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Review

Guiding cell migration through directed extension and stabilization of pseudopodia

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Abstract

Cell migration requires establishment of a single pseudopodium in the direction of movement. Here we highlight recent advances in our understanding of the molecular signaling mechanisms that regulate formation of pseudopodia. We discuss how signal transduction processes are spatially and temporally organized to establish cell polarity through directed extension and stabilization of dominant pseudopodia. We also highlight recent advances in technology that will further the understanding of signaling dynamics specific to pseudopodia extension and cell migration.

Keywords: Pseudopodia; Chemotaxis; Gradient sensing; Cell polarity; Motility; Migration

Introduction

Directed cell movement is an essential component of many critical biological processes including embryonic development, wound repair, and immune surveillance. However, deregulated or inappropriate cell migration can contribute to pathological states such as rheumatoid arthritis and tumor cell metastasis. A better understanding of the fundamental mechanisms that impact cell movement is central to improving therapeutics for the pathological conditions associated with defective cell motility. Directed migration (or chemotaxis) is a carefully orchestrated cellular event that is composed of tightly integrated processes for sensing directional cues, protruding membrane structures, and regulating turnover of adhesive contacts with the underlying substratum. Although many cells randomly protrude membrane structures from their surface when in the presence of a uniform concentration of chemoattractant, in response to a chemoattractant gradient, cells rapidly adopt a persistent morphological polarity with extension of a single pseudopodium in the direction of increasing chemoattractant. The initial detection and continual navigation of cells toward chemoattractant are initiated by membrane-associated receptors that convert extracellular chemoattractant concentrations into highly orchestrated internal signaling events. The receptor-generated signals eventually converge in the localized polymerization of F-actin, which is manifested as an asymmetric reorganization of the actin–myosin cytoskeleton. However, the precise mechanisms of coupling and modulation of signals generated from different membrane receptors to the processes regulating pseudopodia extension are not entirely understood. In this review, we highlight recent insights into the molecular mechanisms that establish formation of a dominant pseudopodium in chemotaxis and discuss perspectives of how cells spatially and temporally integrate these mechanisms to maintain persistent polarity and directed cell migration.

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Protrusion of a pseudopodium initiates morphological polarity

Cells exposed to a gradient of chemoattractant acquire asymmetric polarity marked by protrusion of membrane structures in the direction of increasing chemoattractant concentration. These protrusive structures can be large, sheet-like lamellipodia, or thin, finger-like filopodia, collectively termed pseudopodia. The pseudopodia region immediately after activation contains a large number of new F-actin filaments [1] and it is now well accepted that pseudopodia protrusion, and more generally, changes in cell shape in response to external stimuli are powered, at least in part, by actin polymerization [2]. Indeed, physical analyses show that actin polymerization can provide a protrusive force sufficient to overcome the resistance of the cell membrane [3]. While over the past several years there have been significant advances in understanding the molecular mechanisms that underlie cell migration; however, the complete set of molecules involved in signaling F-actin polymerization to initiate pseudopodia formation is still unknown and the exact relations between them are unclear. Many different molecules have been implicated in F-actin polymerization, pseudopodial dynamics, and organizing the migration machinery of the cell, including small Rho GTPases, phosphatidylinositol-3 kinases (PI(3)Ks), Ca^{2+}-regulated proteins, mitogen-activated protein kinases (ERK/MAPK), protein kinase C (PKC), phospholipase C (PLC), and tyrosine kinases (see recent reviews, Refs. [4–6]). The large number of molecules involved in signaling F-actin polymerization and cell motility is not surprising given the miscellany of cellular activities that must be spatially and temporally orchestrated as a cell migrates in response to a wide array of extracellular cues. Moreover, the overall signaling of motility is additionally complicated by cross-talk between many other signal transduction pathways that control programs of cell survival or death, secretion, and gene transcription. However, despite the apparent complexity, upon stimulation by a chemoattractant gradient, these signaling pathways eventually converge on the hallmark of a chemotactic cell, the establishment of a leading pseudopodium.

Our present understanding of the formation of pseudopodia to achieve directed cell migration is illustrated in Fig. 1. Initially, nonmotile cells are attached to an underlying extracellular matrix (ECM), most likely through integrin receptors on the cell surface. Upon exposure to a soluble gradient of growth factor or chemoattractant, which binds and activates cell surface receptors, cells directionally sense the attractant and respond by local activation and amplification of signaling events on the side facing the gradient [7]. These signals facilitate localized actin polymerization leading to membrane protrusion in the direction of the gradient [8,9]. This marks the first sign of morphological polarity with establishment of a well-defined leading pseudopodium and cell body compartment. Importantly, initial membrane protrusion of a pseudopodium is independent of actual cell body translocation or chemotaxis [2] and is independent of integrins and the ECM [10,11]. However, as discussed below in detail, attachment of the protruding pseudopodium to the substratum stabilizes the pseudopodium, providing positive feedback signals to maintain directional actin polymerization.

While forward protrusion of the leading membrane is driven by actin polymerization, the overall mechanism of cell translocation requires components in addition to pseudopodia protrusion. Formation of adhesive contacts is critical for the generation of a contractile force, providing sites for traction between the supporting ECM and migrating cell [12]. Adhesion complexes are formed at these attachment sites that contain integrin receptors, kinases, adaptor proteins, and other signaling and structural molecules that not only tie the cytoskeletal framework to the ECM but also mediate activation of numerous signaling cascades that include Rho family GTPases and ERK kinases [13]. Myosin II has been shown to interact and link these adhesion complexes with actin filaments to produce a contractile force that serves to pull the cell body forward [14]. This contributes to a net translocation of the cell body forward relative to the pseudopodium. Indeed, pseudopodia that do not establish adhesive contacts to the ECM rapidly retract back into the cell body [10,11]. This suggests that new integrin ligation events at the leading front of the extending membrane provide necessary signals to fine-tune and maintain growth, while suppressing retraction mechanisms. In general, chemotaxis can be viewed as a cyclic process comprised of distinct, but highly integrated, components that include directional sensing, pseudopodium protrusion and stabilization, cell body contraction, and adhesion turnover.

Distinct cellular mechanisms interpret directional cues

While all living cells can sense their extracellular surroundings, directional sensing requires a cell to spatially integrate nonuniform concentrations of stimuli across its body and locate the direction of increasing concentration. The intriguing capability of cells to spatially detect and internally process external stimuli is a fundamental and necessary step in chemotaxis [15]. In the absence of a cue for directionality, cells move randomly and protrude pseudopodia in multiple directions [16]. These cells appear to migrate for a short time, stopping often to change directions. However, in the presence of a gradient of chemoattractant, their movement becomes persistent in the direction of the chemoattractant source guided by a dominant pseudopodium that overrides further, random protrusive events [17]. Recent studies are beginning to shed some light on how external signals are interpreted to produce the formation of a dominant pseudopodium [17,18]. Several lines of evidence suggest that distinct mechanisms control directional sensing to establish intracellular signaling polarity before exhibition of any morpho-

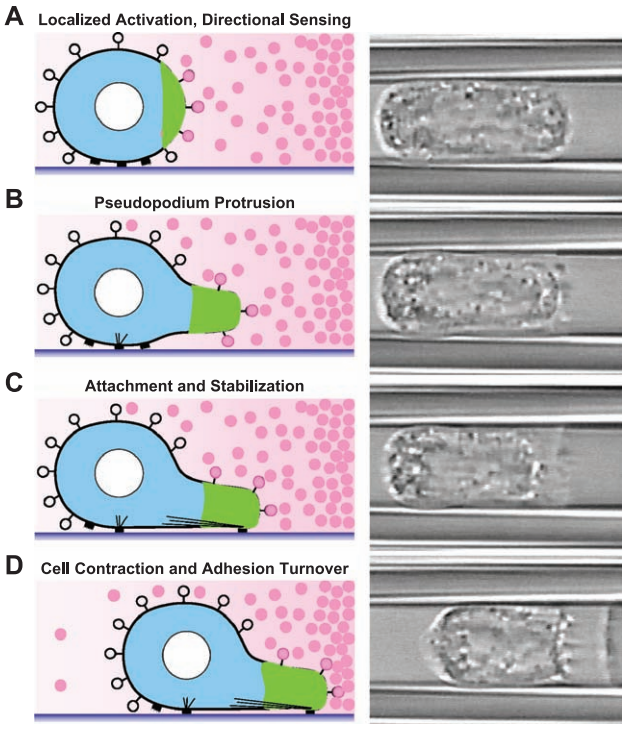

Fig. 1.

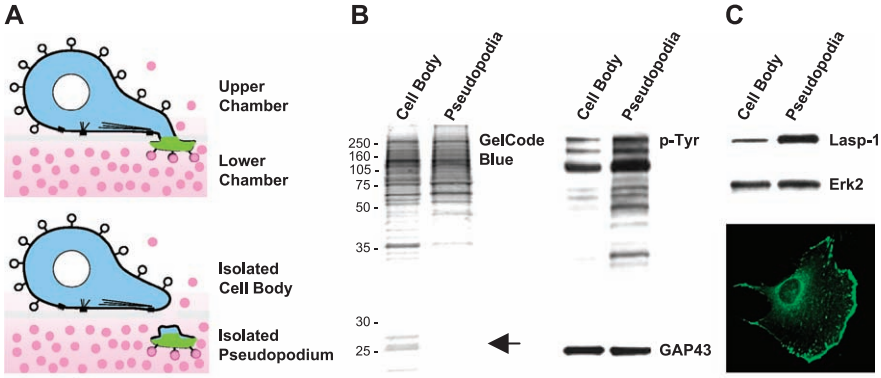

Fig. 2.

Fig. 1. The migration cycle. Schematic of the component processes comprising directed cell migration (left panel) and phase images of a human neutrophil crawling inside a glass micropipette filled with fMLP (right panel). (A) Initially, the cell is attached to a supporting substrate most likely through integrin receptors on the cell surface (squares). Upon exposure to a chemoattractant gradient, directionality is established by localized receptor binding that leads to activation and accumulation of signaling events on the side of the cell facing the highest chemoattractant concentration. (B) The activated signaling events facilitate localized F-actin polymerization leading to membrane protrusion that is independent of integrins and the ECM. (C) The protruding pseudopodium attaches to the ECM, which not only ties the advancing pseudopodium to the supporting substrate but also mediates signaling events that serve to enhance stabilization and maintain pseudopodial extension, presumably via positive feedback mechanisms. (D) A net forward translocation of the cell is achieved as the cell body contracts and releases attachment sites at the rear of the cell. Cell movement then persists in the direction of increasing concentration as the cell continues to sense the gradient and create new extensions and attachment sites continuously coordinated with contraction and adhesion turnover. (Micrographs by D. Chodniewicz and D.V. Zhelev).

Fig. 2. Biochemical separation of polarized cells into the leading pseudopodium and cell body [31]. (A) Schematic depicting the method for isolation of pseudopodia. Cells are allowed to attach to a microporous filter membrane coated with ECM protein. Extension of pseudopodia is initiated by a chemoattractant gradient diffusing from the lower chamber into the upper chamber. Only pseudopodia extend through the small pores in response to the chemoattractant gradient, allowing polarized cells to be fractionated into cell body compartments on the upper surface and pseudopodial compartments on the lower surface for biochemical analysis as previously reported [31]. (B) GelCode Blue stain of total proteins shows that nuclear histones are present only in the cell body compartment demonstrating the pure fractionation of the cell body and pseudopodial fractions. Arrowhead indicates nuclear proteins absent from pseudopodia fraction. Western blot of phosphotyrosine reveals dramatic spatial activation of proteins in purified pseudopodia. (C) Large-scale proteomic and biochemical analysis of fractionated cell body and pseudopodia compartments reveals subcellular localization of proteins, such as Lasp-1, which is confirmed by visualization of GFP-Lasp-1 [41].

logical polarity, even in the absence of a functional actin cytoskeleton [19]. These mechanisms are initiated by localized activation of receptor-mediated signaling pathways at the edge of the cell that faces the chemoattractant source [19,20].

The signaling mechanisms by which cells internally set up polarity and initiate chemotaxis are directly linked to receptor activation patterns; however, the signaling events following asymmetric (or gradient) receptor activation are unclear. The distinct polarized morphology of migrating cells in response to shallow chemoattractant gradients (2–5% difference in concentration across the body of the cell) suggests that directionally sensing cells amplify and accumulate internal signals relative to the external gradient of chemoattractant. However, how these early directionality signals are established and maintained in response to a gradient is poorly understood. Recent investigations indicate that PI(3)K is locally activated on the side of the cell facing the chemoattractant gradient [20]. The activated PI(3)K is responsible for the accumulation of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) lipids that serve to localize other signaling molecules such as pleckstrin homology (PH) domain-containing proteins to the inner face of the plasma membrane [20]. Moreover, PH domain signals are persistently activated in cells exposed to a gradient and only transiently in cells exposed to a uniform concentration of chemoattractant [21]. These findings provided the first evidence for spatial formation and amplification of intracellular signals in cells responding to a chemoattractant gradient, and suggest that cells sensing a chemoattractant gradient activate distinct molecular mechanisms to establish directionality [22].

While signals are activated at the front of the cell during directional sensing, other molecular mechanisms operate to "desensitize" the back of the cell to polarization. In response to a gradient of chemoattractant, the rear of a polarized cell is less responsive toward the chemoattractant when the gradient is repositioned to the back of the cell [19]. This prompted the hypothesis that while signal excitation and amplification occur locally on the side facing the gradient, there is a global inhibition throughout the cell body. Hence, the front facing the gradient will exhibit increased or sustained sensitivity to chemoattractant, while the side and rear of the cell will be less responsive, experiencing inhibition that is greater than the localized activation at the front. This hypothesis implies that excitatory as well as inhibitory mechanisms must be spatially restricted to the anterior and posterior poles of polarized cells. Indeed, molecules such as the phosphoinositide 3-phosphatase (PTEN) have been shown to be restricted from the leading edge and accumulated on the sides and rear of the cell [21], which may explain the apparent polarized sensitivity and desensitization to chemoattractant.

Desensitization is an adaptive cellular response by which cells, after initial receptor stimulation, prevent subsequent activation to either the same stimulus (homologous desensitization) or a different stimulus (heterologous desensitization). Both homologous [23] and heterologous [24] desensitizations are expected to affect the ability of a cell to migrate through tissue in vivo by reducing the cell's apparent threshold of response to chemoattractant. Homologous desensitization involves the coordinated actions of G-protein-coupled receptors serine/threonine kinases (GRKs) and the arrestin family of proteins [25]. During homologous desensitization, GRKs phosphorylate G-protein-coupled receptors (GPCRs) [26], which promote the binding of the receptor to arrestins. Arrestin-bound GPCRs hinder the receptor's coupling to G-proteins and have been shown to result in up to 80% attenuation of receptor signaling [27]. Heterologous desensitization has been linked to receptor cross phosphorylation or from processes distant from the receptor [28], and is a result of action of secondary messengers such as protein kinase A (PKA) or PKC. Desensitization to a simultaneous or sequential exposure to the same of different chemoattractant may play a key role in maintaining directionality, especially in vivo where a migrating cell is exposed to an array of stimuli for prolonged periods of time. This effect has been demonstrated for neutrophil migration in which cells were allowed to crawl in a gradient of one chemoattractant, while another chemoattractant was uniformly present in the experimental chamber. The uniform exposure of neutrophils to *N*-fornyl-Met-Leu-Phe (fMLP) and complement fragment C5a (C5a) desensitized the response of the cell toward gradients of leukotriene B_4 (LTB_4) and interleukin-8 (IL-8) [29]. Interestingly, the same chemoattractants produce a similar desensitization of the neutrophil cytotoxic responses [28]. The molecular mechanisms underlying desensitization likely involve complex biochemical events including partitioning of proteins between the different poles, regulated posttranslational protein modifications, differential protein turnover rates, and spatial and temporal formation of distinct signaling scaffolds.

Examining protrusion of a dominant pseudopodium

The molecular basis for the formation of a pseudopodium, including regulation of F-actin polymerization, microtubule dynamics, and spatiotemporal regulation of signaling molecules that coordinate these processes, has been under intense investigation. Recent progress has been made studying individual cells responding to a point source of chemoattractant diffusing from a micropipette by tracing the spatiotemporal organization of signals using immunofluorescent technology [18–20] or analyzing the dynamics of extending and retracting pseudopods from nonadherent cells [8,11,30]. However, a detailed biochemical examination of these proteins and their binding partners following gradient receptor activation via a micropipette has not been possible due to the lack of cellular material for analysis. To begin to address this issue, a cell fractionation assay that isolates the

leading pseudopodium from its cell body using a microporous filter [31] has been utilized to unravel some of the spatiotemporal details of signaling cascades involved in cell polarization, including mechanisms of protein translocation, activation, posttranslational modifications, and formation of complex multiprotein scaffolds (Fig. 2). This system has the capability to monitor detailed biochemical changes in the front and back of polarized cells responding to a chemoattractant gradient. It has demonstrated, like traditional pseudopodia formation on two-dimensional surfaces, that pseudopodia require localized Cdc42 and Rac activity, focal adhesion kinase (FAK), and p130CAS (CAS) activation [31]. Additionally, such an assay also revealed that ERK mediates pseudopodia extension via regulation of myosin light chain kinase (MLCK) and phosphorylation of myosin light chain (MLC) [14]. The phosphorylation of MLC at its regulatory serine 19 was localized in the pseudopodia and associated with persistent amplification of ERK and MLC kinase activity [32]. In a distinct pathway, RhoA regulated MLC phosphorylation through inhibition of myosin phosphatase activity [32] to control pseudopodial dynamics and contractile forces. Moreover, the specific isolation of cellular protrusions in a state of growth or retraction for protein analysis has provided insight into the regulation of integrin stabilization of pseudopodia, including retraction mechanisms and cellular responses to repulsive cues [32]. Importantly, it is known that in vivo, cells extend pseudopodia projections through small openings in the vasculature [33] and ECM as a necessary process of immune cell intravasation as well as during pathological processes associated with tumor cell metastasis [34]. Therefore, this model recapitulates some of the physiological events associated with cell migration and is ideal for studying detailed molecular signaling mechanisms responsible for this process. Further biochemical analysis of purified pseudopodia and cell body compartments, combined with traditional approaches that visualize pseudopodial dynamics in living cells, such as high-resolution confocal microscopy and time-lapse imaging [31], will considerably advance our understanding of pseudopodial molecules and spatiotemporal mechanisms by which they are regulated to control pseudopodial dynamics.

Maintaining polarity and persistent migration

Initially, the polymerization of F-actin plays a critical role in the extension of a pseudopodium as it extends away from the cell body. However, after reaching a maximum extension, the pseudopodium retracts if it is not stabilized by adhering to a supporting substrate, even in the continued presence of a chemoattractant gradient [11]. Upon stabilization of an advancing pseudopodium by attachment, cell movement commences in the direction of increasing concentration of chemoattractant as the cell continuously creates new extensions and attachment sites at the leading edge of the pseudopodial region [35]. The crawling of the cell is then coupled with cell body contraction and release of attachment sites at the rear of the cell to prevent the cell from being overextended along its crawling path. Clearly then, molecular signals and processes are differentially controlled at the advancing front and trailing rear of a migrating cell. The maintenance of polarity at the leading front appears to be mediated, at least in part, by a set of interlinked positive feedback loops involving Rho GTPases, phosphoinositide 3-kinases, integrins, and microtubules [18]. CAS/Crk and FAK also play key roles, as there is enriched activation of these proteins at the front of polarized cells following integrin attachment [31]. Constitutive activation of these signals prevents pseudopodium retraction when the chemoattractant is removed due to persistent positive signaling through focal adhesions [31]. However, the migration of cells involves more than initial and subsequent sustained polarization. During crawling, cells must persistently maintain their spatial and functional asymmetry even when they change direction, which is common for neutrophils as they follow moving bacteria [36]. Once cells are en route they most likely must make a series of turns to navigate through the extracellular matrix tissues. When a polarized cell makes a large-scale turn in the direction of a new chemotactic signal, it may execute a series of small-scale turns led by the pseudopodium [37] or it may retract the extended pseudopodium and repolarize in the new direction of travel [20]. The factors regulating how a cell "decides" to turn remain to be determined, though recent evidence suggests that different isoforms of PI(3)Ks are involved [38].

Cell migration in vivo is far more elaborate than merely guiding a dominant pseudopodium in the direction of an increasing stimulus. Chemotaxis is further complicated by the potential of being directed by a wide range of different attractants, as well as repulsive cues that can be soluble, diffusible factors or repellent substratum. Additionally, many of the factors that affect cellular behavior may be concomitantly expressed in the extracellular surroundings of a migrating cell. For example, at the site of bacterial infection, a composite of attractive and repulsive cues can be produced by endothelial and epithelial cells, as well as the invading pathogen. While there has been considerable insight over the past several years into the activation of signals in response to a single attractant, important questions remain to be answered as to how cells interpret simultaneous and sequential exposure to multiple attractive and repulsive cues. Evidence indicates that cells have established an intracellular signaling hierarchy that prioritizes activation in opposing chemotactic gradients, allowing for some chemoattractants to dominate others [39]. In addition, cells must distinguish between different concentrations of the same or different stimuli that is complicated by the ability of cells to temporally modulate their responses either positively or negatively, depending on their prior state [40]. This is of particular importance in vivo as cells are exposed to stimuli for prolonged periods

of time, and as a result, desensitization of the cell body is likely to play a key role in cellular navigation by downregulating responses to chemoattractant.

Conclusions and perspectives

Explicating the molecular mechanisms that underlie directed cell migration has proven to be a challenging undertaking, as cell migration is comprised of several complex molecular and cellular processes that are coordinated in both time and space. In response to extracellular cues, cells detect and advance pseudopodia in the direction of movement. The extending pseudopodia adhere to the supporting substrate, providing stable contacts for traction and cell body contraction. The front and back of a migrating cell communicate effectively as new adhesions form at the leading edge and are released at the rear of the cell. In vivo, a model of cell migration is more complex, as a migrating cell recognizes various stimuli at a range of concentrations and differentially processes its external environment into internal signals to maintain persistent navigation. The cellular components comprising chemotaxis including gradient sensing, pseudopodia protrusion, attachment, and detachment have been under intense investigation, however, usually as separate elements. For effective chemotaxis to occur, these processes need to be highly inter-coordinated and regulated by numerous intracellular signaling pathways and dynamic cellular systems. Thus, the future challenge will be to understand not only how each of these complex components is individually regulated, but also how they spatially and temporally communicate with each other to achieve overall chemotaxis. To answer these questions, new technologies are continually emerging to examine spatial and temporal activation of the migratory components, including novel posttranslational specific antibodies, FRET biosensors, and photomanipulative techniques that activate or inactivate signaling molecules. The study of cell migration therefore seems poised for novel breakthroughs by combining these technologies with the new method for large-scale biochemical comparisons of the pseudopodium and cell body compartments. The combination of these approaches will significantly advance our current understanding of how cells establish directionality and maintain persistent navigation. Ultimately, the assembly of information gained from these technologies along with protein structure, proteomics, cell imaging, and signal transduction will provide a meaningful view of pseudopodial dynamics and cell chemotaxis, and provide the informational basis for computer models and rational drug design.

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