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REVIEW

Maintenance and modulation of T cell polarity

Matthew F Krummel[1] & Ian Macara[2]

As T cells move through the lymphatics and tissues, chemokine receptors, adhesion molecules, costimulatory molecules and antigen receptors engage their ligands in the microenvironment and contribute to establishing and maintaining cell polarity. Cytoskeletal assemblies, surface proteins and vesicle traffic are essential components of polarity and probably stabilize the activity of lymphocytes that must negotiate their 'noisy' environment. An additional component of polarity is a family of polarity proteins in T cells that includes Dlg, Scrib and Lgl, as well as a complex of partitioning-defective proteins. Ultimately, the strength of a T cell response may rely on correct T cell polarization. Therefore, loss of polarity regulators or guidance cues may interfere with T cell activation.

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T cells traffic through a signal-rich environment. In the blood, they receive cues from chemokines, which permit attachment and extravasation into tissues. In secondary lymphoid environments, receptors on the T cell surface integrate signals from ligands bound to other cells or to the extracellular matrix and from gradients of soluble mediators. At the sites of effector function, T cells encounter secreted products of activated macrophages, neutrophils and natural killer cells, which may result in polarized or nonpolarized stimulation of cytokine receptors on T cells. Underlying a successful T cell response, then, is the ability to 'prioritize' these cues and subsequently orient effector mechanisms toward the optimal targets. This review discusses polarity 'themes' in T cells and their potential inter-relationships and will postulate how polarity is modulated when T cell receptor (TCR) stimuli are encountered.

Polarity 'themes' in motile lymphocytes

Like a musical partita, the five systems of T cell morphology are variations on a shared polarity 'theme'. The actin cytoskeleton, tubulin cytoskeleton, surface proteins, vesicle traffic and conserved polarity proteins are all polarized relative to the motility axis. Among the best studied of those systems are the actomyosin cytoskeleton and the GTPases associated with promoting actin nucleation (**Fig. 1a**). Actin fibers are inherently polar fibers with their barbed ('plus') end at the site of monomer addition and in the direction of actin-based protrusion. That constitutes the leading edge in lymphocytes and is probably very similar to the lammellipodial regions generated in other motile cell types, in that free actin filaments drive protrusion independently of myosin II crosslinking activities[1]. However, behind the leading edge and extending through the midbody is a zone of high myosin II accumulation[2] in which it is likely that actin is organized by myosin in rods that are mostly parallel. In epithelial cells, this region corresponds to the lamella, and the myosin II in this zone causes retrograde flow of actin filaments and attached membranes toward the rear of the cell[1]. Rearward movement of surface-bound beads toward the uropod has demonstrated that retrograde cortical flow also occurs in T cells[3]. Myosin II 'motors' are probably responsible for retrograde flow, as shown by the accumulation of myosin II at sites of leading edge retraction and the subsequent retrograde flow of those motors into the uropod[2].

The microtubule cytoskeleton and related kinesin and dynein motors comprise a second polarized cytoskeleton (**Fig. 1b**). Like actin filaments, microtubules are inherently polarized. Microtubules are organized around a microtubule-organizing center (MTOC). That arrangement creates another level of polarity, as the MTOC is invariably in the uropod and behind the nucleus.

Surface receptors and perhaps membrane lipid rafts are also polarized such that some are localized toward the uropod, whereas others, activated integrins in particular, are likely to cycle toward and accumulate at the leading edge[4] (**Fig. 1c**). Actomyosin and membrane movement from the front of the cell to the rear of the cell probably accounts for the general accumulation of many surface receptors at the uropod: TCRs, CD2, CD43 and CD44 accumulate at the uropod before activation[5–8]; ICAM, CD43 and CD44 in particular are probably localized as a result of their association with the C terminus–phosphorylated form of ezrin-radixin-moesin (cpERM), which associates with actin[5,6]. In comparison, adhesive receptors such as LFA-1 are selectively retained along the 'midbody' of the cell by adhesion[4]. Cholesterol-rich rafts may also be polarized along with those receptors[9].

Integrins may also be specifically recycled to the leading edge by as-yet-unidentified selective vesicular traffic from the uropod to the leading edge (**Fig. 1d**). Cycling is important, as forward motility requires the internalization of highly adhesive integrins at the front and midbody at the uropod and that they be recycled 'forward' to contribute to new projections. It is highly probable that endocytosis and exocytosis are generally polarized along the axis of movement and also reinforce other systems of polarity by asymmetrically depositing material at one end of the cell or the other.

[1]Department of Pathology, University of California at San Francisco, San Francisco, California 94143-0511, USA. [2]Center for Cell Signaling, Department of Microbiology, University of Virginia School of Medicine, Charlottesville, Virginia 22908-0577, USA. Correspondence should be addressed to M.F.K. (matthew.krummel@ucsf.edu) or I.M. (igm9c@virginia.edu).

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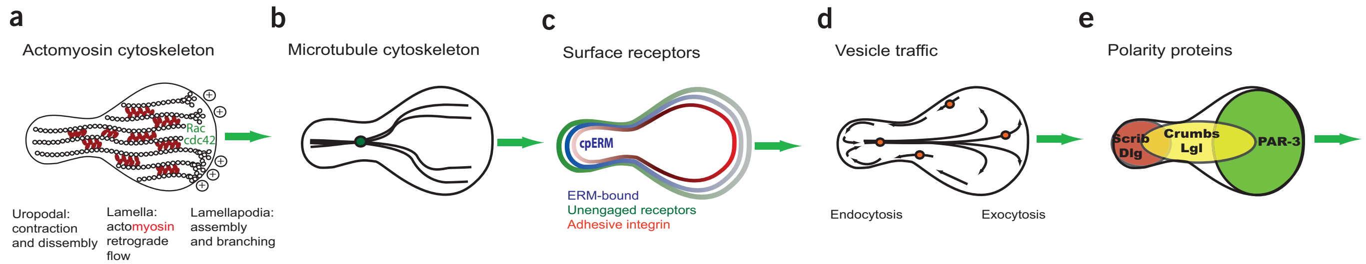

Figure 1 Five polarity systems in motile T lymphocytes. (**a**,**b**) Polarity can be described from the perspective of the actomyosin cytoskeleton (**a**) or tubulin cytoskeleton (**b**), which are polarized relative to the direction of migration (green arrow). (**c**) A front-to-back 'footprint' of integrins on substrates (red shading) and uropodal accumulation of receptors further delineates the polarity. (**d**) Both directional migration and the associated cycling of integrins require the movement of material through the cytoplasm by means of an endocytic-exocytic loop. (**e**) Evidence places evolutionarily conserved polarity proteins in distinct locales during migration.

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Much vesicle movement probably involves the Rab family of proteins that direct the fusion of vesicles with the plasma membrane and also associate with specific myosins or kinesins for directional transport. The targeting and docking of vesicles with membranes involves the recognition of vesicle soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (v-SNAREs) by target SNAREs (t-SNAREs) that are enriched at specific sites along the membrane. SNAREs typically consist of syntaxin and SNAP proteins, some of which have been identified in T lymphocytes[10,11]. Polarized distribution of t-SNAREs at the leading edge of motile T cells remains to be assessed, but would be predicted to 'encourage' delivery of vesicles in the direction of motility.

The proteins scribbled (Scrib), lethal giant larvae (Lgl) and discs large (Dlg), together with partitioning-defective (PAR) polarity proteins (**Fig. 1e**), have emerged as a fifth set of participants regulating many aspects of lymphocyte biology. Scrib, Lgl and Dlg proteins were first identified by genetic screens in flies or worms and are key conserved elements required for polarization of cells in many contexts. For example, Scrib was identified in a screen for genes involved in apical versus basal polarity in drosophila embryonic epithelium, but it also functions in synaptic vesicle dynamics[12] and acts as a tumor suppressor[13]. Scrib interacts genetically with the two other tumor suppressors Lgl and Dlg, which are also essential for maintaining apical versus basal polarity. Somewhat unexpectedly, no existing evidence indicates the involvement of mammalian Scrib protein in the maintenance of apical versus basal polarity, but Scrib has been linked to the regulation of planar polarity of the inner ear epithelium[14], in epithelial cell-cell adhesion[15], in endocytosis and recycling of G protein–coupled receptors (GPCRs)[16] and, most unexpectedly, in T cell polarization[17].

PAR proteins were first identified in a screen for genes affecting the first asymmetric division of the *Caenorhabditis elegans* zygote. Of the six PAR genes that have been cloned, those encoding PAR-3, PAR-6 and atypical protein kinase C (aPKC) interact both genetically and physically. PAR-6 seems to function at least in part as a targeting subunit for aPKC, to which it binds constitutively. The PAR proteins also interact with Scrib, Lgl and Dlg proteins. For example, PAR-6 can recruit Lgl, which is then phosphorylated by aPKC[18–20].

Polarized assemblies in motile lymphocytes

T cells have a directional axis of motility, and a fundamental driving force for the polarization and migration of T cells is the dynamic remodeling of actin cytoskeletal elements. In most cell types that have been studied, remodeling is controlled by the Rho family of GTPases through a plethora of effector proteins. Rac and Cdc42 GTPases, in particular, have been associated with the protrusion of the leading edge and in the orientation of migration (**Fig. 2a**). Overexpression of mutant forms of either GTPase results in loss of polarity and defective chemotaxis toward the chemokine SDF-1 (refs. 21,22). 'Downstream' effectors of Rac and/or Cdc42 include the Wiskott-Aldrich syndrome protein (WASP), neural WASP and the related WAVE (also called Scar) family of proteins, which induce actin nucleation through the complex of Arp2 and Arp3. WASP is required for optimal chemotaxis[23,24], but evidence has indicated WAVE2 is a predominant participant in hematopoietic cell migration[25–27]. WAVE2 is required for actin cap formation at the immunological synapse (IS) and forms a complex with the Hem-1 linker protein[26]. Hem-1 is required for actin protrusions in neutrophils and augments leading-edge identity by inhibiting activation of the myosin light chain at that site[25].

In contrast to Rac and Cdc42, which promote actin nucleation, the GTPase RhoA activates a protein kinase, ROCK, that phosphorylates myosin light chains and thus increases the contraction of actin filaments relative to each other (**Fig. 2b**). Rho is 'downstream' of chemokine GPCRs and is required for increases in integrin affinity[28,29]. Thus, it is likely that in contrast to Rac and Cdc42, Rho proteins located at the site of GPCR signaling activate myosin, which triggers sliding of this membrane patch toward the cell uropod, thereby generating forward thrust in the rest of the cell. Although that model is likely, the system is more complex locally, as a Rac complex with its effector PAK have also been linked to the chemotaxis of both macrophages and T cells[30,31]. As Rac often antagonizes Rho function[32], the details of how these GTPases function in concert remains a mystery.

In T cells, Rho is also required for uropod formation, possibly because of its involvement in augmenting phosphorylation of ERM[33]. That function is important, because cpERM in the uropod may tether a complex that aids in recycling components back to the leading edge through vesicle trafficking mediated by ADP-ribosylation factor (Arf; **Fig. 2c**). Arf family GTPases control vesicle movement during motility in autologous systems[34], although the function of these proteins in T cells has not been established. The PDZ domains of mammalian Scrib associate with the C terminus of the Rac guanine-exchange factor βPIX[35], which in turn binds to GIT1, a scaffold protein with an Arf GTPase-activating protein domain. This Scrib-βPIX-GIT1 complex has been linked to the recycling of GPCRs[16] in autologous systems, suggesting a similar function in T cells. Scrib is localized in the uropod[17], but how does that localization occur? In drosophila neurons, Dlg determines the localization of Scrib, and these two proteins form a complex that is mediated via the scaffold protein 'GUK-holder'[36]. A mammalian homolog of GUK-holder has been identified, but it has not yet been shown to be functionally equivalent to the fly protein, and its expression in T cells is untested. However, human Dlg is targeted to

membranes by its association with ERM[37], and Dlg proteins are found in the T cell uropod alongside cpERM proteins[17]. Notably, Scrib-deficient T cells fail to maintain polarity and cease movement, consistent with requisite involvement of these proteins in a motility cycle[17].

A final functional cassette is probably in the motile cell and consists of PAR-3, Lgl, PAR-6 and aPKC-ζ which are all localized in the midbody[17]. In epithelial cells, Lgl can associate not only with myosin II and PAR-6 but also with syntaxins, which control vesicle delivery to specific target membranes. As mentioned above, PAR-6 seems to function at least in part as a targeting subunit for aPKC, to which it binds constitutively. Thus, in drosophila embryonic epithelial cells, PAR-6 can recruit Lgl, which is then phosphorylated by aPKC[18–20], which in turn triggers disassociation of the complex from the cell cortex. The PAR-6–aPKC complex can also bind to and phosphorylate the ubiquitin E3 ligase Smurf1, one target of which is RhoA[38]. A key function of the PAR-6 polarity cassette might therefore be to trigger local degradation of RhoA, which would reduce actin contractility and block Rho-induced phosphorylation of cpERM proteins. In addition, PAR-6–aPKC binds to and phosphorylates PAR-3 (refs. 39–41). As PAR-3 is capable of binding to and spatially restricting the activity of the Rac guanine-exchange factor Tiam1, the PAR-6–aPKC–PAR-3 interaction probably also regulates actin dynamics[42,43]. *Tiam1* was first identified as a T cell lymphoma invasion and metastasis gene[44]. The association of PAR-3 with LIM kinase (LIMK) may further regulate actin in the area through LIMK regulation of cofilin[43]. Variations in the composition of PAR-6–aPKC complexes may create unique actin dynamics in the midbody that facilitate both forward motility through actomyosin regulation and vesicle trafficking through syntaxin regulation (**Fig. 2d**). The nature of those complexes remain to be elucidated.

Cytoskeletal polarity in the IS

In a signal-rich environment, interrelated feedback systems like those described above are important for stabilizing cellular activity. In contrast, TCR signaling initiates a transformation from high motility to the stable IS[45,46]. How might that signal create the conditions in which proteins are repolarized, and how is a stable IS maintained?

The reorientation of the T cell during engagement with an antigen-presenting cell (APC) and formation of the IS are reminiscent of the switch in polarization that occurs during the epithelialization of mesenchymal cells. In both cases, cells undergo substantial morphological changes, which are driven by remodeling of the actin and microtubule cytoskeletons and by relocalization of polarity proteins. During the mesenchymal-epithelial transition, anterior-posterior polarity is converted into apical-basal polarity. Microtubules and actin reorganize to form bands around the cell periphery, the Rac GTPase redistributes from the leading edge to the lateral membrane, and polarity protein such as PAR-3, Scrib, and Dlg translocate to the cell-cell junctions. A functionally similar switch occurs in T cells: the uropod at the posterior of the T cell disappears[46] and polarity proteins such as Scrib and Dlg transiently redistribute from the uropod to the front of the cell where the IS forms[17,47,48].

A first step in this transformation may be the disassembly of existing cytoskeletal structures (**Fig. 3a**). TCR signaling transiently leads to the dephosphorylation of cpERM, and that modification results in cytoskeletal relaxation, probably because of release of the membrane-actin linkage anchored by ERM[49]. That occurs through Rac activation[49], which may antagonize Rho-induced ERM phosphorylation[33]. Such relaxation may release cell surface receptors and proteins such as Dlg and Scrib, which can then traffic to the leading edge. TCR signaling also induces the calcium-dependent phosphorylation of myosin II (ref. 2). Myosin heavy-chain phosphorylation results in loss of myosin filamentation[50], probably releasing the aligned actin filaments in the midbody and uropod and permitting reorganization of the components formerly located in those regions.

At the leading edge, TCR signaling induces actin complexes and the early assembly of microclusters of TCRs[7,51]. Microclusters probably arise as a result of TCR-induced activation of the Lat and SLP-76 adaptor proteins, which trigger WASP and PAK activation, which promotes actin assembly[52–54] (**Fig. 3b**). However, Dlg, which is found at the center of the contact,

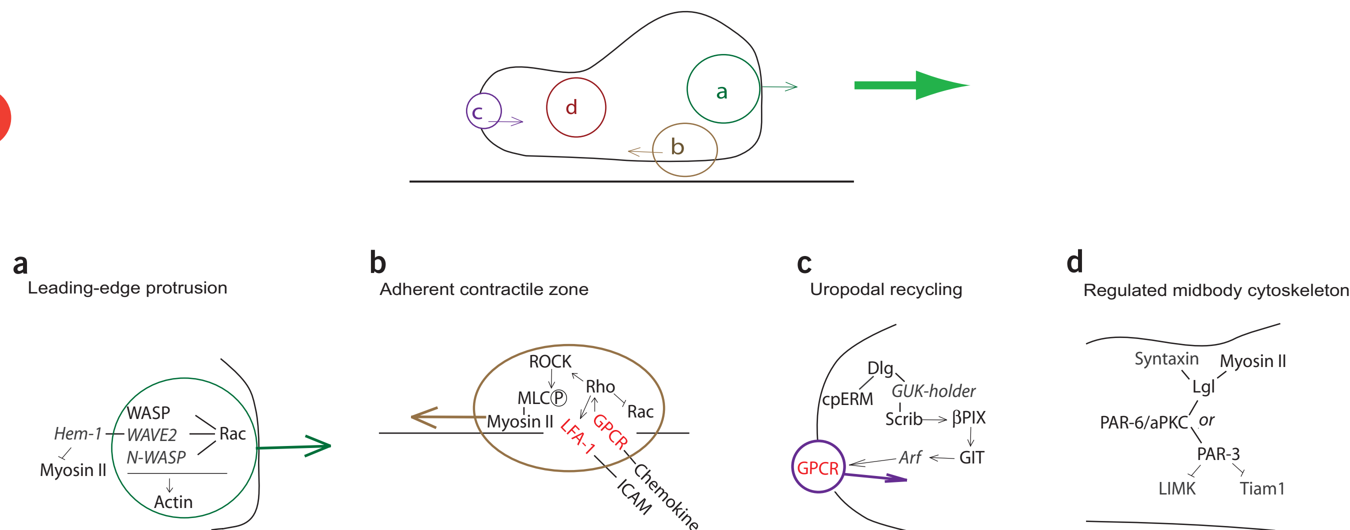

Figure 2 Distinct zones in motile lymphocytes. Various functional compartments serve to stabilize crawling activity. All these molecules have been found in the locations here except those in gray italics, which have not yet been assessed in T cells. (**a**) A protrusive complex that uses a WASP family member to activate actin elongation is probably associated with the Hem-1 molecule in a cassette, which inhibits myosin light-chain activation. N-WASP, neural WASP. (**b**) A contractile cassette 'downstream' of chemokine receptors and integrins may use Rho to activate ROCK and thereby induce myosin II contractility. Additional signaling (such as via phosphatidylinositol-3-OH kinase) is not presented here. MLC, myosin light chain. (**c**) The uropod is proposed to be the site of a cpERM-based internalization 'engine'. The cpERM-mediated recruitment of Dlg may also recruit Scrib via a GUK-holder (not yet identified in T cells). Scrib localization in the uropod in turn may recruit and activate Arf proteins via the assembly of βPIX-GIT complexes. (**d**) PAR-6–aPKC–based cassettes throughout the cytoplasm may prevent actin elongation through PAR-3-mediated binding of LIMK or Tiam1. Alternatively, PAR-6–aPKC complexes can interact with Lgl, which interacts with syntaxins and myosin.

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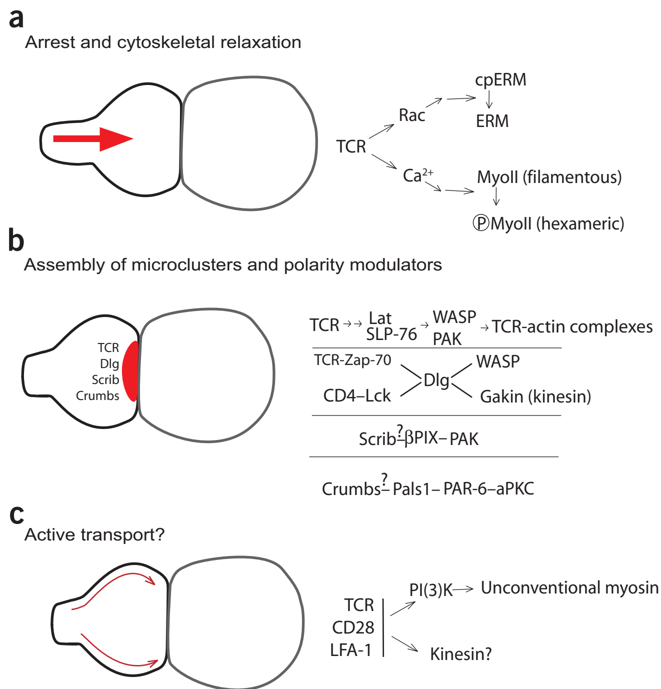

Figure 3 Early events in polarity modulation. Specific multiprotein complexes are suggested to mediate changes in polarity and morphology. (**a**) Cytoskeletal relaxation is thought to occur as a result of cpERM dephosphorylation as well as debundling of myosin II (MyoII) filaments. (**b**) Specific multiprotein complexes then assemble at the T cell–APC membrane contact site. These include TCR-actin complexes, Scrib complexes (perhaps containing βPIX and PAK), TCR-Dlg complexes (perhaps nucleating actin) and crumbs complexes (perhaps recruiting Pals and PAR-6 complexes). Molecules in gray italics have not yet been assessed in T cells, but all others have been localized as presented here. All interactions are not always presented here (for example, Zap70 has been linked to WASP through multiple pathways). (**c**) Active transport is suggested to complete repolarization. Myosin motors may be instrumental in recruiting uropodal components along the membrane or through the cell. Similarly, microtubule-based motors may recruit molecules toward or within the IS. PI(3)K, phosphatidylinositol-3-OH kinase.

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in the central-supramolecular activation cluster (c-SMAC), during the first minutes of contact[17,47,48,55], is probably another participant in this dynamic process. Dlg associates directly with the Src homology 3 domains of both the Zap70 and Lck tyrosine kinases[48,55]. In that way, Dlg probably senses the proximity between Lck and Zap70 that occurs during clustering of TCR and CD4 proteins and might even promote further aggregation of those proteins (**Fig. 3b**). Dlg also interacts with microtubule kinesins[56] and thus may act to initiate repolarization of the microtubule-based cytoskeleton. Finally, the Lck-Zap70-Dlg complex also seems to include WASP[48], and formation of that complex may help to move WASP into proximity of active Cdc42. Transient phosphorylation of Zap70 and Lck when the TCR is activated may trigger conformational changes in that complex and promote its alteration from a motile to an IS form. Loss of Dlg from the IS at later times[17] is consistent with loss of active Lck in the late IS[57]. It is perhaps notable that elimination of Dlg by means of short hairpin RNA in cell lines can alternatively augment[47] or attenuate[48] T cell activation. Those observations are perhaps indicative of the importance of polarity in making T cells receptive to signals in the first place or, alternatively, in stabilizing the newly emerging signal, and those disparate results may reflect the polarized nature of the stimulation provided in the various experiments.

Like Dlg, Scrib moves from the uropod, is transiently localized in the nascent IS and subsequently moves to a position mainly outside the IS[17]. In the IS, Scrib may function to recruit the Rac and Cdc42 guanine-exchange factor βPIX (**Fig. 3b**). That protein has been shown to recruit PAK to the IS in T cells[58]. Therefore, defects in Scrib-deficient T cells[17] and in T cells expressing dominant negative PAK[59] may reflect a requirement for a Scrib-βPIX-PAK complex during T cell activation.

Finally, the regulation of PAR-6-based cassettes may represent a more complex mechanism of influencing local polarity in response to signaling. The PAR-6–aPKC heterodimer can bind several alternative partners through its PDZ domain, including Lgl, PAR-3 and Pals1 (protein associated with Lin7)[60]. In eukaryotes, Pals1 is recruited to the cell surface by crumbs, the mammalian homolog of Lin7, and functions to recruit the PAR-6–aPKC complex to the cell cortex. In the case of T cell IS, crumbs1 is constitutively localized in the IS[17] and thus may lend 'apical identity' to this surface (**Fig. 3b**). The mechanism by which crumbs is localized to the IS, however, is yet to be determined.

Release of cytoskeletal tension (**Fig. 3a**) and assembly of polarity complexes (**Fig. 3b**) are probably supplemented by an active transport mechanism that moves proteins from the former uropod into the IS (**Fig. 3c**). Surface movements exceeding the rate of diffusion have been noted for TCRs and for beads moving along the cell surface[61] and have been shown to depend on phosphatidylinositol-3-OH kinase and myosin[3]. Conversely, movement of SLP-76 into the central IS seems to rely on microtubules[62], suggesting that a microtubule-based movement, perhaps mediated by a kinesin, also functions to reorient critical signaling proteins.

Stabilization of polarity in the IS

In a stable IS, actin and actomyosin contraction is diverted away from rapid motility and functions in maintaining the contact. In that configuration, the bulk of TCRs and many other receptors are polarized toward the IS[3,10,61,63,64]. However, a second pool of those receptors often accumulates at the opposite end of the cell, in what is referred to as the 'distal pole complex'[65]. Once established, that asymmetric distribution is apparently stable for many hours.

Despite being relatively nonmotile, engaged lymphocytes continuously expand and contract away from the c-SMAC in the peripheral SMAC (p-SMAC) and distal SMAC (d-SMAC) regions, where new peptide–major histocompatibility complex ligands might be encountered and where microclusters of TCRs continue to form[51,66]. One likely model proposes that actin fibers initiating at or beyond microclusters in the p-SMAC elongate into the c-SMAC, perhaps as a result of WASP activation 'downstream' of TCR signals[67] (**Fig. 4a**). Engaged integrins in the p-SMAC might also promote activation of Cdc42 and WASP, as well as of aPKC, as they do in migrating astrocytes[68]. The elongating actin fibers might encounter resistance both from elements in the p-SMAC (generating a retrograde direction of movement that ultimately returns to the c-SMAC) and from pSMAC-localized myosin motors (which 'tug' on fibers, pulling them backward into the c-SMAC). A selective 'clutch' mechanism, perhaps provided by CD2-associated protein, which has been associated with TCR internalization[69], may allow microclusters to attach to this moving 'radial fiber' and become localized in the central IS.

Integrins and the microtubule cytoskeleton are also repolarized after TCR engagement, and the MTOC faces the IS (**Fig. 4b,c**). The general mechanisms underlying MTOC movement are complicated and probably vary among cell types. For instance, in 3T3 fibroblasts initiating movement into a wound, the MTOC actually remains stationary while the nucleus is pushed backward by retrograde actin flow, thereby placing the MTOC in front of the nucleus. PAR-6 and aPKC are needed to hold the MTOC in position, whereas Cdc42, myotonic dystrophy–related kinase and myosin function are required for nuclear relocation[70]. Conversely, in astrocytes, the MTOC is actively pulled forward by microtubules by a mechanism that depends on Cdc42, PAR-6–aPKC and dynein and that involves capture of

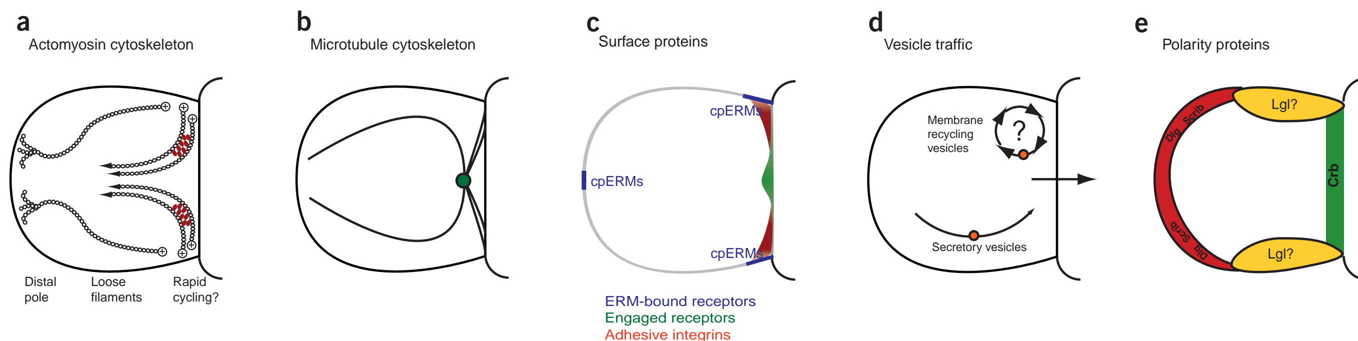

Figure 4 Five polarity systems in established T cell couples. As in **Figure 1**, polarity can be viewed from the perspective of five different types of events. (**a**,**b**) The hypothesized 'radial fibers' of the actin cytoskeleton (**a**) and tubulin cytoskeleton (**b**) represent the profound changes in cytoskeletal reorganization that occur. (**c**) Integrins and signaling receptors are ultimately arrayed mainly in the IS as overlapping densities. (**d**) Many types of vesicles are transported toward the IS, and we suggest the existence of an additional recycling pool at or near the c-SMAC. (**e**) Polarity proteins are arrayed in patterns that presumably reinforce IS stability.

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the microtubule 'plus' ends at the leading edge of the cell by adenomatosis polyposis coli protein and the polarity protein Dlg1 (refs. 68,71,72). The mechanism of MTOC reorientation in T cells remains to be elucidated.

Polarized endocytosis and exocytosis in the IS

Controlled internalization and delivery of vesicles between the cytoplasm and the plasma membrane is a key component regulating the polarity and function of motile as well as engaged T cells. For example, in cytotoxic T lymphocytes, secretory vesicles containing cytotoxic granules line up alongside the TCR in the IS[73]. Secretory vesicles bearing the cytokines interleukin 2 and interferon-γ also line up facing the APC, although others (such as those containing tumor necrosis factor) do not demonstrate obvious directional 'preferences'[11]. Secretory vesicles bearing interleukin 2 and interferon-γ localize together with vesicles defined by Rab proteins as well as by the v-SNARE syntaxin 6 (ref. 11).

Vesicular release is probably also important for the reinforcement or modulation of signaling at the IS. Studies of Lck[74], Lat[75], TCR[10] and CTLA-4 (ref. 76) has shown that vesicles bearing those components of the proximal signaling cascade traffic toward the center of the IS, where they presumably fuse with the plasma membrane and deliver vesicular content. Cytokine receptors as well as 'housekeeping' proteins such as transferrin are similarly brought to the IS[64,77]. The recycling of TCRs in vesicles may be essential for TCR accumulation at the IS[10], although direct movement of the TCR into the IS along the plasma membrane[3,61] also occurs. SNAP-23 and syntaxin 4, both of which are components of t-SNAREs, are enriched in plasma membrane regions at or near the TCR accumulation in the IS, whereas vesicles bearing cellubrevin and v-SNAREs localize on vesicles inside the cytoplasm, juxtaposed to the IS[10]. In summary, these observations suggest the existence of an exocytic machine that delivers proteins into the IS.

Perhaps equally important to exocytosis is the uptake of proteins by endocytosis in regions adjacent to or in the IS. It is believed that internalization occurs at the center of the IS to facilitate TCR degradation[69,78]. Alterations in CD2-associated protein, for example, result in decreased TCR internalization[69,79], and this protein has been associated with ubiquitin E3 ligases such as Cbl. However, more than simple degradation may be occurring at the IS. Continuous ruffling of membrane in the peripheral region as well as coalescing flow toward the central zone may require consistent reintroduction of membrane at the far outer domains. We thus suggest that a vesicular cycle (**Fig. 4d**) may serve to recycle proteins through the IS during serial receptor-ligand engagements.

How does exocytosis coordinate with the other polarity participants present in the established IS? Lgl associates with syntaxin-4 in mammalian cells[80] and may therefore influence the placement of t-SNARE on the plasma membrane in the peripheral IS, into which proteins may be recycled (**Fig. 4e**). Lgl protein may associate with cellular myosins, and such an interaction may influence the placement of adjacent t-SNAREs and thereby also influence the adhesiveness of integrins. Different t-SNAREs are required for the sorting of vesicles to distinct regions of the plasma membrane. For example, syntaxin-4 is involved in apical sorting, whereas syntaxin-3 is necessary for basolateral sorting. Distinct Rab proteins have also been associated with that. For example, Rab17 seems to be required specifically for apical sorting[81]. Rab13 has a specialized function in the recycling and delivery of transmembrane proteins that form the tight junctions of epithelial cells, such as occludin[82]. In cytotoxic T lymphocytes, the delivery of lytic granules to the IS depends on Rab27a, which is important for regulated secretion in several cell types[73], and IS-localized secretory vesicles containing interleukin 2 are associated with Rab3d and Rab19 (ref. 11). Although syntaxin-4 accumulates along the IS near the TCR[10], the specialized Rab proteins involved in recycling membranes at the IS are not yet known.

Future perspectives

Although re-establishing polarity during signaling remains a focus of ongoing work in this area, there is yet another arena in which polarity and its modification is likely to be potentially more clinically relevant. Underlying normal T cell surveillance is presumably a coordinated series of cues for the T cell to follow. Although the organization of those cues remains to be completely elucidated, it is certain that chemokine gradients into and throughout organs serve to orient T cell polarity (**Fig. 1**). The presence and density of integrin substrates provides a potential 'highway' on which T cells might 'crawl'. The presentation of chemokines together on an integrin receptor–bearing surface[83] or to glycosaminoglycans on APCs[84] may also influence T cell function. Two-photon imaging studies have suggested that T cells might travel along high endothelial venules[85], perhaps 'entranced' by the unique combination of immobilized chemokines and a local high density of adhesion molecules.

A remaining issue is whether 'immune-privileged' sites, such as tumors or chronic inflammatory sites, might provide a defective series of motility cues that accounts in part for poor T cell surveillance and tolerance to antigens produced in these locations. Tumors, for example, co-opt both chemokine and matrix-remodeling mechanisms that influence the chemotactic and adhesive pathways discussed above. Might tumors create T cell disorder by overexpressing some cues while minimizing others? T cells are known to be much less reactive in their uropod[46], and loss of polarity, such as by loss of Scrib, is associated with inhibition of signaling competence[17]. Notably, depletion of Dlg by means of short hairpin

RNA results in overactive T cells in culture[47], whereas for primary T cell blasts stimulated with APC plus antigen, loss of Dlg attenuates activation[48]. Perhaps the difference lies in the polarized cues that accompany the signals received in those disparate stimulation conditions. As future experiments examine tolerant environments in greater depth, it may be important to consider the possibilities that a T cell might well fail to become active or might remain tolerant precisely because of a disorganized array of conflicting polarity cues that effectively 'freezes' the T cell in a state of inactivity.

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