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Running with Neighbors: Coordinating Cell Migration and Cell-Cell Adhesion

Caitlin Collins and W. James Nelson

Department of Biology, Stanford University, Stanford, CA 94305, U.S.A

Abstract

Coordinated movement of large groups of cells is required for many biological processes, such as gastrulation and wound healing. During collective cell migration, cell-cell and cell-extracellular matrix (ECM) adhesions must be integrated so that cells maintain strong interactions with neighboring cells and the underlying substratum. Initiation and maintenance of cadherin adhesions at cell-cell junctions and integrin-based cell-ECM adhesions require integration of mechanical cues, dynamic regulation of the actin cytoskeleton, and input from specific signaling cascades, including Rho family GTPases. Here, we summarize recent advances made in understanding the interplay between these pathways at cadherin- and integrin-based adhesions during collective cell migration and highlight outstanding questions that remain in the field.

Introduction

The coordinated movement of groups of cells, termed collective cell migration, is critical for many biological processes at nearly all stages of life. In development, groups of cells move in a coordinated manner during gastrulation when the blastula is reorganized into a multilayer tissue comprised of the three germ layers [1,2]. During neurogenesis, neural crest cells migrate to distant regions of the embryo as loosely connected strands of cells [3,4]. Other forms of collective cell migration require coordinated movements of large sheets of cells, such as closing a wound following injury [5].

Collective cell migration is also prevalent in certain disease states, such as cancer. The classic view of cancer metastasis is that of single cells undergoing an epithelial to mesenchymal transition (EMT) and adopting a migratory phenotype [6]. However, collective cell migration is also recognized as a well-established mode of metastasis for certain types of tumors, especially carcinomas [7].

Coordinated movement of large groups of cells is tightly regulated, as cells maintain strong, yet dynamic adhesions with both neighboring cells and the ECM. Cells within cohesive tissues have cadherin-based adhesions at cell-cell junctions [8] and integrin-based focal

Address correspondence to: W. James Nelson (wjnelson@stanford.edu).

Present Address: 318 Campus Drive, James H. Clark Center – W200, Stanford, CA 94305

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adhesions at cell-ECM contacts [9]. Cadherin- and integrin-based adhesions are large, multi-protein complexes that function as structural, mechanical, and signaling hubs whose functions must be integrated to coordinate cell migration and cell-cell adhesion [8,9]. The importance of “cadherin-integrin crosstalk” has been recognized for decades [10,11], yet only recently have advances been made in understanding the biophysical properties, biochemical signals and mechanisms that govern transitions between migration and cell-cell adhesion [12–14]. This review will highlight recent advances made in understanding force transmission, actin dynamics, and Rho GTPases at cadherin and integrin adhesions, and how signals arising from both adhesions are integrated during collective cell migration.

Biophysical properties of cell-cell and cell-ECM adhesions

Both cell-cell and cell-ECM adhesions are force-bearing structures that withstand and respond to piconewton to nanonewton forces from the surrounding environment (neighboring cells or the substratum) [15–17]. Focal adhesions grow in response to applied force [18], and traction stresses generated by focal adhesions are influenced by the rigidity of the substratum [19]. Cadherin adhesions are also mechanosensitive structures. Cadherins are under constitutive tension [16,17], and cadherin-based adhesions are reinforced upon force application [20–22]. Thus mechanical force regulates the size of both cadherin and integrin junctions [18,22].

Forces at cell-cell and cell-ECM adhesions are intimately connected with each other. The amount of tension that develops at cell-cell junctions can be influenced by the composition, rigidity, and organization of the ECM [23–25]. For example, pairs of Madin-Darby canine kidney (MDCK) cells adhering to collagen I-coated polyacrylamide gels generate higher tension at cell-cell contacts compared to pairs of cells on a fibronectin-coated gel [23]. Substrate rigidity can also influence how integrins affect cadherin function. Using micropatterned substrates designed with islands of ECM surrounded by E-cadherin, Tsai et al demonstrated that MCF-7 cell adhesion to ECM inhibited formation of cadherin adhesions on rigid micropatterned substrates (5 MPa), while cadherin adhesions were still able to form when cells adhered to softer substrates (60 kPa) [25]. However, rigidity-dependent inhibition of cadherin adhesion is cell-type specific, or may be a hallmark of cancerous cells, as integrin adhesion on stiff substrates does not block cadherin adhesion in MDCK cells [25]. Another study, using ECM micropatterned in various geometries, demonstrated that ECM organization influences cell-cell contact positioning and generation of intra- and inter-cellular tension; cell-cell junctions formed away from ECM contacts are stabilized due to low intra- and inter-cellular force generation [24].

Cadherin-based adhesions also influence traction forces at cell-ECM contacts. In the absence of cadherin-based adhesions, a group of cells exerts traction stresses throughout the colony. However, when cadherin-mediated adhesion is induced, traction stresses at cell-ECM adhesions reorganize and become highly localized at the periphery of the colony [26]. These observations raise the question: how are forces tightly regulated at specific cellular locations to maintain homeostasis? A model of cellular tensegrity suggests that a pre-stressed actin cytoskeleton could provide rapid and directed transmission of forces across

the cell [27], and changes in actin dynamics could facilitate the reorganization of those forces.

Actin-associated proteins, such as vinculin, may facilitate force transduction at both adhesions. Vinculin is recruited to cadherin adhesions in a force-dependent manner and is required for mechanical reinforcement of the adhesion [20,21]. Furthermore, the interaction between vinculin and the cadherin-catenin complex is stabilized by a tension-induced conformational change in α -catenin that exposes a vinculin binding site [28]. Single molecule studies have shown that binding of vinculin to α -catenin dramatically increases the lifetime of an unfolded conformation of α -catenin [29•], and that force is required for strong binding between F-actin and α -catenin in the cadherin complex [30••]. Force-dependent recruitment of vinculin to cell-cell junctions likely stabilizes the junction by increasing the lifetime of the interaction between cadherin adhesions and the actin cytoskeleton, thereby priming the cadherin adhesion as a mechanosensitive structure. In addition, vinculin has a well-documented role in anchoring the actin cytoskeleton to focal adhesions; for example, it associates with the focal adhesion protein talin in a force-dependent manner [31,32], and strengthens adhesions by increasing the interaction between integrin adhesions and the actin cytoskeleton [31,33].

In light of evidence indicating that vinculin plays a critical role in force transduction at both adhesive structures, it becomes apparent the subcellular localization of the protein must be finely regulated based on changes in cell mechanics. Recent work demonstrated that mechanical tension on E-cadherin activates Abl kinase resulting in phosphorylation of vinculin and its preferential recruitment to cell-cell junctions, rather focal adhesions [34••]. These data indicate that the actin cytoskeleton acts as a global transducer of mechanical force, and that specific signaling cascades and post-translational modifications on mechanosensitive proteins (such as vinculin) fine-tune force transduction at specific subcellular locations to maintain mechanical homeostasis in multicellular tissues (Figure 1).

Actin dynamics and signaling pathways at cell-cell and cell-ECM adhesions

The formation and maintenance of cell-cell and cell-ECM adhesions require dynamic changes in the actin cytoskeleton. During cell migration, directed actin polymerization at the leading edge drives lamellipodial protrusions forward, and actomyosin tension generates traction stresses and contractility that facilitate tail retraction [35]. Similarly, cell-cell adhesions require dynamic regulation of the actin cytoskeleton, and photobleaching studies have indicated that a large fraction of actin filaments at cell-cell contacts is highly dynamic [36,37]. Given that the actin cytoskeleton is highly dynamic at both types of adhesions, actin dynamics must be tightly regulated in multicellular groups.

The roles of specific actin regulators in collective cell migration remain largely unexplored. However, a recent study revealed that the actin regulatory protein lamellipodin (Lpd) facilitates collective cell migration by influencing the activity of the Scar/WAVE complex [38••]. Lpd directly interacts with Rac and the Scar/WAVE complex to promote lamellipodia extension and cell migration in fibroblasts in culture, but also has clear roles in collective cell migration *in vivo*. Knockdown of Lpd in *Xenopus* or of the *Drosophila*

ortholog of Lpd (Pico) impairs neural crest cell migration and oocyte border cell migration, respectively [38••]. The mechanisms of how Lpd (and other actin regulators) integrate actin dynamics at cell-cell and cell-ECM adhesions during collective cell migration remain largely unexplored. However, it is possible that upstream signaling molecules (such as Rho family GTPases) may influence the subcellular localization and activity of actin regulatory proteins to fine-tune the strength of cell-cell or cell-ECM adhesions.

Rho family GTPases are master regulators of the actin cytoskeleton, and play key roles in assembly and maintenance of cell-cell contacts and cell migration. Initial studies revealed that cell-cell adhesion results in decreased RhoA activity and increased Rac and Cdc42 activities [39–41], and later work indicated that precise spatiotemporal control of GTPase activity is critical for development of mature cell-cell contacts [42]. Maintenance of mature cell-cell contacts requires basal levels of Rho GTPase signaling, which support dynamic remodeling of the junction via recycling of cadherins and local actin polymerization [43]. Similarly, cell migration requires dynamic changes in GTPase activity. Cdc42, Rac, and RhoA are active at the leading edge of a migrating cell and play distinct roles in cytoskeletal regulation. Cdc42 is classically known to initiate formation of filopodia which are important for determining migration direction but can also indirectly activate Rac [44]. Rac promotes lamellipodia formation and branched actin organization via its effectors, WAVE and Arp2/3, and RhoA activity at the leading edge has been proposed to initiate actin polymerization at the leading edge through regulation of the Diaphanous family of formins [44,45]. RhoA activity at the lateral and rear edges of the cell is also required for generation of actomyosin contractility [45,46]. Given Since Rho family that GTPases are required for cadherin and integrin function, their activity must be regulated to transitions between cell-cell adhesion and cell migration.

The activities of Rho GTPases at cadherin or integrin-based adhesions could be regulated by the subcellular localization of: 1) the GTPase itself (including its regulation through cytosolic sequestration by RhoGDIs), and 2) guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) that locally regulate GTPase activity. In line with the second hypothesis, many GEFs and GAPs are required for both cell-cell adhesion and cell-cell migration, and recent studies have identified specific roles for GEFs/GAPs in regulating adhesive crosstalk. For example, the Rac GEF Tiam1 promotes either cell-cell adhesion or migration in an ECM-dependent manner [47]. Tiam1 localizes to lamellipodia and promotes cell migration in Ras-transformed MDCK-f3 fibroblast-like cells plated on collagen. In contrast, Tiam1 is highly localized to cell-cell adhesions and inhibits migration in MDCK-f3 cells adhering to fibronectin or laminin by restoring cadherin-dependent adhesion. Thus, dynamic spatial regulation of Tiam1 influences Rac activity at distinct cellular sites to promote either cell-cell adhesion or cell migration. The molecular mechanism involved in the spatial regulation of Tiam1 is not well understood, although PI3-kinase (PI3K) activity is important for Tiam1 activity at both cell-cell and cell-matrix adhesions [47]. Another example of crosstalk can be found in p190B RhoGAP (p190B). p190B associates with p120-catenin at adherens junctions and influences RhoA activity at cell-cell and cell-ECM adhesions in T47D breast epithelial cells cultured in a 3D collagen gel [48•]. When T47D epithelial cells are grown in a 3D collagen gel attached to the substrate, the matrix is able to resist cell-generated force, as attachment to the substrate

restrains the matrix and permits development of isometric tension. However, if the collagen gel is released from the substrate, the matrix cannot resist force applied by cells, and the gel contracts due to cell-generated tension. In compliant (floating) gels, p190B associates with p120-catenin and inhibits RhoA at cell-cell contacts thereby changing the balance of RhoA activity at cell-cell vs. cell-ECM contacts. In contrast, when T47D cells are grown in a stiff (attached) 3D collagen gel, p190B and p120-catenin dissociate from cadherin, which releases a pool of previously inactive RhoA from cell-cell contacts and results in enhanced RhoA activity at cell-ECM contacts. Thus, p190B holds RhoA inactive at junctions in cells within soft matrices, and dissociation of the GAP from cell-cell contacts promotes recruitment of RhoA to cell-ECM contacts within stiff matrices. While it is possible that a Rho GEF may be spatially sequestered at cell-matrix contacts to permit local activation of the RhoA at that site in stiff matrices, a specific GEF has not yet been shown to facilitate RhoA activation in this pathway.

While we highlight Tiam1 and p190B as regulators of cadherin-integrin crosstalk, many other regulators of GTPase activity are involved. For example, The Dock-Elmo complex (a bipartite GEF with activity for Rac and Cdc42) was originally identified to play a role in integrin-based cell spreading and migration [49,50]. However, a recent screen to identify proteins required for cadherin-based adhesion (in the absence of integrin-based adhesions) identified Elmo2 [51]. A follow-up study revealed a Dock-Elmo complex, consisting of Dock1 and Elmo2, that locally regulates actin dynamics and Rho GTPase during the initiation of cadherin-mediated cell-cell adhesion [52]. In addition, numerous GEFs and GAPs have been identified as components of both cadherin and integrin adhesomes [53,54] and are also prime candidates to be studied in the future. Furthermore, while we have focused on the Rho family of GTPases and regulators of GTPase activity as mediators of cadherin-integrin crosstalk, many other kinases and GTPases (ex: Src, FAK, Rap1) also mediate signaling between the two adhesion sites and have been described in detail elsewhere [12,55–58].

Given that some of the same signaling molecules are required for both cell-cell and cell-ECM adhesions, the following questions arise: how do cells integrate signaling arising from both types of adhesions, and how are these signals coordinated during complex cellular behaviors, such as collective cell migration? Recently, reductionist approaches (microfabricated substrates or biophysical assays) have been used to dissect the crosstalk between cell-cell and cell-ECM adhesions [25,59]. From these studies, it is apparent that integrins can influence cadherin adhesion organization and function, and *vice versa*. Microfabricated substrates patterned with collagen IV and the extracellular domain of E-cadherin revealed that cadherin-mediated adhesion suppresses integrin-based lamellipodia activity in a dose-dependent manner (as the concentration of E-cadherin increases, the effect on integrin-based membrane dynamics increases) [59]. Down-regulation of integrin-based activity also occurred when desmosomal cadherin adhesion was engaged using a similar assay [60]. These findings may provide insight into a potential mechanism of cadherin-integrin adhesion crosstalk. For example, an increase in cadherin engagement during cell-cell contact formation may serve as a signal to titrate signaling proteins, which are normally shared between cell-cell and cell-ECM adhesions, to cell-cell junctions. This would limit the

availability of these proteins to act on effectors at integrin-based adhesions and diminish their capacity to promote cell migration. Microfabricated surfaces that allow for fine control of spatial organization, concentration, and identity of specific adhesion proteins provide excellent platforms to further elucidate molecular mechanisms of adhesive crosstalk.

Integration of biophysical and biochemical signals during collective cell migration

During collective cell migration, signaling cascades arising from cell-cell and cell-ECM adhesions must be spatially regulated and coordinated, and recent studies have started to address how these signals are integrated in more complex biological processes. For example, integrins, Rac, and PI3K are upregulated in leader cells during collective cell migration [61]; N-cadherin spatially polarizes these signaling cascades to the free (non-contacting) edge of migrating cells by locally excluding $\alpha 5$ integrin at cell-cell junctions, which decreases integrin-dependent PI3K and Rac activity at those sites [62•]. In a separate study, paramagnetic beads coated with the extracellular domain of C-cadherin (the predominant cadherin in *Xenopus* embryos) were used to apply force on cadherin adhesions in isolated, migrating mesendoderm cells. This led to recruitment of plakoglobin and intermediate filaments to the cadherin adhesion, and polarized protrusive activity at the opposite side of the cell, which resulted in cells migrating away from the direction of the applied force [63]. Further studies are required to determine whether cadherins provide spatial cues for additional signaling cascades.

Recent advances in microscopy have enabled calculations of intercellular forces during collective cell migration. Monolayer stress microscopy, which permits the measurement of local stresses within a monolayer, revealed a large heterogeneity in intercellular forces within a monolayer of migrating MDCK epithelial cells, but strong local correlations in groups of cells. Perturbation of cell-cell contacts by calcium chelation or addition of an E-cadherin function-blocking antibody disrupted local intercellular force correlations and coordinated migration of monolayer [64]. These results indicate that E-cadherin is specifically required for coordinating cues that govern coordinated movement of epithelial cells. A follow-up study systematically tested the contribution of individual cadherin isoforms (E-cadherin, P-cadherin, and N-cadherin) in monolayer force transmission in MCF10A cells revealed an unexpected result. P-cadherin is responsible for intercellular force transmission, while E-cadherin influences the rate of intercellular force generation [65••]. Interestingly, in the absence of E-cadherin, P-cadherin also triggers mechanotransduction events and undergoes vinculin-dependent reinforcement. These results indicate the E-cadherin and P-cadherin share a similar mechanotransduction pathway, although E-cadherin is dominant when both isoforms are present. These results also highlight the importance of multiple cadherin isoforms in monolayer force transmission.

Rho family GTPase activities have also been examined in epithelial monolayers. In a wound healing assay, rat liver epithelial (IAR-2) cells migrate to close a wound using finger-like projections as specific cells at the edge of the wound take on a 'leader cell' identity [66]. In a separate study utilizing micro-posts to measure traction forces between cells and the substratum, Reffay et al reported that the highest traction stresses in MDCK cells are exerted

by leader cells at the tips of migration fingers. High traction stresses also co-localized with increase RhoA activity and phospho-myosin light chain, and hence active myosin II [67]. Strikingly, the traction stress pattern of the entire monolayer was reminiscent of force distribution found within a single cell [67••], indicating that the entire monolayer was acting as a mechanical entity. How are force and signaling cascades coordinated throughout a multicellular structure? As noted above, E-cadherin is involved in coordinating local intercellular forces throughout a monolayer [64], and other studies indicate that E-cadherin engagement regulates RhoA signaling at cell junctions [68]. An attractive hypothesis, therefore, is that E-cadherin engagement is required to coordinate traction stresses and RhoA activity in a migrating monolayer, but further experiments need to be performed to specifically test this idea.

The hypothesis that cadherins play a central role coordinating changes in mechanical forces and Rac signaling during collective cell migration is supported by recent studies of border cell migration in the *Drosophila* oocyte [69••]. A FRET-based biosensor, which measures mechanical tension in the cytoplasmic domain of E-cadherin, revealed asymmetric force distribution across E-cadherin in migrating border cells, such that E-cadherin at the front of the cell cluster was under more tension than E-cadherin in cells at the rear of the cluster. Force across E-cadherin initiated a mechanical feed forward loop that amplified and polarized Rac signaling to the front of the cell cluster to promote coordinated migration [69••]. More recent work has identified a mechanotransduction pathway involving Rac and the tumor suppressor protein merlin that influences collective cell migration in MDCK cells [70]. Initial polarization of Rac activity and lamellipodia extension in leader cells increases intercellular stress at the leader cell-follower cell junction and results in force-induced translocation of merlin from junctions to the cytoplasm; polarized Rac activation and formation of a cryptic lamellipod in the follower cell required merlin translocation [70••]. Thus, force-dependent regulation of merlin localization and Rac activity enables long-range coordination of mechanical forces and GTPase activity in a migrating monolayer. While the role of cadherin was not specifically tested in force transmission in this system, the finding that E-cadherin is required for Rac polarization in the *Drosophila* oocyte model system indicates that cadherin may be required for force-dependent translocation of merlin from cell-cell contacts to the cytoplasm [69].

Bringing all of these data together, we propose a model whereby cadherins play multiples roles in coordinating collective cell migration. Upon cell-cell contact, cadherins on neighboring cells are engaged, and organized cell-cell adhesions begin to assemble. Integrin-based traction forces at the substratum are then reorganized (reduced) as tension is transferred to cell-cell adhesions and intercellular forces increase, which results in further growth and reinforcement of cadherin-based adhesions. As the size of cell-cell adhesions increases and become more prominent, we hypothesize that proteins required for strong cell-cell adhesion (vinculin, Rho family GTPases, regulators of actin dynamics) are titrated to the growing cadherin adhesions. The full establishment of cell-cell adhesions results in a balance of both forces and localization of key structural and signaling proteins between cadherin- and integrin-based adhesions that coordinate collective cell migration (Figure 2). While the titration of signaling molecules is one mechanism for integrating signals between

cadherin- and integrin-based adhesions during collective cell migration, other mechanisms also contribute to adhesive crosstalk, as multiple studies have also highlighted the importance of force transmission via a tensile actin cytoskeleton and precise spatiotemporal activation of specific signaling cascades during collective cell migration [64,67,70].

Conclusions

Collective cell migration is central to nearly all stages of life and contributes to disease states, such as cancer metastasis. A comprehensive understanding of mechanisms of collective cell migration is required to understand basic biological processes during development and may also reveal new avenues for cancer therapies. Recent work has indicated that the coordinated organization of traction and intercellular forces cadherin- and integrin-based adhesion complexes is required for coordinated movement of large groups of cells, and spatiotemporal regulation of Rho family GTPases and actin dynamics are required for collective cell migration. However, further studies are required to understand how these pathways are integrated between cadherin- and integrin-based adhesions in collective cell migration.

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Figure 1. Integration of forces at cell-cell and cell-ECM adhesions

In multicellular tissues, mechanical forces are balanced between cell-cell and cell-ECM adhesion, and tension on either structure reinforces the adhesion. At cell-cell junctions, mechanical force strengthens the interaction between the cadherin-catenin complex and the actin cytoskeleton. Similarly, force on cell-ECM adhesions, results in recruitment of actin binding proteins that reinforce association with actin filaments. Some key actin binding proteins, such as vinculin, are critical for force transduction at both adhesive structures, and the subcellular localization of such proteins must be regulated to fine-tune force balance at specific adhesions.

Figure 2. Coordination of cell-cell and cell-ECM adhesions in multicellular groups and collective cell migration

A) In individual migrating cells, GTPases at the leading edge of the lamellipodia drive actin polymerization and the formation of nascent focal contacts. RhoA activity promotes stress fiber formation, contractility and tail retraction. B) During formation of a naïve cell-cell contact, small cadherin adhesions form and begin recruiting regulators of GTPases and actin dynamics. Focal adhesions reorganize as force is transferred to the growing cell-cell adhesion. C) As cadherin adhesions grow in size and strength, we hypothesize that key signaling molecules (such as GAPs and GEFs) and regulators of actin dynamics are titrated to cell-cell adhesions. Cellular forces are balanced between cell-cell and cell-ECM adhesions to reach mechanical homeostasis. D) During collective cell migration of large groups of cells, cadherin adhesions enable local correlations in intercellular forces, and Rho GTPase activity is spatially regulated to facilitate coordinated movement of the monolayer.