Recent advances in the biology of WASP and WIP

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Abstract WASP, the product of the gene mutated in Wiskott–Aldrich syndrome, is expressed only in hematopoietic cells and is the archetype of a family of proteins that include N-WASP and Scar/WAVE. WASP plays a critical role in T cell activation and actin reorganization. WASP has multiple protein-interacting domains. Through its *N*-terminal EVH1 domain WASP binds to its partner WASP interacting protein (WIP) and through its *C*-terminal end it interacts with and activates the Arp2/3 complex. In lymphocytes, most of WASP is sequestered with WIP and binding to WIP is essential for the stability of WASP. The central proline-rich region of WASP serves as docking site to several adaptor proteins. Through these multiple interactions WASP integrates many cellular signals to actin cytoskeleton remodeling. In this review, we have summarized recent developments in the biology of WASP and the role of WIP in regulating WASP function. We also discuss WASP-independent functions of WIP.

Keywords Wiskott-Aldrich syndrome · WASP · WIP · Lymphocytes · Actin cytoskeleton

Introduction

Wiskott–Aldrich syndrome (WAS) is an X-linked immunodeficiency, characterized by recurrent infections, eczema, and thrombocytopenia. T-lymphocytes from WAS patients fail to proliferate and to secrete IL-2 after anti-CD3 stimulation. Lymphocytes of WAS patients show cytoskeletal abnormalities. The disease is caused by mutations in the WASP gene [1]. X-linked thrombocytopenia (XLT) is a variant of WAS that is caused by missense mutations in WASP. Residual levels of WASP in XLT T cells support normal T cell function. However, WASP is absent in XLT platelets resulting in the bleeding diathesis. In the last decade investigations into the function of WASP family of proteins has been

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explosive and have offered new insights into the function of WASP, especially signaling to the actin cytoskeleton. The molecular and cellular functions of the WASP family of proteins and the clinical correlates have been summarized in several excellent reviews [2–4]. In hematopoietic cells most of the WASP is sequestered as a constitutive complex with WIP (WASP interacting protein) and WIP has been demonstrated to stabilize WASP [5]. Most of the published missense mutations (76 out of 80) in WAS and XLT are located in the WIP-binding domain of WASP [6, 7]. Thus, mutations of WASP that prevent binding to WIP are a major cause of disease in a large subset of WAS/XLT patients [6, 8]. In addition to stabilization, a number of other cellular functions of WASP may depend on its association with WIP. Here we provide an overview on the recent advances in the biology of WASP and WIP with emphasis on the chaperone function of WIP and discuss it in relation to WAS.

WASP and WIP family of proteins

WASP

WASP is the first identified member of a family of proteins involved in signaling and cytoskeletal organization that includes N-WASP and Scar/WAVE [9–12]. WASP homologues have been identified in many eukaryotes from yeast to mammals (Table 1). WASP and its closest homologue, N-WASP, play a critical role in linking cellular signals that activate Cdc42 to the actin cytoskeleton. WASP and N-WASP are both structurally and functionally very similar in as much as one protein can substitute for the other especially in many in vitro assays. WASP is expressed only in hematopoietic cells [1], whereas N-WASP is ubiquitously expressed [11]. It is most likely that WASP and N-WASP evolved from the same ancestral gene as paralogues, yet have retained remarkable similitude in most of their functions and their mode of regulation. This notion implies that each protein must have unique functions; throughout this review we will attempt to point out the similarities as well as the differences between WASP and N-WASP functions. The SCAR/WAVE proteins show enough structural difference to form a different subclass and will not be discussed in this review.

Domain architecture of WASP family of proteins

Both WASP and N-WASP possess an Ena-Vasp homology domain (EVH1), also referred to as WASP homology domain 1 (WH1), a Cdc42/Rac GTPase binding domain (GBD), a proline-rich domain, a G-actin-binding verprolin homology (V) domain, a cofilin homology (C) domain and a C-terminal acidic (A) segment (Fig. 1). The yeast homolog of WASP/N-WASP, Las17p, differs from WASP/N-WASP in that it does not have the GBD. Recently a protein that is functionally similar to WASP called WHAMM has been identified. Much like WASP, the C-terminal half of WHAMM has a proline-rich region followed by V (also called WH2), C, and A region [13]. It remains to be seen if more proteins similar to WHAMM will be identified and thus would form yet another subclass of WASP family of proteins. In resting cells, WASP/N-WASP proteins exist in an auto-inhibited inactive state. Many signals that activate WASP/N-WASP proteins relieve this autoinhibition: these include binding of Cdc42 to the GBD domain, phosphorylation, and binding of SH3 domain containing proteins to the proline-rich region.

Table 1 Representative examples of members of the WASP family of proteins

Organism	Protein	NCBI accession number
Homo sapiens	WASP	NM_000377.1
	N-WASP	NM_003941
	WAVE1	NM_001024936
	WAVE2	NM_006990
	WAVE3	NM_006646
	WHAMM	NM_001080435
Mus musculus	WASp	NM_009515
	N-WASp	NM_028459
	WAVE1	NM_031877
Gallus gallus	Wasl (N-WASP)	XM_415994
Xenopus tropicalis	Wasl (N-WASP)	NM_001001206
Danio rerio	Wasl (N-WASP)	NM_201059
D. melanogaster	DmWASp (N-WASP) and isoforms	NM_143380
C. elegans	Wsp-1 (N-WASP)	NM_171393
Saccharomyces cerevisiae	Las17	D78487
Schizosaccharomyces pombe	Wsp1	NM_001020185

Note: A table representing the WIP family members was published recently [20]

WIP

WIP is a proline-rich 503 amino acid long protein (human WIP) that shows high sequence similarity at its *N*-terminal end to the yeast polarity development protein verprolin [14, 15]. We showed that WIP binds actin via an actin-binding motif (KLKK) within its WH2 domain and binds WASP via its carboxy terminal end (a.a. 416–488). NMR studies have precisely mapped the WASP binding sites on human WIP to encompass amino acids 461–485 [16, 17]. WIP has three ABM2 (actin-based mobility 2) profilin binding motifs. In addition to binding profilin, WIP binds the adapter protein Nck [18] and CrkL [19]. There is 95% identity between human and murine WIP. Since the discovery of WIP two more proteins, namely CR16 (corticosteroid responsive) and WIRE/WICH (WIP related/WIP CR16 homologous), belonging to this family have been described.

WIP is ubiquitously expressed when compared to WASP but is expressed at a higher level in lymphoid cells [14]. A complex of WIP–WASP or WIP–N-WASP is readily detected in resting cells. Over the past 10 years it has been established that WIP is a multifunctional protein [20]; however, details of many of its biological functions are far from complete. The demonstrated functions of WIP are summarized in the next section "Effect of WASP and WIP family of proteins on the actin-based cytoskeleton".

Effect of WASP and WIP family of proteins on the actin-based cytoskeleton

WASP and the actin cytoskeleton

WASP has profound effect on the cellular actin dynamics. Ectopic expression of WASP in a variety of cells including pig aortic epithelial cells (PAE) cells resulted in massive deposition of actin containing clusters [21]. The acidic region (A region) of WASP family
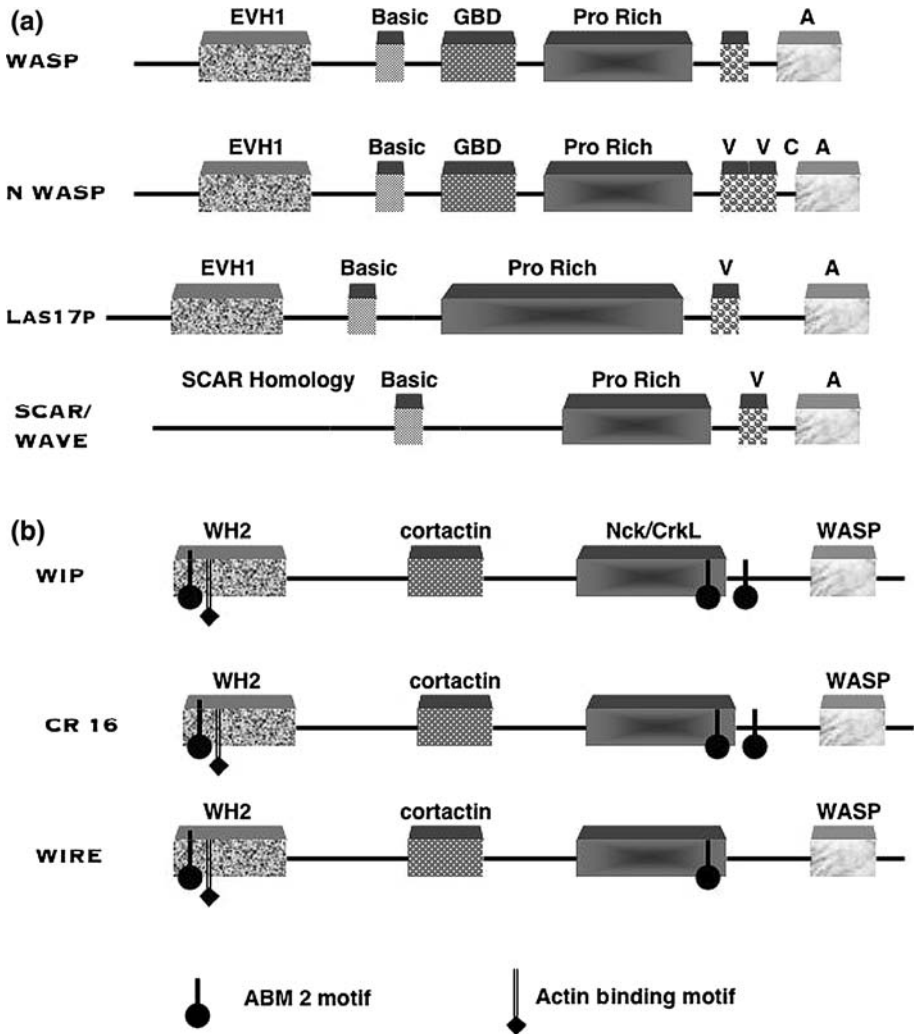
Fig. 1 Schematic showing the motifs in WASP family **a** and WIP family **b** of proteins

of proteins binds to and activates the Arp 2/3 complex, a hetero-multimer of 7 proteins that is conserved in eukaryotes, and results in actin nucleation [22, 23]. WASP and N-WASP bind G-actin via the V domain [24, 25]. Binding of Cdc42-GTP to N-WASP causes a conformational change that allows its VCA domain to interact with the Arp2/3 complex and initiate actin assembly; this is further enhanced by phosphatidylinositol 4,5-bisphosphate (PIP2) [24, 25]. Phosphorylation at tyrosine 291 of WASP also enhances its ability to induce Arp 2/3 complex mediated actin polymerization and filopodia formation by "opening up" the WASP molecule [26–28]. Over-expression of N-WASP potentiates filopodium formation in fibroblasts micro-injected with an active form of Cdc42 (Cdc42-V12) [29]. Microinjection of WIP induces filopodium formation. This is abrogated by co-injection of anti-N-WASP antibody [30]. More importantly, microinjection of anti-WIP antibody inhibits Cdc42 mediated filopodia in bradykinin-stimulated fibroblasts indicating

that N-WASP and WIP act in consort to induce filopodia. Furthermore, megakaryocytes from WAS patients are deficient in filopodium formation [31] and macrophages derived from these patients have difficulty in assembling podosomal structures [32].

WASP plays a critical role in T cell, B cell, monocyte, dendritic cell, and platelet function by linking surface receptor signaling to actin reorganization [33]. T cells from WAS patients and WASP KO mice fail to spread, cap their TCR, proliferate and secrete IL-2 in response to TCR triggering by immobilized anti-CD3 [34]. In T cells, most of the WASP is associated with WIP. WIP−/− T cells have ∼10% of the WASP content compared to WT cells. On the contrary, WIP level is not affected in WASP−/− cells. WIP functions exclusively to stabilize WASP since the level of other proteins investigated, N-WASP, NF-AT, and actin do not show difference between WT and WIP−/− cells indicating that association with WIP is critical for the stability of WASP in cells [5, 35, 36]. WIP inhibits N-WASP/WASP activity in vitro and most likely in vivo (see section "WIP inhibits Cdc42-mediated activation of N-WASP") [30], multiple cellular signals converge to relieve WIP inhibition of N-WASP/WASP in activated cells.

WIP is an important regulator of the actin cytoskeleton in cells

Verprolin is an actin and myosin binding WIP homolog in yeast. Verprolin-deficient yeast show defect in cell growth, cytoskeletal organization, endocytosis and cell polarity [15]. Introduction of human WIP into verprolin-deficient yeast corrects all of these defects. WIP overexpression in human B cell lines causes an increase in cellular F-actin content and induces the formation of subcortical patches of actin [14]. WIP is important for filopodium formation [30, 37], and has been reported to play a role in actin tail generation by vaccinia virus based on studies using dominant negative mutants [38]. The cortical actin network is disrupted in WIP-deficient lymphocytes [39]. Finally, we have shown that WIP stabilizes actin filaments [30].

WIP inhibits Cdc42-mediated activation of N-WASP

In vitro studies have shown that WIP binds to and inhibits WASP/N-WASP activity most probably by stabilizing their inactive closed conformation [30]. In Xenopus oocyte lysates N-WASP exists in a constitutive complex with WIP. This complex does not exhibit basal Arp2/3 activation activity similar to purified recombinant N-WASP further indicating that WIP functions to keep N-WASP/WASP proteins in their inactive conformation [40]. Recently, we have demonstrated that in T cells almost all of WASP is bound to WIP [5]. Upon T cell activation, WIP is phosphorylated by PKCθ [19]. Initial interpretation of studies on WASP activation following CD3 engagement suggested that phosphorylation of WIP on S488 caused the WASP–WIP complex to dissociate, freeing WASP from the inhibitory effect of WIP. Recent observations using S488-specific monoclonal antibody has unequivocally demonstrated that CD3 stimulation indeed causes phosphorylation of S488 following CD3 stimulation, but not dissociation of the WASP–WIP complex [41]. Our previously observed dissociation of the WASP–WIP complex is an artifact due to the fact that the anti-WIP polyclonal antibody used to immunoprecipitate the WASP–WIP complex was raised against a sequence immediately adjacent to the WASP binding site of WIP, since immunoprecipitation using anti-WIP mAb that recognizes an epitope distal from the WASP binding site did not confirm the dissociation of the WASP–WIP complex [36, 42]. The best interpretation of the currently available data is that the WASP–WIP complex undergoes a change in molecular conformation whereby WIP no longer exerts its

inhibitory effect. Further biochemical and biophysical studies are required to precisely define the molecular rearrangement of the WASP–WIP complex following TCR/CD3 engagement.

Activation of WASP/N-WASP by intracellular pathogens

Many intracellular pathogens effectively use cellular actin polymerizing machinery for invasion, intracellular motility, and for cellular egress. Vaccinia virus was shown to require WIP and N-WASP for cellular invasion [38]. Shigella requires N-WASP for intracellular motility [43]. The Shigella protein IcsA recruits N-WASP; however, this event is alone not sufficient as recruitment of another protein, TOCA-1, is required to relieve N-WASP from its auto-inhibited state such that it can initiate actin assembly to generate motile force [44]. Another interesting example is cell invasion by enterohemorrahagic *E. coli* (EHEC) into cells. A protein expressed on EHEC, EspFu, is critical for cell invasion by the pathogen. EspFu assumes an amphipathic helix that binds to the GBD domain and displaces the VCA domain, thus activating WASP/N-WASP proteins [45, 46]. Although EspFu may activate both WASP and N-WASP in vitro, probably activation of N-WASP is of more physiological consequence since WASP is not expressed at sites of invasion by EHEC. Nonetheless, these studies underscore the striking similarities in the cell biology of WASP and N-WASP while highlighting the fact that each may have independent function in specialized cells.

Role of WASP and WIP in immune cell function

T and B cells

WASP plays a critical role in T cell activation and in the reorganization of the actin cytoskeleton following TCR engagement [34, 47]. This is evidenced by the markedly impaired proliferation of T cells from WAS patients and WASP−/− mice stimulated with anti-CD3 mAb and by their decreased ability to form caps and to increase their F-actin content following TCR/CD3 ligation [34, 48]. The importance of WASP to T cell signaling is further underscored by the fact that WASP is recruited to the immunological synapse (IS) [19, 49]. IS formation is an important step in coupling TCR engagement to T cell activation. The WASP protein interacts with many adapter proteins including Grb2 [50], PSTPIP1, and CD2AP [51]. Interaction with the latter two proteins appears to recruit WASP to the IS [51]. Finally, WASP interacts with its multifunctional ligand WIP. This interaction also helps to recruit WASP to the IS [19]. IS is defined as the area of interaction between T cells and antigen presenting cells and is a symmetrical structure that is demarcated into three zones called SMACS (Supra-molecular activation clusters) [52]. TCR localizes to the central or c-SMAC and is critical for TCR originated signaling and endocytosis of TCR. This is surrounded by peripheral-SMAC (p-SMAC) that contains leukocyte adhesion molecules such as LFA-1/ICAM, which are in turn connected to the cytoskeleton by talin. Distal or d-SMAC radially surrounds the p-SMAC and is enriched in CD45. Antigen recognition by naïve T cells involves stable IS formation alternating with T cell migration. The T-cell migration is driven by PKCθ, the predominant isoform of PKC expressed in T cells and which localizes to the p-SMAC [53]. This is achieved by PKCθ mediated breakage of IS symmetry leading to slowing of cell motility. Reformation of IS following T cell migration requires WASP. With initial contact with APC, WASP−/− T cells form a normal IS; once this IS is disturbed, they are unable to re-establish it unless PKCθ is inhibited. Therefore, WASP and PKCθ act as opposing forces to achieve a

delicate balance between IS stability and cell motility and is critical for antigen recognition by naïve T cells [53].

In contrast to the functions of WASP and WIP in T cells, relatively less is known about the function of WASP in B cells, although WASP is expressed at high levels in B cells. Soon after its discovery it was noted that WASP associates with Bruton's tyrosine kinase (BTK) [54] and has been proposed to a substrate of BTK [55]; however, the functional significance has not been clarified. WASP was shown to be involved in cytoskeletal regulation of B cells especially for the formation of microvilli on B cell surface [56] and was shown to be critical for the motility of B cells. Indeed, WASP has been shown to be necessary for all aspects of B cells function including adhesion, migration, and homing [57, 58]. Recently, WASP was demonstrated to be critical for the development of marginal zone (naïve) B cells [59]. WASP-deficient marginal zone B cells fail to respond to sphingosine-1-phosphate, an important chemoattractant for the localization of B cells to the marginal zone. WASP−/− mice display diminished response to the T-independent antigen, TNP-ficoll. Together these findings indicate that WASP is not only necessary for the development, but also to B cells functions.

WASP is critical for the development and function of Tregs

Recently, it was observed that WASP is critical for the development of $CD4^+$ $CD25^+$ $Fox3p^+$ regulatory T cells (Tregs) [60]. Although T cell number per se in the thymus and peripheral lymphoid organs is not significantly altered in WASP knockout mice (WASP−/−), these mice had decreased Tregs both in the thymus and in the peripheral lymphoid organs. Treg cells derived from WASP−/− mice were shown to have diminished suppressor activity. Since autoimmune colitis is often observed due to imbalance in the ratio between effector T cells and suppressor T cells, Snapper and coworkers have argued that the inflammatory bowel disease that is always observed in WASP KO mice is due to reduced number and function of Tregs [59].

Constitutively active mutants of WASP affect mitosis and cytokinesis

It is clear that WASP affects many cellular functions by its effect on actin dynamics. This is highlighted by various studies on cells derived from WAS patients and from WASP−/− mice. X-linked neutropenia is caused by activating WASP mutations, in that these mutations result in constitutively active WASP protein. Mutation of Ile 294 to Thr results in XLT; expression of WASP with this mutation causes uncontrolled ectopic actin polymerization that resulted in decreased proliferation and apoptosis. Filamentous actin was abnormally localized causing aborted mitosis, chromosomal aberrations, and multinucleated cells [61].

WIP deficiency attenuates T cell function(s)

Proliferation of T-cells in response to plate-bound anti-CD3 was abolished in WIP−/− T lymphocytes over a wide range of concentrations tested [39]. In contrast, T cells proliferated normally to stimulation with PMA and ionomycin, which bypass receptor signaling. These results suggest that WIP is essential for T cell activation via TCR/CD3. T-cells from WIP−/− mice are deficient in anti-CD3 induced actin polymerization and conjugate formation with superantigen presenting B-cells or anti-CD3 containing lipid bilayers.

Absence of WIP results is poorly organized immune synapse and diminished recruitment of WASP to the immune synapse [19]. WASP is essential for the formation of podosomes in macrophages [32] and in dedritic cells [35] and WIP has been shown to be essential for the localization of WASP to the forming podosomes [35, 62].

WIP is a chaperone for WASP

WASP protein, but not mRNA, levels were severely diminished in T cells from WIP−/− mice. WASP protein level was increased by re-introduction of WIP into these cells. The WASP binding domain of WIP was shown to protect WASP from degradation by calpain in vitro. Treatment with calpain and proteasome inhibitors increased WASP levels in T cells from WIP−/− mice and in lymphocytes from two WAS patients with the missense mutations R86H and A134T that disrupt WIP binding [5]. These results demonstrate that WIP stabilizes WASP and missense mutations that affect WIP binding affect the stability of WASP. This concept is highly relevant clinically because of the observation that point mutations in WASP that abolish WIP binding result in WAS [5, 8]. In this context, it is noteworthy to reiterate that in the majority of WAS patients the point mutations in WASP localize to the WIP binding region [33].

WIP is essential for mast cell and NK cell activity

Bone marrow derived mast cells from WIP−/− mice show impaired degranulation and fail to secrete IL-6 following high affinity FcεR ligation [63]. The actin cytoskeleton was largely normal in WIP−/− mast cells but the kinetics of FcεR induced cell shape changes were altered. Interestingly, knockdown of WIP completely inhibited NK cell toxicity [64]. WIP associates with lytic granules and absence of WIP inhibited lytic granule polarization. The precise mechanism of the role of WIP in mast cell and NK cell function is not clear; however, it is clear that WIP is involved by as yet unknown mechanism in the transport of secretory/lytic granules in these cell types. A functional link with proteins that interact with the actin cytoskeleton likely underlies the role of WIP in degranulation (see section "WASP and WIP form multiprotein complex").

WASP and WIP form multiprotein complex

Activation of NK cells results in the formation of a multiprotein complex containing WASP, actin, myosin IIA and myosin light chains 2 and 3, and WIP [65]. As in T cells, WIP phosphorylation is primarily effected by PKCθ and phosphorylation coincides with the formation of the multiprotein complex. This complex polarizes to the NK cell-target cell contact site. Inhibitory signaling through killer cell immunoglobulin-like receptor (KIR2-DL1) prevents the assembly and the localization of this complex indicating that this complex is necessary for killer cell activity [64, 66]. N-WASP was shown to exist as a multiprotein complex in bovine brain lysates and in Xenopus oocyte lysates [40] along with WIP and TOCA-1. Fractionation on hydrophobic affinity column showed that WIP but not TOCA-1 co-fractionates with N-WASP, suggesting that WIP binds to N-WASP with stronger affinity. N-WASP bound to WIP was shown to be not active in actin polymerization assays unless Cdc42 and TOCA-1 were present suggesting that WIP also serves to keep N-WASP in an inactive state in vivo. Several adaptor proteins such as Nck/CrkL/Grb2 [19, 67, 68] have been shown to associate with WASP/N-WASP proteins;

therefore, it is not difficult to envisage that, in addition to forming constitutive multiprotein complexes in cells with WIP, actin and myosin WASP/N-WASP proteins engage in the formation of a number of transient signaling complexes, depending on cell type and sub-cellular location.

Functions of WIP homologs

Relatively less is known about the biology of either CR16 or WIRE/WICH compared to WIP. CR16 was shown to form a tight complex with N-WASP in bovine brain [69]. Unlike WIP, CR16 does not affect the kinetics of Arp2/3 complex mediated actin polymerization induced by N-WASP. Recently, the phenotype of CR16 knockout mice was published. Surprisingly, these mice do not display any defects in the central nervous system, but show male specific sterility [70]. WIRE/WICH protein was identified simultaneously by two different groups [71, 72]. WIRE localizes to the actin filaments in PAE cells. Cells ectopically expressing WIRE produced intense ruffles and co-expression of WASP with WIRE in PAE cells resulted in the relocalization of WASP to the actin containing motile structures in response to PDGFβ stimulation. WASP is not expressed in non-hematopoietic cells; therefore, the significance of this finding needs evaluation. However, N-WASP may substitute functionally for WASP; perhaps WIRE may localize N-WASP to actin containing motile structures. Interestingly, WIRE also binds to Nckβ, an adaptor protein involved in signaling to PDGF receptor [71]. In addition, overexpression of WIRE was shown to inhibit endocytosis of PDGF receptor [73].

Concluding remarks

We have shown that T cells from WIP−/− mice, like those from WASP−/− mice, fail to proliferate, secrete IL-2, or increase their F-actin content after TCR ligation and have a defect in in vivo homing. Furthermore, WIP−/− mice display a progressive immuno-logical disorder that resembles that of WASP−/− T cells. In addition, WIP−/− T cells, but not WASP−/− T cells, have a disrupted actin cytoskeleton and are deficient in IS for-mation, proliferation to antigen and soluble anti-CD3, and in vitro chemotaxis to SDF-1α [74], suggesting an independent role for WIP in T cell function. However, because WASP protein level is severely diminished in T cells from WIP−/− mice, it is not possible to define the individual contribution of isolated WIP deficiency to the phenotype of WIP KO mice. Nevertheless, our observations that the defect in T cell activation, chemotaxis, and in vivo homing of WASP/WIP DKO mice is more severe than that of WASP KO mice ([74] and S. LeBras and R. Geha, unpublished) strongly argue that WIP plays an important role in T cell activation independently of WASP.

It is clear from the aforementioned arguments that WASP and WIP are multifunctional proteins that strongly influence the actin dynamics in cells. WIP has an additional special function of stabilizing WASP. Increasing evidence demonstrates that WASP and WIP are part of multiprotein macromolecular complexes. Studies to date have reported only the stable high affinity constitutive complexes. Given the multitude of protein with which WASP and WIP interact, it is likely that WASP and WIP engage in transient complexes that are temporally and spatially regulated in cells. While many functions have been described for the WASP/N-WASP and WIP family members, their interrelationship and the full range of their functions remain to be discovered.

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