Zhang S., Han J., Sell M. A., Chernoff J., Knaus R. J., Ulevitch R. J. et al. (1995) Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. J. Biol. Chem. **270:** 23934–23936

46 Bagrodia S., Derijard R., Davis R. J. and Cerione R. A. (1995) Cdc42 and PAK-mediated signaling leads to jun kinase and p38 mitogen-activated protein kinase activation. J. Biol. Chem. **270:** 27995–27998

47 Nobes C. D. and Hall A. (1995) Rho, rac and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia and filopodia. Cell **81:** 53–62

48 Kozma R., Ahmed S., Best A. and Lim L. (1995) The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblast. Mol. Cell. Biol. **15:** 1942–1952

49 Von Andrian U. H., Hassien S. R., Nelson R. D., Erlandsen S. L. and Butcher E. C. (1995) A central role for microvillus receptor presentation in leukocyte adhesion under flow. Cell **82:** 989–999

50 Miki H., Sasaki T., Takai Y. and Takenawa T. (1998) Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. Nature **391:** 93–96

51 Lamarche N., Tapon N., Stowers L., Burbelo P. D., Aspenstrom P., Bridges T. et al. (1996) Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade. Cell **87:** 519–529

52 She H. Y., Rockow S., Tang J., Nishimura R., Skolnik E. Y., Chen M. et al. (1997) Wiskott-Aldrich syndrome protein is associated with the adapter protein Grb2 and the epidermal growth factor receptor in living cells. Mol. Biol. Cell. **8:** 1709–1721

53 Finan P. M., Soames C. J., Wilson L., Nelson D. L., Stewart D. M., Truong O. et al. (1996) Identification of regions of the Wiskott-Aldrich syndrome protein responsible for association with selected Src homology 3 domains. J. Biol. Chem. **271:** 26291–26295

54 Spencer S., Dowbenko D., Cheng J., Li W., Brush J., Utzig S. et al. (1997) PSTPIP: a tyrosine phosphorylated cleavage furrow-associated protein that is a substrate for a PEST tyrosine phosphatase. J. Cell. Biol. **138:** 845–860

55 Ramesh N., Anton I. M., Hartwig J. H. and Geha R. S. (1997) WIP, a protein associated with Wiskott-Aldrich syndrome protein, induces actin polymerization and redistribution in lymphoid cells. Proc. Natl. Acad. Sci. USA **94:** 14671–14676

56 Klausner R. D. and Samelson L. E. (1991) T cell antigen receptor activation pathways: the tyrosine kinase connection. Cell **64:** 875–878

57 Reth M. (1989) Antigen receptor tail clue. Nature **338:** 383–384

58 Weiss A. and Littman D. R. (1994) Signal transduction by lymphocyte antigen receptors. Cell **76:** 263–274

59 Valge V. E., Wong J. G. P., Datlof B. M., Sinskey A. J. and Rao A. (1988) Protein kinase C is required for responses to T cell receptor ligands but not to interleukin-2 in T cells. Cell **55:** 101–112

60 Yoshikai Y., Anatoniou D., Clark S. P., Yanagi Y., Sangster R., Van den Elsen P. et al. (1984) Sequence and expression of transcripts of the human T-cell receptor beta-chain genes. Nature **312:** 521–524

61 Alberola-Ila J., Forbush K. A., Seger R., Krebs E. G. and Perlmutter R. M. (1995) Selective requirement for MAP kinase activation in thymocyte differentiation. Nature **373:** 620–623

62 Bustelo X. R., Ledbetter J. A. and Barbacid M. (1992) Product of vav proto-oncogene defines a new class of tyrosine protein kinase substrates. Nature **356:** 68–71

63 Boguski M. S. and McCormick F. (1993) Proteins regulating Ras and its relatives. Nature **366:** 643–654

64 Katzav S., Sutherland M., Packham G., Yi T. and Weiss A. (1994) The protein tyrosine kinase ZAP-70 can associate withthe SH2 domain of proto-Vav. J. Biol. Chem. **269:** 32579–32585
65 Fischer K. D., Zmuldzinas A., Gardner S., Barbacid M., Bernstein A. and Guidos C. (1996) Defective T-cell receptor signalling and positive selection of Vav-deficient CD4+ CD8+ thymocytes. Nature **374:** 474–477
66 Crespo P., Bustelo X. R., Aaronson D. S., Coso O. A., Lopez-Barahona M., Barbacid M. et al. (1996) Rac-1 dependent stimulation of the JNK/SAPK signaling pathway by Vav. Oncogene **13:** 455–477
67 Crespo P., Schuebel K. E., Ostrom A. A., Gutkind J. S. and Bustelo X. R. (1997) Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. Nature **385:** 169–172
68 Schwartz R. H. (1992) Costimulation of T lymphocytes: the role of CD28, CTLA-4 and B7/BB1 in interleukin-2 production and immunotherapy. Cell **71:** 1065–1068
69 Clipstone N. A. and Crabtree G. R. (1992) Identification of calcineurin as a key signalling enzyme in T cell activation. Nature **357:** 695–697
70 Downward J., Graves J. D., Warne P. H., Rayter S. and Cantrell D. A (1990) Stimulation of $p21^{ras}$ upon T-cell activation. Nature **346:** 719–723
71 Su B., Jacinto E., Hibi M., Kallunki T., Karin M. and Neriah-Ben Y. (1994) JNK is involved in signal integration during costimulation of T lymphocytes. Cell **77:** 727–736
72 Schlessinger J. (1988) Signal transduction by allosteric receptor oligomerization. Trends Biochem. Sci. **13:** 443–447
73 Spencer D. M., Wandless T. J., Schreiber S. L. and Crabtree G. R. (1993) Controlling signal transduction with synthetic ligands. Science **262:** 1019–1024