T-cell-receptor-dependent actin regulatory mechanisms

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Summary

Following stimulation, T cells undergo marked changes in actin architecture that are required for productive immune responses. T-cell-receptor-dependent reorganization of the actin cytoskeleton is necessary for the formation of the immunological synapse at the T-cell–antigen-presenting-cell contact site and the distal pole complex at the opposite face of the T cell. Convergence of specific signaling molecules within these two plasma membrane domains facilitates downstream signaling events leading to full T-cell activation. Recent studies have identified many of the relevant actin-regulatory proteins, and significant progress has been made in our understanding of how these proteins choreograph molecular movements associated with T-cell activation. Proteins such as WASp, WAVE2, HS1 and cofilin direct the formation of a cortical actin scaffold at the immune synapse, while actin-binding proteins such as ezrin and moesin direct binding of signaling molecules to actin filaments within the distal pole complex.

Key words: Actin, T cells, Lymphocyte, WASp, WAVE, HS1, Cofilin, Ezrin, Moesin, Immune synapse, Distal pole complex

Introduction

Upon encountering an antigen-presenting cell (APC), a T cell undergoes dramatic structural changes. Migration ceases, the uropod retracts, and the leading edge flattens against the surface of the APC. The microtubule array and associated secretory apparatus move to the site of cell-cell contact, setting the stage for polarized secretion of cytokines or cytotoxins toward the appropriate target cell. APC binding also induces polymerization of actin filaments beneath the area of cell-cell contact, generating a structural support that stabilizes integrin-dependent adhesive interactions between the T cell and APC and promotes the assembly of an ordered array of signaling molecules called the immunological synapse (IS) (Grakoui et al., 1999; Monks et al., 1997; Shaw and Dustin, 1997). The architecture of the IS is variable and depends on many factors (reviewed by Friedl et al., 2005; Jacobelli et al., 2004). In its most ordered, mature form, the IS is organized into a central domain containing the clustered TCR-MHC-peptide complexes, surrounded by a ring of adhesion molecules and a second ring containing the inhibitory phosphatase CD45. Analysis of protein dynamics during IS formation indicate the TCR and associated signaling molecules initially form stable microclusters that are actively engaged in signaling. Initial formation of these microclusters and their subsequent translocation to the central region of the IS are dependent on an intact actin cytoskeleton (Campi et al., 2005; Varma et al., 2006), controlled in part by the Rho family GTPase Cdc42 (Tskvitaria-Fuller et al., 2006). Once formed, these signaling complexes are highly stable and are largely unaffected by actin-depolymerizing agents. In parallel with formation of the IS, a second actin-dependent protein complex, the distal pole complex (DPC), forms at the opposite face of the T cell (Allenspach et al., 2001; Cullinan et al., 2002; Ludford-Menting et al., 2005). Together, these protein complexes facilitate and fine-tune the sustained signaling required for full T-cell activation.

Although the importance of actin remodeling for T-cell activation has been recognized for many years, the relevant actin regulatory proteins have only recently begun to be identified. Here, we discuss recent advances in our understanding of several key players, focusing on WASp, WAVE2 and HS1, which direct Arp2/3-complex-dependent actin polymerization, cofilin, which induces actin severing and depolymerization, and ezrin and moesin, which link cargo molecules to actin filaments in a regulated fashion (see Fig. 1).

Arp2/3-dependent actin polymerization at the IS

In T cells, as in other cell types, a major regulator of actin dynamics is the Arp2/3 complex, a seven-subunit complex that induces the polymerization of branched actin filaments (May, 2001; Mullins et al., 1998; Higgs and Pollard, 2001). Of the Arp2/3-activating factors known to function in T cells, WASp, the 54 kDa hematopoietic-cell-specific form of the Cdc42 effector protein N-WASp, is by far the best studied. The pathways leading to WASp activation at the IS are relatively well understood, and several excellent reviews are available (Mullins, 2000; Stradal et al., 2004; Zalevsky et al., 2001). We therefore focus here on new data regarding two other Arp2/3-activating proteins, WAVE2 and HS1.

The WAVE complex

Mammals express three WAVE isoforms (Ibarra et al., 2005). WAVE1 and WAVE3 expression is strong in brain, whereas WAVE2 is widely expressed and the primary isoform in T cells

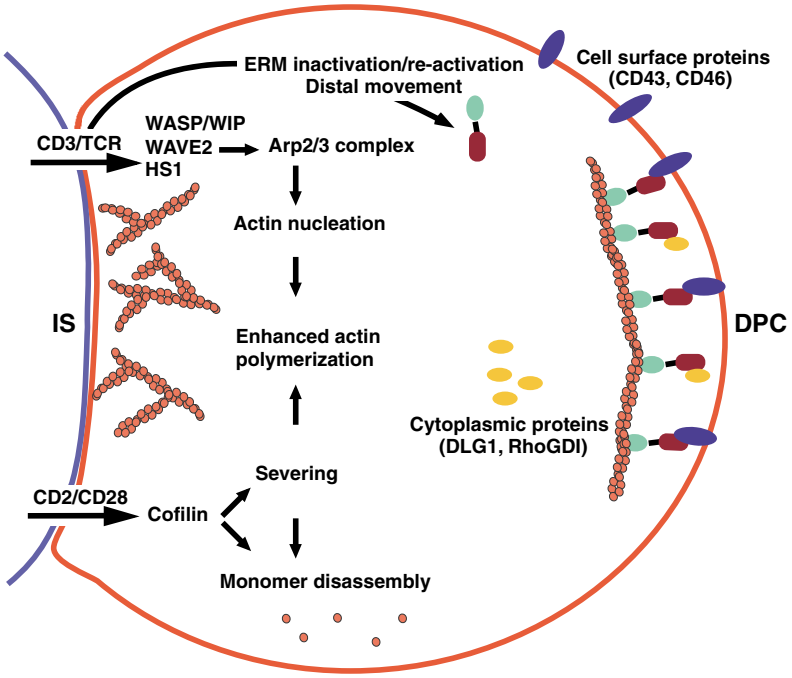

Fig. 1. Actin regulatory pathways during T-cell activation by antigen-presenting cells. The drawing shows the formation of the immunological synapse (IS) at the T-cell–APC interface, and the distal pole complex (DPC) at the opposite face of the T cell. Both complexes are organized in part by actin filaments. TCR stimulation recruits the actin regulators WASp/WIP, WAVE2 and HS1 to the IS, and pathways involving specific kinases, adapter molecules and Rho GTPases activate Arp2/3-complex-dependent polymerization of branched actin filaments. In parallel, engagement of the co-stimulatory molecules CD2 and CD28 induces dephosphorylation and activation of cofilin. The severing activity of cofilin disassembles filaments to monomers and generates new barbed ends for filament elongation, a process that further enhances actin polymerization at the IS. TCR stimulation also induces transient dephosphorylation of the ERM proteins ezrin and moesin, leading to the release of these proteins from actin filaments and plasma membrane binding proteins. Subsequent rephosphorylation of ERM proteins is coupled by unknown mechanisms to their movement away from the site of TCR engagement. This process allows ERM proteins to attach to their cargo molecules (which include both cell surface and cytoplasmic proteins), near the IS and then sweep these molecules rearward to form the DPC.

(Nolz et al., 2006; Suetsugu et al., 1999). WAVE proteins are structurally similar to WASp (Fig. 2) and, like WASp, they interact with G-actin and the Arp2/3 complex through their C-terminal VCA domains (Stradal et al., 2004). This interaction induces a conformational change in the Arp2/3 complex, thereby stimulating localized polymerization of branched actin filaments. The remainder of the molecule is dedicated to adapter functions. The N-terminal WAVE homology domain (WHD) mediates interactions with the Abl-tyrosine-kinase-interacting scaffold molecules Abi1 and Abi2 (Innocenti et al., 2005), and the basic region binds to PIP_3 (Oikawa et al., 2004). The proline-rich region interacts with the Rac1 effector molecule IRSp53 (Miki et al., 2000), thereby coupling WAVE function to Rac1 activation. WAVE is present as a complex containing four other proteins: Abi1/2, the Rac effector protein PIR121 (also known as Sra-1), Nap1 or its hematopoietic form HEM1, and HSPC300 (Eden et al., 2002; Steffen et al., 2004). The complex seems to be organized around a core of Nap1 and Abi1/2, which recruits PIR121 and WAVE-HSPC300. Interestingly, all of the subunits are only present in vivo as a complex, with the exception of HSPC300, which is also found in a free pool (Gautreau et al., 2004). WAVE protein stability is dependent on its interactions with other components of the complex; loss of expression of individual complex components leads to loss of WAVE protein, presumably via degradation (Djakovic et al., 2006; Le et al., 2006; Nolz et al., 2006).

Despite the structural similarities between WASp and WAVE proteins, they are regulated very differently. WASp is regulated primarily by auto-inhibition that is relieved by direct binding of the GTPase Cdc42 to its GTPase-binding domain (Higgs and Pollard, 2001; Mullins, 2000). By contrast, although WAVE proteins activate the Arp2/3 complex downstream of GTP-loaded Rac1 (Miki et al., 1998), they have no GTPase-binding domain and are active in the purified state (Takenawa and Miki, 2001). WAVE function is probably regulated at least in part by components of the WAVE complex, but how this occurs is controversial. Initial in vitro experiments indicated that interaction with other WAVE complex components represses WAVE activity (Eden et al., 2002). This idea was reinforced by genetic studies in *Drosophila* (Bogdan and Klambt, 2003) and *Dictyostelium* (Blagg et al., 2003), in which knocking out WAVE-complex components led to excessive WAVE-mediated actin polymerization. However, studies in cultured cells suggest that the complex activates rather than represses WAVE (Ibarra et al., 2005; Nolz et al., 2006; Zipfel et al., 2006). Recent studies raise the possibility that post-translational modification also regulates WAVE function. TCR-stimulated tyrosine phosphorylation of WAVE2 by Abl results in increased actin polymerization (Leng et al., 2005). WAVE2 is also phosphorylated on serine and/or threonine residues in response to TCR ligation (Nolz et al., 2006), but how this affects WAVE activity remains unknown.

Recent experiments have begun to elucidate the role of the WAVE complex in T-cell activation. Many WAVE complex components localize to the IS (Nolz et al., 2006; Zipfel et al., 2006) and to the edges of lamellipodia in T cells spreading in response to TCR ligation (Zipfel et al., 2006). Localization of WAVE to the IS requires interaction with Abi proteins (Zipfel et al., 2006), but the role of other WAVE complex components is unknown. Depletion of WAVE2 or Abi by RNAi inhibits F-actin accumulation at the IS and formation of lamellipodia in T cells spreading in response to TCR ligation (Nolz et al., 2006; Zipfel et al., 2006). In addition, integrin-mediated adhesion following TCR engagement is inhibited by depletion of WAVE2, HEM1 or PIR121. Both adhesion to APCs (conjugate formation) and adhesion to fibronectin are affected by WAVE2 depletion, which indicates that the WAVE complex functions in the inside-out signaling that activates integrins (Mayne et al., 2004; Nolz et al., 2006).

In addition to cytoskeletal abnormalities, T cells from *Abi1*$^{+/-}$*Abi2*$^{-/-}$ mice show proliferative defects and reduced expression of the autocrine growth factor IL-2 in response to TCR crosslinking (Zipfel et al., 2006). Similarly, knocking down WAVE2 in Jurkat T cells leads to defects in transcriptional activation of NF-AT elements within the *IL-2* promoter (Nolz et al., 2006). NF-AT activation is highly Ca^{2+} dependent, and analysis of Ca^{2+} signaling in these cells revealed defects in coupling of intracellular store depletion to activation of Ca^{2+} influx though Ca^{2+}-release-activated Ca^{2+} (CRAC) channels (Nolz et al., 2006). It is difficult to prove that these signaling defects stem from abnormal actin dynamics. However, WAVE complex suppression has effects similar to those of treatment with cytochalasin D (Holsinger et al., 1998), which suggests a causal linkage. Thus, these findings raise the interesting prospect that CRAC channel activation is an actin-dependent process. Given recent advances identifying CRAC channel proteins (Prakriya et al., 2006), this possibility will soon be testable.

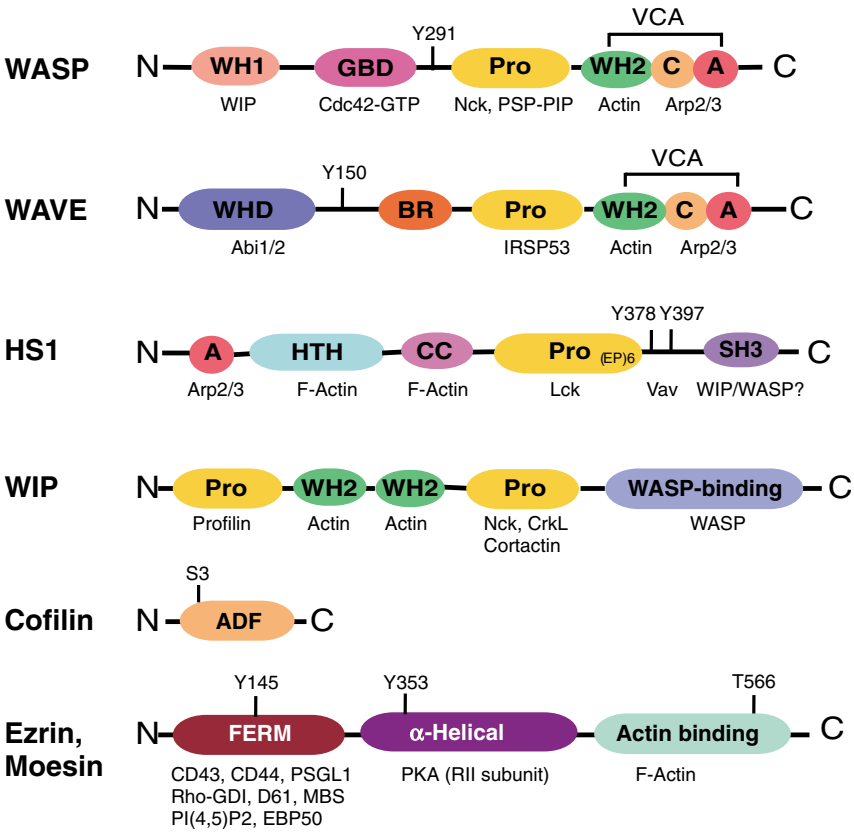

Fig. 2. Domain structures of actin regulatory proteins important for T-cell activation. Binding partners are shown below the domain with which they associate. Sites of phosphorylation that have been implicated in regulating protein function are indicated. Regions with sequence and functional similarity are similarly colored. Note, for example, that WASp and WAVE proteins share a conserved VCA domain responsible for binding actin monomers and the Arp2/3 complex. This domain consists of three subdomains: the WASp-homology 2 (WH2)/verprolin region, a cofilin homology region and an acidic region. HS1 contains only the acidic subdomain within its N-terminal acidic region (NTA), whereas WIP contains two copies of the WH2/verprolin subdomain. Note also the prevalence of proline-rich regions (Pro) among actin regulatory proteins at the IS; these regions are responsible for numerous interactions with SH3-domain-containing proteins involved in T-cell signaling. WH1, WASp homology domain 1; GBD, GTPase binding domain; WHD, WAVE homology domain; BR, basic region; HTH, helix-turn-helix (cortactin) repeat region; CC, coiled-coil region; SH3, Src homology 3 domain; ADF, actin depolymerizing factor/cofilin; FERM, Band 4.1, ezrin, radixin moesin homology domain.

HS1

HS1 is a 53 kDa hematopoietic-cell-specific form of the more broadly expressed actin regulatory protein cortactin. The N-terminal acidic (NTA) regions of HS1 and cortactin show similarity to the acidic region within the VCA domains of WASp and WAVE and contain the conserved DDW motif needed for Arp2/3 binding (Fig. 2). However, HS1 and cortactin lack cofilin and verprolin homology regions, and they make more limited contacts with the Arp2/3 complex. In vitro studies show that the induction of Arp2/3-dependent actin polymerization by HS1 and cortactin is comparatively weak and indicate that these proteins function primarily by stabilizing branched actin filaments (Uruno et al., 2003; Weaver et al., 2002).

Actin regulation by HS1 depends upon an F-actin-binding region containing 3.5 37-residue helix-turn-helix (HTH) repeats followed by a short coiled-coil (CC) region (Hao et al., 2005). Both the HTH and CC regions of HS1 are required for its ability to activate Arp2/3-dependent actin polymerization, and deletion of the HTH region destabilizes binding to the Arp2/3 complex. This suggests that one role of the HTH/CC region is to align the NTA region with its Arp2/3 binding sites on nascent actin branches. The functional importance of the HTH/CC region is underscored by its clinical significance: a mutation leading to loss of a single HTH repeat from HS1 is associated with Systemic Lupus Erythematosus (Sawabe et al., 2003). C-terminal to the HTH/CC region of HS1 is a proline-rich region that interacts with the SH3 domain of Lck (Takemoto et al., 1995). As described below, this region is also involved in regulation of HS1 by tyrosine phosphorylation. Intriguingly, very near the regulatory tyrosine residues is a polymorphic region containing glutamic acid-proline repeats (EP6 or EP8) that is genetically linked to Systemic Lupus Erythematosus (Otsuka et al., 2004). Expression of the disease-associated HS1 variant in B cells leads to enhanced apoptosis, but it is currently unclear how it affects cytoskeletal dynamics. At the C-terminus of HS1 is an SH3 domain. In cortactin, this domain binds WIP, N-WASp and other proline-rich proteins, such as dynamin 2, CD2AP, and Fgd-1 (Daly, 2004). In vitro studies show that binding of the WASp-interacting protein WIP stimulates the ability of cortactin to drive Arp2/3-dependent actin polymerization (Kinley et al., 2003). Whether HS1 also binds to WIP remains an important unresolved question (Gomez et al., 2006).

HS1-deficient mice do not exhibit major defects in lymphocyte development, but immunoreceptor-induced proliferation of lymphocytes is impaired (Gomez et al., 1997; Taniuchi et al., 1995). Studies in HS1-deficient T cells reveal that although TCR-stimulated tyrosine phosphorylation events are intact, the cells exhibit defects in Ca^{2+} influx and activation of NF-AT and NF-κB elements within the *IL-2* promoter. F-actin polymerizes at the IS in these cells, but disassembles within a few minutes of conjugate formation (Gomez et al., 2006). Video analysis of GFP-actin dynamics shows that lamellipodia are unstable and disorganized, which is consistent with biochemical models in which HS1 stabilizes branched actin filaments. Thus, T cells lacking each of the three Arp2/3-dependent actin regulators found at the IS exhibit distinct phenotypes: WASp-deficient cells show no gross defects in actin responses, cells lacking HS1 extend unstable lamellipodia, and cells lacking WAVE2 fail to extend lamellipodia altogether (Gomez et al., 2006; Nolz et al., 2006). These findings indicate that these proteins play unique and complementary roles in shaping the actin response at the IS.

Although the regulation of HS1 activity remains uncharacterized, tyrosine phosphorylation is likely to be important (Fusaki et al., 1996; Hutchcroft et al., 1998; Takemoto et al., 1995; Yamanashi et al., 1993). In B cells, tyrosine phosphorylation drives HS1 into lipid rafts, where it colocalizes with B-cell receptor, WASp and Arp2/3 complex (Hao et al., 2004; Yamanashi et al., 1997). In T cells, HS1 is rapidly phosphorylated at tyrosine 378 and 397 after TCR engagement. Phosphorylation is required for recruitment of HS1 to the IS (Gomez et al., 2006). In addition, phosphorylation creates binding sites for SH2-domain-containing proteins, including Lck, Vav1 and PLCγ1 (Gomez et al., 2006). Although the functional consequences of binding to Lck and PLCγ1 are yet to be determined, interactions with Vav1 stabilize its localization to the IS. Since Vav1 is a guanine nucleotide exchange factor for Rac1 and Cdc42, this adapter function represents another mechanism through which HS1 contributes to the regulation of actin dynamics at the IS.

Cofilin

Cofilin (Fig. 2), a ubiquitously expressed 19 kDa actin-binding protein, is crucial for efficient actin polymerization (Chan et al., 2000; Ichetovkin et al., 2002). In vitro, cofilin binds to G- and F-actin and induces actin severing and depolymerization (Carlier et al., 1997; Lappalainen and Drubin, 1997). Moreover, binding of cofilin to F-actin leads to alterations in the F-actin filament twist, which promotes severing and influences the binding of actin to other molecules (McGough et al., 1997). The short filament fragments generated by cofilin severing are probably stabilized and crosslinked by the Arp2/3 complex (Eddy et al., 1997). The actin-binding capacity of cofilin is negatively regulated by phosphorylation at Ser3 (Moriyama et al., 1996). The serine kinases LIM motif-containing protein kinases (LIMK) 1 and 2, testicular protein kinases (TESK) 1 and 2, and Nck-interacting-kinase-related kinase/Nck-interacting kinase-like embryo-specific kinase (NRK/NESK) phosphorylate cofilin (Arber et al., 1998; Nakano et al., 2003; Toshima et al., 2001; Yang et al., 1998). Protein phosphatases that are able to dephosphorylate cofilin include PP1, PP2A (Ambach et al., 2000), the slingshot phosphatases (Ohta et al., 2003) and chronophin (Gohla et al., 2005).

Cofilin localizes to the periphery of the IS and plays a crucial role in T-cell activation. (Eibert et al., 2004). Blocking cofilin-actin interaction during T-cell stimulation impairs IS formation and inhibits proliferation and cytokine production (Eibert et al., 2004). In resting human peripheral blood T cells, cofilin exists in its inactive Ser3-phosphorylated form. Co-stimulation of the TCR together with the co-receptors CD2 or CD28, but not TCR triggering alone, induces dephosphorylation of cofilin, resulting in its activation (Samstag et al., 1994; Samstag et al., 1992). Since co-stimulation contributes decisively to sustained actin dynamics at the T-cell–APC contact zone (Tskvitaria-Fuller et al., 2003), activation of cofilin may represent a key mechanism by which co-stimulatory signaling promotes T-cell activation (Samstag et al., 2003).

The pathways controlling cofilin activity during T-cell activation remain to be fully elucidated, but recent studies have revealed several key players. PP1 and PP2A have been implicated in dephosphorylation of cofilin in response to CD3/CD28 ligation (Ambach et al., 2000) and LIMK has been shown to couple early tyrosine phosphorylation events to actin remodeling in cytotoxic T-cell conjugates (Lou et al., 2001). The slingshot phosphatase SSH1L has also been found to regulate cofilin activity in response to chemokine stimulation (Nishita et al., 2005); however, a role for slingshot phosphatases in the response to TCR engagement has so far not been demonstrated. Finally, a recent report shows that the combined activities of MAPK/ERK kinase (MEK) and PI3K downstream of Ras are required to induce cofilin dephosphorylation (Wabnitz et al., 2006). An important goal for future investigations will be to understand how cofilin function at the IS is coordinated with that of the Arp2/3-dependent actin regulators.

Other actin regulatory proteins at the IS

The list of proteins known to control actin dynamics at the IS continues to grow. Other players include WIP (Fig. 2) and Ena/VASP family members such as EVL (reviewed by Billadeau and Burkhardt, 2006). Members of the formin family are also likely to play a role. In addition to proteins that control actin directly, new upstream regulatory molecules continue to be identified. One intriguing new player is Ezh2, a polycomb group protein best known for its role in control of gene expression by histone methylation. Recently, Su et al. (Su et al., 2005) showed that a cytosolic Ezh2 complex associates with Vav1 and acts upstream of Cdc42 to control actin polymerization at the IS. Although the targets of Ezh2 activity remain unknown, its identification as an actin regulator suggests the involvement of novel regulatory pathways involving lysine methylation. Another new upstream factor is discs large 1 (Dlg-1, also called SAP-97) a PDZ-domain-containing protein of the MAGUK family. Dlg-1 associates with several key signaling molecules, including WASp, and suppression of Dlg-1 disrupts TCR-induced actin polymerization and clustering of CD3 and GM-1 glycosphingolipid at the IS (Round et al., 2005; Xavier et al., 1998). How Dlg-1 affects actin dynamics is not understood. Interestingly, several reports indicate that Dlg-1 localizes only transiently to the IS, and that it moves to the distal T-cell pole a few minutes after APC engagement (Cullinan et al., 2002; Ludford-Menting et al., 2005; Xavier et al., 1998) (also see below). Thus, Dlg-1 is likely to control early aspects of actin regulation at the IS.

An important issue for the future will be to understand the extent to which individual proteins control actin polymerization at the IS vs. actin polymerization at the leading edge of the migrating T cell. For example, WASp and WIP clearly play a role in both processes (Gallego et al., 2006; Haddad et al., 2001). By contrast, coronin 1, an Arp2/3 complex inhibitory factor, is required for T-cell migration and homing, but apparently plays little or no role in controlling actin dynamics at the IS (Foger et al., 2006). These findings highlight the overlapping but distinct functions of individual actin regulators in controlling distinct T-cell responses.

ERM proteins and the distal pole complex

The concept that actin dynamics facilitate T-cell activation by controlling the segregation of signaling molecules assumes that two processes occur upon TCR engagement: (1) the regulated polymerization and redistribution of actin filaments to form a cortical scaffold; (2) the segregation of key signaling molecules along this scaffold by regulated association with motor proteins and actin-binding proteins. With the exception of the interactions between integrins and the actin-binding protein talin, we still know very little about how signaling molecules associate with actin filaments at the IS. Ironically, much more has been learned about actin-dependent removal of signaling proteins from the IS. One of the clearest cases where actin-binding proteins control the distribution of signaling molecules to promote T-cell activation is the organization of the DPC by ezrin and moesin.

Ezrin and moesin, closely related members of the ezrin, radixin, moesin (ERM) family, link cell surface and cytosolic proteins to the actin cytoskeleton in a regulated fashion [the third family member, radixin, is not expressed in lymphocytes (Shcherbina et al., 1999)]. Each of these ~80 kDa proteins has an N-terminal FERM domain linked to a C-terminal F-actin-binding domain by a flexible coiled-coil region (Fig. 2). The FERM domain can bind directly to a series of basic residues in the cytoplasmic tails of many cell surface molecules, including CD43, CD44, ICAM-1 and ICAM-2, and PSGL-1 (Alonso-Lebrero et al., 2000; Serrador et al., 1998; Tsukita et al., 1994; Yonemura et al., 1998), as well as cytoplasmic proteins such as Rho-GDI and the adapter protein EBP-50 (Takahashi et al., 1997; Reczek et al., 1997). An important feature of ERM proteins is that the N- and C-termini can bind to one another, which generates an auto-inhibited conformation that cannot bind cargo molecules or actin filaments (Pearson et al., 2000). Linker function is activated by binding of the molecule to PIP_2 and/or by phosphorylation of a specific threonine residue within the actin-binding domain (Thr566 in ezrin) (Fievet et al., 2004).

ERM proteins are expressed in virtually all cell types, and disruption of their function results in the loss of specialized structures such as microvilli and neuronal growth cones, and inhibits cell-cell and cell-matrix adhesion (Bonilha et al., 1999; Crepaldi et al., 1997; Hiscox and Jiang, 1999; Paglini et al., 1998; Takeuchi et al., 1994). The first evidence that ERM proteins play an important role in T-cell activation came from studies on CD43, a large, highly charged mucin that functions in part as a negative regulator of T-cell activation. Interaction with antigen-presenting cells leads to removal of CD43 from the IS region and, in some cases, to its concentration in a cap-like structure distal to the site of TCR engagement (Allenspach et al., 2001; Delon et al., 2001; Revy et al., 2001; Roumier et al., 2001; Savage et al., 2002; Sperling et al., 1998; Stoll et al., 2002). The distal movement of CD43 is an active process that relies on interaction of its cytoplasmic tail with ezrin and moesin (Allenspach et al., 2001; Delon et al., 2001; Savage et al., 2002). Ezrin and moesin colocalize with CD43 at the distal T-cell pole, and disruption of the interaction, either by overexpression of the ezrin FERM domain or by mutation of the relevant amino acids in CD43, leads to loss of CD43 movement and disruption of some aspects of T-cell activation. Other known ERM-binding proteins (e.g. Rho-GDI and Dlg-1) colocalize with CD43 and ERM proteins, forming the DPC (Allenspach et al., 2001; Cullinan et al., 2002).

Formation of the DPC is a tightly regulated process. Within seconds after a T cell contacts an APC, ERM proteins undergo dephosphorylation at the activating threonine (Delon et al., 2001). The resulting dissociation of ERM proteins from the plasma membrane is thought to facilitate T-cell–APC adhesion by allowing enhanced lateral mobility of cell surface proteins (Faure et al., 2004). About 3 minutes after TCR engagement, ERM proteins undergo re-phosphorylation at the activating threonine. This process begins at the cell-cell contact site and moves outward as a wave, during which the ERM proteins and their cargo move toward the distal pole. This process is thought to allow ERM proteins to reattach to cargo molecules near the nascent IS, and then sweep these molecules rearward.

In addition to their role in regulating responses to APC binding, ERM proteins play a role in T-cell extravasation. In resting T cells, ERM proteins stabilize microvillar structures. Treatment with chemokines induces rapid threonine dephosphorylation of ERM proteins, resulting in microvillar collapse (Brown et al., 2003). This event leads to the exposure of integrins that were sequestered in microvillar crypts and has thus been proposed to mediate the transition from low-affinity selectin-mediated adhesion associated with tethering and rolling to high-affinity interactions required for extravasation and movement through tissues. The identification of the kinases and phosphatases that control these events is an area of much interest. The kinase that phosphorylates the activating threonine has been elusive. Rho-kinase and PKCθ have been implicated in vitro; however, they do not appear to be the relevant kinases in T cells. More progress has been made in our understanding of the pathways that lead to dephosphorylation. A Vav1/Rac-dependent pathway is required, and pharmacological studies suggest that a PP1/PP2A phosphatase is involved (Faure et al., 2004; Nijhara et al., 2004).

Ezrin is also regulated by tyrosine phosphorylation. In epithelial cells, phosphorylation at Y145 and Y353 occurs in response to growth factor receptor ligation (Krieg and Hunter, 1992; Paglini et al., 1998), and mutation of these residues leads to decreased growth-factor-induced motility (Paglini et al., 1998). Phosphorylation at Y353 controls interactions with specific binding partners, including the p85 regulatory subunit of PI3-kinase (PI3K) (Dransfield et al., 1997; Gautreau et al., 1999). Thus, superimposed on the on-off control of ezrin linker function is an additional level of control, at the level of binding to specific cargo molecules. In T cells, ezrin is transiently tyrosine phosphorylated upon ligation of the TCR and/or CD4 in a process that depends on Lck and CD45 (Autero et al., 2003; Egerton et al., 1992; Thuillier et al., 1994). Lck can

phosphorylate ezrin at Y145 in vitro (Autero et al., 2003); however, it is not known whether (or where) Lck directly phosphorylates ezrin in T cells, nor is the involvement of other kinases understood. The functional consequences of ezrin tyrosine phosphorylation in T cells remain untested. Interestingly, Y353, the regulated cargo-binding site in ezrin, is not conserved in moesin (or radixin). This raises the possibility that at least some aspects of ezrin and moesin function in T cells are distinct.

Formation of the DPC is clearly important for T-cell activation; its disruption by overexpression of a dominant negative ERM mutant leads to defects in signaling pathways leading to production of IL-2 and IFN-γ (Allenspach et al., 2001). However, the mechanism by which the DPC functions remains to be elucidated. The composition of the DPC indicates that it may serve as a sink for negative regulators of activation events occurring at the IS. CD43 negatively regulates T-cell activation, and expression of a CD43 mutant that fails to be sequestered in the DPC because it cannot interact with ERM proteins leads to further suppression of IL-2 production (Tong et al., 2004). Rho-GDI can inhibit Rho-GTPase activation, and its sequestration in the DPC could therefore facilitate activation of Rho-GTPases at the IS. Phosphodiesterase PDE4B2, which also moves distally upon T-cell activation, downregulates cAMP production (Arp et al., 2003). CD148, which behaves similarly, is a FERM-domain containing tyrosine phosphatase that potently inhibits T-cell activation when targeted to the IS (Lin and Weiss, 2003). CD46, a co-stimulatory molecule that directs regulatory T-cell development and serves as a pathogen receptor, is also present within the DPC (Ludford-Menting et al., 2005), although how this relates to the role of the DPC as a sink for negative regulators of T-cell activation is not clear.

In addition to negative regulators of T-cell activation, the DPC contains Dlg-1 and scribble, PDZ-domain proteins associated with the establishment of asymmetry and cell polarity in epithelial cells, neurons and migrating fibroblasts (Cullinan et al., 2002; Ludford-Menting et al., 2005). In these systems, polarized localization of PDZ proteins is accompanied by polarization of ERM proteins, which are in turn required for the appropriate polarized distribution of cell surface and cytoplasmic proteins to which they bind (Bretscher et al., 2002; Polesello and Payre, 2004). The presence of these proteins suggests that the DPC functions in overall establishment of T-cell polarity. Indeed, Ludford-Menting et al. (Ludford-Menting et al., 2005) showed that RNAi-mediated suppression of scribble in a murine T-cell line leads to defects in uropod formation, chemotaxis and conjugate formation. Of course, the proposed roles of the DPC in controlling T-cell polarity and activation need not be mutually exclusive. Two studies on Dlg-1 function in T cells report conflicting results but clearly implicate Dlg-1 in regulation of T-cell signaling, NF-AT activation and cytokine production (Round et al., 2005; Xavier et al., 2004). Important areas for future investigation will be the role of individual DPC constituents and the relationship between T-cell polarity and activation.

Conclusions and Perspectives

Only a decade ago, our understanding of the role of actin in T-cell activation was limited to images showing that F-actin accumulation at the T-cell–APC interaction site correlates with T-cell activation and to studies showing that actin-depolymerizing agents perturb specific aspects of this process. Today, the list of actin regulators and actin-binding proteins known to function during T-cell activation is growing rapidly, and we are beginning to understand the complex networks that coordinate the function of these molecules. In addition, we have learned that T-cell activation involves the formation of not one but two actin-dependent signaling complexes, both of which are required for T-cell activation to proceed normally. As in any young field, however, more questions have been raised than answered. What controls protein segregation within the IS? How are actin filaments at the DPC different from those at the IS? What drives the physical segregation of these domains? And why do perturbations in actin regulators lead to immunodeficiency on one hand and autoimmunity on the other? Ultimately, answering these questions will mean finding ways to ask how the cell interprets four-dimensional cytoskeletal dynamics to modulate gene expression and effector function.

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