The tumor suppressor Lgl1 regulates front-rear polarity of migrating cells

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Cell migration is a highly integrated, multistep process that plays an important role in physiological and pathological processes. The migrating cell is highly polarized, with complex regulatory pathways that integrate its component processes spatially and temporally.\(^1\)

The *Drosophila* tumor suppressor, Lethal (2) giant larvae (Lgl), regulates apical-basal polarity in epithelia and asymmetric cell division.\(^2\) But little is known about the role of Lgl in establishing cell polarity in migrating cells. Recently, we showed that the mammalian Lgl1 interacts directly with non-muscle myosin IIA (NMIIA), inhibiting its ability to assemble into filaments in vitro.\(^3\) Lgl1 also regulates the cellular localization of NMIIA, the maturation of focal adhesions, and cell migration.\(^3\) We further showed that phosphorylation of Lgl1 by aPKC\(_\zeta\) prevents its interaction with NMIIA and is important for Lgl1 and acto-NMII cytoskeleton cellular organization.\(^4\) Lgl1 is a critical downstream target of the Par6-aPKC cell polarity complex; we showed that Lgl1 forms two distinct complexes in vivo, Lgl1-NMIIA and Lgl1-Par6-aPKC\(_\zeta\) in different cellular compartments.\(^4\) We further showed that aPKC\(_\zeta\) and NMIIA compete to bind directly to Lgl1 through the same domain. These data provide new insights into the role of Lgl1, NMIIA, and Par6-aPKC\(_\zeta\) in establishing front-rear polarity in migrating cells. In this commentary, I discuss the role of Lgl1 in the regulation of the acto-NMII cytoskeleton and its regulation by the Par6-aPKC\(_\zeta\) polarity complex, and how Lgl1 activity may contribute to the establishment of front-rear polarity in migrating cells.

Cell migration is a highly integrated multistep process that orchestrates embryonic morphogenesis, contributes to tissue repair and regeneration, and drives disease progression in cancer. A cell must be polarized in order to migrate, which means that molecular processes at the front and the back of a moving cell are different. Cell polarity is important for distinguishing between random cell migration, in which cells migrate in all directions in a non-coordinated manner, and directed cell migration, in which cells respond to polarizing cues to migrate in a given direction. Although in both cases, cell polarity is required to generate a front-rear axis, polarizing cues stabilize the front–rear axis determining the extent of persistent directional cell movement.\(^3\)

Many different external cues initiate front-rear polarity, including growth factors and the extracellular matrix. Conversion of these cues into directional migration requires global changes in cell organization by protein complexes and signaling pathways that control the actomyosin cytoskeleton. Non-muscle myosin II (NMII) is an actin-based motor that converts chemical energy into force and movement, and thus functions as a key regulator of the eukaryotic cytoskeleton. NMII is an important motor protein present in all cell types; it participates in crucial processes, including cytokinesis, surface attachment, and cell movement.\(^5\) NMII molecules are comprised of two heavy chains of 230 kDa, two 20 kDa regulatory light chains (RLCs) that regulate NMII activity, and two 17 kDa essential light chains (ELCs) that stabilize the heavy chain structure (Fig. 1A). NMII heavy chain is composed of a globular...
Figure 1. (A) Structure of NMII and filament formation. The subunit and domain structure of NMII, which forms a dimer through interactions between the α-helical coiled-coil rod domains. The globular head domain contains the actin-binding regions and the enzymatic Mg$^{2+}$-ATPase motor domains. The essential light chains (ELCs) and the regulatory light chains (RLCs) bind to the heavy chains at the lever arms that link the head and rod domains. NMII molecules assemble into bipolar filaments through interactions between their rod domains. These filaments bind to actin through their head domains, and the ATPase activity of the head enables a conformational change that moves actin filaments in an anti-parallel manner. Bipolar NMII filaments link actin filaments together in thick bundles that form cellular structures such as stress fibers. (B) Lgl1 inhibits NMIIA filament assembly. The assembly process is governed by electrostatic interactions between adjacent α-helical coiled-coil rods containing alternating charged regions with specific periodicity (blue and red positively and negatively charged regions, respectively). Lgl1 binds to a negatively charged region of NMIIA, and conversely, NMIIA interacts with the positively charged region of Lgl1. Thus, the interaction between Lgl1 and NMIIA is electrostatic. Lgl1 binding to a NMIIA monomer masks its negatively charged region, inhibiting its ability to interact with another monomer and assemble into filaments. In addition, Lgl1 binding domain in NMIIA resides between assembly competence domains 1 and 2, that are crucial for NMII filament assembly.31
head containing the actin-binding and force-generating ATPase domains, followed by a large coiled-coil rod that terminates with a short non-helical tailpiece. To carry out its cellular functions, NMII assembles into dimers and higher order filaments by interactions between the coiled-coil regions of the heavy chains. The assembly process is governed by electrostatic interactions between adjacent coiled-coil rods containing alternating charged regions with specific periodicity (Fig. 1B). The assembly process is enhanced by activation of the motor domain through RLCs phosphorylation. Three isoforms of NMII (termed NMIIA, NMIIB, and NMIIC) have been identified in mammals. Although NMII isoforms share somewhat overlapping roles, each isoform has distinctive tissue distribution and specific functions, which are mediated by the C-terminus of the NMII heavy chain (Fig. 1A). These observations point to the importance of the NMII tail in modulating the spatiotemporal localization of NMII molecules and suggest that regulation by means of the tail could modulate NMII localization and activity. NMIIA is important for neural growth cone retraction and is distributed to the front of migrating endothelial cells; NMIIB participates in growth cone advancement and was detected in the retracting tails of migrating endothelial cells. Furthermore, NMIIA and NMIIB have opposite effects on motility, as depletion of NMIIA leads to increased motility, whereas NMIIB depletion hinders motility. In migrating fibroblasts, the NMII isoforms have different roles in cell polarization. NMIIA is dynamic and assembles actomyosin bundles in protrusions. By contrast, NMIIB incorporates into preformed actin bundles and remains stationary, defining the center and rear of the migrating cell. In this manner, the cooperative functions of NMIIA and NMIIB induce big, non-dynamic actomyosin structures that define the non-protrusive parts of the cell, whereas dynamic filaments in protruding regions of the cell are comprised of NMIIA alone.

The Drosophila tumor suppressor, Lgl, an evolutionarily conserved and widely expressed cytoskeletal protein, is indispensable for the establishment and maintenance of polarized epithelia and for cell polarity associated with asymmetric cell division of neuroblasts during fly development. Lgl is implicated in cell migration, and loss of Lgl inhibits dorsal closure. Furthermore, loss of Lgl leads to invasive cell behavior in the Drosophila follicular epithelium during border cell migration. Conversely, in transformed human epithelial cells, overexpression of Lgl1 inhibits migration. Lgl has also been implicated in mouse embryonic fibroblast migration. The function of Lgl in polarized cell migration, however, has not been studied in detail. Biochemical and genetic analyses suggest that the Drosophila Lgl is the component of the cytoskeleton that interacts with NMII, and that this interaction is regulated by the phosphorylation of Lgl. In Lgl mutant neuroblasts, the neuronal differentiation factor Miranda, did not localize asymmetrically in mitotic neuroblasts, but rather distributed uniformly throughout the cortex as well as in the cytoplasm. Elimination of NMII expression restored the basal localization of Miranda. Thus, Lgl and NMII act antagonistically in the basal targeting of cell fate determinants. It was proposed that Lgl acts to restrict NMII to the apical cortex of neuroblasts during prometaphase and metaphase of mitosis, where it acts to exclude cell fate determinants. However, the importance of Lgl in NMII regulation and thereby for F-actin filament contractility in cell polarization remains an unresolved issue. Moreover, the role of Lgl was studied mainly in the polarity of epithelial cells, and therefore the mechanism by which Lgl contributes to the establishment of migrating cell polarity is poorly understood. In our recent studies we reported new findings on the role of Lgl1, NMII, and ParGαPKCζ in establishing cell polarity in migrating cells.

Front-back polarization of migrating cells results in two distinct regions: a protrusive area in the direction of migration and a retracting rear (Fig. 2). NMIIA and NMIIB reside outside of protrusions and are largely absent from the lamellipodium, acting at a distance to regulate cell protrusion, signaling, and maturation of nascent adhesions. MIIB also controls the dynamics and size of adhesions in central regions of the cell and contributes to retraction and adhesion disassembly at the rear. In contrast, MIIB establishes front-back polarity (Fig. 2). Our studies provide a clue to the differential roles played by NMIIA and NMIIB in establishing front-back polarity in migrating cells. We showed that Lgl1 interacts directly with NMIIA both in vivo and in vitro, inhibiting its filament assembly in vitro (Fig. 1B). The binding site of Lgl1 to NMIIA is localized to the tail coiled-coil region, between the domains that are critical for NMII filament assembly (Fig. 1B). Furthermore, Lgl1 localization to the leading edge of the cell and depletion of Lgl1 expression result in the unexpected presence of NMII in the lamellipodium and the leading edge of the cell. This is consistent with the findings that asymmetric segregation in Drosophila neuroblasts is achieved in part by the restriction of NMII to the apical cortex by Lgl. Recently we found that Lgl1 did not interact with NMIIB, indicating that NMIIB regulation with regard to Lgl1 is different from that of NMIIA (Dahan and Ravid, unpublished data). Based on these data we propose that Lgl1 interacts with NMIIA in the lamellipodium inhibiting NMIIA filament assembly in this region, thereby confining its activity to the lamella. Lgl1 also affects the size and number of focal adhesions as well as cell polarity, membrane dynamics, and the rate of migrating cells. NMIIA mediates several important component processes that drive migration, including the initiation and maturation of adhesion sites. We showed that in cells depleted of Lgl1 there was a marked increase in the number of small nascent focal adhesions and a decrease in the number of large mature focal adhesions. NMII is dispensable for the assembly and disassembly of nascent adhesions inside the lamellipodium. Actomyosin bundles containing only MIIB mediate initial adhesion maturation, whereas the incorporation of MIIB enlarges and stabilizes them. The maturation of adhesions in the lamellipodium, seems to depend on the level of active...
NMIIA. But the mechanism by which NMIIA mediates focal adhesion maturation is not completely understood. We propose that during protrusion, adhesions initially assemble as puncta in the lamellipodium; their formation is driven by actin polymerization. In the lamellum, the activities of both actin and NMIIA are necessary for the initial elongation and maturation of the adhesions. The complex Lgl1-Par6-aPKCζ resides in the leading edge of the cell. Upon phosphorylation by aPKCζ, Lgl1 is released from the cell leading edge to the lamellipodium, where it undergoes dephosphorylation, allowing its interaction with NMIIA and inhibiting NMIIA filament assembly. Lgl1 forms in two distinct complexes, NMIIA-Lgl1 and Par6-aPKCζ-Lgl1, because NMIIA and aPKCζ compete to bind to the same region on Lgl1. The lamellipodial aPKCζ phosphorylates NMIIA, preventing its assembly in this region, thus restricting its activity to the cell trailing edge.

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Lgl1-depleted cells present three unique characterizations during migration: elongated shape, detachment from the cell sheet, and migration in different directions – in contrast with control cells that move as one sheet in one direction. This behavior of loss of cell-cell contact and independent migration of detached cells into the wound space is characteristic of tumor cells. Indeed, Lgl mutant cells form metastatic tumors in Drosophila. We propose that cell polarity of migrating cells and maintenance of cell-cell contact are achieved at least in part by exclusion of NMIIA from the leading edge of the cell by Lgl1. Failure to exclude NMIIA from the leading edge may result in the appearance of non-polarized cells, detachment from the cell sheet, and independent cell migration.

The aPKC-Par6 complex is involved in establishing the apical-basal polarity of epithelial cells. A direct interaction between basolateral Lgl and the apical Par6-aPKC complex has been demonstrated in Drosophila and mammalian epithelial cells. Lgl can be phosphorylated in the Lgl-Par6-aPKC complex by a process that requires five closely spaced serine residues located in aPKC consensus phosphorylation sites. The functional significance of phosphorylation of Lgl by the Par6-aPKC complex is suggested by the observation that a mutant of Lgl lacking the five serine residues reduces the polarization of cells in response to wounding. Furthermore, phosphorylation of the Drosophila Lgl by aPKC is required for the exclusion of Lgl from the apical region in epithelial cells. In a genetic study in Drosophila, reduction in aPKC levels suppressed the development of Lgl mutant phenotypes, such as cell polarity defects and tumorigenesis. In human cancers, overexpression of aPKC at the membrane results in a cytosolic accumulation of Lgl, suggesting that membrane-bound Lgl is necessary for tissue homeostasis. Moreover, studies using Drosophila or Xenopus embryos indicate that mutual inhibition between apical aPKC and basolateral Lgl is important for maintaining epithelial membrane polarity. These observations suggest the importance of an antagonistic interaction of Lgl with the apical Par6-aPKC complex for the development of polarized membrane domains in epithelial cells. The mechanisms by which Lgl phosphorylation modifies its function remain to be established. Our recent observation may shed light on the role of Lgl phosphorylation by aPKC in establishing cell polarity of migrating cells. We found that aPKCζ is necessary for proper cellular localization of Lgl. Lgl expressed in aPKCζ knockout cells (aPKCζ−/− cells) presented aberrant cellular localization, and Lgl was confined to the cell cortex, forming a
shell-like structure around the cell. Similarly, expression of an unphosphorylatable form of Lgl1 (Lgl1\(^{Ala}\)) was restricted mainly to the cell cortex. In contrast, a phosphorylation-regulated form of Lgl1 (Lgl1\(^{WT}\)) localized to the leading edge of the cell. Furthermore, a phosphomimetic form of Lgl1 (Lgl1\(^{Ala}\)) diffused throughout the cell and was completely absent from the leading edge. These cellular localization properties were also reflected by the degree of Lgl1 phospho-mutant association with the cytoskeleton. Furthermore, the extent of Lgl1 phosphorylation also affects the level of NMIIA association with the cytoskeleton. Ectopic expression of Lgl1\(^{WT}\) decreased the amount of NMIIA associated with the cytoskeleton, and expression of Lgl1\(^{Ala}\) decreased it further. We conclude that the effect of the decreased amounts of cytoskeletal NMIIA by expression of Lgl1\(^{WT}\) or Lgl1\(^{Ala}\) reflects the increased amounts of non-filamentous NMIIA. We propose that the phosphorylation of Lgl1 by aPKC\(_{\alpha}\) regulates its binding to NMIIA. The localization properties of Lgl1 in aPKC\(_{\alpha}\)--Lgl1 may indicate that the phosphorylation of Lgl1 by aPKC\(_{\alpha}\) takes place in the leading edge of the cell, where the complex Lgl1--Par6--aPKC\(_{\alpha}\) is localized (Fig. 2). We propose that upon phosphorylation by aPKC\(_{\alpha}\), Lgl1 is released from the leading edge of the cell to the lamellipodium, and therefore phospho-Lgl1 is unable to bind NMIIA. In the lamellipodium, phospho-Lgl1 undergoes dephosphorylation by unknown phosphatase, allowing the interaction between Lgl1 and NMIIA, and inhibiting NMIIA filament assembly (Fig. 2).

Lgl interacts directly with both Par6 and aPKC to form a multiprotein complex. Par6 can associate with Lgl both through its PDZ domain and through an N-terminal segment. This association may be bridged by aPKC, which binds directly to both Par6 and Lgl. Although the importance of the Lgl--Par6--aPKC complex in the formation of apical--basal polarity in Drosophila is well established, its importance in establishing front-rear cell polarization is not understood. We showed that in polarized migrating cells the complex Par6--aPKC\(_{\alpha}\)--Lgl1 localized to the leading edge of the cell. We further showed that Par6--aPKC\(_{\alpha}\)--Lgl1 complex formation is regulated by aPKC\(_{\alpha}\) phosphorylation of Lgl1. We propose that the interaction between Lgl1 and the asymmetrically localized Par6--aPKC\(_{\alpha}\) complex may localize and phosphorylate a subpopulation of Lgl1, thereby creating a gradient of Lgl1 activity in the cell (Fig. 2). In vivo Lgl1 exists in two distinct complexes, NMIIA-Lgl1 and Par6--aPKC\(_{\alpha}\)--Lgl1, which are affected by the state of Lgl1 phosphorylation. The formation of two discrete complexes is explained by our finding that NMIIA and aPKC\(_{\alpha}\) compete to bind to the same region on Lgl1. This behavior may ensure the cellular localization of the two complexes to different cellular compartments, thereby establishing front-rear polarization in migrating cells. We propose that Lgl1 in the leading edge of the cell is in an unphosphorylated state and therefore forms a complex with Par6--aPKC\(_{\alpha}\) in this region. It is possible that non-filamentous NMIIA forms a complex with unphosphorylated Lgl1 at the lamellipodium, preventing NMIIA from assembling into filaments, thus allowing polymerization of F-actin in that region. In contrast, filamentous NMIIA that is unbound to Lgl1 is found in the lamellum, where together with F-actin it forms the stress fibers required for attachment/detachment of migrating cells. Several years ago we showed that upon EGF stimulation, aPKC\(_{\alpha}\) phosphorylates NMIIB but not NMIIA, leading to slower filament assembly of NMIIB. Furthermore, a decrease in aPKC\(_{\alpha}\) expression alters the localization and organization of NMIIB after EGF stimulation, causing it to become more diffuse and not localized to the cell cortex. These results were confirmed using aPKC\(_{\alpha}\)--Lgl1 cells, we found that the amount of NMIIB that is associated with the cytoskeleton is higher than that in control cells (Dahan and Ravid, unpublished data). These results indicate that aPKC\(_{\alpha}\) regulates NMIIA filament assembly indirectly by phosphorylation of Lgl1 and that it regulates NMIIB directly. Furthermore, aPKC\(_{\alpha}\) has a positive effect on NMIIA assembly because phosphorylation of Lgl1 results in Lgl1 inhibition and NMIIA filament assembly. We propose a model for the role of Lgl1--NMIIA and Lgl1--Par6--aPKC\(_{\alpha}\) in establishing front-rear polarization in migrating cells (Fig. 2).

Cell leading edge, the very front region of the cell; lamellipodium, a broad, flat cell protrusion containing a branched dendritic actin-network; lamellum, localizes behind the lamellipodium and contains acto--NMII stress fibers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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