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Feedback signaling controls leading-edge formation during chemotaxis

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Chemotactic cells translate shallow chemoattractant gradients into a highly polarized intracellular response that includes the localized production of PI(3,4,5)P₃ on the side of the cell facing the highest chemoattractant concentration. Research over the past decade began to uncover the molecular mechanisms involved in this localized signal amplification controlling the leading edge of chemotaxing cells. These mechanisms have been shown to involve multiple positive feedback loops, in which the PI(3,4,5)P₃ signal amplifies itself independently of the original stimulus, as well as inhibitory signals that restrict PI(3,4,5)P₃ to the leading edge, thereby creating a steep intracellular PI(3,4,5)P₃ gradient. Molecules involved in positive feedback signaling at the leading edge include the small G-proteins Rac and Ras, phosphatidylinositol-3 kinase and F-actin, as part of interlinked feedback loops that lead to a robust production of PI(3,4,5)P₃.

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Introduction

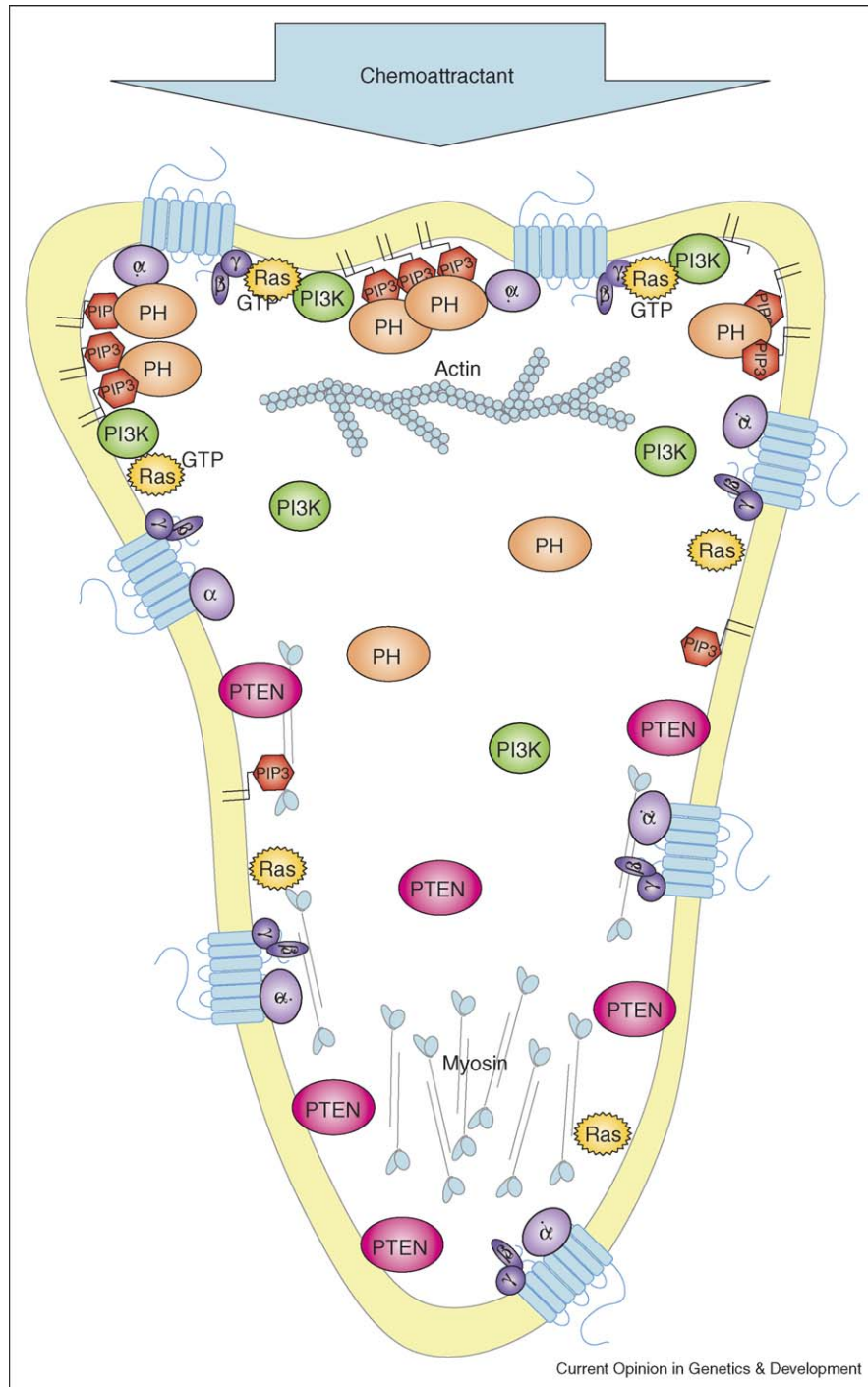
Chemotaxis is the ability of cells to detect and move towards the source of a chemoattractant signal. Such a directional migration is essential for a variety of cellular processes, including cell movement during development, immune responses and wound healing, in addition to the metastasis of tumor cells. Studies performed with highly motile leukocytes and *Dictyostelium* amoebae have led to our current understanding of directed cell movement. In response to a chemoattractant, these cells rapidly polarize in the direction of the signal, forming a pseudopod on the side exposed to the highest chemoattractant concentration, and a uropod or a posterior domain on the opposite side of the cell; these structures become the leading and trailing edges of the chemotaxing cell, respectively [1,2]. The formation of the leading edge results from localized

chemoattractant-induced F-actin assembly, whereas the cell's sides and posterior become enriched in assembled myosin II. Regulation of these cytoskeletal components during chemotaxis provides the driving and contractile forces required for cell motility [3]. Cells are able to detect and respond to chemoattractant concentrations differing by as little as 2–5% between the front and the back of the cell. These differences are then translated by the cell into a steep intracellular gradient of signaling components, leading to an asymmetric cellular response [4].

In both leukocytes and *Dictyostelium*, one of the first asymmetric responses to chemoattractant stimulation is the localized accumulation of phosphatidylinositol-3,4,5-triphosphate [PI(3,4,5)P₃], the product of class I phosphatidylinositol-3 kinases (PI3Ks), at the site of the new leading edge [5,6]. In *Dictyostelium*, this localized production of PI(3,4,5)P₃ is produced through the localized activation of Ras, an upstream activator of PI3K, the localized accumulation of PI3K, and the delocalization of the PI(3,4,5)P₃ 3-phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) from this site (Figure 1). The reciprocal localization and delocalization of PI3K and PTEN restricts PI(3,4,5)P₃ to the newly forming leading edge and thus helps generate and maintain a steep intracellular anterior–posterior PI(3,4,5)P₃ gradient. This localized enrichment of PI(3,4,5)P₃ at the leading-edge membrane results in the recruitment and activation of proteins that preferentially bind to PI(3,4,5)P₃, including proteins that contain a plextrin-homology (PH) domain (e.g. protein kinase B [Akt/PKB], guanine nucleotide exchange factors [GEFs] for Rac [RacGEFs], *Dictyostelium* PH domain-containing protein A [PhdA] and the cytosolic regulator of adenylyl cyclase [CRAC]) or proteins that contain lysine-rich PI(3,4,5)P₃-binding motifs, such as those found in the Wiskott-Aldrich syndrome protein (WASP) and the WASP-family verprolin homology (WAVE)/suppressor of cAMP receptor (SCAR) proteins [7,8,9^{••},10–19].

Many of the PI(3,4,5)P₃-binding proteins mentioned above are linked to the remodeling of the actin cytoskeleton [3]. Indeed, evidence suggests that chemoattractant-induced F-actin polymerization is tightly coupled to PI(3,4,5)P₃ accumulation. Inhibition of PI(3,4,5)P₃ production in *Dictyostelium*, either by disruption of class I PI3Ks or by treatment with the PI3K inhibitor LY294002, is accompanied by a defect in the second peak of chemoattractant-mediated F-actin polymerization; by contrast, PI(3,4,5)P₃ production in addition to the F-actin polymerization response are increased and prolonged in

Figure 1



Schematic illustration of the spatial localization of key components implicated in directed cell movement. Seven-transmembrane chemoattractant receptors and heterotrimeric G-proteins (α , β and γ) are evenly distributed along the cell's perimeter, their activation levels reflecting that of the extracellular gradient of chemoattractant. The small G-protein Ras displays uniform localization along the cell cortex, but its activated form (Ras GTP) is predominantly found at the leading edge. PI3K, PI(3,4,5)P₃, PH-domain containing proteins and F-actin are enriched at the leading edge, whereas PTEN and myosin localize to the lateral sides and posterior of chemotaxing cells.

pten-null cells [16,20,21]. Furthermore, expression of myr-PI3K (a membrane-targeted form of PI3K that distributes uniformly on the plasma membrane) induces the formation of multiple, functional pseudopodia along the plasma membrane of chemotaxing cells, directly linking PI3K activity to pseudopod formation [22]. Similar phenotypes are observed in *pten*-null cells, wherein PI(3,4,5)P₃ accumulates along the cell's entire cortex. The chemoattractant-induced F-actin response is highly correlated with the temporal and spatial accumulation of PI(3,4,5)P₃, as reflected by the translocation of specific PH domains to the membrane [23]. However, although there is clear evidence that PI(3,4,5)P₃ is linked to cell polarization and directional movement, cells treated with the PI3K inhibitor LY294002 are able to effectively move up a steep chemoattractant gradient if given sufficient time to polarize [24] (K Takeda and RA Firtel, unpublished).

In neutrophils as well as *Dictyostelium*, the chemoattractant signal is detected by seven-transmembrane receptors and transmitted to heterotrimeric G-proteins. Studies using green fluorescent protein (GFP) fusion proteins in these systems determined that the receptors stay uniformly distributed along the plasma membrane of chemotaxing cells [25,26], and the distribution as well as activation of the G proteins directly reflect the shallow extracellular gradient, hence the receptor occupancy (Figure 1) [27–29,30**]. These observations suggest that the localized responses and amplification at the leading edge are not due to differences in receptor distribution or localized G-protein signaling; rather, they must be generated after activation of heterotrimeric G-protein and before the localized accumulation of PI(3,4,5)P₃. How PI(3,4,5)P₃ production is locally amplified in the chemotaxing cell is still not completely understood. Recent evidence suggests that this is partly achieved through positive feedback loops, acting as self-organizing and auto-amplifying signals. In this review, we discuss these recent findings, which lead to a new model of chemotactic signaling in which positive feedback loops play a central role in signal amplification leading to an asymmetric cellular response and cell polarity.

A combination of localized feedback signaling and global response

Models of mechanisms in which small differences in a stimulus are amplified through positive feedback loops have been proposed to explain the biological pattern formation observed in developing tissues and in polarization of individual cells, as well as in directional sensing [31,32]. These early models suggest the presence of an intrinsic pattern formation system comprising two opposing signaling cues, both derived from the original stimulus: a stimulatory signal with a local self-enhancing component acting over a short range, restricted by a long-range inhibitory signal. Much evidence in various developmental systems has confirmed the importance of

autocatalytic feedback loops combined with inhibitory signals in developmental patterning [32]. Recent evidence suggests that single chemotactic cells acquire their polarity and signaling asymmetry in a similar fashion, giving rise to a model in which chemoattractant-regulated PI(3,4,5)P₃ is tightly controlled by PI3K and PTEN activities as part of local excitation and global inhibition (LEGI) mechanisms [4,33–35,36*]. The recent advances leading to our current understanding of eukaryotic chemotaxis indeed favor a model in which LEGI mechanisms underlie the behavior of a chemotaxing cell. This model does not consider a role for spontaneous polarization in gradient sensing, as did another recent model based on the mutual inhibition of frontness and backness pathways [37]; nevertheless, the LEGI model takes into account that the spatial distribution of the response depends on the relative chemoattractant gradient and that the rear of a cell remains responsive to further stimulation [36*]. This contrasts with models predicting a steady-state response that is invariant with respect to the mean value and relative gradient of chemoattractant concentration [31,38]; also with those that cannot account for the cell's ability to sense gradients of increasing average concentration [39]; and with models that suggest that the rear of the cell becomes inhibited and so would not be able to sense further stimuli [40].

The LEGI model suggests that a balance between excitation and inhibition processes controls the membrane binding and activity of PI3K and PTEN. For PI3K, excitation would reflect local levels of receptor occupancy, leading to the recruitment and activation of the enzyme. Conversely, global inhibition, determined by the average receptor occupancy, counteracts these effects. For PTEN, local excitation would decrease its association with the membrane, whereas global inhibition would restore binding [34]. Chemoattractant-induced targeting of *Dictyostelium* PI3K at the plasma membrane is mediated by its hydrophilic N-terminus independently of its Ras binding domain, even though the latter is required for activation [22]. By contrast, association of PTEN to the plasma membrane involves a putative N-terminal PI(4,5)P₂-binding motif [21]. Upon gradient sensing, the membrane-associated PTEN is delocalized from the developing leading edge but stays associated with the lateral and rear parts of the cell [21,22,41]. Preferential localization of PTEN at the back of chemotactic neutrophils has also been reported [42]. By constitutively dephosphorylating PI(3,4,5)P₃ into PI(4,5)P₂, PTEN therefore restricts the diffusion of PI(3,4,5)P₃ from the leading edge of chemotactic cells [21,22]. It was then postulated that, in order to subsist the inhibitory action of PTEN, the persistent accumulation of PI(3,4,5)P₃ at the leading edge could result from positive feedback loops leading to signal amplification [33]. Consequently, as suggested in early models of gradient sensing, the competition between the self-enhancing reaction

(i.e. PI(3,4,5)P₃ production) and the antagonistic reaction (i.e. PTEN activity) will only be won by the cortical region of the cell exposed to the highest concentration of the external graded chemoattractant, thereby determining the site of the leading edge [31]. Signal amplification processes appear to be crucial for the initiation of a chemotactic response, especially when considering chemotaxing cells in their natural context, in which they need to respond to weak and long-range diffusing signals.

The idea that there exists a PI(3,4,5)P₃ amplification mechanism involving positive feedback loops derives from studies in which exogenously delivered PI(3,4,5)P₃ was able to induce cell polarity and F-actin polymerization in neutrophils, through the activation of endogenous PI3K and production of new PI(3,4,5)P₃, implicating Rho GTPases and F-actin-dependent pathway [43–45]. Studies in *Dictyostelium* (see below) revealed the existence of a similar pathway that involves Rac, Ras, PI3K and F-actin, and which mediates leading-edge formation [9,46^{••},47]. When *Dictyostelium* cells are suddenly exposed to saturating concentrations of cAMP, the observed PI(3,4,5)P₃ response is biphasic: an initial uniform response peaks at around 10 seconds and is followed by a second response occurring in patches of PI(3,4,5)P₃ around the cell membrane [48]. These patches are proposed to be similar to the single patch of PI(3,4,5)P₃ present at the leading edge of chemotaxing cells in a cAMP gradient, and they persist as long as the stimulus is present. On the basis of the observation that the probability of patch formation is determined by the cAMP stimulus, whereas the size, lifetime and intensity of the patches seems to be independent of the stimulus concentration, the PI(3,4,5)P₃ patches were also proposed to be self-organizing in the sense that they appear to determine their own structure [49[•]]. Moreover, in most cases, the formation of PI(3,4,5)P₃ patches is followed by pseudopod extension, suggesting that these patches organize the actin cytoskeleton. Interestingly, uniform chemoattractant stimulation of *Dictyostelium* cells and neutrophils also induces a similar biphasic actin polymerization response: an initial rapid phase in which motility stops and the cell rounds up, and a second slow phase in which pseudopodia are extended from local regions of the cell's perimeter [23,48,50,51]. Studies performed in *Dictyostelium* suggest that the self-organizing patches of PI(3,4,5)P₃, in addition to the second phase of actin polymerization and Rac activation, are more sensitive to perturbations of PI3K activity [9^{••},23,48]. Two phases of PI(3,4,5)P₃ production were also observed when cells were abruptly exposed to stable cAMP gradients: an initial transient and asymmetric response around the cell membrane, followed by a second phase producing a highly polarized distribution of PI(3,4,5)P₃ [30^{••}]. Altogether, these observations suggest a number of things: that gradient sensing first involves a rapid and transient response to a chemoattractant; that this might serve to activate the gradient machinery; and that this is followed by a slower

response that amplifies differences in receptor occupancy, thereby achieving a highly polarized PI(3,4,5)P₃ response through positive feedback signaling. In fact, mathematical simulations of systems with such interlinking fast and slow positive feedback loops suggested that they create a dual-time switch that is both rapidly inducible and resistant to noise in the upstream signalling system, leading to reliable cell decisions [52[•]].

The presence of a two-step process of PI(3,4,5)P₃ production also suggests the presence of rapidly activated negative feedback loops as part of a desensitization machinery that enables the cells to adapt to different stimulatory conditions. In fact, a recent quantitative study suggests that a stronger cAMP stimulation triggers not only a stronger G-protein activation but also a faster adaptation of the downstream response [30^{••}]. Furthermore, the same study shows that, although abrupt exposure of cells to stable cAMP gradients induces persistent G-protein activation throughout the entire cell surface, the PI(3,4,5)P₃ response displays the previously described biphasic temporal pattern at the front of the cell. These observations reveal the presence of a signal-induced, locally acting inhibitor of PI3K signaling; this would act in addition to the 'global inhibition' mechanism that had been implied previously from models of directional sensing and which was suggested to play roles in establishing a steep intracellular PI(3,4,5)P₃ gradient as well as in adaptation. Although suggestions of the biochemical components responsible for the inhibitory process remain speculative, a combination of studies performed in neutrophils and *Dictyostelium* over the past five years is beginning to uncover the nature of the positive feedback loops implicated in gradient sensing and signal amplification at the leading edge of chemotaxing cells.

Positive feedback loops in directional sensing versus cell polarity

Several lines of evidence point to a directional sensing mechanism that does not require global cell polarity. Polarized cells have distinct fronts and rears defined by the accumulation of specific molecules, such as actin and myosin, and differences in their sensitivity at the two ends [34]. *Dictyostelium* cells treated with the actin polymerization inhibitor latrunculin, which creates a symmetrical and spherical cell without pseudopodia, still display significant localization of PI3K and amplification of PI(3,4,5)P₃ upon either uniform or graded cAMP stimulation [53[•]]. In a similar manner, latrunculin treatment of neutrophils only partially reduced the chemoattractant-induced PI3K activation and PI(3,4,5)P₃ production, even though the response was less asymmetric than the one in untreated cells [54]. It was found that latrunculin-treated *Dictyostelium* cells could accumulate PI3K and PI(3,4,5)P₃ on both ends and PTEN in the middle when two chemoattractant-filled micropipettes were placed on opposite sides of the cells, indicating that they do not have

predefined fronts and backs. Rather, a cell can form two fronts and a 'back' at the midline, reminiscent of a dividing cell [53[•],55]. By contrast, polarized cells are incapable of stably responding simultaneously at the front and back [27]. It is important to note that, in these studies, the level of the inhibitory effect of latrunculin on actin polymerization was not quantified. Hence, one cannot rule out the possibility that inhibition of F-actin assembly in these studies was incomplete and that remaining F-actin polymerization was sufficient to generate the partial PI3K localization and PI(3,4,5)P₃ production observed in these latrunculin-treated cells. In fact, another study, in which latrunculin inhibition of actin polymerization was carefully monitored, suggested the presence of a basal level of PI3K at the plasma membrane of *Dictyostelium* cells, independent of chemoattractant stimulation, and that the *de novo* recruitment of PI3K requires newly synthesized F-actin [46^{••}]. Furthermore, a recent report suggests that relatively low PI3K activity, resulting in a small increase in PI(3,4,5)P₃ levels, is sufficient to achieve maximal translocation of the PH domain-containing protein CRAC to the plasma membrane [24].

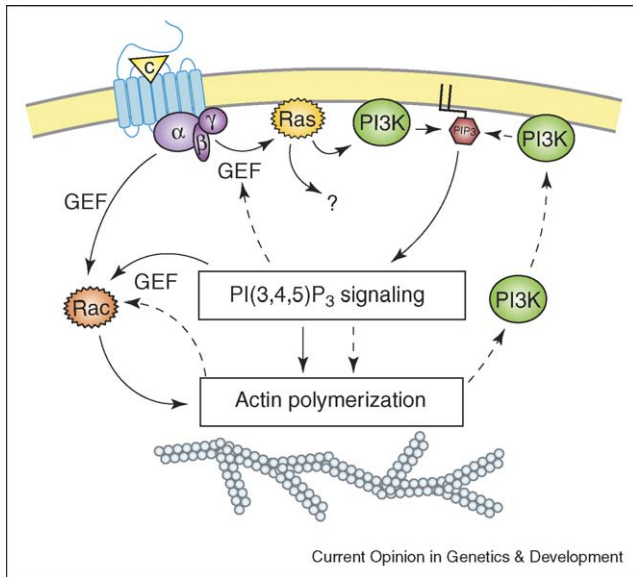
One can then postulate that, even though robust PI3K translocation to the membrane requires F-actin polymerization, directional sensing could occur in the absence of a functional cytoskeleton because the preexisting membrane pool of the enzyme could spatially amplify the PI(3,4,5)P₃ response to levels sufficient to induce the asymmetrical localization of some PH domain-containing proteins. It could be further hypothesized that, depending on the nature of the PH domain-containing protein or any other protein that is responsive to PI(3,4,5)P₃, proteins might respond differently depending upon their binding affinity for PI(3,4,5)P₃ and/or the cytoskeleton. Some proteins might be involved in directional sensing whereas others could be implicated in signal amplification and cell polarity. Consistent with different thresholds of signals eliciting an overlapping set of responses, GFP-conjugated proteins with different PI(3,4,5)P₃-binding PH domains can give distinct spatial and temporal read-outs of the same underlying PI(3,4,5)P₃ signal, enabling distinct biological responses to one signal [6[•]]. Moreover, genetic studies have suggested that different PH domain-containing proteins have different functions in modulating polarity and directionality: *Dictyostelium* Akt/PKB is involved in the establishment of cell polarity [7], CRAC regulates adenylyl cyclase activation as well as chemotaxis [56], whereas PhdA and RacGEFs might directly regulate the site of F-actin polymerization [9^{••},16].

Other evidence supporting the idea that gradient sensing can be separated from cellular polarity comes from the observation that cAMP stimulation of latrunculin-immobilized *Dictyostelium* cells induced the same biphasic PI(3,4,5)P₃ response as in control cells, including the formation of multiple self-organizing patches of

PI(3,4,5)P₃ [49[•]]. Interestingly, local PI(3,4,5)P₃-patch formation has been proposed to dominate chemotaxis of fibroblasts and dendritic cells, which move in shallow platelet-derived growth factor and C5a gradients respectively [57[•]]. It was shown that small and localized transient patches of PI(3,4,5)P₃ drive localized F-actin polymerization and local lamellipod extension, resulting in small discrete turns in the direction of the gradient. Even though a role for the actin cytoskeleton in the formation of these PI(3,4,5)P₃ patches was not investigated, these results suggest that spatial sensing is localized and is independent of global cell polarization.

Insights into a potential positive feedback loop working to locally amplify the PI(3,4,5)P₃ production come from studies revealing that chemoattractant stimulation of *Dictyostelium* cells is rapidly translated into a transient activation of the Ras family of small G-proteins, which seems both to precede and to be independent of PI3K activity [46^{••},58]. Furthermore, activated Ras exclusively localizes to the presumptive leading edge of chemotaxing cells when cells are placed in a chemoattractant gradient. Interestingly, PI3K activity is significantly reduced either in cells lacking the Aimless RasGEF or by the overexpression of a Ras dominant-negative mutant [46^{••}]. Along with the finding that PI3K activation requires a functional Ras-binding domain [22], these observations suggest that Ras proteins act upstream and might directly regulate PI3K activity at the leading edge of chemotaxing cells. Moreover, cAMP stimulation of latrunculin-treated cells induces a strong Ras activation and a significant translocation of PH domain-containing proteins, whereas both PI3K activity and translocation to the cortex are drastically reduced in comparison with in untreated cells [46^{••}]. However, it was also observed that, although Ras activation appears to require neither PI3K activity nor F-actin polymerization, the Ras activation level is reduced in the presence of a PI3K inhibitor or latrunculin, suggesting that Ras operates downstream as well as upstream of PI3K and F-actin. Furthermore, spontaneous Ras activation is upregulated in randomly moving *pten*-null cells, suggesting that Ras itself is partly regulated by events involving PI(3,4,5)P₃ and F-actin polymerization. These studies suggested that Ras and PI3K activation are tightly regulated through a positive feedback loop (Figure 2) [46^{••}]. A feedback loop between preexisting membrane pools of PI3K and Ras could explain the actin-independent directional sensing whereas, as observed in other studies, localization of PI3K and PI(3,4,5)P₃ are enhanced further in cells with a functional cytoskeleton. This suggests that differences in gradient amplification between latrunculin-treated and -untreated cells represent feedback from the cytoskeleton of a polarized cell to the signaling apparatus [34,46^{••},53[•],54]. The self-organizing nature of the Ras-PI3K circuit is supported by the observation that its spontaneous activation, presumably originating from the random interaction of activated Ras molecules with

Figure 2



Positive feedback loops amplify the chemoattractant signal to produce a strong PI(3,4,5)P₃ response. Chemoattractant (c) stimulation induces the heterotrimeric G-proteins (α, β and γ)-dependent activation of the small G-proteins Rac and Ras. Activated Rac then stimulates F-actin polymerization, and Ras activates a membrane-bound pool of PI3K, thereby inducing PI(3,4,5)P₃ production. PI(3,4,5)P₃ signaling then leads to increased F-actin polymerization, and both events participate in enhancing the Rac activity by promoting the localized activation of RacGEFs. PI(3,4,5)P₃ is also believed to increase Ras activation. F-actin assembly then promotes the translocation of cytoplasmic PI3K to the cell cortex, which increases the localized PI3K activity, leading to robust PI(3,4,5)P₃ production. Other as yet unidentified Ras effectors (?) are also likely to participate in this amplification process. The solid lines represent the first signaling events, whereas the dashed lines stand for positive feedback signaling.

membrane-localized PI3K at the inner surface of the plasma membrane, might be the basis of random cell movement (AT Sasaki, C Janetopoulos, K Takeda, S Lee, LW Sundenheimer, R Meili, PN Devreotes and RA Firtel, unpublished). Sasaki *et al.* postulate that, during chemotaxis, chemoattractant-induced G-protein signaling causes biased localized activation of this same intrinsic Ras–PI3K circuit, which controls random cell movement, and which is then amplified through F-actin.

Actin-dependent positive feedback loops

The exact role of actin polymerization in the proposed Ras–PI3K feedback loop implicated in *Dictyostelium* chemotaxis is not known. However, accumulating evidence from studies in both neutrophils and *Dictyostelium* underlines an important role for Rac GTPases in actin-dependent positive feedback loops implicated in amplifying the PI(3,4,5)P₃ response at the leading edge of chemotactic cells. Small GTPases of the Rho family are key regulators of the actin–myosin cytoskeleton during chemotaxis. In neutrophils, RhoA controls the retraction of the posterior,

through actin–myosin contractility, whereas Cdc42 and Rac guide the direction of the response and regulate actin polymerization at the front [59]. A role for Rho GTPases in a self-organizing actin–PI3K–PI(3,4,5)P₃ feedback loop was suggested following the observation that latrunculin treatment as well as pharmacological inhibition of PI3K or Rho GTPases significantly blocks the PI(3,4,5)P₃ accumulation induced not only by the chemoattractant but also by exogenously delivered PI(3,4,5)P₃ [44,45]. It was later shown that Rac activity, but not that of Cdc42 or RhoA, is necessary and sufficient for the observed chemoattractant-stimulated accumulation of PI(3,4,5)P₃ and actin polymers in neutrophils. Although Cdc42 does not participate in the PI(3,4,5)P₃ feedback loop, Cdc42 appears to determine where PI(3,4,5)P₃, F-actin and active Rac accumulate [60]. The presence of a feedback loop between PI3K and Rac provides an explanation for apparently contradicting reports in which PI3K activity was shown to function upstream of Rac and Cdc42 activation [61–63], and Rac and Cdc42 have been shown to function upstream of PI(3,4,5)P₃ generation [64,65]. Interestingly, expression of a constitutively active Rac is insufficient to promote an asymmetric response, suggesting that both chemoattractant and exogenous PI(3,4,5)P₃ trigger the polarity-inducing machinery by mechanisms either upstream of or parallel to Rac activation [60].

Dictyostelium Rac GTPases co-localize with F-actin and regulate its assembly at the leading edge of chemotactic cells [66–68]. Interestingly, activation of the RacB isoform exhibits a biphasic kinetic paralleling that of F-actin polymerization, in which the second peak of RacB activation shows a dependence on PI3K activity [9**]. The same study identified a RacGEF specific for RacB, RacGEF1, which localizes to sites of F-actin polymerization. Inhibition of this localization reduces RacB activation, suggesting the presence of a feedback loop from RacB through F-actin polymerization to RacGEF1. RacGEFs are also expected to play a role in the Rac-dependent feedback loop in neutrophils, particularly in the arc of the loop where PI(3,4,5)P₃ activates Rac. Several PI(3,4,5)P₃-dependent GEFs were indeed shown to regulate leukocyte chemotaxis, including Vav [10,11], the PI(3,4,5)P₃-sensitive Rac GEF P-REX1 [12–14], the protein downstream of CRK-2 (DOCK2) [69], and DOCK180 [70]. The molecular mechanisms by which Rac could promote accumulation of PI(3,4,5)P₃ are less intuitive. In light of the recent finding that actin polymerization increases the translocation of PI3K to the plasma membrane of chemotactic *Dictyostelium* cells [46**], one could propose that the contribution of Rac to PI(3,4,5)P₃ accumulation is a consequence of its role in actin polymerization. Rac effectors of the WAVE/SCAR and WASP family of proteins, which regulate F-actin polymerization through their action on the Arp2–Arp3 complex, are also likely to play a role in that feedback loop, because some of them were shown to be regulated by PI(3,4,5)P₃ [71,72].

Conclusions

The reports of the past few years enable us to better understand the molecular mechanisms underlying directional sensing and signal amplification in chemotactic cells, and to propose a new model in which chemoattractants simultaneously trigger two G-protein-dependent stimulatory pathways: the Ras–PI3K pathway, which is involved in directional sensing; and the Rac pathway, which promotes actin polymerization. Through positive feedback loops and signaling crosstalk linking PI(3,4,5)P₃ to Rac to F-actin polymerization and back to PI3K activity, these two pathways work together to optimally amplify and maintain the PI(3,4,5)P₃ asymmetry in chemotactic cells (Figure 2). Positive feedback signaling thus seems to be the mechanism by which chemotactic cells locally amplify the chemoattractant signal, enabling them to interpret and respond to weak and shallow gradients of chemoattractants. However, many questions remain unanswered, such as what regulates the activation of Ras and its location, as well as how PI(3,4,5)P₃ regulates Ras and PI3K activities. Future studies should focus on these questions and also on identifying the signal desensitization mechanisms, because adaptation also seems to play a crucial role in directional sensing. Indeed, the findings that many components involved in the gradient-sensing machinery present a biphasic response to chemoattractant stimulation, and that the second phase is implicated in the polarization and amplification of the response, suggest that attenuation of the strong initial response might enable a cell to interpret the extracellular gradient by accentuating the differences in receptor occupancy.

Update

Recent work has demonstrated that the steady-state localization of PH domain-containing proteins at the leading edge of chemotaxing cells is dynamically maintained by rapid recycling of individual proteins [73[•]]. The dynamic nature of the interaction between PH domains and PI(3,4,5)P₃ probably confers sensitivity and plasticity to the system by enabling the rapid redistribution of the PH domain-containing proteins. Consequently, the reported differences in spatial and temporal patterns of membrane localization of distinct PH domain-containing proteins [6[•]] could be explained by a difference in the kinetics of their association and dissociation from the plasma membrane, which would be dictated by their individual affinity for PI(3,4,5)P₃.

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