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REVIEW

Actin dynamics at the immunological synapse

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Abstract: Actins are a family of highly conserved proteins that play determinant roles in a variety of biological processes including: cell shape; migration; endocytosis/exocytosis; and adhesion. The dynamics of actin polymerization and depolymerization at specific cellular locations is tightly regulated and defines the cellular roles of actin. Here, we offer a perspective on the roles played by actin regulator molecules during the formation of the immunological synapse (IS) which is defined as the structure formed by the interaction between lymphocytes and antigen-presenting cells (APC). Proper actin dynamics at the IS are absolutely necessary for correct lymphocyte activation and alterations of such actin dynamics result in immune diseases.

Keywords: actin, lymphocytes, immunological synapse, arp2/3, actin regulators

Introduction

One of the central processes in the induction of an immune response is the formation of a cell-cell contact between lymphocytes (naïve T cells, natural killer cells or B cells) and antigen-presenting cells (APCs) such as dendritic cells (DCs), macrophages, B cells and certain target cells.[1,2] T lymphocytes exert a key role in immunity, mainly through the production of cytokines and the destruction of antigen-bearing cells. The interaction between T cells and APCs includes the recognition of the major histocompatibility complex (MHC)-bearing molecules by the T cell receptor (TCR) and directs T cell activation; the structure formed at the T cell/APC contact zone is known as the immunological synapse (IS).[1,3,4] TCR stimulation during the formation of the IS induces major morphological and functional changes in T cells, including rearrangements of actin and tubulin at the T cell/APC contact zone to increase the interacting area.[3,4] Active accumulation of actin at the IS is absolutely necessary for lymphocyte activation.[5,6]

The IS has been demonstrated to occur *in vivo*[7] and consists of concentric rings of molecular aggregates. The TCR-MHC complexes are concentrated in a central cluster called the central supramolecular activation cluster, or cSMAC.[8] This is surrounded by an outer ring containing adhesion proteins such as integrins and their ligands, and called the peripheral supramolecular activation cluster, or pSMAC.[8] The pSMAC is supported by an F-actin ring formed after a polar local actin polymerization[5] and existent microfilaments reorganization[9] (Figure 1). Polar actin polymerization in T cells early in the formation of the IS creates a lamellipodium at the intercellular contact zone that increases the interacting surface area.[10] Microfilament reorganization generates a structure called an "actin cloud", specifically induced by the outside-in

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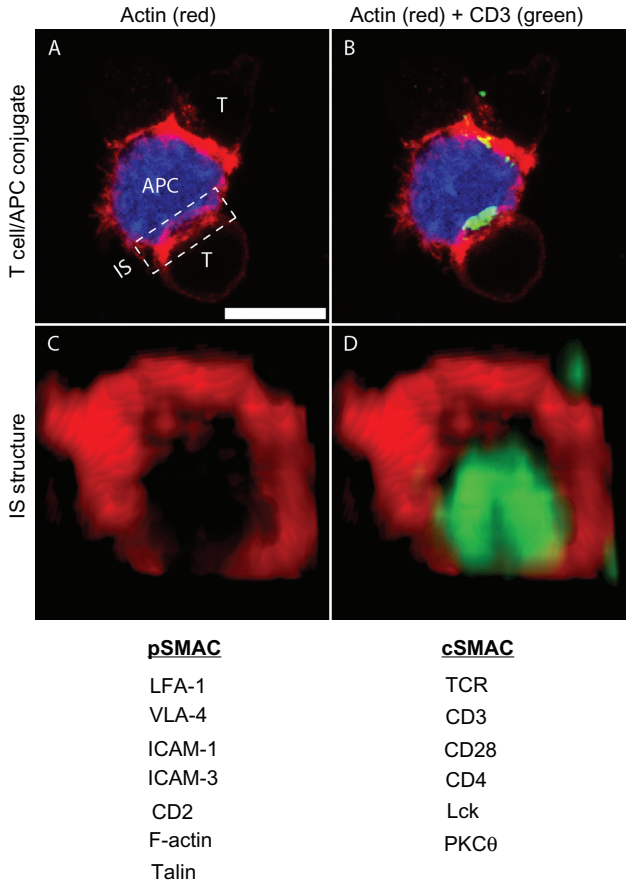

Figure 1 Known structure of the IS.

Notes: (**A**) and (**B**), Confocal image showing T cells forming conjugates with an APC (blue). Actin, detected with phalloidin, is shown in red, and CD3 (in B), is shown in green. (**C**) and (**D**), 3D reconstruction of the structure of the IS indicated by boxed area in (**A**). Note that actin (red) forms the typical ring-like structure corresponding to the pSMAC while CD3 (green) is restricted to the central part of the IS (cSMAC). Scale bar = 10 μm. Proteins located either in the cSMAC or the pSMAC are indicated below. CD4 can be also observed at the pSMAC. Images were acquired by the authors using a Leica SP-5 confocal microscope and treated using ImageJ software.

Abbreviations: SMAC, supramolecular activation cluster; IS, immunological synapse; APC, antigen presenting cell; LFA, lymphocyte function-associated antigen; VLA, very late antigen; ICAM, inter-cellular adhesion molecule; PKC, protein kinase C; TCR, T cell receptor; CD, cluster of differentiation.

signals derived from integrin engagement and dependent upon c-Jun N-terminal kinase (JNK) activation, which lowers the threshold of TCR ligation for subsequent T cell activation.[9]

Actin microfilaments then form diverse associations with different transmembrane proteins, directing their movement to produce the observed segregation of proteins in concentric rings. After molecular domain segregation, the actin cytoskeleton plays an important role in the maturation and stabilization of the IS, allowing the translocation of the microtubular organizing centre (MTOC) towards the APC contact zone.[11,12] This sustained cell-cell contact assures sustained stimulation and allows the triggering of parallel signaling pathways that influence the final outcome of different functional programs. Defective actin polymerization at the IS leads to a deficient T cell activation, Ca^{2+} influx, cytokine secretion and T cell proliferation.[6,13,14]

The dynamics of actin polymerization/depolymerization at specific cellular locations is tightly regulated and defines actin's multiple cellular roles. In addition to its involvement in IS formation, actin plays an essential role in remarkably diverse cellular processes, including: cell shape; motility; adhesion; endocytosis/exocytosis; vesicle trafficking; and cytokinesis. In the present review we offer a perspective on the roles played by actin regulator molecules during the formation of the IS (Figure 2). These molecules control the dynamics and cellular location of actin polymerization, and determine the structure of actin networks.

Actin filaments

Actin, a 43 kDa ATPase, is the most abundant protein in most eukaryotic cells, accounting for up to one fifth of the total cellular protein in muscle cells and occurring at concentrations between 100 and 500 μM in non-muscle cells.[15] In vertebrates three main groups of actin isoforms, α, β and γ, have been identified. α actins are found in muscle and are the most important components of the contractile machinery. β and γ actins are found in many cell types as components of the actin cytoskeleton. Actins are highly conserved proteins, with 90% amino acid identity between β actin from humans and the yeast *Saccharomyces cerevisiae*.

Actin occurs in two forms, monomeric globular actin (G-actin) and polymeric filamentous actin (F-actin). Each asymmetric F-actin filament possesses a fast growing barbed end and a slower growing pointed end that are distinguishable by their structural characteristics and kinetic properties. *In vitro*, actin can self-assemble spontaneously in a process requiring adenosine triphosphate (ATP) hydrolysis. This spontaneous initiation of actin-filament formation first requires the assembly of three actin monomers into a stable actin oligomer, in a process called nucleation.[16] Spontaneous nucleation is kinetically unfavored because the actin dimer/trimer intermediates are very unstable and rapidly dissociate; therefore slower by several orders of magnitude than actin polymerization observed intracellularly.[17] In cells, actin nucleation and filament elongation is enhanced by a variety of actin nucleators, including: the Arp2/3 complex and its large family of nucleation promoting factors (NPFs);[16–20] formins;[21–24] Spire;[25] Cobl;[26] bacterial VopL and VopF;[27,28] TARP;[29] and Lmod.[30]

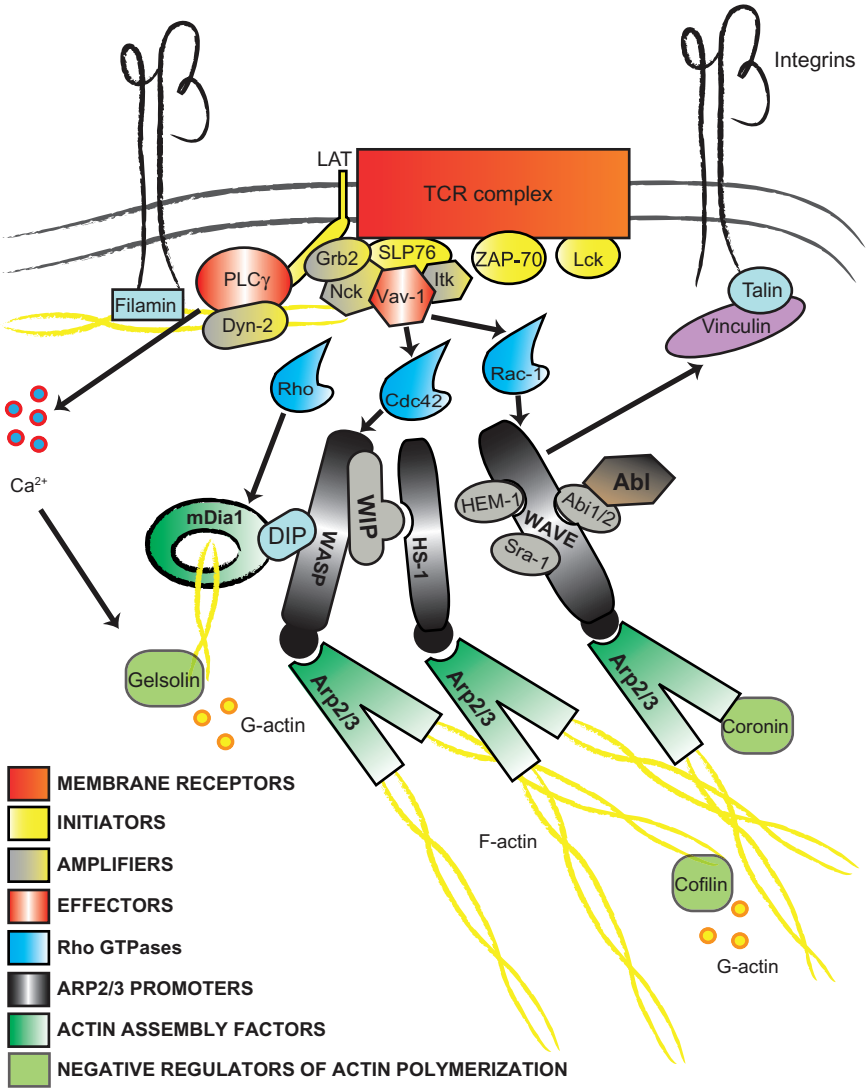

Figure 2 Signaling pathways leading to actin polymerization at the IS.

Notes: Following antigen-mediated activation of the TCR, Lck activates ZAP-70, which phosphorylates LAT, which in turn recruits PLCγ-1 and SLP76. PLCγ1 catalyzes the generation of IP_3, which mobilizes Ca^{2+}. SLP76 serves as a scaffold for actin-regulatory proteins such as Itk, Nck and Vav-1. Nck recruits several Arp2/3 promoting factors such as WASP and WIP, thereby regulating the actin-polymerization activity of the Arp2/3 complex. Vav-1 is Tyr-phosphorylated by Lck, activating its GEF activity. Vav-1 is a critical regulator of actin dynamics, T cell activation and cytokine production, activating the small Rho GTPases Rac1 and Cdc42, which in their GTP-bound state activate WAVE and WASP during the actin rearrangements observed upon TCR engagement. HS-1 is activated by TCR proximal kinases, thereby promoting actin polymerization via Arp2/3. In addition, HS-1 associates with Vav-1 and PLCγ, and the interaction of HS-1 with Vav-1 is necessary for stable localization of Vav-1 at the IS. Integrin activation at the IS is mediated by the WAVE complex through the recruitment of vinculin and talin. WAVE2 forms a complex with the scaffold proteins Abi-1 and Abi-2, HEM-1/Nap-1, and PIR121/Sra-1. Abi proteins promote Abl-dependent phosphorylation of WAVE2. Dynamin-2 interacts with PLCγ, Vav-1, WASP and Abi-1/2 and promotes actin polymerization (in order to simplify the figure it is depicted interacting only with PLCγ). Actin depolymerization factors such as gelsolin and cofilin, as well as inhibitors of Arp2/3 complex such as coronin are also recruited to the IS maintaining the highly dynamic process of polymerization/depolymerization of the actin cytoskeleton at the IS.

Abbreviations: WASP, Wiskott–Aldrich syndrome protein; PLC, phospholipase C; Abl, Abelson interactor protein; Abi, Abelson interactor protein; HS-1, hematopoietic lineage cell-specific protein-1; GTPase, guanine triphosphatase; WAVE, WASP/verprolin-homologous; TCR, T cell receptor.

Molecular regulators of actin polymerization/depolymerization at the IS

Actin nucleators

Filament nucleators are in general not closely related. However, with the exception of formins they all use the Wiskott–Aldrich syndrome protein (WASP)-Homology 2 domain (WH2 or W), a small and versatile actin-binding motif, for interaction with actin. A common architecture, found in Spire, Cobl and VopL/VopF, consists of tandem W domains that bind three to four actin monomers to form a nucleus. Formins are unique in that they use the formin-homology 2 (FH2) domain for interaction with actin and promote not only nucleation, but also processive barbed end elongation.[19]

Arp2/3 complex/NPFs

The actin-related protein (Arp)2/3 complex was first purified more than 15 years ago from *Acanthamoeba castellanii*.[31] It consists of a stable assembly of seven polypeptides, including two actin-related proteins (Arp2 and Arp3). The remaining five subunits are referred as ARPC1 (actin-related protein complex-1), ARPC2, ARPC3, ARPC4 and ARPC5.[16] The Arp2/3 complex by itself has low nucleation activity, and the complex needs to be activated by NPFs in order to promote actin nucleation and polymerization. Once activated, the Arp2/3 complex initiates the formation of a new (daughter) filament that emerges from an existing (mother) filament in a y-branch configuration with a 70° angle, that can be observed as a network of branching filaments.[20,32,33] This coupling of nucleation and branching by the Arp2/3 complex is central to its functions *in vivo*; it is this type of actin network that forms the lamellipodium structure in the T cell that embraces the APC.[10] Arp2/3 thus plays a predominant role in F-actin accumulation at the IS; and accordingly depletion of Arp2 or Arp3 abrogates lamellipodium formation, although Arp2- or Arp3- deficient cells can still polymerize F-actin to form filopodia.[34] Several NPFs play important roles in the actin polymerization observed at the IS (Figure 2):

HS-1 (hematopoietic lineage cell-specific protein-1) is the hematopoietic specific form of the ubiquitous cortactin. It can bind both F-actin and Arp2/3 through its N-terminal domains, promoting Arp2/3 nucleator activity and stabilizing filament branch-points.[35] In addition, HS-1 associates constitutively with lymphocyte cell-specific protein-tyrosine kinase (Lck) and may also interact through its C-terminal SH3 domain with WASP-interacting protein (WIP), N-Wasp, dynamin-2 and CD2AP, as cortactin does. This would imply an adaptor function for HS-1 as a promoter of the formation of Arp2/3 activator complexes. HS-1 is not essential for actin polymerization at the IS; however, actin polymerization in HS-1 deficient T cells is disorganized and does not sustain the lamellipodium structure. HS-1 deficient T cells consequently cannot support conjugate formation for more than a few minutes, which is not enough for proper T cell activation.[36] HS-1 is recruited to the IS rapidly after TCR engagement by Tyr-phosphorylation. This phosphorylation is essential for HS-1-mediated actin regulation at the IS and requires both Lck and zeta-associated protein of 70kDa (ZAP-70), since cells lacking either of these kinases fail to phosphorylate HS-1. Recently, the non-receptor Abelson tyrosine kinase (Abl) has been identified as the kinase responsible for HS-1 Tyr-phosphorylation during T cell activation.[37] Phosphorylated HS-1 associates directly with Vav-1 and phospholipase Cγ1 (PLCγ1) through their SH2 domains. Interaction of HS-1 with Vav-1 is essential for the stable localization of Vav-1 at the IS. Hence recruitment of HS-1 to the IS is unaffected in Vav-1-silenced T cells, whereas HS-1-silenced T cells can only recruit Vav-1 to the IS for a short time.[36] The kinetics of the removal of Vav-1 from the IS after the first contact with the APC closely parallels that of F-actin. HS-1 is thus a limited Arp2/3 activator whose main function is to stabilize F-actin branch-points and localize key signaling proteins such as Vav-1 at the IS.

WASP is the hematopoietic specific form of the Cdc42 effector N-WASP. Although WASP is involved in actin dynamics upon Cdc42 activation, its recruitment towards the IS is Cdc42-independent, and instead requires interaction with the kinase Nck.[38,39] WASP interacts constitutively with WIP and Cdc42 through its N-terminal region. Cdc42 activation (Cdc42-GTP) unfolds WASP, exposing its C-terminal region. The exposed C-terminal VCA region binds actin monomers and the cofilin-homology and acidic motifs bind Arp2/3 complex. In addition, WIP interacts with actin monomers, profilin and other actin regulators such as cortactin and Nck. Consequently, Nck targets WASP/WIP complex to the IS, in the proximity to Vav-1, which activates Cdc42. Cdc42 subsequently activates WASP/WIP, enhancing its interaction with Arp2/3, profilin and actin monomers. The WASP/WIP complex promotes Arp2/3 actin nucleation by providing a local pool of actin monomers.

WASP-deficient mice develop a severe immunodeficiency characterized by defective migration and activation of T cells, although T cell development is normal. This phenotype reflects substitution of some WASP functions by N-WASP, since wasp−/−/n-wasp−/− double knockouts have more pronounced peripheral T cell defects and impaired thymocyte development.[40] WIP is also essential for WASP function, since its suppression leads to proteolytic degradation of WASP; levels of WASP in wip−/− T cells are less than 10% of normal.[41] However, some reports indicate that WASP-deficient T cells still polymerize F-actin at the IS although they produce interleukin-2 (IL-2) inefficiently,[42] indicating that WASP is involved in other actin-independent pathways that allow T cell activation.

Recent studies show that WASP is required to stabilize the IS, rather than for the initial actin polymerization.[43] Furthermore, protein kinase C θ (PKCθ) promotes T cell motility and IS destabilization,[43] suggesting that WASP and PKCθ may have opposite effects on the IS. Moreover, PKCθ has been shown to phosphorylate WIP, enhancing its interaction with actin and myosin II during natural killer (NK) cell

activation.[44] Interaction of WIP with myosin II does not require association with WASP.[44] Thus when WIP-actin-myosin complexes are also formed at the IS, PKCθ might destabilize the IS by promoting the WIP-myosin II interaction.

The WAVE/Scar family regulates actin dynamics downstream of Ras-related C3 botulinum toxin substrate 1 (Rac1). In T lymphocytes WASP/Verprolin-homologous 2 (WAVE2) has been identified as the main promoter of Arp2/3 activity after TCR engagement, since its suppression impairs actin accumulation and the generation of the lamellipodium or any other F-actin structure.[45,46] WAVE2 is constitutively active and its recruitment to the IS depends on Rac-1 activation. WAVE2 interacts with Arp2/3, triggering a conformational change in the Arp2/3 complex that stimulates its nucleation activity. In addition, WAVE2 binds to G-actin, providing monomers for new microfilaments. WAVE2 occurs in cells only as part of a high molecular weight complex with other proteins such as insulin receptor substrate protein 53 (IRSp53), the scaffold proteins Abelson interactor protein-1 (Abi-1) and Abi-2, hematopoietic protein-1/Nck-associated protein 1 (HEM-1/Nap-1), and Rac1-associated protein-1 (PIR121/Sra-1).[47] None of these components exists *in vivo* in a free form, and suppression of any one leads to loss of the whole WAVE complex, presumably by protein degradation.[45] IRSp53 and Sra-1 are Rac-1 effectors couple WAVE function to Rac-1 activation. Abi proteins facilitate Abl-WAVE interaction. Abl tyrosine kinase phosphorylates WAVE2 in response to TCR activation, increasing actin polymerization.[48] Furthermore, Abl-Abi is required for normal targeting of the WAVE complex to the IS.[37,46] Depletion of WAVE2 or Abi impairs F-actin accumulation, lamellipodium formation and T cell activation upon TCR ligation. The function of WAVE in actin dynamics depends on the binding of the complex to Arp2/3, whereas its function in T cell activation depends on its ability to activate Ca^{2+} entry in an Arp2/3 independent manner.[49] Furthermore, the WAVE complex activates integrins at the IS by recruiting vinculin and talin[49] and during adhesion to fibronectin.[50] A vinculin mutant that cannot interact with Arp2/3 is unable to regulate integrins, and interaction of vinculin with Arp2/3 requires Arp 2/3-bound WAVE2.[49] WAVE2 thus enables the cell to integrate lamellipodium formation with integrin activation upon TCR engagement.

Formins are a class of actin nucleators that are independent of Arp2/3. Formins function as dimers that upon activation by Rho GTPases catalyze the elongation of non-branched microfilaments at the barbed ends[51] (Figure 2). Actin monomers are provided through the association of formins with profilins. In addition, formins impede the binding of actin-capping proteins to the actin filament barbed ends; and have been implicated in many linear structures such as filopodia, stress fibers and the contractile ring formed during cytokinesis.[10] Formins also participate in MTOC localization during mitosis and cellular migration, demonstrating a function in the coordination of microfilaments and microtubules.[52] Remarkably, it has been reported that IQ-motif-containing GTPase (guanine triphosphate, [GTP]) activating protein-1 (IQGAP1), which links microtubule plus-ends to cortical actin in non-hematopoietic cells, is required for MTOC polarization after TCR engagement.[34] In addition, the formin mammalian diaphanous-1 (mDia1) interacts with IQGAP1 in macrophages during phagocytic cup formation.[53] Furthermore, the two formins known to colocalize with F-actin at the IS, mDia1 and formin like-1 (FMNL-1), while dispensable for lamellipodium formation and F-actin accumulation, are necessary for MTOC translocation towards the IS.[34,54] Overexpression of constitutively active mDia1 in T cells increases actin polymerization but blocks TCR induced spreading and T cell motility.[55]

The similarity of the T cell defects in mDia1 and WASP mutants suggests that formins and Arp2/3 act on the same pathways. Indeed, WASP stability depends on mDia1 expression since WASP levels are dramatically reduced by proteosomal degradation in mDia1-depleted T cells.[56] Other evidence also suggests a link between Arp2/3 regulation and formins. For example, the mDia-interacting protein (DIP/WISH) can bind to WASP,[57] and some formins have been shown to interact with the WAVE complex via HEM-1.[58] Moreover, Arp2/3-depleted T cells form WAVE2-enriched filopodia in response to TCR ligation, whereas WAVE2-depleted cells are unable to generate any F-actin structure.[34,45] It is therefore possible that WAVE2 enhances Arp2/3-independent F-actin polymerization, perhaps through its association with formins.

Spire proteins are a newly discovered family of actin nucleators that promote non-branched microfilament assembly.[10] These proteins can affect global cytoskeleton dynamics by crosslinking microtubules and microfilaments.[59] Spire also inhibits formin-mediated actin nucleation through direct association with formin1.[25] However, Spire has not been studied in T cells so its functions at the IS are unknown; as is also the case for other actin nucleators such as Cobl, TARP and Lmod.

Ena/Vasp proteins are a family of actin regulators that favor microfilament elongation by impeding the binding of actin-capping proteins at the barbed ends. They also bind to profilin at sites of actin polymerization.[60] Ena/VASP-like protein (Evl) and vasodilator-stimulated phosphoprotein

(VASP) are the predominant members of the Ena/Vasp family expressed in T cells. Although these proteins are important for filopodia extension, they seem to play only a supporting role at the IS. Their suppression does not significantly affect F-actin polymerization or Arp2/3 localization at the IS.[3,60] However, Evl is found at the tips of cellular projections that contact the APC and also at the tips of filopodia.[61] Evl and Vasp interact with Rap1-GTP-interacting adapter molecule (RIAM), an important Ras-related protein-1 (Rap-1) effector involved in integrin activation in T cells, suggesting that they might play a role in integrin activation.[62]

Negative regulation of F-actin assembly at the IS

Formation of lamellipodal structures at the IS is accomplished mainly by the assembly of branched microfilaments; however, in order to generate a dynamic structure, the F-actin assembly must be balanced by mechanisms that counteract microfilament elongation. Several negative regulators of F-actin have been described in T cells (Figure 2):

Cofilin is a small ubiquitously-expressed protein that is essential for cell survival. Cofilin functions by severing actin filaments and sequestering actin monomers in a Ca^{2+} independent manner. Although most reports associated cofilin activation with F-actin depolymerization, this relationship does not always hold. Indeed, cofilin activity can favor F-actin polymerization, since cofilin activity generates actin monomers and new barbed ends where active Arp2/3 complexes can associate and promote F-actin elongation. Therefore, the final outcome of cofilin activity depends on the state of activation of other actin regulators such as WAVE and WASP complexes.

Phosphorylated cofilin is unable to bind to actin and is consequently inactive. In many cell types cofilin inactivation is mediated by phosphorylation at Ser3 by LIM kinases, which in T cells are activated by Rho-associated protein kinase (ROCK) and cAMP-dependent protein kinase (PAK). Cofilin is mostly inactive in resting T cells, and is activated during APC recognition by a costimulatory signal generated through CD2 or CD28, which activates cofilin dephosphorylation by the protein phosphatases PP1 and PP2.[63] Cofilin dephosphorylation is detected early after stimulation and requires mitogen-activated protein kinase kinase (MEKK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) activation. Cofilin activity does not seem to be necessary for the initial formation of the lamellipodium at the IS;[64,65] however, impairing cofilin activity in T cells with specific blocking peptides alters conjugate formation and subsequent T cell activation and proliferation.[66]

Coronin1 can bind to the Arp2/3 complex and inhibits its F-actin polymerization capacity. Coronin1 has been implicated in T cell homeostasis and is required for migration and homing in response to chemokines.[67] Coronin-1 A-deficient mice show defects in cytokine production in T cells after TCR activation and increased F-actin accumulation at the IS.[68] However, coronin1 seems to be dispensable for F-actin accumulation at the IS.[67]

Gelsolin is a microfilament severing factor positively regulated by Ca^{2+} and inhibited by phosphoinositides.[69,70] In addition, gelsolin can block the elongation of microfilaments by binding actin filament barbed ends. Since TCR engagement elevates intracellular Ca^{2+}, this protein should be activated in response to APC recognition. Gelsolin is important for leukocyte phagocytosis and Tyr-phosphorylation upon TCR engagement.[71,72]

Actin-related proteins recently implicated in actin dynamics at the IS

HIP-55, which is also called drebrin-like protein or actin-binding protein 1 (Abp-1), has been found to translocate to the IS, where it contributes to TCR internalization by linking the TCR to the actin cytoskeleton.[73,74] Actin-binding protein 1 (Abp-1 or HIP-55) may also collaborate with ADAP (adhesion- and degranulation-promoting adaptor protein) in the generation of the "actin-cloud" structure. In this regard, HIP-55 has been involved in JNK activation and may be important for T cell activation in conditions of low antigen presentation.[9]

Drebrin is an actin-binding protein structurally related to HIP-55 that regulates actin dynamics during dendritic and axonal growth in neurons.[75] Drebrin competes with tropomyosin for actin binding, thereby modulating myosin interactions with microfilaments and the actin severing activity of gelsolin in neuronal protrusions.[76–78] In addition, drebrin expression has recently been detected in T cells and shown to be relocalized to the IS, where it is required for the correct recruitment of actin and the chemokine receptor CXC chemokine receptor 4 (CXCR4) to the pSMAC and for subsequent complete T cell activation.[79]

Dynamin2 belongs to a family of large GTPases involved in membrane organization and receptor internalization.[80] Dynamin2 is the only dynamin isoform expressed in T cells, and is involved in pre-TCR internalization in thymocytes although not in TCR internalization after

stimulation in Jurkat T cells.[81,82] Interestingly, dynamin2 is required for F-actin accumulation at the IS and couples TCR signaling to the activation of JNK and ERK.[81] Dynamin2 is also involved in Ca^{2+} mobilization after TCR engagement and interacts directly with PLCγ1 and Vav-1.[81,83] Moreover, dynamin 2 has been proposed to engage in several interactions with actin-regulatory proteins through its C-terminal domain. For instance, several reports have shown its interaction with cortactin and Nck, and indirectly with the WASP-WIP complex[84,85] (Figure 2). Dynamin2 also associates with Abi1/2 adaptor proteins and therefore with the WAVE2/Rac-1 complex.[86,87] This multiplicity of interactions suggests that dynamin2 might be able to crosslink numerous Arp2/3 promoter factors and signaling proteins.

Filamin A is an important actin-crosslinker that interacts with CD28 at the IS and is important for T cell activation after antigen recognition.[88,89] Filamin A also connects integrins with the actin cytoskeleton.[90,91]

EZH2 (enhancer of zeste homologue 2) is a methyltransferase that has been shown to interact with Vav-1 and regulates TCR-induced F-actin polymerization.[92] Deletion of ezh2 in T cells blocks thymocyte development and Cdc42 activity, similar to the phenotype of vav-1−/− mice.[92] These observations point to methylation as a novel regulatory post-ranslational mechanism in actin dynamics.

Proteins excluded from the IS

Some actin-linking adaptor proteins are dynamically excluded from the IS because their presence would impair the signaling triggered by TCR ligation, usually through steric inhibition or phosphatase activity (Figure 3).

ERMs (ezrin-radixin-moesin) constitutes a family of proteins that bind actin filaments through their C-terminal domains and connect them to transmembrane receptors through their N-terminal domains. Both ezrin and moesin are expressed in T cells, where they cross-link the actin cytoskeleton with molecules such as cluster of differentiation (CD) CD43, CD44, inter-cellular adhesion molecule (ICAM)-1,2,3, P-selectin glycoprotein ligand-1(PSGL-1), L-selectin, EWI-2 and CD81.[93–96] ERMs are folded in an inactive conformation unless they are phosphorylated on a specific threonine in the C-terminal domain. Phosphorylation induces a conformational change that exposes both the C-terminal and N-terminal binding domains.[97] Binding of phosphatidylinositol 4,5-bisphosphate (PIP2) maintains ERMs in an active state by stabilizing the open conformation.[97]

Upon TCR engagement ERMs are transiently dephosphorylated via the Vav-1-Rac-1 pathway.[98] This inactivation of ERMs decreases cellular rigidity and favors T-APC conjugation.[98] ERMs are absent from the APC contact area in the mature IS, and are accompanied by membrane receptors such as CD43 and PSGL-1,[99–101] which are actively excluded from the IS. ERM-mediated exclusion of CD43 from the IS has been shown to be important for T cell activation.[102,103] However, another study reports that ezrin is located at the IS, where it interacts and recruits ZAP-70, whereas moesin is dephosphorylated upon TCR engagement and removed from the IS together with CD43.[104] Ezrin is also regulated by Tyr-phosphorylation, apparently by lymphocyte cell-specific protein-tyrosine kinase (Lck), upon TCR or CD4 engagement.[105] Ezrin and moesin thus appear to be differentially regulated in T cells. Nevertheless, a recent report proposes that although they can be differentially phosphorylated and distributed, ezrin and moesin both promote T cell activation, since suppression of both proteins provokes a more pronounced decrease in IL-2 production than suppression of each protein individually.[106]

Dlg-1 and Scribble are PDZ domain-containing adaptor proteins which play important roles in cellular polarity of several cell types.[107,108] In T cells, both proteins are recruited together with moesin to the distal pole of the T cell involved in the IS.[109] Suppression of scribble in a mouse T cell line impairs conjugate formation, uropod formation and chemotaxis.[108] Dlg-1 seems to contribute to T cell activation by promoting nuclear factor of activated T-cells (NF-AT) activation and cytokine production,[110] and to play a role in actin dynamics at the IS through interaction with Lck-Zap70 and WASP.[111]

T cell signaling at the IS resulting in actin polymerization

The connections between TCR complexes and the actin cytoskeleton that allow TCR recruitment to the cSMAC remain elusive. Some studies suggest CD3ε as a possible linker because of its interaction through its proline-rich motif with the cytoskeletal adaptor Nck[112] or with the actin binding protein epidermal growth factor receptor kinase substrate 8 (EPS8).[113] Other interactions between the actin cytoskeleton and proteins segregated at the IS are better known; for example, the interaction of integrins with talin and vinculin. Vinculin is a multidomain protein that can bring together talin, F-actin and Arp2/3 at the IS.[49]

The correct activation of effector T cells requires the coordinated triggering of highly regulated and

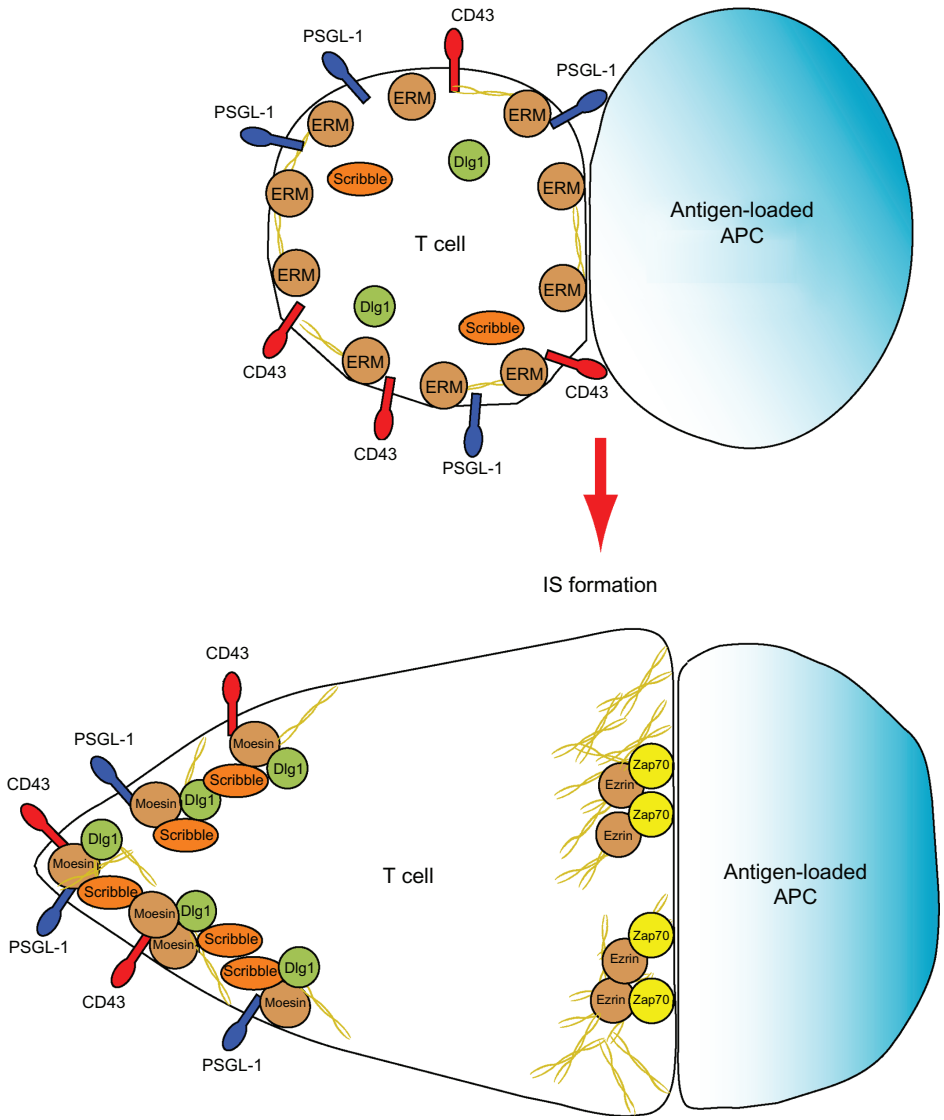

Figure 3 Moesin which links the actin cytoskeleton with membrane receptors is actively excluded from the IS together with their partners CD43 and PSGL-1.
After **TCR** activation, moesin is transiently dephosphorylated being therefore inactivated. Moesin/CD43 exclusion of the IS is a relevant step in order to achieve a full T cell activation. However, ezrin polarizes towards the IS where it interacts and recruits ZAP-70. Dlg-1 and Scribble are recruited together with moesin to the distal pole of the T cell during IS formation.

Abbreviations: PSGL-1, P-selectin glycoprotein ligand 1; CD, cluster of differentiation; WASP, Wiskott–Aldrich syndrome protein; PLC, phospholipase C; Abl, Abelson tyrosine kinase; Abi, Abelson interactor protein; HS-1, hematopoietic lineage cell-specific protein-1; GTPase, guanine triphosphatase; WAVE, WASP/verprolin-homologous; TCR, T cell receptor.

related signaling pathways. To achieve this, the cell uses multidomain proteins that are able to participate in several protein complexes implicated in different pathways. Vav-1, for instance, regulates the Rho GTPases Rac and Cdc42, is involved in actin remodeling by activating WAVE and WASP Arp2/3 promoter complexes, and is also essential for the signaling pathways that lead to activation of nuclear factor of activated t-cells (NFAT), nuclear factor kappa-B (NFkB), and Ca^{2+} mobilization, triggering T cell activation.[6,114,115]

The initial engagement of the TCR induces immediate activation of tyrosine kinases, mobilization of Ca^{2+} and polymerization of actin, all of which precede IS formation.[3,116] First, Lck activates ZAP-70, which in turn phosphorylates several tyrosines of the adaptor protein, linker for activation of T-cells (LAT). Phosphorylated LAT recruits two main factors, phospholipase Cγ-1 (PLCγ-1) and SH2 domain-containing leukocyte protein of 76 kDa (SLP76) (through the adaptor GRB2-related adaptor protein 2 [GADS]). PLCγ-1catalyzes the generation of inositol trisphosphate (IP_3), which induces a Ca^{2+} mobilization that regulates both T cell activation and actin polymerization at the IS. Furthermore, SLP76 interacts with IL2-inducible T-cell kinase (Itk),

non-catalytic region of tyrosine kinase (Nck) and Vav-1. Once Itk is recruited to this complex at the adaptor protein plasma membrane, it is phosphorylated and activated by Lck. However, the kinase activity of Itk is not needed for F-actin regulation since a kinase-dead Itk mutant rescues normal actin polymerization in Itk-silenced cells.[117] Itk is constitutively associated with Vav-1,[117] suggesting that its role in actin polymerization depends on its function as an adaptor protein for the association of SLP76 and Vav-1.[118] Nck interacts with several Arp2/3 promoting factors such as WASP and WIP, mediating the formation of active complexes that trigger actin polymerization. Lastly, Vav-1 is Tyr-phosphorylated by Lck, which activates its guanine nucleotide exchange factor (GEF) activity. Vav-1 is a critical regulator of actin dynamics, T cell activation and cytokine production. Vav-1 can activate both Rac1 and Cdc42; however, a GEF-inactive Vav-1 mutant rescues the defects generated by Vav-1 deficiency regarding Rac1 inactivation,[119] indicating that it only directly activates Cdc42 during actin reorganization upon TCR engagement. In general, Rac1 is responsible of the initial actin rearrangements while the maintenance of F-actin accumulation at the IS is mainly due to Cdc42 activity. Together with F-actin remodellations, the tubulin cytoskeleton gets reorganized by positioning the MTOC towards the IS, which delivers the secretory machinery close to the APC.[120,121] In addition, the role of tubulin deacetylase HDAC-6 in controlling microtubules stability, MTOC translocation and IS formation has been reported.[122]

An important regulatory mechanism of many actin-related proteins is the binding of phospholipids, which in turn modulates their activities and induces the recruitment of PH-bearing proteins to the plasma membrane.[123–125] Some of these proteins are: gelsolin and other actin-severing proteins; ERMs; filamin A and α-actinin. In addition, the Arp2/3 promoters, WASP and WAVE2 are activated by these inositol phospholipids.[126,127] These effects explain why PI3K activation via PKC is determinant for the signaling leading to both T cell activation and actin dynamics.[128]

Integrins are another class of proteins that upon activation at the IS are able to trigger signals that contribute to T cell activation.[3,116]

Actin dynamics in other immunological synapses

This review has focused in actin reorganization during IS formation between naïve T cells and APC. However, other intercellular contacts between cells of the immune system involve the generation of IS.

NK cells and CTLs

NK cells contact with potential target cells to scan their repertoire of MHC molecules. If the majority of the NK activating receptors are engaged during this cell-cell contact, an activating or lytic IS is formed, inducing a local accumulation of F-actin.[129] In contrast, if the majority of the NK inhibitory receptors are engaged, a non-lytic IS forms, with no F-actin accumulation and with exclusion of CD43, CD45 and ezrin.[129] Non-lytic IS disrupts actin polymerization via the dephosphorylation of Vav-1 and ERMs, whereas an activating IS promotes the phosphorylation of both Vav-1 and ERM.[130] Suppression of Arp2/3, HS-1, WASP, WIP or formin hDia1 blocks F-actin accumulation and NK cell cytotoxicity,[131–134] demonstrating that actin regulators are essential during a lytic IS. In addition, myosin II is constitutively associated with lytic granules, establishing its link to the actin cytoskeleton.[135] Myosin II inhibition abrogates the translocation of lytic granules towards the IS and the subsequent release of granzymes.[136]

Cytotoxic T lymphocytes (CTLs) also organize their cytoskeleton and membrane proteins in an IS when they come into contact with target cells. However, in this case lytic granule recruitment and secretion are actin-independent and microtubule-dependent.[121] In fact, the MTOC moves and contacts the plasma membrane at the cSMAC, and actin and IQGAP1 are cleared away from the IS in order to facilitate granule delivery.[121] Furthermore, the cSMAC of the IS of CTLs contains a signaling and a secretory subdomain; the secretory domain is functionally connected with tubulin cytoskeleton and MTOC.[137,138]

DCs and B cells

The actin cytoskeleton of APCs is involved in antigen processing[139] prior to contact with T cells, and is responsible for the recruitment of MHC-II and ICAMs to the APC side of the IS.[140–142] The regulation of the cytoskeleton during antigen presentation differs between B cells and dendritic cells (DCs).[143,144] In B lymphocytes F-actin polymerization requires Rho and Rac activation, though not WASP, whereas in DCs actin polymerization is Rho-GTPase-independent and WASP–dependent.[144,145] However, these data appear to contradict a previous study that claimed that specific inhibition of Rho reduces DC conjugation with T cells.[146] Disruption of the B cell actin cytoskeleton abrogates IS formation and T cell activation, whereas inhibition of PKC only impairs T cell activation.[147] In contrast, induction of actin depolymerization in DCs significantly increases T cell activation.[144]

Actin polymerization in B lymphocytes is critically regulated by spleen tyrosine kinase (Syk) and Rac2,[148,149] while formation of the pSMAC requires Rap activation.[150] Moreover, HIP-55 in B cells interacts with dynamin2 and is an essential adaptor that couples B cell receptor signaling and antigen processing pathways to the actin cytoskeleton.[151]

Virological synapses

Viruses and other pathogens take advantage of the actin cytoskeleton and IS formation in order to gain access to target cells.[152] For instance, human imunodeficiency virus-1 (HIV-1) induces CD4 and CXCR4 clustering by promoting the interaction of filamin A with the cytoplasmic tail of both receptors.[153] HIV-1 also triggers signaling via CD4 and CXCR4 that activates PIP2 production, moesin activation and cofilin dephosphorylation.[154–156] Other actin regulators are direct targets of HIV-1, interacting with viral proteins such as DIP, the GEF of Rho GTPases, Vav-1, Rac and Cdc42.[111,157–160] HIV-1 thus hijacks the cellular signaling pathways that control actin dynamics[161] and, conversely, the alteration of cellular cytoskeleton regulators such as moesin, filamin, dynamin, dlg-1 or Arp2/3 interferes with HIV-1 infection.[158,162,163]

Role of the actin cytoskeleton in health and disease

A number of genetic defects have been identified in genes encoding actin-regulatory and binding molecules. For instance, mutation in one of the actin-binding domains of HS-1 is associated with systemic lupus erythematosus,[164] and apparently linked to enhanced signaling through the B cell receptor.[164] Other HS-1 genetic polymorphisms are also linked to systemic lupus erythematosus and to enhanced activation and apoptosis of B cells.[165] Furthermore, WASP mutations (mostly in the WIP-binding region) lead to the Wiskott-Aldrich syndrome, a severe immunodeficiency.[166] Homozygous mutations of coronin-1 have also been detected in patients suffering from severe combined immunodeficiency characterized by deficient T lymphocytes although normal B and NK cells.[167,168] Moreover, overexpression of EZH2 has been detected in several human malignancies.[169–171] Finally, mutations of DOCK8, a GEF of the RhoGTPases related to DOCK2, have been found to cause a novel variant of combined immunodeficiency.[172] Recently, DOCK8 mutations have been found to disrupt ICAM-1 accumulation at the IS on B cells affecting their survival[173] and impair T cell activation and eosinophil homeostasis causing a severe cellular immunodeficiency.[174] All the immunodeficiencies that have been associated with the mutated DOCK8 present high levels of IgE pointing to a B-cell dysfunction.[172,174]

Concluding remarks and future perspectives

Polarized polymerization of actin at the cell to cell contact (Figure 1) is required for full maturation of the IS. Addition of cytochalasin D or depletion of cytoskeletal regulators impedes T cell activation after APC contact.[1,175,176] Nevertheless, the crucial link between TCR activation and polarized actin polymerization is still unclear, and very little is known about the actin structure at the IS. Resolving the ultrastructure of the actin cytoskeleton at the IS and the intimate interactions with its actin-binding partners is absolutely essential to understand the function of IS and other biological processes that require actin polymerization. Although the Protein Data Bank contains several crystal structures of the actin monomer, including its interactions with various actin-binding proteins, there is as yet no atomic resolution structure for F-actin.[15,177] One of the most useful studies used a rotational and translational search to fit a G-actin crystal structure into the helical filament structure detected in the x-ray fiber diffraction pattern of an oriented F-actin gel.[178] More recently, advances in high-resolution microscopy techniques such as cryo-electron tomography have produced several other models.[33,179–181] The images acquired by electron tomography can be combined and used to fit the G-actin crystal structure into the obtained volumes,[182] and since crystal structures of several actin-binding proteins are available it is possible to generate models of their intimate interaction with F-actin. The interaction of the Arp2/3 complex with F-actin and the formation of the Y-branched actin filaments have been described.[181] To provide a more complete view of the structure of actin interacting with its partners, to integrate the structural analysis with the dynamic properties of the actin cytoskeleton, and to provide a better understanding of actin function, it will be necessary to combine high-resolution microscopy techniques with biochemical and live-cell imaging studies.

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Disclosures

The authors report no conflicts of interest relevant to this research.

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