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Regulation of Cytoskeletal Dynamics at the Immune Synapse: New Stars Join the Actin Troupe

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Abstract

Reorganization of actin cytoskeletal dynamics plays a critical role in controlling T-lymphocyte activation and effector functions. Interaction of T-cell receptors (TCR) with appropriate major histocompatibility complex–peptide complexes on antigen-presenting cells results in the activation of signaling cascades, leading to the accumulation of F-actin at the cell–cell contact site. This event is required for the formation and stabilization of the immune synapse (IS), a cellular structure essential for the modulation of T-cell responses. Analysis of actin cyto-skeletal dynamics following engagement of the TCR has largely focused on the Arp2/3 regulator, WASp, because of its early identification and its association with human disease. However, recent studies have shown equally important roles for several additional actin regulatory proteins. In this review, we turn the spotlight on the expanding cast of actin regulatory proteins, which coordinate actin dynamics at the IS.

Keywords

actin; cofilin; HS1; immune synapse; signaling; T cell; VASP; WASp; WAVE; WIP

Activation of T lymphocytes by antigen-presenting cells (APCs) induces dramatic polarization of the T-cell cyto-skeleton and associated signaling molecules. Within a few minutes of cell–cell contact, actin filaments are polymerized beneath the contact site and the T-cell microtubule-organizing center reorients to face the bound APCs. This process was recognized as a hallmark of productive lymphocyte engagement well over two decades ago (1,2). Since that time, the contact site between the T cell and the APC has been the subject of intense study. Pharmacological studies showed that actin reorganization is indispensable for T-cell activation and effector function, and it soon became clear that one role of actin remodeling is to organize proteins involved in the activation of T-cell receptors (TCR) into a specialized signaling domain, the immune synapse (IS) (3–5). Despite significant progress in understanding the dynamics of T-cell signaling assemblies [reviewed by Bromley et al. (6), Tooley et al. (7), Lin et al. (8) and Friedl et al. (9)], progress in identifying and understanding the immediate effectors of actin dynamics has lagged. However, the field has recently begun to see rapid progress, driven largely by advances in our understanding of actin biology in nonhematopoietic cells.

In many respects, the cytoskeletal dynamics triggered by TCR engagement resemble events occurring in the lamellipodium of a migrating cell, where protrusion is driven largely by the assembly of actin monomers into filaments. Studies on bacterial comet tails have led to the identification of a core set of requisite proteins that can also account for actin dynamics during

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lamellipodial extension: actin, profilin, cofilin, capping protein, Arp2/3 complex and an Arp2/3 complex activator. The 'dendritic nucleation/array treadmilling' hypothesis put forward by Pollard and Borisy explains how these proteins function together in mammalian cells (10): 1) nucleation-promoting factors stimulate the Arp2/3 complex to initiate a new filament as a branch on a preexisting filament; 2) filaments grow transiently by addition of ATP-actin monomer from the actin-profilin pool to the barbed end, and this growth pushes the cell membrane forward; 3) binding of capping proteins to the barbed end of growing filaments terminates elongation and promotes branching and 4) as filaments age, ATP is hydrolyzed and hydrolysis and dissociation of γ -phosphate lead to debranching and association with ADF (actin depolymerizing factor)/ cofilin, which severs the filaments and promotes monomer dissociation. Recent studies on T cells have implicated some of the core actin regulatory proteins in controlling IS dynamics and formation. Through these studies, we are beginning to understand how upstream signaling events coordinate actin dynamics during T-cell activation.

WASp and WASp-Interacting Protein

The best-studied actin regulatory protein in T cells is WASp, a 54-kDa, hematopoietic-specific homologue of the Cdc42 effector protein, N-WASp. WASp was cloned as the gene responsible for Wiskott-Aldrich syndrome (WAS), a severe X-linked immunodeficiency disorder [reviewed by Snapper and Rosen (11)]. T cells from patients with WAS and from WASp-deficient mice show defects in TCR-induced activation events and actin polymerization (12–15). WASp has a C-terminal VCA region, comprising a WASp-homology 2 motif (WH2; also called a verprolin-homology domain) that binds actin monomer, a cofilin-homology motif that binds and activates the Arp2/3 complex and an acidic motif that also participates in Arp2/3 binding (Figure 1A). Like other Arp2/3-dependent actin-nucleation-promoting factors, WASp also works by presenting actin monomers to preformed Arp2/Arp3 dimers, catalyzing the formation of new actin filaments on the sides of the preexisting filaments (16). WASp interacts with Cdc42-GTP through its guanosine-triphosphatase-binding domain (GBD) (17). In the absence of Cdc42, the VCA region folds back on the GBD, inhibiting WASp activity (18). Binding of Cdc42-GTP releases the VCA region, allowing Arp2/3 complex activation. Between the GBD and the VCA domains lies a prolinerich region that targets WASp to the IS by means of interactions with Nck and prolineserine-threonine phosphatase-interacting protein 1 (PST-PIP) (19,20,21). The WASp-homology 1 motif (WH1) domain at the N-terminus of WASp mediates binding to WASp-interacting protein (WIP) (22).

The pathways leading to WASp activation at the IS are relatively well understood (Figure 1C). The engagement of TCR induces localized accumulation of Cdc42-GTP through a pathway involving TCR, Lck, Zap-70, Itk, SLP-76 and Vav1 (19,20,23). This brings Cdc42-GTP into proximity with WASp, inducing the conformational changes associated with WASp activation. WASp can also be activated through tyrosine phosphorylation by the Src kinase Fyn (24). *In vitro* studies show that interactions with Cdc42 and Src kinases activate WASp in a highly synergistic fashion (25).

Although there is broad agreement that WASp is required for signaling events that lead to T-cell cytokine expression, questions have recently emerged about the relative importance of WASp for T-cell actin responses. T cells from WASp knockout mice exhibit profound defects in conjugate formation and IS architecture in some experimental systems (21,24) but not others (26–28). Moreover, RNAi-mediated suppression of WASp in the Jurkat T-cell line showed little effect on actin responses under conditions where suppression of other actin regulatory proteins caused profound actin defects (29). Finally, although one of the early pieces of evidence pointing to WASp as an actin regulatory molecule was the abnormally smooth structure of WAS lymphocytes (11); a recent study found no difference in microvillar length or density in freshly isolated WAS lymphocytes (30). One possible explanation for the

apparently modest actin defects in WASp-deficient T cells is compensation by N-WASP. Although N-WASP expression in T cells is very low, this protein may nonetheless be sufficient for some actin-dependent events. Definitive resolution of this question must await the generation of mice lacking both molecules in the T-cell lineage.

One key to understanding WASP function almost certainly lies in understanding its binding partner, the 54-kD verprolin family protein, WASp-interacting protein (WIP). The C-terminal domain of WIP binds the WH1 domain of WASp, forming an obligate heterodimer in resting T cells (22,31). Overexpression of WIP leads to increased filamentous actin (F-actin) content and formation of filopodia (22,32). T cells from WIP^{-/-} mice develop normally but fail to proliferate, secrete the T-cell autocrine growth factor interleukin (IL)-2 or increase their F-actin content after TCR ligation (33). They exhibit defective conjugation, profoundly disorganized actin at the IS and significant defects in chemotaxis and homing to lymphoid organs (34). WIP has a proline-rich core region, flanked by an actin regulatory N-terminus and a C-terminal WASp-binding region (Figure 1B). The N-terminus, which contains two globular actin (G-actin)-binding WH2 motifs, also binds actin filaments and likely mediates the ability of WIP to stabilize F-actin (32). The prolinerich region binds to SH3-domain-containing proteins including Nck and cortactin (35,36).

WASP-interacting protein and its homologues (CR16, WIRE) affect actin dynamics both through interactions with WASp and on their own (32,37). For example, WIP can synergize with Vav to enhance activation of NF-AT/AP-1 transcription elements within the IL-2 promoter, through both WASp-dependent and WASp-independent pathways (38). *In vitro*, WIP inhibits the ability of N-WASP to drive actin polymerization, and it has been proposed that WIP stabilizes the autoinhibited conformation of WASp (32). In T cells, however, WIP stimulates WASp-dependent actin polymerization (22,32), and it may contribute to WASp recruitment to the TCR signaling complex (31). WASp-interacting protein may also positively regulate WASp function by co-ordinating its interaction with other actin regulatory proteins such as the cortactin homologue, HS1.

One key role of WIP is to stabilize WASp from proteolytic degradation. T cells from WIP^{-/-} mice express only 10% of normal WASp levels (34), and suppression of WIP using RNAi leads to loss of WASp protein (E. Carrizosa, S. Li and J. K. Burkhardt, unpublished data). These new findings explain the long-standing observation that WAS patients with mutations in the WIP-binding region express little or no WASp protein (39,40). Indeed, the vast majority of WAS mutations map to the WIP-binding region, and many perturb WASp–WIP binding (11). Interestingly, mice deficient for both WIP and WASp show significantly more severe defects in T-cell homing and chemotaxis than either of the single knockouts, indicating that the small amount of residual WASp must be functionally significant (34). The synergistic phenotype observed in WASp–WIP double knockout mice points to a degree of functional redundancy between these two proteins and re-emphasizes that WIP plays an important role in T cells, independent of its effects on WASp.

The WAVE Complex

In parallel with the WIP/WASP complex, which functions downstream of Cdc42, the WAVE/Scar family of proteins regulate actin responses downstream of Rac. Like WASp, WAVE/Scar proteins can directly interact with G-actin and Arp2/3 complex by means of their C-terminal VCA domains, leading to the formation of a branched actin filament meshwork (41). Of the three structurally similar WAVE isoforms (WAVE1–3), WAVE2 shows the broadest expression pattern and is the primary isoform found in T cells (29). In addition to the VCA domain, WAVE proteins contain an N-terminal WAVE homology domain involved in interaction with the Abl tyrosine kinase-interacting scaffold molecules, Abi1 or Abi2 (42), a

basic region involved in membrane targeting to PIP3 (43) and a proline-rich sequence that interacts with the Rac1 effector molecule, IRSp53 (Figure 2A). WAVE proteins are present in cells as part of a high molecular weight complex, which includes Abi1 or Abi2, IRSp53, the Nck-associated protein, Nap1 (44,45) or its hematopoietic homologue, HEM1 (29), the Rac effector protein Sra-1/PIR121 and HSPC300 (45). This complex can be recruited by Rac1-GTP to promote actin polymerization at the leading edge of stimulated cells (Figure 2B). Inhibiting expression of Abi proteins, WAVE, PIR121/Sra-1 or Nap1/HEM1 by RNAi leads to a loss not only of Rac1-induced actin remodeling but also of other members of the complex (29,42,44).

Although WASp and WAVE proteins are structurally similar, they are regulated very differently (41). WAVE lacks a GBD region and therefore does not form an autoinhibited fold and does not bind Rac1 directly (16). Instead, Rac1-GTP interacts with WAVE by binding to Sra-1 (46) or a related isoform, PIR121 (44) (Figure 2B). In addition to being targeted to sites of Rac1-GTP accumulation, recent evidence suggests that WAVE activity may be regulated in several ways. For example, TCR-stimulated tyrosine phosphorylation of WAVE2 by Abl reportedly results in increased actin polymerization activity (47). On TCR ligation, WAVE proteins are also phosphorylated on serine and threonine residues in an extracellular signal-regulated kinase (ERK)-dependent and protein kinase C-dependent manner (29), presenting additional possibilities for posttranslational control.

Many WAVE complex components localize to the IS, including Abi1, Abi2, WAVE1, WAVE2 and HEM1 (29,48). Additionally, both WAVE2 and Abi1 are found at the periphery of the lamellipod of T cells spreading in response to TCR ligation (48; J. Nolz and D. D. Billadeau, unpublished data). This is in contrast to WASp, which initially colocalizes with the activated TCR and then relocates to a diffuse circumferential ring but never localizes to the leading edge of the spreading T cell (49). Exactly how the WAVE complex is targeted to the contact site between T cell and APC is currently being unraveled. Studies using RNAi-mediated depletion of Abi proteins or expression of Abi1 mutants that are unable to interact with WAVE suggest that targeting involves a co-operative interaction between Abi and Wave (48). Remarkably, WAVE complex targeting to the IS has not been formally shown to require interactions with Rac1-GTP. Nonetheless, lessons from non-hematopoietic cells indicate that Rac1-GTP interactions with Sra-1/PIR121 and/or IRSp53 will prove to be involved.

Consistent with its role in regulating *de novo* actin polymerization in other cell types, the WAVE complex is critical for actin polymerization in T cells. Depletion of WAVE2 or Abi by RNAi leads to decreased lamellipodia formation and F-actin accumulation at the IS (29, 48). In addition, integrin-mediated adhesion following TCR engagement, an actin-regulated process, was inhibited by RNAi-mediated depletion of WAVE2, HEM1 or PIR121. Interestingly, comparative studies of T cells deficient for WASp or WAVE2 suggest that IS formation and integrin function during T-cell activation depend more heavily on the WAVE complex than on WASp (29). Notably, the adhesion defects in WAVE2-suppressed T cells can be rescued by wildtype WAVE2 but not by a WAVE2 mutant lacking its VCA domain (J. C. Nolz, Y. Shimizu and D. D. Billadeau, unpublished data). This finding indicates that WAVE2-dependent actin polymerization is required.

T cells from mice lacking Abi2 and heterozygous for Abi1 show a defect in proliferation in response to TCR ligation (48). This defect may stem from the failure to produce the T-cell growth factor, IL-2, as WAVE2-suppressed cells showed normal proximal signaling events but dramatic defects in TCR-stimulated IL-2 promoter activation (29). This phenotype is very similar to the effects of cytochalasin D (50), in keeping with the view that the effects are because of loss of F-actin *per se*. Further analysis of WAVE2-suppressed cells showed that the WAVE2 complex is required for optimal Ca^{++} entry through calcium release-activated calcium (CRAC)

channels in response to intracellular store depletion, resulting in the failure to properly activate Ca^{++} -dependent NF-AT transcriptional elements within the IL-2 promoter (29). Future experiments aimed at determining whether WAVE2-dependent CRAC channel activation is an actin-dependent process will provide valuable insight into the mechanism by which this elusive channel is regulated following TCR engagement.

HS1

The third Arp2/3-complex-activating factor to be identified at the IS, HS1, differs from WASp and WAVE2 in that it is not regulated by Rho family guanosine triphosphatases. HS1 is the 75-Mr, hematopoietic-specific homologue of the ubiquitously expressed protein cortactin. HS1 and cortactin share an N-terminal acidic (NTA) region, with limited homology to the VCA regions of WASp and WAVE (Figure 3). This region includes the DDW residues required for Arp2/3 binding but lacks cofilin and verprolin-homology motifs, and it makes more limited contacts with Arp2/3 complex (51). Adjacent to the NTA region is an F-actin-binding region composed of 37-amino-acid helix-turn-helix (HTH) repeats (repeated 6.5 times in cortactin and 3.5 times in HS1), followed (in HS1 but not in cortactin) by a coiled-coil (CC) region that also binds F-actin (52). While HS1 and cortactin can catalyse actin polymerization in an Arp2/3-complex-dependent manner, they do so relatively weakly compared with WASp (53). Instead, these proteins are thought to promote net actin polymerization by bridging between Arp2/3 complex and actin filaments, thereby prolonging the lifetime of branched actin structures (54). Both the HTH and the CC regions of HS1 are required for its ability to induce Arp2/3-dependent actin polymerization, and deletion of the HTH region destabilizes interactions with Arp2/3 complex in cell extracts (55). This suggests that one role of the HTH/CC region is to bring the NTA region into correct alignment with Arp2/3-complex-binding sites on nascent actin branches.

C-terminal to the HTH/CC region of HS1 is an extended proline-rich region that binds to the SH3 domain of Lck (56). Within this region are six glutamate–proline (EP) repeats. Interestingly, a polymorphic HS1 allele in humans encoding an additional two EP repeats is genetically linked to systemic lupus erythematosus (57). Expression of this variant is associated with increased antigen-receptor-induced apoptosis (57), but there is currently no information about how cytoskeletal dynamics are affected. The C-terminus of HS1 contains an SH3 domain. In cortactin, this domain was shown to bind WIP and N-WASp and also other proline-rich actin regulatory proteins (58). WASp-interacting protein stimulates the ability of cortactin to mediate Arp2/3-complex-dependent actin polymerization *in vitro* (36). Whether HS1 also binds to WIP and WASp remains unclear. We found that the SH3 domain of HS1 binds *in vitro* to WIP, but we have so far failed to detect this interaction in cell lysates (59).

Although HS1-deficient mice do not exhibit major defects in lymphocyte development (60), immunoreceptor-induced proliferation of B and T cells is impaired. In addition, these mice exhibit significant defects in immunoreceptor-induced apoptosis. T cells from these mice and Jurkat T cells in which HS1 is suppressed using RNAi show defective actin responses at the IS (59). Signaling studies showed that while early tyrosine phosphorylation events are intact, these cells exhibit defects in TCR-stimulated Ca^{++} influx and activation of NF-AT and NFkB elements within the IL-2 promoter. Interestingly, parallel video analysis of green fluorescent protein (GFP)-actin expressing Jurkat T cells deficient for WAVE2, HS1 and WASp show clear phenotypic differences [29,59; E. Comiskey and J. K. Burkhardt, unpublished data]. Cells deficient for WASp show no gross defects in TCR-stimulated actin responses, while cells lacking WAVE2 fail to spread and extend lamellipodia altogether (29). Cells lacking HS1 show an intermediate phenotype: they extend actin-rich lamellipodia, but these structures are disorganized and unstable (59). This suggests that HS1 is not required for actin polymerization at the IS but rather for stabilization of actin-rich structures at this site. In keeping with this

interpretation, kinetic analysis of conjugates formed with HS1-deficient cells showed nearly normal actin responses at early times, with loss of actin by 3–5 min after cell–cell contact.

Although relatively little is known about the regulation of HS1 function, it seems clear that tyrosine phosphorylation will play an important role. HS1 is a major target for tyrosine phosphorylation after immunoreceptor ligation (56,61,62). In T cells, HS1 phosphorylation occurs predominantly at Y378 and 397 (59). This event, which requires both the Src family kinase Lck and the downstream Syk family kinase ZAP-70, is required for recruitment of HS1 to the IS. These findings are consistent with studies in B cells, which show that phosphorylation of HS1 at the same sites drives HS1 recruitment into lipid raft aggregates, where it colocalizes with BCR, WASp and Arp2/3 complex (63,64). Phosphorylation of HS1 creates binding sites for SH2-domain-containing proteins, including Src kinases (61,65), Vav1 and PLC γ 1 (59). The functional consequences of HS1 binding to Lck and PLC γ 1 are yet to be determined, but interactions with Vav1 stabilize Vav1 recruitment at the IS. As Vav1 activates both Rac1 and Cdc42, this interaction might represent an important point of cross talk among Arp2/3-dependent actin regulatory pathways.

Ena/VASP Proteins

The Ena/VASP proteins represent a conserved family of actin regulatory proteins consisting of EVH1 and EVH2 domains and a central proline-rich region (66). These proteins associate with the barbed ends of actin filaments, impede binding of actincapping proteins and interact with profilin at sites of actin polymerization. Of the three Ena/VASP family members, Evl and VASP are primarily expressed in T cells. Evl colocalizes to the site of TCR ligation and can be found within a TCR-inducible complex that includes WASp, the Rac/Cdc42 GTP exchange factor Vav and the adapters SLP-76, Nck and ADAP (to which it binds directly through its EVH1 domain) (67). Evidence that Evl and VASP participate in regulation of actin responses in T cells comes from two sets of studies. First, expression of the EVH1-binding domain of the bacterial protein ActA fused to GFP was found to inhibit Evl and F-actin accumulation at the site of TCR ligation (67). In contrast, the localization of Arp3, SLAP and WASp was unaffected, suggesting that Evl is required for the accumulation/stabilization of F-actin at the IS. Second, recent work has identified the Rap1 effector molecule, RIAM, as an Evl/VASP- and profilin-binding partner (68,69). Overexpression of RIAM in T cells induced integrin activation/adhesion and cell spreading on fibronectin, whereas RNAi toward RIAM resulted in loss of membrane-bound Rap1-GTP and an overall decrease in F-actin (68). Thus, this protein may be a key regulator of Rap1-induced adhesion and actin reorganization in T cells. Although these findings point to a role for VASP and/or Evl in regulating T-cell actin dynamics and integrin-mediated adhesion, definitive studies involving T cells deficient in these proteins need to be performed. In preliminary studies using RNAi to suppress expression of Evl and VASP independently or together, we have so far failed to detect major effects on actin responses at the IS (T. S. Gomez, Y.-P. Huang, J. K. Burkhardt and D. D. Billadeau, unpublished data). Thus, these proteins may function only in a supporting role.

Cofilin

Cofilin is a 19-kDa protein that is ubiquitously expressed in eukaryotic cells and is required for cell survival. As shown in Figure 4, the primary activities of cofilin are to sever actin filaments and dissociate actin monomers (70). Paradoxically, severing creates new barbed ends that serve as sites for elongation, and these newly polymerized filaments are preferential sites for the formation of branches by the Arp2/3 complex (71). The role of cofilin in fostering actin polymerization versus depolymerization is essentially controlled by activators of the Arp 2/3 complex because in the absence of active Arp2/3 complex, cofilin activity results in net dissociation of actin monomers.

Like WASp, cofilin has been known to be important for T-cell function for many years. However, the role of cofilin in T-cell signaling has been much more difficult to study as it is essential for cell survival. Recently, the Samstag group has used cell-permeant peptides that block cofilin binding to F-actin to test the effects on T-cell activation (72). These peptides partially inhibit conjugate formation, and the conjugates that do form show loss of cofilin and CD2 but not TCR from the IS. Analysis of downstream events shows defects in the upregulation of T-cell activation markers, in proliferation and in the production of several cytokines including IL-2, interferon- γ and IL-10.

Cofilin is regulated by phosphorylation at serine 3, which inactivates actin binding (70). In resting, untransformed T cells, cofilin is present in the cytoplasm and is largely phosphorylated and inactive. Following costimulatory signaling through CD2 or CD28 (but not TCR ligation alone), cofilin is dephosphorylated, which activates the protein and drives its transient association with F-actin (73,74). Although dephosphorylation of cofilin is necessary for actin binding, it is not sufficient and other undefined signals are also required (74). In superantigen-induced T cell-B cell conjugates, cofilin localizes to the peripheral region of the IS (72). The mechanism of cofilin targeting to the IS has not been tested but likely occurs as a direct result of actin binding.

The regulatory pathways that control cofilin activation during T-cell signaling are not well defined. In nonlymphoid cells, cofilin is typically phosphorylated through LIM kinases and related TES kinases and is dephosphorylated by members of the slingshot family of phosphatases (70). LIM kinase-1 and the slingshot proteins SSH1L are known to regulate cofilin activity in migrating T cells responding to chemokines (75), and LIM kinase is required to couple early tyrosine phosphorylation events to actin remodeling in cytotoxic T lymphocyte conjugates (76). PP1 and PP2A have also been implicated in dephosphorylating cofilin in response to CD3/CD28 ligation (73). A related unresolved issue concerns *when* cofilin functions during T cell signaling. In CD2 crosslinking studies, the earliest dephosphorylation and association of the cofilin with the actin cytoskeleton was detected in about 15 min after receptor stimulation and persisted for about 4 h (74). This suggests that cofilin may be dispensable for early signaling events but that it may also play a role in stabilization of conjugates and in sustained signaling processes.

Ensemble Acting

For many years, control of actin at the IS was thought to be a ‘one-protein show’, starring WASp. The cast has grown substantially in just a few years and is destined to continue to grow as additional actin regulatory proteins identified in nonhematopoietic cells are explored in T cells. Members of the formin family, for example, are waiting in the wings. Going forward, cell biologists studying the IS will be confronted with understanding the stage direction of T cell signaling. We must learn how actin binding, severing and nucleating proteins interact at the IS in response to specific cues and how these proteins direct T cell activation and effector functions by co-ordinating temporal/ spatial actin cytoskeletal changes.

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Figure 1. WASp/WIP complex function at the IS

A) Domain structure and activation of WASp. In unstimulated cells, WASp assumes an autoinhibited conformation, in which the C-terminal VCA region interacts with the GBD. Binding of Cdc42-GTP, often in conjunction with phosphorylation of WASp tyrosine 291, induces a conformational change, allowing WASp to activate Arp2/3 complex function. B) Domain structure of WIP. C) Model of WASp/WIP function at the IS. WASp and WIP are present in an obligate heterodimer generated by interaction of their N- and C-termini, respectively. WASp recruitment is mediated by the adapters SLP-76 and Nck, bringing it into proximity with Cdc42-GTP and Src kinases in the TCR signaling complex. Independent interaction of WIP with the TCR signaling complex (not shown) occurs through binding to

ZAP-70 and CrkL (31). WIP and WASp likely function coordinately, with WASp activating Arp2/3-complex-induced actin polymerization and WIP stabilizing actin filaments and perhaps also facilitating polymerization.

Figure 2. WAVE complex function at the IS

A) Domain architecture of the WAVE family of proteins. Similar to WASp, the WAVE proteins stimulate *de novo* actin polymerization through their VCA domains. A recent report suggests that WAVE2 actin polymerization activity is positively regulated through phosphorylation of tyrosine 150 by the Abl tyrosine kinase (47). B) Model of WAVE protein recruitment to the IS following TCR engagement. On TCR engagement, numerous intracellular signaling cascades are activated, among which are Rho family GEFs that lead to the GTP-loading of Rac1 and subsequent localization of the WAVE macromolecular complex. While the most obvious GEFs to carry out this function in T cells would be Vav1, neither Vav1 nor Rac1 has been formally shown to participate in the recruitment of the WAVE complex to the activated receptor in T cells. In addition, although the BR and Rac1 effector molecule IRSp53 have been shown to regulate the localization of WAVE to the plasma membrane in other cell types, their role in regulating the recruitment of the WAVE complex to the IS is yet to be determined. Last, recent evidence indicates that the WAVE2 complex regulates TCR-stimulated integrin activation and couples Ca²⁺ intracellular store release to capacitive Ca²⁺ entrance through the CRAC channel. However, it is currently unknown how these events are controlled by the WAVE2 complex. GEFs, GTP exchange factors.

Figure 3. HS1 function at the IS

A) Domain structure of HS1. The N-terminal acidic region (A) has homology to the C-terminus of WASp and WAVE proteins and can interact with Arp2/3 complex in a limited fashion. However, actin regulatory activity is also dependent on the 3.5-HTH (cortactin) repeats and the neighboring CC region. The remainder of the protein has adapter-like properties. B) Model of HS1 function at the IS. HS1 binds Lck in resting T cells through its proline-rich region. On TCR engagement, HS1 is tyrosine phosphorylated at Y378/397, most likely by ZAP-70, stabilizing binding to Lck. This phosphorylation directs recruitment of HS1 to the IS through as yet unidentified binding partners. HS1 interacts with F-actin filaments and Arp 2/3 complex, stabilizing branched actin structures. In addition, HS1 stabilizes Vav1 recruitment at the IS, thereby fostering ongoing Arp2/3 complex activation by Cdc42/Rac effectors such as WASp and WAVE2. It remains to be determined whether HS1 binds WASp and/or WIP directly and exactly how the function of these proteins is co-ordinated at the IS.

Figure 4. Cofilin function at the IS

Cofilin is regulated by LIM-kinase-dependent phosphorylation at serine 3, which inactivates the protein. In T cells, ligation of the costimulatory molecules CD2 or CD28 induces cofilin dephosphorylation in a PI3-kinase-dependent manner, most likely by PP1/PP2 phosphatases or the slingshot phosphatase SSH1L. Once activated, cofilin binds to ADP-actin and severs the actin filament and/or depolymerizes actin monomers. The resulting monomers interact with profilin, which promotes the exchange of ADP for ATP. Profilin-ATP-actin complexes are then available for assembly at filament barbed ends. Under conditions where Arp2/3-complex-activating factors such as WASp and WAVE2 are active, this process stimulates the formation of new actin branches. Note that the effects of cofilin on filament elongation and branching are not distinct events but are two manifestations of the same activity.