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Leukocyte polarization in cell migration and immune interactions

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Cell migration plays a key role in a wide variety of biological phenomena. This process is particularly important for leukocyte function and the inflammatory response. Prior to migration leukocytes undergo polarization, with the formation of a lamellipodium at the leading edge and a uropod at the trailing edge. This cell shape allows them to convert cytoskeletal forces into net cell-body displacement. Leukocyte chemoattractants, including chemokines, provide directional cues for leukocyte motility, and concomitantly induce polarization. Chemoattractant receptors, integrins and other adhesion molecules, cytoskeletal proteins and intracellular regulatory molecules change their cellular localization during cell polarization. A complex system of signal transduction molecules, including tyrosine kinases, lipid kinases, second messengers and members of the Rho family of small GTPases is thought to regulate the cytoskeletal rearrangements underlying leukocyte polarization and migration. The elucidation of the mechanisms and signals that control this complex reorganization will lead to a better understanding of critical questions in cell biology of leukocyte migration and polarity.
Keywords: cell migration/immune interactions/leukocytes/polarization/signaling

Introduction

Leukocytes emigrate from the bloodstream across the wall of microvessels. The attraction of leukocytes to tissues is an essential step in the inflammatory phenomenon and the host response to infection. The first requirement for a cell to initiate migration is the acquisition of a polarized morphology that enables it to turn intracellularly generated forces into net cell locomotion. We offer a perspective on cell polarization, emphasizing its role in the migratory pattern and effector functions of leukocytes. Specifically, this review deals with trafficking signals, receptor redistribution at the advancing front and uropod of migrating leukocytes, cytoskeletal reorganization and regulatory intracellular signaling pathways. The biochemical properties of the proteins and receptors involved as well as the diversity of functional roles of the various families of chemokines, adhesion and regulatory molecules are not described here in detail. Readers interested in further discussions of these topics are referred to excellent and recent reviews (Bretscher, 1996; Butcher and Picker, 1996; Drubin and Nelson, 1996; Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996; Rollins, 1997; Baggiolini, 1998; Hall, 1998; Mermall *et al*., 1998; Ward *et al*., 1998).

Chemokines, trafficking signals for leukocyte migration

Cells migrating in a tissue encounter multiple chemoattractant signals that can potentially guide their path (Foxman *et al*., 1997). The number of known leukocyte attractants has expanded nowadays with the characterization of the extensive family of chemokines, which largely control leukocyte migration (Luster, 1998). Chemokines are released by leukocytes and other cells in response to different stimuli, induce chemotaxis of different leukocyte types and regulate the adhesive properties of leukocyte integrins (Oppenheim *et al*., 1991; Lukacs *et al*., 1995; Rollins, 1997; Baggiolini, 1998). The large superfamily of chemokines consists of four subfamilies which display 2–4 highly conserved N-terminal cysteines. Most of them fall into the α or C-X-C (including SDF-1, IL-8/NAP-1, IP-10 and MGSA/gro) groups and the β or C-C (RANTES, MCP-1, -2 and -3, MIP-1α and β) groups, whereas there is only one C (lymphotactin) and one CX_3C (fraktalkine) chemokine described so far (Baggiolini *et al*., 1997; Rollins, 1997). Like classical leukocyte chemoattractants such as complement C5a, or bacterial *N*-formyl peptides, chemokines bind to heptahelical receptors coupled to heterotrimeric G proteins. At least 15 chemokine receptors (CKR) have been cloned and classified into CCR, CXCR, XCR, and CX_3C receptors (Baggiolini *et al*., 1997; Luster, 1998; Ward *et al*., 1998). Chemokines attract different leukocyte cell subsets depending on the chemokine-receptor pattern of expression, which is heterogeneous among leukocytes and appears to be tightly regulated at the transcriptional level (Loetscher *et al*., 1996). In addition to their role as regulators of leukocyte migration, chemokines play a role in angiogenesis, tissue morphogenesis, hematopoiesis or tumor cell growth, and their receptors are clearly involved in the infection of $CD4^+$ cells by human immunodeficiency virus (HIV) (reviewed in Littman, 1998; Luster, 1998; Ward *et al*., 1998).

The binding of chemokines to their receptors is followed by the involvement of heterotrimeric G proteins, adenylyl cyclase, phospholipases (PL) C, A, D, protein tyrosine and serine/threonine kinases, lipid kinases (as the phosphatidylinositol 3-kinase or PI3K), the Rho family of small GTPases (Reif and Cantrell, 1998; Ward *et al*., 1998), and the triggering of intracellular second messengers such

as cAMP, phosphoinositides and calcium. One of most impressive effects of chemokines on leukocytes are the morphological changes: the cytoskeleton is rearranged, integrin-mediated focal adhesions are formed, and the cell binds and detaches from the substrate in a coordinated manner with extension and retraction of pseudopods to execute the directional migration (Bokoch, 1995; Ward *et al*., 1998).

Polarization of leukocytes during migration and immune cell–cell interactions

An early event in the leukocyte polarization induced by chemoattractants is a change in filamentous F-actin distribution from a radial symmetry around the cell to its concentration at a particular region, resulting in the switch from a spherical to a polarized shape (Howard and Oresajo, 1985; Coates *et al*., 1992). Chemokines are responsible for the polarization of leukocytes with the establishment of two different regions: the leading edge and the uropod (del Pozo *et al*., 1995). In early studies, Wilkinson described the polarized morphology of leukocytes as similar to that of migrating amoebae: a leading edge followed by the cell body, and the tail or uropod (Wilkinson, 1986). The uropod, which is not observed in other migrating eukaryotic cells, is a pseudopod-like projection that represents a specialized structure with important motility and adhesive functions. Extension of active membrane processes, including both lamellipodia and filopodia, takes place primarily around the cell front, as a consequence of polarization of F-actin (Lauffenburger and Horwitz, 1996). Cells emit variable numbers of lamella, but only the dominant one contacts the substrate, expands and directs the locomotion. Contractions at the junction of the lamella and cell body and at the rear of the cell modify the cell shape, inducing a flat anterior portion of the polarized cell and a narrow tail (Stossel, 1993). The speed of cell migration depends on the rate at which the adhesive membrane extends the cell forward, and tends to correlate inversely with contractile force. Retraction at the rear of the cell is followed after a short lag by extension at the front, if cells migrate slowly as fibroblasts or endothelial cells (1 μm/min). By contrast, if lamellar extension and retraction occur in a very coordinated manner, the cell size and shape are maintained during locomotion, as is observed in fish keratocytes (10 μm/min), which display a gliding mode of locomotion. Leukocytes exhibit a migration speed intermediate between fibroblasts and keratocytes.

Cell polarity is inherent to crawling lymphocytes prior to contact with antigen-presenting cells (APCs) or target cells, although it also plays a role during immune cell–cell interactions. Crawling T cells recognize and bind APCs through their leading edge (Kupfer and Singer, 1989), showing an enhanced sensitivity to antigen in this area compared with the trailing edge of the cell (Negulescu *et al*., 1996). The subsequent engagement of T-cell antigen receptor (TcR) by peptide–major histocompatibility complex (MHC) delivers a stop signal for migration, which is essential for the formation of the contact cap, and this clusters CD3–TcR complex and other co-stimulatory molecules, required for antigen-specific recognition and triggering of an immune–effector function (Dustin *et al*., 1997). Likewise, cytolytic T lymphocytes and natural killer (NK) cells, maintain a polarized shape when they are bound to their targets. Furthermore, the polarized secretion of cytolytic granules is required for proper cell-killing function, and the prevention of cell polarization blocks the formation of effector–target cell conjugates and cell-mediated cytotoxicity (Lang *et al*., 1996; Nieto *et al*., 1998).

Receptor redistribution during leukocyte polarization and migration

Leukocyte polarization occurs during a variety of cell–cell interactions, such as antigen presentation, target-cell recognition, or leukocyte binding to endothelial cells (Campanero *et al*., 1993; Kupfer *et al*., 1994; del Pozo *et al*., 1995; Helander *et al*., 1996; Negulescu *et al*., 1996). The surface molecules which mediate these processes correspond to cell-adhesion receptors (Springer, 1994).

The polarity of T cells following contact with an APC has been well characterized on the basis of plasma membrane protein clustering, cytoskeletal and organellar reorganization, and cytokine secretion (Kupfer and Singer, 1989). Clustered plasma membrane receptors include several molecules such as CD3, TcR, leukocyte function-associated antigen 1 (LFA-1), CD28, CD2 and CD4 (Monks *et al*., 1997; Dustin *et al*., 1998; Kaga *et al*., 1998). The three-dimensional analysis of the cell contacts shows that the CD3/TcR and LFA-1 receptors are organized into distinct and segregated spatial domains (Monks *et al*., 1998).

The leading edge

In migrating lymphocytes, actin is distributed in a linear cortical array over most of lymphocyte cell body, but is concentrated at the cell leading edge and contact zones (Campanero *et al*., 1994; Stowers *et al*., 1995; Friedl *et al*., 1998; Lowin-Kropf *et al*., 1998). Different receptors are concentrated at the cell advancing front (Figure 1), such as the αvβ3 integrin (Lawson and Maxfield, 1996), the receptor for the urokinase plasminogen activator (uPAR) (Estreicher *et al*., 1990; Kindzelskii *et al*., 1996), as well as receptors for the chemoattractant formyl-methionil-leucil-phenylalanine (fMLP) (in neutrophils) (Sullivan *et al*., 1984; Mackay *et al*., 1991), or chemokines (CCR2 and CCR5 in lymphocytes) (Nieto *et al*., 1997). The redistribution of CKR to the leading edge of migrating lymphocytes helps us to understand the mechanism of chemotaxis, a mechanism which is not yet fully elucidated. CKR redistribution to the cell leading edge is triggered by different chemotactic factors that act through distinct membrane receptors, including cytokines IL-2 and IL-15, and chemokines, as well as other T-cell polarization-inducing agents (Nieto *et al*., 1997). IL-2 also induces lamellipodia and membrane ruffling at the leading edge of T cells (Arrieumerlou *et al*., 1998). Therefore, the polarization of CKR in leukocytes correlates with the acquisition of a migrating phenotype and seems to be independent of the chemoattractant stimulus. Nevertheless, the redistribution of chemosensory receptors to the advancing front of migrating cells does not seem to be a general finding in other cellular systems. In this regard, the cAMP chemoattractant receptors of *Dictyostelium discoideum*

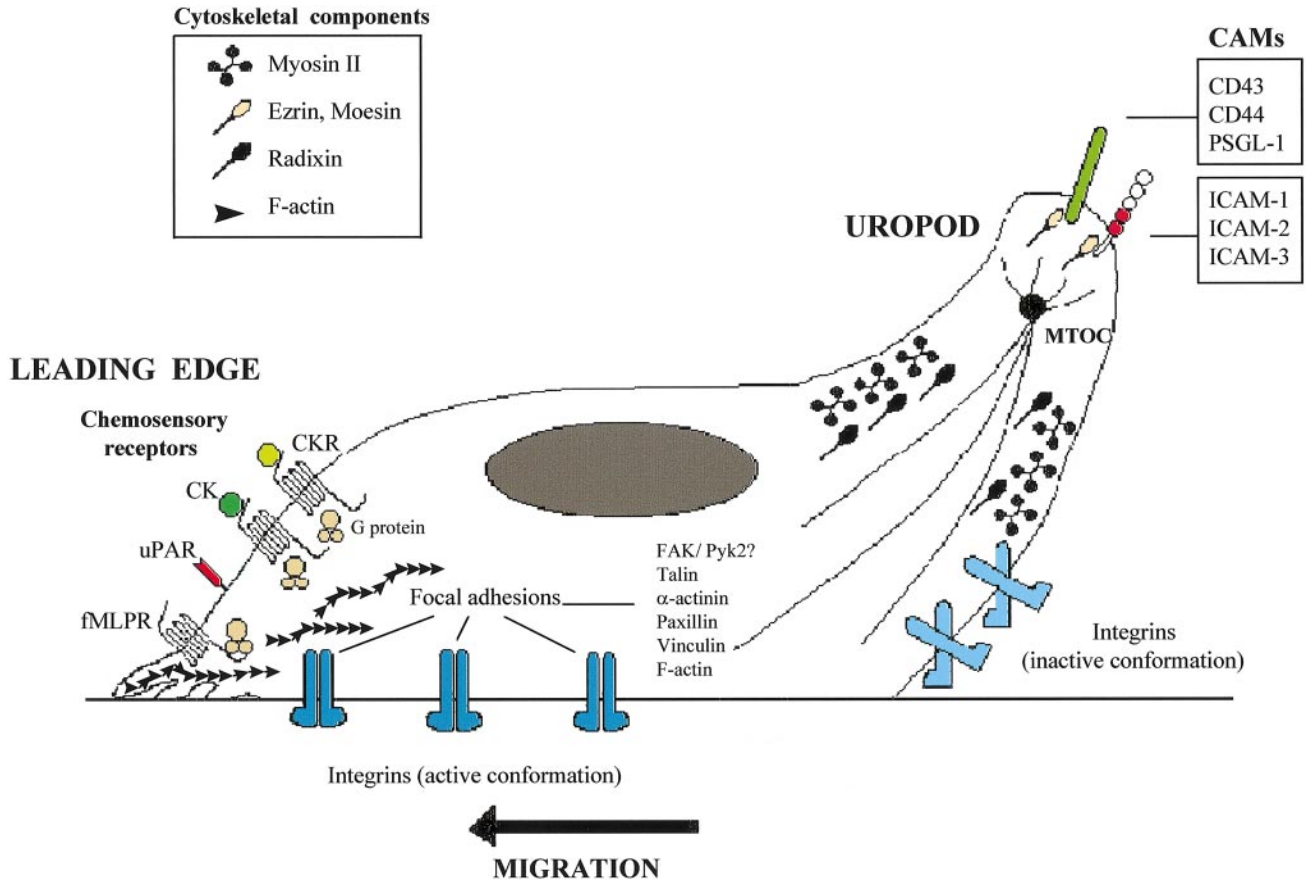

Fig. 1. Scheme for the establishment of two poles in migrating leukocytes and redistribution of chemoattractant receptors, adhesion molecules and cytoskeletal elements. See text for details.

remain evenly distributed on the cell surface and all of its projections during chemotaxis (Xiao *et al*., 1997).

The uropod

While the events described above occur at the leading edge, several adhesion molecules accumulate at the trailing edge or uropod (Figure 1). Depending on the leukocyte subset, they include intercellular adhesion molecules (ICAMs), CD2, CD44, CD43, P-selective glycoprotein ligand 1 (PSGL-1), L-selectin, Mac-1 and FcγR-IIIb (Rosenman *et al*., 1993; del Pozo *et al*., 1995; Lorant *et al*., 1995; Kindzelskii *et al*., 1996). The leukocyte integrin Mac-1 is associated with uPAR in the membrane of resting non-polarized neutrophils, but they dissociate during cell polarization. Mac-1 redistributes towards the uropod while uPAR accumulates at the lamellipodia of migrating neutrophils (Kindzelski *et al*., 1996). In migrating lymphocytes, the β1 and β2 integrins are located in the area of the cell body which is in contact with the extracellular matrix (ECM) or endothelial cells (Campanero *et al*., 1994; del Pozo *et al*., 1995). In a three-dimensional type I collagen matrix model of migration, the β1 integrins of resting peripheral blood lymphocytes (which mainly correspond to the fibronectin receptors VLA-4 and VLA-5) are redistributed towards the uropod (Friedl *et al*., 1998).

Several pieces of evidence indicate that the uropod is an important adhesive structure. First, a plethora of adhesion molecules are concentrated in this structure during cell migration. Secondly, the uropod may anchor the cell to the ECM fibers while the cell front pulls forward the cell body during migration of T cells into a three-dimensional collagen lattice (Friedl *et al*., 1998). Thirdly, the adhesion molecules that are accumulated in the uropod support the binding of other cells, as described for PSGL-1 in neutrophil–platelet interactions (Dore *et al*., 1996) and for ICAMs in the interactions of activated and memory T lymphocytes with bystander T cells (del Pozo *et al*., 1997). Fourthly, the uropod enhances leukocyte recruitment and transendothelial migration (del Pozo *et al*., 1997). Furthermore, uropod formation facilitates migration through constricted spaces in three-dimensional matrix assays (Ratner *et al*., 1997). Therefore, an endogenous polarity and asymmetry is established in migrating leukocytes with the generation of different and specialized cell domains: the receptors involved in the detection of the chemoattractant gradient are located at one pole (the leading edge), while the adhesion receptors, which facilitate migration and are implicated in the recruitment of bystander leukocytes, are concentrated at the other pole (the uropod).

Cytoskeletal reorganization

The binding of the T cell to an APC induces the rapid reorientation of the T-cell cytoskeleton, which includes the formation of a tight collar of polymerized actin at the T cell–APC interface and the relocalization of the microtubule-organizing center (MTOC) towards the bound APC (Kupfer and Singer, 1989; Kupfer *et al*., 1994; Dustin

et al., 1997). This polarization is dependent on the TcR recognition of peptide–MHC and seems to contribute to the specificity of the immune response. F-actin accumulation at the cell–cell interface is suggested to stabilize and facilitate continuous TcR–antigen interactions (Valitutti *et al*., 1995), whereas the reorientation of MTOC allows the concentration of cytokines and cytotoxic mediators by repositioning the T-cell secretory apparatus into close proximity with the APC (Kupfer *et al*., 1994). The induction of cytoskeletal rearrangements is dependent of the immunoreceptor tyrosine-based activation motifs (ITAMs) present in TcR-ζ and CD3 chains, and requires ITAM phosphorylation by the src tyrosine kinase lck (Lowin-Kropf *et al*., 1998). In addition, a novel adaptor protein (CD2 AP) is likely to be required for the recruitment of adhesion receptors to the contact area and their linkage to the cytoskeleton (Dustin *et al*., 1998).

Migration and polarization involves a drastic reconfiguration of the tubulin cytoskeleton. As mentioned above, during cognate immune interactions the migration is stopped by a signal dependent on TcR occupancy (Dustin *et al*., 1997). Several important elements such as the MTOC have a different orientation in migrating or immune-interacting polarized cells (Dustin *et al*., 1997, Ratner *et al*., 1997; Serrador *et al*., 1997). During lymphocyte migration into a three-dimensional collagen lattice, the uropod forms near the MTOC, and when the uropod buds out, the outspread microtubule complex retracts into its narrow lumen, collapsing into a thin compact sheaf. Retraction of the relatively rigid microtubules into the streamlined uropod increases the deformability of T cells and facilitates their migration through constricted spaces (Ratner *et al*., 1997).

During migration, the actin network is stably linked to the surface receptors which are interacting with the substratum. Acto–myosin-based contraction of this network pulls the cell to move forward. Continuous movement of the cell is possible because the network is uninterruptedly regenerated by assembly at the leading edge and disassembly at the rear (Mitchison and Cramer, 1996; Heidemann and Buxbaum, 1998). The motor protein myosin plays an important role in migration in many different cell types, including those from *Drosophila*, *Dictyostelium* and amoebae (Pasternak *et al*., 1989; Mitchison and Cramer, 1996). In myosin II-deficient amoebae, the migration is slow (Wessels *et al*., 1988), the transport of beads attached to the membrane is delayed at the uropodial area, and the formation of ultrathin lamella at the cell tail is diminished, suggesting that the force generated by myosin II acts strongly at the uropod (Jay *et al*., 1995). Consistent with this idea, myosin II is highly concentrated in posterior regions in *Dictyostelium* (Rubino *et al*., 1984) and in a more intermediate position in 3T3 fibroblasts (Conrad *et al*., 1993). Similarly, linear arrays of myosin II are located within the uropod neck of polarized T lymphocytes (Figure 1), and the myosin-disrupting agent butanedione monoxime prevents the cell polarization and membrane receptor redistribution induced by chemotactic cytokines (del Pozo *et al*., 1995; Nieto *et al*., 1998). Therefore, the myosin motor seems to play an important role in the driving of adhesion receptors towards the uropod and in the generation of this cellular structure. Furthermore, myosin is clearly involved in other polarity phenomena such as capping and cytokinesis (Lauffenburger and Horwitz, 1996).

Membrane interactions with the cytoskeleton are necessary for the formation of specialized protrusions such as lamellipodia or filopodia (Nobes and Hall, 1995). Since very few integral membrane proteins interact directly with the actin-based cytoskeleton, it is very likely that accessory proteins function as molecular linkers between the actin–cytoskeleton and the plasma membrane; possible candidates for this role are the closely-related ERM proteins (ezrin, radixin and moesin). The ERM proteins are able to bind to actin through their C-terminal region, concentrate at the inner surface of plasma membrane, and interact by their N-terminal half with the cytoplasmic tail of plasma membrane receptors. They may therefore serve as general actin filament–plasma membrane linkers (Tsukita *et al*., 1997). Ezrin, radixin and moesin are preferentially located at cell-surface protrusions such as microvilli, filopodia, microspikes, focal adhesions and membrane ruffles (Tsukita *et al*., 1997). The integral membrane receptors CD43 and CD44 are associated with one or more of these proteins in different protrusive elements of distinct cell types (Yonemura *et al*., 1993; Tsukita *et al*., 1994; Serrador *et al*., 1997; Vaheri *et al*., 1997; Yonemura *et al*., 1998). In T lymphocytes induced to polarize by chemokines, radixin colocalizes with myosin II in the neck of the uropod, while moesin is preferentially located at the uropod tip, interacting with the cytoplasmic tail of ICAM-3, CD43 and CD44 (Figure 1). These molecular associations increase during the clustering of these molecules to the uropod within the cell polarization process (Serrador *et al*., 1997).

Less is known about the membrane–cytoskeletal interactions which drive chemosensory receptors towards the cell leading edge. In neutrophils, the association with actin of the receptors for formyl peptide chemoattractants has been described and a possible role in the regulation of chemoattractant-induced activation or actin polymerization was suggested (Jesaitis *et al*., 1993). However, its relationship to neutrophil polarization and migration remains undetermined. As described above, F-actin is weakly localized to uropods of migrating cells, but concentrates at leading edges, along with α-actinin, vinculin and talin (Campanero *et al*., 1994). The assembly of F-actin into stress fibers anchored to focal adhesion complexes typically described in fibroblasts (Burridge *et al*., 1988) has never been reported in leukocytes. T cells migrate very rapidly (7–7.5 μm/min) compared with fibroblasts (1 μm/min) (Niggemann *et al*., 1997), and for this reason the interactions of lymphocytes with the ECM may be too transient to support the assembly of completely formed focal adhesions. Therefore, in T cells the high migration speed might be inversely correlated with focal adhesion formation (Palecek *et al*., 1997).

Intracellular signaling pathways

As mentioned above, a complex and still not completely defined array of signaling cascades are induced through the CKR, regulating cytoskeletal rearrangement, integrin-dependent adhesions, and cell displacement. A number of these signals is involved in the government of cell polarization (Figure 2), and we are only just beginning to

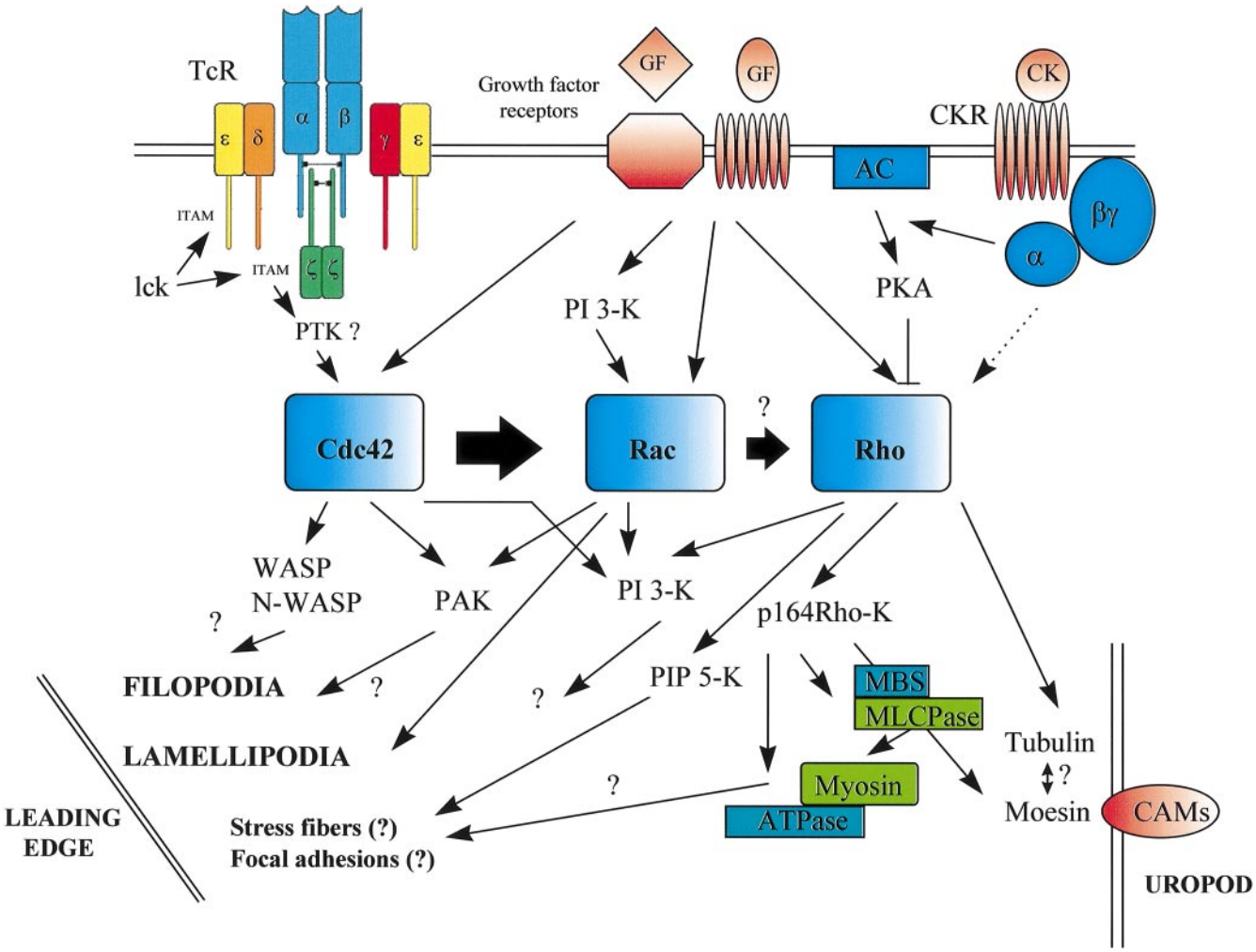

Fig. 2. Schematic representation of the potential signaling pathways involved in the regulation of cell polarization and redistribution of cell-surface receptors.

understand them. Most of the studies on this topic have been performed in cell systems other than lymphocytes (fibroblasts, yeasts) but signaling seems to be conserved to some extent among different cellular types. The role of second messengers, such as calcium or cAMP, protein kinases such as PKA or PKC, lipid kinases such as PI3K, and Rho family small GTPases is discussed.

Calcium

Many roles for calcium in cell motility and polarity have been proposed, from the regulation of actin-binding proteins to the stimulation of myosin II-based contraction (Stossel, 1993). Free intracellular calcium concentrations are low at the leading edge and high at the cell rear in migrating leukocytes (Brundage *et al*., 1991; Hahn *et al*., 1992; Gilbert *et al*., 1994), further suggesting that myosin II (located in the uropod neck)-based contraction mainly takes place at the cell rear, where the release of cell-substrate attachment must occur. Calcium also seems to have an important role in the replenishment of adhesion receptors (integrins) at the leading edge, which is necessary since as the cell advances receptors attached to the substrate move backwards. Therefore, a mechanism is required to return them from the rear regions of the cell towards its front. Two hypothesis have been raised, the existence of a polarized endocytic cycle and the forward diffusion in the plane of the membrane (Bretscher, 1996). Lawson and Maxfield (1996) observed that in crawling neutrophils the surface integrin αvβ3 concentrated in the leading edge is continuously replenished via the endocytic cycle from the rear of the cell. The release of αvβ3 from the trailing edge requires intracellular calcium transients, since when it is depleted or clamped, the cell is unable to detach and stops, and the αvβ3 accumulates at the rear pole, adhered to vitronectin. However, the extension of protrusions in the leading edge is not impaired in the absence of calcium transients. The calcium fluctuations act through the calcium/calmodulin-regulated serine/threonine phosphatase calcineurin, since its inhibition produces the same effects as calcium depletion (Hendey *et al*., 1992). In chinese hamster ovary cells, the calcium-dependent protease calpain is also involved since its inhibition prevents cell migration by stabilizing cytoskeletal linkages and decreasing the integrin release from the cell membrane, and consequently the rate of retraction of the cell rear (Huttenlocher *et al*., 1997; Palecek *et al*., 1998). An important question that arises is whether chemotactic receptors follow a similar endocytic cycle and calcium regulates this possible process with evident implications for understanding the mechanism of chemotaxis. In this

regard, it is well known that endocytosis of CKR exists as a mechanism of desensitization (Baggiolini *et al*., 1997).

Ca^{2+} also regulates the dynamics of T-cell morphology and cell–cell contacts during cognate immune interactions of T cells with APC (Negulescu *et al*., 1996). When the intracellular calcium is clamped, immobilization and prolonged contact with the APC and cell rounding occur via a calcineurin-independent mechanism.

cAMP

Although chemokines diminish intracellular cAMP levels in some systems where their receptors are overexpressed (Myers *et al*., 1995), they appear to increase levels in leukocytes (Mantell *et al*., 1995; M.Nieto, M.A.del Pozo and F.Sánchez-Madrid, unpublished observations). This probably depends on the Gβγ dimers, Gα subunits and especially on the adenylyl cyclase isoforms expressed by these cells (Taussig and Gilman, 1995; Hamm and Gilchrist, 1996). Elevated levels of cAMP diminish neutrophil adhesion to endothelial cells (Derian *et al*., 1995), thus pointing out a modulatory role for cAMP in phenomena taking place at the cell leading edge. On the other hand, this second messenger as well as the cAMP-dependent protein kinase (PKA) have been reported to be involved in cell polarization and uropod formation induced by chemokines; cAMP analogs induce uropod development whereas an specific PKA inhibitor (H-89) prevents its formation (del Pozo *et al*., 1995). Moreover, PKA inhibition causes a dramatic decrease in lymphocyte random motility and chemokine-directed chemotaxis (M.A.del Pozo, M.Vincente-Manzanares, R.Tejedor, J.Serrador and F.Sanchez-Madrid, manuscript submitted). In addition to its ability to phosphorylate certain cytoskeletal proteins (Selden and Pollard, 1983; Kammer *et al*., 1988), PKA can also phosphorylate RhoA leading to inactivation of this GTPase (Lang *et al*., 1996), thus suggesting a connection between chemokine receptors and Rho GTPases. In this regard, both PKA activation and RhoA inactivation causes lymphocyte polarization and adhesion molecule clustering at the uropod (del Pozo *et al*., 1995). Furthermore, ERM proteins function as anchoring proteins of PKA (Dransfield *et al*., 1997), thus suggesting that PKA could mediate the polarization of both ERM and adhesion molecules. However, the possible location of PKA at the uropod of polarized cells, bound to ERM proteins, remains to be determined.

Phosphatidylinositol 3-kinase

Phosphatidylinositol 3-kinase (PI3-K) induces the phosphorylation of phosphatidylinositol lipids involved in cytoskeletal rearrangements triggered by chemoattractants (Bokoch, 1995). It is required for the cytoskeletal polarization of T cells contacted by an APC (Stowers *et al*., 1995). Chemokines activate a PI3-K-dependent signaling cascade implicated in the regulation of chemotaxis and modulation of integrin affinity (Kundra *et al*., 1994; Wennstrom *et al*., 1994; Hartwig *et al*., 1995). In this regard, the chemotaxis and polarization of T cells induced by RANTES is blocked by PI3-K inhibitors (Turner *et al*., 1995). The chemotactic cytokine IL-2, which is also a critical growth factor for T cells, triggers actin polymerization in the leading lamella. The signaling cascade-regulating membrane ruffling induced by IL-2 involves sequentially PI3-K and Rac (Arrieumerlou *et al*., 1998). The connections between PI3-K and Rho GTPases are complex and will be discussed below.

Protein kinases

Tyrosine kinases are involved in the formation and stabilization of cell attachments in migrating fibroblasts (Huttenlocher *et al*., 1995). Upon integrin clustering at the focal adhesions, several proteins localized therein such as FAK, paxillin and tensin become tyrosine phosphorylated, and inhibitors of tyrosine-phosphorylation diminish migration (Burridge *et al*., 1992; Schaller and Parsons, 1994). In migrating lymphocytes, focal adhesion kinase (FAK) is located mostly in the leading edge, and also became tyrosine phosphorylated (Entschladen *et al*., 1997). As in fibroblasts, inhibitors of tyrosine phosphorylation reduce the locomotor activity of lymphocytes. However, FAK is not expressed in some leukocyte subsets such as monocytes and macrophages, and, in fact, its highly related calcium-dependent tyrosine kinase (CADTK) or Pyk2 is the predominant form of cytoskeletal-associated PTK in hematopoietic cells, including monocytes, T cells, B cells and NK cells (Avraham and Avraham, 1997; Li *et al*., 1998). Furthermore, CKR signals via Pyk2 (Davis *et al*., 1997; Dikic *et al*., 1998). Whether Pyk2 is asymmetrically localized in migrating leukocytes and whether it is involved in the regulation of chemotaxis is currently undetermined. In fibroblasts, PKC is present in focal adhesions in the leading edge, and its activation is implicated in focal-adhesion formation (Woods *et al*., 1992). However, in migrating lymphocytes, PKC is redistributed near or in the uropod, where colocalizes with its substrate MARCKS (myristoylated, alanine-rich C kinase substrate), and with adhesion receptors and cytoskeletal proteins such as radixin and myosin II (Entschladen *et al*., 1997; Serrador *et al*., 1997). In addition, a cross-talk between PKC and PKA has been described for the regulation of lymphocyte polarization (del Pozo *et al*., 1995). Therefore, FAK is localized in the leading edge of lymphoid cells, and may regulate the direction of migration, whereas PKC may be involved in the formation of uropod and the regulation of associated adhesion molecules. Other studies on the cellular localization of several PKC isoforms during the T cell–APC conjugate interaction revealed that only PKC-θ is translocated to the site of cell contact. Hence, the location of PKC, at least of this isoform, seems to be different during cognate immune interactions (cell contact area) and migration (uropod). Furthermore, a selective increase in the activity of PKC-θ is induced during the antigen-specific cell conjugation (Monks *et al*., 1997, 1998). PKC-θ has been recently identified as a major kinase with specificity for the moesin actin-binding sequence, and with activation under non-classical conditions (Pietromonaco *et al*., 1998). Whether PKC-θ is regulating the receptor-membrane association of moesin during cell polarization is an issue that deserves further investigation.

Rho family of GTPases

The Rho family of small GTPases plays a major role in regulating the actin cytoskeleton that organizes the membrane protrusions and focal adhesions (Hall, 1998). Rho family of GTPases seem also to regulate cell polarity during leukocyte migration. Rho is involved in the

leukocyte adhesion through integrins induced by chemoattractants (Laudanna *et al*., 1996), and in LFA-1/ICAM-1-dependent leukocyte aggregation (Tominaga *et al*., 1993). Moreover, a role for Rho GTPases in determining cellular polarity has been suggested or demonstrated for budding and mating in yeasts (Chant, 1996; Drgonová *et al*., 1996; Drubin and Nelson, 1996), embryonic development in *Drosophila* and *Caeorhabditis elegans* (Eaton *et al*., 1995; Zipkin *et al*., 1997), and mammary epithelial cells (Keely *et al*., 1997).

Several studies have suggested a major role for these GTPases in the control of leukocyte polarity (Figure 2). Cdc42 can regulate the polarization of actin and microtubules towards APCs in T cells (Stowers *et al*., 1995), a mechanism possibly initiated by tyrosine phosphorylation of the ITAMs present in TcR-ζ and CD3 (Lowin-Kropf *et al*., 1998), thus suggesting a link between the TcR-signaling and the Rho GTPases. In macrophages, Rac and Cdc42 regulate the formation of lamellipodia and filopodia, respectively; Rho is activated downstream of Rac and induces cell contraction (Allen *et al*., 1997). In an elegant study of macrophage chemotaxis induced by colony stimulating factor-1 (CSF-1), Allen and co-workers showed that constitutively activated RhoA, Rac1 and Cdc42 as well as dominant-negative RhoA and Rac1 inhibit cell migration, whereas cells microinjected with dominant-negative Cdc42 are able to migrate but do not polarize in the direction of the gradient, therefore abolishing chemotaxis towards CSF-1 (Allen *et al*., 1998). This important finding underscores the major role of Cdc42 in the control of directional migration of leukocytes. In T lymphocytes, overexpression of activated mutants of each GTPase, or activation of the endogenous proteins impair lymphocyte polarization, random migration and chemokine (SDF-1)-directed migration. Strikingly, dominant-negative overexpressed proteins induce a polarized phenotype in T cells with concentration of adhesion receptors and ERM proteins in the uropod (del Pozo *et al*., manuscript submitted). Therefore, it is very likely that the process of adhesion/deadhesion must be coordinated through cycles of GTPase activity (GTP-bound) and inactivity (GDP-bound) to produce cell motility. In addition, it is tempting to speculate that Rho GTPases could be activated in the lymphocyte leading edge, regulating the actin polymerization required for cell advance, and inactivated in the posterior pole, inducing uropod formation. In this regard, it has been proposed that in fibroblasts Rho is activated in the advancing front but it must be inhibited to allow rear release (Huttenlocher *et al*., 1995; Lauffenburger and Horwitz, 1996). Accordingly, an asymmetric spatial distribution of Rho GTPases and some of their effector proteins occurs in budding yeast (Drgonová *et al*., 1996; Evangelista *et al*., 1997). On the other hand, a new member of the Rho family, Rnd3/RhoE, has been demonstrated to inhibit the formation of fibroblast actin stress fibers, membrane ruffles and focal adhesions (Nobes *et al*., 1998), but its role on leukocyte polarization is unknown. Rho GTPases have been involved in other polarization process such as the cytokinesis (Dutartre *et al*., 1996; Hall, 1998), and both ERM proteins and adhesion receptors have been localized in the cleavage furrow (Yonemura *et al*., 1993), suggesting a role for these GTPases as general regulators of cell polarity.

The mechanisms through which Rho GTPases exert their effects are not completely elucidated, although a great number of effectors have been described (Hall, 1998) and actin-binding proteins are leading candidates (Hall, 1998; Reif and Cantrell, 1998). Possible intermediaries are tyrosine kinases such as FAK or Pyk2, and lipid kinases such as PIP5-K (Chong *et al*., 1994) and PI3-K (Figure 2). PIP5-K regulates the production of phosphatidylinositol phosphatase (PIP_2), which is implicated in the assembly of actin filaments. The understanding of the connection between PI3K and Rho GTPases remains fragmentary. PI3K seems to activate Rac in the *ruffling* pathway but not in the c-Jun N-terminal kinase mitogen-activated protein (JNK MAP) kinase pathway. Moreover, PI3K apparently does not activate Rac in T lymphocytes (Reif and Cantrell., 1998). Nevertheless, PI3K inhibitors abolish polarization and chemotaxis (Stowers *et al*., 1995; Turner *et al*., 1995). Therefore, it is feasible that GTPase-induced effects could be mediated by multimolecular complexes and not by linear pathways of biochemical cascades, as described for Cdc42 in yeast (reviewed in Hall, 1998).

The molecular mechanism by which Cdc42 generates a polarized phenotype in response to chemoattractants might involve members of the p21-activated kinase (PAK) family or WASP (Figure 2). PAK1 induces the rapid formation of polarized filopodia and membrane ruffles (Sells *et al*., 1997), and its homolog in *Saccharomyces cerevisiae* Ste20 seems to act on the myosin I homologs Myo3 and Myo5 (Goodson *et al*., 1996). WASP induces the formation of Cdc42-dependent actin clusters (Symons *et al*., 1996) and patients suffering from the Wiskott–Aldrich syndrome are defective in T-cell surface microvilli (Molina *et al*., 1992) and chemotactic responses (Ochs *et al*., 1980). In addition, its relative N-WASP induces the formation of filopodia (Miki *et al*., 1998).

Another signal transduction molecule which could be involved in the down-regulation of cell polarization and motility by Rho GTPases is the p164 Rho-associated kinase or Rho-kinase (Rho-K), an effector of Rho that has been also shown to interact with Rac (Joneson *et al*., 1996). This molecule induces the formation of focal adhesions and stress fibers (Matsui *et al*., 1996; Amano *et al*., 1997) and binds to the myosin-binding subunit (MBS) of myosin light chain phosphatase (MLCPase), phosphorylating it and causing a decrease in its activity (Kimura *et al*., 1996). Recently it has been described that the MBS of MLCPase is associated with moesin, and that moesin phosphorylation is regulated by both Rho-K and MLCPase (Fukata *et al*., 1998; Matsui *et al*., 1998). ERM proteins, which act as actin filament/plasma membrane linkers, seem to be regulated by Rho and Rho-K by interfering with the intramolecular and/or intermolecular head-to-tail association of ERM proteins (Hirao *et al*., 1996; Matsui *et al*., 1998). In addition, ERM proteins are essential for both Rho- and Rac-induced cytoskeletal effects in permeabilized cells (Mackay *et al*., 1997). Together, these observations suggest a possible connection among myosin, ERM proteins and adhesion molecules with the morphological changes induced by Rho GTPase activation and the down-regulation of uropod formation during cell polarization and migration (Figure 2). Myosin could form a complex with moesin, and perhaps with other ERM proteins, through the myosin-binding subunit of myosin phosphatase, which could be phosphorylated or dephosphorylated by Rho GTPases (through Rho-K or other target

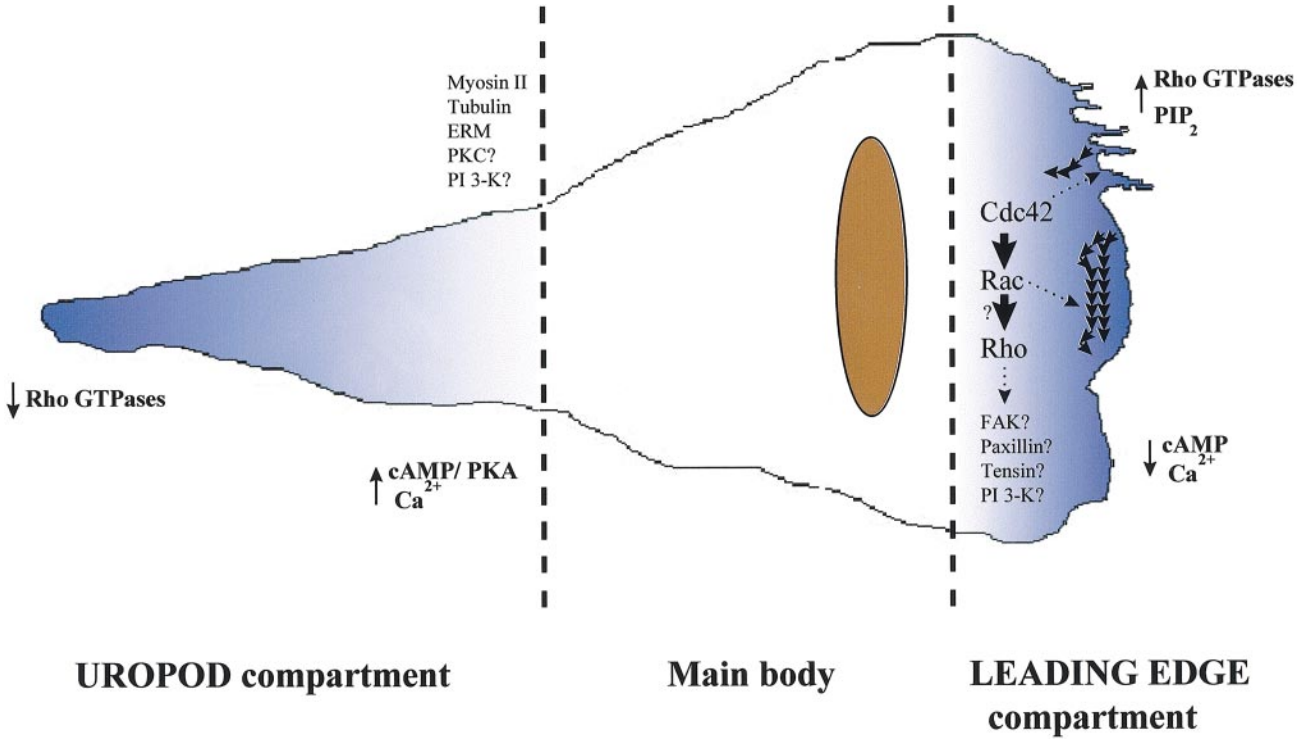

Fig. 3. Schematic model of the potential mechanisms of regulation of cell polarization and establishment of two compartments in migrating leukocytes.

effectors) thus regulating its association with adhesion molecules located in the plasma membrane. Another essential cytoskeletal component, tubulin, could also participate in this complex. During T-lymphocyte polarization, the MTOC determines the site of uropod budding (Ratner *et al*., 1997) and a remarkable meshwork of microtubules is directed from an MTOC located in the distal part of the uropod (Serrador *et al*., 1997). Tubulin has been postulated to interact with myosin (Mermall *et al*., 1998) and it could also associate direct or indirectly with moesin–adhesion molecule complexes. Moreover, Rho GTPase acts as a regulator which selectively estabilizes microtubules during cell polarization (Cook *et al*., 1998). Further analysis of the physiological relationships between CKR, PKA, Rho GTPases, myosin, tubulin, ERM proteins and adhesion molecules will lead to a better understanding of the mechanisms controlling lymphocyte polarization and motility.

Conclusions

Cell polarization is a characteristic feature of migrating leukocytes which is triggered by chemotactic stimuli. Two clearly distinct compartments are established in the cell (Figure 3). The leading edge, where chemoattractant receptors, activated integrins, uPAR, FAK and other molecules are concentrated, and is thought to act as a sensor compartment to detect the chemotactic gradient and guide directionally the leukocyte migration. At the trailing edge, the uropod, where adhesion receptors, ERM proteins, myosin II, tubulin cytoskeleton and PKC among others are accumulated, plays a pivotal adhesive role and facilitates cell migration. In the other model of leukocyte polarization which occurs during the cognate immune interactions, redistribution of some elements of these compartments is maintained in the T cell, whereas others, such as MTOC or PKC-θ, are reoriented towards the contact site with the APC or target cell. Although information on signals governing these cellular processes is rapidly increasing, how all the involved phenomena are coordinated remain elusive. An attractive possibility is that gradients of second messengers as well as an asymmetrical cell distribution of regulatory and motor proteins might exist. A combination of low calcium and cAMP, and high phosphoinositide levels and Rho GTPases activity at the cell leading edge would increase integrin affinity and activate proteins that cross-link actin filaments, facilitating the establishment of adhesions and membrane protrusions (lamellipodia and filopodia). On the other hand, high calcium and cAMP concentrations and low Rho GTPases activity at the cell rear would activate proteins that disrupt actin filaments (Janmey, 1994) and enhance myosin II contractions to promote release of adhesions, and uropod formation.

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