Small GTPases 5, e28997; May 2014; © 2014 Landes Bioscience

Rho GTPases in collective cell migration

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Keywords: Rac, Rho, cell adhesion, cell migration, collective migration

The family of Rho GTPases are intracellular signal transducers that link cell surface signals to multiple intracellular responses. They are best known for their role in regulating actin dynamics required for cell migration, but in addition control cell-cell adhesion, polarization, vesicle trafficking, and the cell cycle. The roles of Rho GTPases in single mesenchymal cell migration are well established and rely on Cdc42- and Rac-dependent cell protrusion of a leading edge, coupled to Rho-dependent contractility required to move the cell body forward. In cells migrating collectively, cell-cell junctions are maintained, and migrating leader cells are mechanically coupled to, and coordinate, migration with follower cells. Recent evidence suggests that Rho GTPases provide multifunctional input to collective cell polarization, cell-cell interaction, and migration. Here, we discuss the role of Rho GTPases in initiating and maintaining front-rear, apical-basal cell polarization, mechanotransduction, and cell-cell junction stability between leader and follower cells, and how these roles are integrated in collective migration. Thereby, spatiotemporal fine-tuning of Rho GTPases within the same cell and among cells in the cell group are crucial in controlling potentially conflicting, divergent cell adhesion and cytoskeletal functions to achieve supracellular coordination and mechanocoupling.

Introduction

Cell migration is a fundamental process controlling cell position, fate decisions, and function in morphogenesis, immune function, regeneration, and cancer.[1,2] To translocate the cell body, cells undergo a cyclic process to maintain bipolarity and extend a leading and trailing edge, followed by adhesive interaction with tissue substrate and actomysosin mediated contraction which leads to the forward gliding of the cell rear.[1,3-5] Polarized cell extension and contraction are mediated by the actin cytoskeleton, which generates shape change and, via adhesion receptors, connects to the extracellular tissue environment.[5-7] Besides single-cell migration, which propels the movement of individual cells, collective cell migration of cells that retain cell-cell adhesion and communication supports the movement of multicellular units along or within tissues.[8,9] Collective migration depends upon and causes complex alterations of tissue organization and function, including the formation and regeneration of skin, tubulogenesis of epithelial tubules and glands, vascular sprouting, and complex organ remodeling, including the formation of a germ cell niche in the *Drosophila* ovary and development of the lateral line, the balance organ in zebrafish.[10-12] In pathological contexts, collective cell migration underlies the deep tissue invasion of solid cancers.[8,13] Similar to single-cell migration, collective movements depend upon actomyosin-dependent front-rear asymmetry. In multicellular groups, leading cells polarize by protruding anterior leading pseudopods, which engage with the tissue substrate by adhesive and proteolytic interactions, while the rear pole and lateral sides retain cadherin-based cell-cell adhesion and mechanocoupling to follower cells.[14,15] Likewise, follower cells exhibit front-rear polarity with lateral and basal portions of cohesive cell groups form so-called cryptic lamellipodia, which extend toward the direction of migration, engage with substrate and generate traction with cell-cell junctions, which remain intact in the direct vicinity.[16] This enables cells inside the group to actively migrate and generate traction toward the substrate[17]. Thus, collective cell migration is a specialized and complex cell migration mode that combines cell movement with "supracellular" polarity, cell-cell junction stability, and coordinated multicellular migration.[10]

Rho GTPases are important upstream regulators of actin polymerization and actomyosin contractility, linking outside signals received from adhesion, chemokine, and/or receptor tyrosine kinase receptors to cytoskeletal dynamics.[4,14,18] Thereby Rho GTPases control mechanosensory cell functions, including cell adhesion, polarity, contractility, as well as cell-cell junction regulation in a tissue-context dependent manner. The roles of Rho GTPases in single-cell migration, particularly cell polarization and protrusion formation, and cell contractility are well established[1], yet their dual role in controlling both cell kinetics and cell-cell junctions in collective cell movements adds additional complexity. We here summarize key functions of Rho GTPases in collective cell migration, with focus on their contribution to leader cell polarity, cell-cell junction stability and turnover, and multicellular coordination during morphogenesis and cancer.

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Submitted: 12/06/2013; Revised: 04/23/2014; Accepted: 04/23/2014; Published Online: 05/09/2014
http://dx.doi.org/10.4161/sgtp.28997

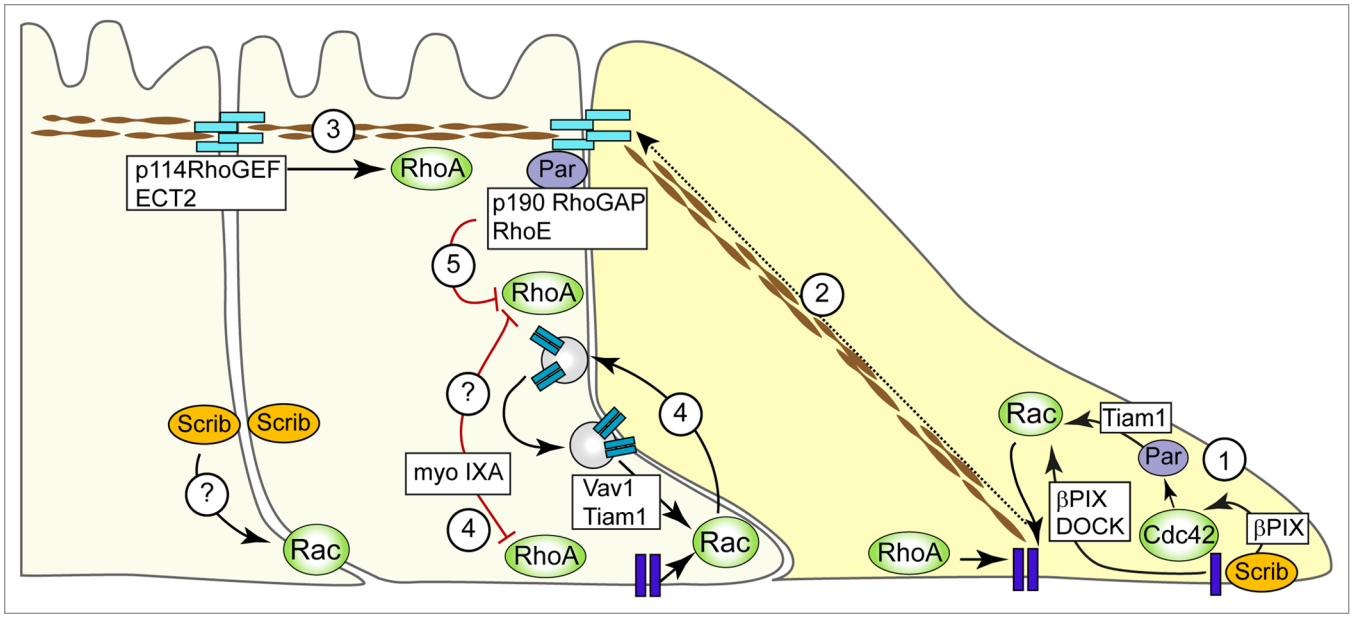

Figure 1. Spatial segregation of Rho GTPase signaling in collective migration. (1) Sustained activation of Cdc42 and Rac upstream and downstream of ECM adhesion promotes protrusion formation. (2) Mechanocoupling of traction forces to the apical junctional complex. (3) Actomyosin contractility in conjunction with Rho activation by ECT2 and p114RhoGEF. (4) Increased Rac activation at cryptic lamellipodia by a combination of ECM-mediated Rac activation and Rac activation due to increased recycling and de novo formation of adherens junctions. In parallel, Rho activity is downregulated at cryptic lamellipodia by myosin IXA, which may also inhibit Rho activity at lateral junctions. (5) Rho activation at lateral junctions is inhibited by p190 RhoGAP, or via p120 catenin or Rnd3/RhoE.

Rho GTPase Regulation and Basic Functions in Cell Migration

Rho GTPases belong to the family of Ras-like GTPases, the activity of which is regulated by a cyclic switch between an inactive GDP-bound and an active GTP-bound state.[18,19] Activation of Rho GTPases is controlled by guanine exchange factors (GEFs) that promote GTP-loading in response to extracellular cues. Upstream regulators of GEFs include growth factor and cytokine receptors, integrins, and cadherins.[18] As antagonists to GEFs, GTPase activating proteins (GAPs) inactivate Rho GTPases through their conserved catalytic GAP domain which hydrolyses GTP to GDP. Most GAPs also execute other functions, including additional GAP function or GEF activity toward other small GTPases, or function as myosin motor. The upstream signals engaging RhoGAPs are poorly defined.[20] Rho GTPases are further inhibited by Rho guanine nucleotide dissociation inhibitors (GDIs), which bind the prenyl membrane anchor of GTPases and prevent their translocation to the plasma membrane, thereby retaining Rho GTPases in inactive state and sequestered in the cytosol.[21,22]

Key mechanosensory cell functions controlled by Rho GTPases include protrusion formation and front-rear polarity, actomyosin contractility, and the turnover of cell-matrix and cell-cell adhesions, which jointly contribute to the type and efficacy of cell migration. In moving cells, at least three types of cell protrusions are mediated by Rho GTPases. Filopodia, thin membrane protrusions containing parallel actin bundles for mechanosensory probing of the environment, are predominantly controlled by Cdc42, through the Mammalian Diaphanous-related (mDia) formin mDia2, which nucleates and elongates actin filaments, and IRSp53, which bundles actin filaments.[4] Lamellipodia, sheet-like protrusions that provide adhesion to substrate, are controlled by active Rac1, Cdc42, RhoA and RhoC[4,6] (**Fig. 1**). Cdc42 controls cell polarization and promotes extension by stabilizing the microtubule cytoskeleton.[23] Rac regulates branched actin network assembly and extension toward the leading edge through WAVE and Arp2/3.[5,24] Actin branching is further promoted by cofilin, which is activated downstream of the Rac-PAK-LIMK axis[25] or via RhoC-ROCK-LIMK.[6] Cofilin severs actin filaments at protrusions and thereby provides free barbed ends of existing actin filaments, which enhances Arp2/3-mediated extension of lamellipodia.[6] Through Pak, Rac further supports integrin-based adhesion to ECM and mechanical stabilization of forward protruding lamellipodia.[26] As third principal protrusion type, membrane blebbing results from a two-step process of initial bleb-like membrane protrusion with secondary stabilization of the bleb by the cortical actin network.[27,28] Membrane blebbing depends upon intracytoplasmic hydrostatic pressure, mediated by RhoA and downstream actomyosin contraction.[29-31]

Besides cell protrusions at the leading edge, Rho GTPases control cell contractility at the trailing edge of moving cells. RhoA activates ROCK, which engages myosin light chain kinase and myosin II for actomyosin contraction, preferentially at lateral and rear cell portions.[32] Thereby RhoA controls the retraction

of the tail in migrating single cells and in cell groups, and likely, the lateral mechanocoupling toward cadherin-based cell-cell adhesions.[32,33]

In both 2D and 3D models for collective cell migration, Rho GTPases initiate and maintain leader and follower cell function, interaction with substrate, cell-cell cohesion and supracellular coordination. In individually moving cells, Rac and Cdc42 activity, controlling polarized actin polymerization, is spatiotemporally separated from Rho-dependent actomyosin engagement and cell contraction, which ensures each cell region to function simultaneously and in vicinity. Cell regions or subregions of active Rac show limited Rho activity, and vice versa, as consequence of reciprocal feed-back and mutual inhibition. Downstream of Rac and Cdc42, Par6 and atypical PKC (aPKC) engage the ubiquitin ligase Smurf1 which degrades Rho and thereby limits local Rho availability at the leading edge.[34] In addition, Rac inactivates Rho by activating p190 RhoGAP, either via direct interaction,[35] or indirectly, via engagement of Par6/aPKC[36] or p120catenin along cell-cell junctions.[37] Likewise, the Rac effectors Pak1 and Pak4 promote the phosphorylation and inactivation of a set of RhoGEFs including p115-RhoGEF,[38] GEF-H1,[39,40] PDZ-RhoGEF[41] and Net1,[42] which limits Rho activation.

Conversely, Rho limits Rac either via direct engagement of the Rac inhibitors FilGAP[43] and ARHGAP22,[44]or via ROCK which inhibits recruitment of the Cdc42/Rac GEF βPIX to cell-matrix adhesions.[45] ROCK further inhibits the assembly of the polarity complex Par3/Par6, which limits Rac and thereby prevents ectopic protrusions near the cell rear or cell-cell junctions.[46] Thus, through negative cross-talk, potentially conflicting functions between Rho GTPases are distributed to and define functional subcellular compartments, including protrusions vs. cell-cell junctions, apical vs. basal zones, and actomysosin-dependent contractile vs. actomyosin-independent regions in protrusions or cell-cell junctions.

Cellular Principles of Collective Cell Migration

As initiating event of collective cell migration, cells at the edge of the cell group acquire ligand-mediated contact with tissue structures via matrix-binding adhesion receptors, particularly integrins, as well as (co)receptors, including syndecans, CD44, and chemokine and cytokine receptors engaging with ECM-bound migration-promoting chemokine and cytokines.[14,47] These combined signals promote outward polarization and adhesion of leading and marginal cells to the ECM.[16,47,48] Whereas in moving cell monolayer sheets all cells retain contact to the basal substrate, cells within inner portions of 3D groups lack the signaling input from ECM and adhere exclusively to neighbor cells. These cell-cell junctions mostly involve homophilic cadherin-cadherin adhesive junctions, but may also include immunoglobulin family members with homophilic or heterophilic binding, such as N-CAM, L1-CAM, ALCAM, Ephrins/Eph receptors, desmosomal proteins, and integrins.[10,48] Similar to non-moving stable epithelia, moving epithelial cell sheets commonly maintain exclusive apicobasal polarity and thereby polarize toward the ECM interface by unilateral adhesion and deposition of ECM components, such as basement membrane proteins.[11] Both downregulation and overexpression of cadherins inhibits collective migration,[49-51] suggesting precise control of cadherin-based adherens junctions as prerequisite of collective movement. Thus, overlapping adhesion systems maintain multicellular mechanocoupling and integration between the cytoskeleton of individual cells and contribute to supracellular coordination.[47,52]

Rho GTPases and Front-Rear Polarity in Leader Cells

The role of Rho GTPases in leader cell functions is mostly derived from single cell migration assays, addressing general rules of front-rear polarity and cell sheets moving across 2D surfaces (**Table 1**). When studied in 3D models for collective cell migration or tubulogenesis,[10,11] Rho GTPases and their downstream effectors likely impose additional complexity and cross-talk to accommodate the spatial constraints of 3D tissue invasion.

Leader cells maintain an intrinsically bipolar state, with the protruding leading edge oriented toward the ECM and the rear engaged with cell-cell connections to follower cells.[14,53,54] The protruding leading edge is initially determined by Cdc42- and Rac-mediated polarization and actin polymerization and Rho-mediated actomyosin contraction for adhesion stabilization several micrometer rearward of the cell front.[4,55] The rear pole of a leader cell is determined by mechanosignaling from cell-cell junctions, and higher forces at the rear support actomyosin contractility to move the cell rear and the cell-cell junction forward,[15] which further minimizes potentially counterproductive ectopic protrusions.[53] The process and underlying signals counteracting actin polymerization and cell protrusion formation at cell-cell junctions are termed contact-inhibition of migration.[53]

Leader cells, via adhesion complexes and actomyosin contraction, generate force toward the substrate and thus control tensional regulation of ECM alignment.[56,57] Leader cells further reorganize ECM through degradation by surface-associated proteases, such as MT1-MMP or localized secretion of soluble proteases including MMP9, to generate space and enable the formation of ECM neo-tracks to accommodate and guide collective cell strands.[58,59] In collectively migrating squamous carcinoma cells LIMK and cofilin regulate invadopodia formation and stability, and delivery of MMP9 to invadopodia, thereby promoting focal proteolysis and proteolytic path generation, possibly downstream of ROCK.[59] In single tumor cells, LIMK/cofilin are regulated upstream by spatiotemporally-controlled RhoC/ROCK[6] or Rac/Pak activity.[25] Alternatively, local MMP-mediated proteolysis in single osteosarcoma cells depends on localized recruitment of βPIX to focal adhesions by the linker protein α-parvin followed by Rac and Pak activation.[60] As special case, heterologous cells with proteolytic capacity, such as activated fibroblasts, may provide leader cell function, by generating proteolytic ECM paths in a Rho GTPase- and MMP-dependent manner.[61,62]

Table 1. Model systems for collective cell migration and identified roles of Rho GTPases

Model	Type of collective migration	Biological and/or application context	Roles of Rho GTPases	References
Cell-sheet migration (2D, in vitro)	Migration of cohesive cells as 2D monolayer in vitro	Simplified model for epithelial and/or endothelial cell migration, wound healing	Rac, Rho in leading cells: DDR1/Rho axis reduces actomyosin contractility and stabilizes cell-cell junctions; sheet migration requires p114RhoGEF-dependent myosin II contractility	47,49,136,137
Convergent extension movement (2D in vivo)	Migration of multilayered cell sheets	Gastrulation	Wnt-driven Rac and Rho engagement controlling filopodia, protrusion, polarity and protrusion lifespan	138,139
Tubulogenesis (3D, in vitro, in vivo)	Epithelial collective sprouting and tube formation, including lumen formation.	Morphogenesis, gland development (mammary, salivary, kidney)	Rac-dependent multicellular budding tip composed of several positionally instable leader cells followed by positionally stable cells forming the duct with inner lumen and outward basement membrane deposition; Rho-dependent restriction of leader cells. Rac-dependent maintenance of cell-cell adhesion	54,110,115,140
Invasion of collective strands (3D, in vitro)	Finger-like sprouting of multicellular strand	Epithelial tubulogenesis, vascular sprouting, strand-like cancer cell invasion	Low Rho activity stabilizes cell-cell junction; active Rho favors conversion to single-cell migration	83,103
Tumor explant invasion (3D, in vitro)	3D invasion	Cancer, personalized medicine	Rac-dependent leader cell selection; Rho dependent engagement of heterologous leader cells (fibroblasts); silencing of Rho along cell-cell junctions	62,83,141

Rac and Cdc42 activity is controlled by upstream chemokine and cytokine receptor and adhesion receptor signaling. Early matrix adhesions engage integrin binding to ECM and focal adhesion formation followed by Cdc42 and Rac activation through βPIX, Tiam1 and DOCK-family GEFs, which sustains protrusive activity.[4] Individually migrating or loosely connected astrocyte monolayers rely upon Cdc42 for leading edge polarization and migration[63,64] and on Rac for sustained protrusion formation,[23] suggesting non-redundant roles for Cdc42 and Rac in leading edge dynamics. In border cell clusters moving in the *Drosophila* ovary, local activation of Rac, using a photoactivatable analog of Rac, is sufficient to induce cell protrusion and maintain leader cell polarization and migration.[65] This state as leader cell is transient, as decrease of Rac activity is rapidly followed by loss of guidance ability and the emergence of ectopic protrusions in other cells of the group.[65,66] While the role of Cdc42 initiation of these ectopic protrusions was not addressed in these models, a recent screen suggests that both Rac and Cdc42 are sufficient to promote cell collective migration of endothelial cell sheets.[47] Thus, sustained Rac and Cdc42 signaling cooperate in leader cell polarity and directional persistence of moving cell groups.

Upstream effectors of Rac and Cdc42 in maintaining front-rear polarity in both single-cell and collective cell migration include conserved Scribble and Par polarity protein complexes. The Scribble complex consists of Scribble, Discs large and Lethal giant larvae and localizes to the leading edge of migrating cells in response to integrin engagement where it controls activation of Cdc42 and recruitment of Rac via βPIX.[63,67-69] The integrin/Scribble complex/Cdc42/Rac axis drives the leading edge of both 2D epithelial and endothelial cell sheets and 3D sprouting and matrix invasion models,[64,69-74] indicating a key function in collective polarity. Scribble is further recruited to both adherens and tight junctions by E-cadherin[75,76] and ZO1/2,[77,78] where it contributes to junction stability through βPIX, Rac, and Pak signaling.[75,79,80] The Par complex, composed of Par3, Par6, and aPKC, is initiated by active Cdc42 binding to Par6, which recruits Par3 and aPKC to fulfill a dual function by locally (1) activating Rac and Cdc42, and (2) inhibiting Rho.[74,81-83] Par3 engages the RacGEF Tiam1, and this complex is recruited to integrin adhesions by talin, leading to a talin-dependent activation of Rac, which initiates a leading edge and promotes the formation of focal adhesions.[84,85] Lastly, Par3/aPKC stabilize cell-surface integrin levels by activating Numb, which locally inhibits the endocytosis of integrins.[86]

Rho GTPases in Cell-Cell Junction Stability in Moving Cells

In both stable epithelia and moving cell groups, functionally stable and mechanotransducing cell-cell junctions are maintained by complementary mechanisms. These include Rac- and Cdc42-mediated formation and dynamic maintenance of junctions, Rho silencing to minimize mechanical friction along the junction, and, in subregions, Rho-mediated actin cable formation for intercellular mechanocoupling.

The apical junctional complex, located at the apical end of the lateral membrane of epithelial cells comprises adherens and tight junctions associated with contractile actin filaments. Its formation, maintenance and turnover is tightly controlled by Rho GTPases.[87] E-cadherin-based adherens junctions form de novo by touching of lamillipodia of adjacent cells, followed by

ligation of E-cadherins in trans. Initial E-cadherin engagement initiates transient Rac activation, via PI3 kinase and the RacGEFs Vav2 and Tiam1.[88,89] Rac, in turn, activates WAVE and Arp2/3 to initiate branched actin network formation required for lateral expansion of junctions.[90] Rho-mediated actomyosin contractility at the outer edges of emerging cell-cell adhesions supports junctional maturation.[91,92] Likewise, tight junction formation is promoted by cell-cell adhesion through local p114RhoGEF and Rho activation and downstream myosin II and actomyosin effector activity.[93] In addition, tight junction biogenesis relies on local Rac activation, which is mediated by Par3-dependent recruitment of the RacGEF Tiam1.[81] How and in which sequence these pathways contribute to tight junction formation, and whether they crosstalk with each other is unclear.

As dynamic endpoint of adherens junction maturation and maintenance of junction stability, both Rac and Rho remain engaged at moderate and/or sub region-controlled level.[94] To maintain junctional adhesion, established adherens junctions undergo a constant rate of cyclic remodeling by endocytosis and recycling of cadherins.[95] Rac-dependent actin dynamics involved in de novo formation of adherens junctions thus remain active but are counterbalanced by RhoGTPase-mediated turnover of junctional proteins. Downstream of Cdc42 and Par6, the Arp2/3 complex and CIP4 induce initiation and scission of endocytic vesicles, thereby promoting endocytosis of E-cadherin in *Drosophila* embryonic epithelial cells.[96,97] Likely concurrently, centralspindlin engages Rho near cell-cell junctions by two distinct mechanisms, including (1) the engagement of α-catenin to recruit the RhoGEF ECT2, which activates Rho, and (2) the inhibition of 190RhoGAP, which prevents Rho deactivation.[98] Active Rho contributes to junction stabilization, perhaps by promoting formin-mediated actin nucleation.[94] Consequently, the balance of Rac/Cdc42 vs. Rho activity at junctions determines junction formation, maturation and stability, or dissolution.

Collectively moving cells suppress actomyosin contractility along "inner" cell-cell junctions, likely to enable cadherin turnover and reduce friction along cell-cell junctions.[51] Indeed, high activity of Rho and actomyosin contractility disrupt both adherens and tight junctions and compromise epithelial barrier functions.[87,99] Inhibition of Rho activity and actomyosin contractility along cell-cell junctions depends upon Par3 and Par6, which are recruited to cell-cell junctions by E-cadherin and/or discoidin domain receptor 1 and engage RhoE/Rnd3, which in turn activates p190RhoGAP to silence the Rho-ROCK-MLC axis and actomyosin contraction.[83,100] Besides through RhoE/Rnd3, Rho activity is further inhibited by active Rac and downstream p190RhoGAP, which are recruited to junctions by p120 catenin.[37,83] Consistently, the Par complex is indispensable for group cohesion and coordination during collective migration of *Drosophila* border cells[101] and of sprouting endothelia,[102] although the role for silencing junctional Rho activity was not analyzed in these models. In aggregate, these data suggest that cell-cell junctions depend upon a fine balance of actin-based contractility and relaxation. As consequence of increasing either turnover or contractility, cell-cell adhesions are downregulated allowing cells to move individually.[83,103]

Whereas inner portions of cell-cell junctions appear to maintain low levels of tensile stress, moving cell sheets maintain substantial mechanocoupling and actomyosin contractility along cell junctions of the apical junctional complex. The resulting contractility is supracellular and allows cells within moving sheets to maintain collective mechanocoupling of directional traction forces, transmitted by cadherins.[15,17,104,105] Such supracellular coordination relies on RhoA-ROCK-dependent actomyosin cables extending along apical cell-cell junctions over multiple cell bodies.[106] In cell-junction free areas, such as the marginal cells of advancing sheets or wounded epithelia, relatively high Rho-ROCK-myosin II activity likely increases the formation of actomyosin cables, to coordinate cell-sheet contractility by a purse-string like mechanism and to inhibit leader cell formation.[107,108] Consequently, inhibition of the Rho-ROCK axis disrupts intercellular mechanocoupling and coordination, in both 2D and 3D models of collective cell migration.[107,109,110] As second, possibly related location of intercellular actomyosin cables, the circular periphery of cell clusters relies upon myosin activation downstream of Cdc42 and MRCK.[83] Likely, any interface of moving cell groups toward an outward fluid or air environment depends upon mechanical rigor to shield the group and enable supracellular contractility.

Rho GTPases in Apical Basal Polarity in Moving Cells

In both stationary and moving epithelia, apical-basal polarity is important for apical lumen formation and barrier function, and basal assembly of a basement membrane.[11,111] In non-moving, stable epithelia, Rho GTPases contribute to apical-basal polarity by cooperating with the Scrib, Par polarity, and Crumbs complexes. Cdc42 activates Par6, which, through downstream activation of aPKC and phosphorylation of Lgl, releases Lgl from a Par6/aPKC complex.[112] This recruits Lgl to Scrib and Dlg to form the Scrib complex at the basolateral surface,[112] and binding of Par6/aPKC to Par3 which forms the active Par complex at the apical and/or tight junctional area.[81,112] The Par complex also interacts with the transmembrane protein Crumbs, which, together with PATJ and PALS, forms the Crumbs complex, which localizes to the apical surface by unclear mechanisms.[81,113] The dichotomy of Scribble vs. Par/Crumbs distribution determines apical and basolateral membrane identity and asymmetry of membrane-associated protein complexes, organelles and cytoskeletal organization.[114]

Multiple upstream signals regulate polarity complex formation and distribution in collectively moving cells, including ECM-derived signals from the basement membrane which engage β1 integrins, Par3-dependent Rac or Cdc42 activation and negative cross-talk to Rho inhibiting the Rho-ROCK-myosin II axis.[115-119] Mechanisms driving initial cell polarity downstream of the polarity complexes remain poorly

defined, but include phosphoinosites and regulators of vesicle traffic.[111] As consequence of cytoskeletal polarization, collective migration is coordinated with ECM protein deposition and remodeling required for the formation of basement membrane. Moving cell sheets or tubules deposit the basement membrane of the developing *Drosophila* eggchamber[120] or along epithelial acini,[121] highlighting how collective movement may contribute to polarization, tissue formation and patterning. Once established, both cell-cell and cell-ECM adhesions maintain and reinforce cell polarization.[114] Consistently, in moving 2D epithelial sheets and *Drosophila* border cells, cell-cell adhesions maintain apical basal polarization of follower cells toward the underlying substrate, whereas leader cells exhibit a combination of apicobasal and front-rear polarization[12,101,122] (Zegers, unpublished results).

In differentiating epithelia moving through 3D environments, apical-basal polarity is partly abandoned in early branching morphogenesis of mammary and kidney epithelial cells but reestablished in follower cells with lumen formation and stabilization of the duct.[11] In tumors, apical-basal polarity is lost with dedifferentiation, resulting in non-polarized multicellular masses that extend into the connective tissue stroma.[123] Likely, in collective cancer cell invasion, cell-cell junctions are loosened, as indicated by intravital studies showing increased E-cadherin dynamics and decreased adhesion strength downstream of signaling via β1 integrin, Src and Fak.[51] Thus, in a context-dependent manner, collective cell migration may result in varying levels of apical-basal polarity with apical lumen formation and basolateral basement membrane assembly.

Rho GTPases in Front-Rear Polarity of Follower Cells

The forward movement of single (leader) cells is coupled to the collective motion of follower cells. The limited traction force of leader cells exerts drag force to follower cells which, on its own, is not sufficient to move a multicellular group forward.[17,124,125] Individual follower cells thus maintain and integrate two types of mechanocoupling, including (1) traction from neighboring cells, mostly mediated by apical junctional complexes and (2) by actively generating traction through basal adhesion and force transmission to the substrate.[17,124,126]

In contrast to leader cells, which show high Rho activity and associated traction forces toward the substrate mostly at the front of the cell,[107] follower cells generate both pulling- and pushing-type mechanotransduction forces to the substrate as well as varying degree of Rho activity.[17,107] The forces impacting follower cell kinetics likely represent an integrated force map from leading cells pulling, follower cell mechanocoupling toward the basal substratum, and supracellular mechanocoupling along cell-cell junctions between neighboring cells in a "tug-of-war"-like manner.[124,127] While moving, follower cells develop cryptic lamellipodia at their basal surface which generate protrusions along the substrate and underneath neighbor cells toward the direction of migration,[12,16] the relevance of which for force generation yet remains to be resolved. In moving 2D sheets, Rac activity in both leading and trailing cells is required for basal actin polymerization and sheet migration,[33,128] which indicates that Rac drives the formation of cryptic lamellipodia

The molecular integration of dynamic protrusion formation in direct vicinity to intact cell-cell junctions and apical-basal polarization signaling remains unclear. Formation of cryptic lamellipodia at the cell basis does not depend on RhoA-ROCK-myosin II pathways;[16] however, mechanocoupling between leader and follower cells depends upon apical myosin II, suggesting a vertical separation between basal Rac and apical Rho dominance.[129,130] Thereby cryptic lamellipodia may reflect a perpetual state of nascent cell-cell junctions with high Rac-driven cytoskeletal dynamics that simultaneously engage with and extend along ECM substrate. Consistently, the unconventional myosin IXA, an actin-binding RhoGAP involved in collective, but not single cell migration, colocalizes with myosin II in nascent adherens junctions[131] and contributes to both, cryptic lamellipodia and stable cell-cell junctions.[132] This function in downregulating Rho activity may enable both cryptic lamellipodia and non-contractile cell-cell junction in vicinity. The basolateral interface thus combines features of front-rear asymmetry and mechanotransduction, through cryptic lamellipodia, and apicobasal polarity with polarized protein deposition and tissue scaffold assembly.

Concluding Remarks

In contrast to single-cell migration, collective movement combines themes of mechanotransduction, epithelial biology, tissue formation and remodeling, and intercellular communication to an exciting, but also challenging interdisciplinary theme. Because interference with one signaling pathway potentially affects different modules of multicellular mechanotransduction and coordination, mechanistic insight is often blurred by complex phenotypes with yet very similar end-points, such as loss of migration efficacy or cell dispersion. Thus, although general functions of Rho GTPases in regulating supracellular shape, interaction with ECM and mechanotransduction are well established, global interference with Rho GTPases cause complex phenotypes by compromising several key functions concurrently. Spatiotemporally defined approaches such as the use of FRET-based biosensors for Rho GTPase activity or mechanotransduction, or optogenetic and laser microablation approaches enable the in-context probing for partial functions of specific Rho GTPase family members in cell mechanics and migration in a subcellular defined manner.[24,106,133-135] When combined with molecular interference, RhoA biosensor imaging and the use of micropillar substrate reveal the supracellular mechanocoupling as function of RhoA in leader cells.[107] Combining such precision molecular interference with mechanical and FRET-based activity readout will allow to establish the kinetic mosaic of Rac/Cdc42 and distinct Rho subfamily members in intracellular and intercellular subregions. This will delineate regions of exclusive vs. joint

activity of GTPases that balance cell dynamics with cell-cell junction stability in collectively moving cells (**Fig. 1**).

Open questions on collective polarity further include mechanisms regulating front protrusion of budding-type invasion into 3D environments without dedicated leader cell, whereby multiple cells form the front row on a rotating, interchangeable manner[54]; the upstream effectors that maintain spatial separation of concurrently active Rho GTPases, including GEFs and GAPs; and the spatiotemporal specialization toward downstream effectors that execute dedicated non-redundant functions in a cell-type specific manner, including cytoskeletal regulators as well as effectors of other cellular pathways such as microtubule dynamics and cytoskeletal interaction with the nucleus.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

M.Z. is supported by the European Commission FP7-PEOPLE-2011-IIF (302067). P.F. is supported by the Netherlands Science Organization (NWO-VICI 918.11.626) and the Cancer Genomics Center, Netherlands.

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