Collective cell migration driven by filopodia—New insights from the social behavior of myotubes

Maik C. Bischoff | Sven Bogdan

Institute of Physiology and Pathophysiology, Department of Molecular Cell Physiology, Philipps-University Marburg, Marburg, Germany

Correspondence
Sven Bogdan, Institute of Physiology and Pathophysiology, Department of Molecular Cell Physiology, Philipps-University Marburg, Marburg, Germany. Email: sven.bogdan@staff.uni-marburg.de

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Abstract
Collective migration is a key process that is critical during development, as well as in physiological and pathophysiological processes including tissue repair, wound healing and cancer. Studies in genetic model organisms have made important contributions to our current understanding of the mechanisms that shape cells into different tissues during morphogenesis. Recent advances in high-resolution and live-cell-imaging techniques provided new insights into the social behavior of cells based on careful visual observations within the context of a living tissue. In this review, we will compare Drosophila testis nascent myotube migration with established in vivo model systems, elucidate similarities, new features and principles in collective cell migration.

KEYWORDS
actin dynamics, collective cell migration, contact inhibition of locomotion, contact stimulation of migration, Drosophila, filopodia, myotubes

INTRODUCTION
In the early 50s, Michael Abercrombie and Joan Heaysman observed in chick heart explants how fibroblasts seem to repel each other—a process they termed contact inhibition of locomotion.[1] In observing what they called “the social behavior of cells,” they became pioneers in collective cell migration research. Collective migration is a highly coordinated process that is critical in nearly all stages of animal development, in wound closure as well as in cancer.[2] Studies in various model organisms allowed researchers to acquire most existing knowledge of collective cell dynamics.[3] Unlike cell culture-based approaches, these systems allow for the examination of collective cell behavior in a physiological context of a living organism. Testis nascent myotube migration is a new model system for collective cell migration that was recently discovered.[4] In this review, we will compare it with established in vivo model systems, elucidate similarities, new features and concepts of self-regulation in collective cell migration.

First, different modes of cell motility and protrusive mechanisms will be introduced to set the testis myotube migration system in a broader context. Secondly, some well-established model systems for collective cell migration and the testis myotube migration system will be outlined. There will be a detailed comparison between Xenopus neural crest cell migration and myotube migration, as there are many similarities but also some important differences. In the third section, we will discuss key concepts of self-regulation in collective cell migration such as contact-inhibition of locomotion (CIL) and contact-stimulation of migration (CSM), two related phenomena employed by mesenchymal cell groups such as neural crest and myotubes, respectively. Finally, actomyosin-dependent “purse string” dynamics will be addressed and how testis myotubes use actin cables to cooperatively close gaps during collective migration.
THE MIGRATORY PLASTICITY OF CELLS

Different modes and mechanisms of cell motility

The molecular principles of collective cell migration share many features with the directed migration of individual cells. In principle, cellular locomotion can be divided into two categories: swimming and crawling. Flagellate-powered swimming is certainly the ancestral form originating from prokaryotes. A type of swimming, that is based on actin-derived peristalsis, was recently characterized in Drosophila fat cells and may be used by many metazoan cell types.[5] Nevertheless, crawling-based types of locomotion seem to be most common during development and homeostasis.

A prominent form of protrusion of crawling cell types is lamellipodium, a thin sheet-like structure composed of a dense branched actin network used by many cells to crawl on flat surfaces through matrix adhesion.[6,7] Variations are employed in 1D (along a single fiber), 2D and 3D environments (Figure 1A, B)[8] contributing to epithelial and mesenchymal collective cell migration as well.[9] In contrast, blebbing and lobopod-based motility—which is used in 2D and 3D—is based on actomyosin-driven contractility. The cell utilizes its internal hydrostatic pressure by controlled rupture of the cortical actin-cytoskeleton or by loosening of the cortex–membrane connection to cause the membrane to bulge outwards (Figure 1A, B).[10] Lobopodia are blunt cylindrically shaped protrusions structures—used in 3D-migration—that are similar to blebs but contain integrin-based adhesions connecting them to the extracellular matrix (ECM), like lamellipodia (Figure 1B).[11] So far, blebbing and lobopodia have only been described in single cell migration.[12]

Lamellipodia-based cell migration

Since the initial discovery by Michael Abercrombie and colleagues,[13–16] we have gained a profound understanding of the underlying processes driving lamellipodia-based cell migration (Figure 1C). Lamellipodial branched actin network not only generates the pushing force but also provides the mechanical support by focalized integrin-mediated matrix adhesions.[17,18]

Persistent lamellipodial protrusions as a major force drive mesenchymal cell migration both in 2D and 3D environments depending on the locally restricted branching activity of the actin nucleator Arp2/3-complex, which in turn is controlled by its nucleation promoting factors (NPFs) such as the WAVE regulatory complex (WRC) and WASp.[19] The Arp2/3 complex creates new barbed ends at the lamellipodium, that can be polymerized by other actin-nucleators and elongators like Formins and Ena/VASP proteins.[20,21] The treadmilling of the actin cytoskeleton is attached to the extracellular substrate via transmembrane integrin-based adhesions. This "molecular clutch" causes the F-actin flow to advance the leading edge, rather than treadmilling rearwards.[22] The small GTPases Rac, Cdc42, and RhoA differentially regulate cytoskeletal dynamics and cell migration.[23] Rac-GTP activates the WRC to promote Arp2/3-mediated branched actin nucleation and thereby defines the front of a migrating mesenchymal cell, a process that could be dissected in detail, recently.[24,25] Cdc42-GTP in turn activates the Arp2/3 complex via WASp.[26] In contrast, RhoA-GTP defines a cell's rear mainly by activating myosin II via the downstream kinase Rock.[27] Additionally, Rac, Cdc42 and Rho regulate integrin-based adhesion maturation throughout the cell.[28] Matrix adhesions are initially assembled at the front of lamellipodia as so-called nascent adhesions. Triggered by myosin II activity, they further form into focal complexes at the lamella region. Accompanied by the binding of α-integrin, focal complexes can mature into focal adhesions at the trailing edge (Figure 1C).[29] Rac and Cdc42 control nascent adhesion and focal complex assembly, whereas RhoA is responsible for the maturation of focal adhesions.[28,30–32] A recent study demonstrates that, conversely, integrin-based adhesions can also affect Rho-GTPase activity. Analysis of Rac1-GAPs and -GEFs in a large systemic approach, revealed that Rac1-GEFs are associated with nascent adhesions. In contrast, Rac1-GAPs are closely associated with focal complexes. Thereby, matrix adhesions safeguard the local activation of Rac1 that in turn regulates nascent adhesion assembly and maturation.[33]

Filopodia-based cell migration

A basic concept of the lamellipodium implies that actin polymerization drives the protrusion of the membrane. This principle also underlies other well-known protrusions, such as filopodia, long finger-like protrusions, which are thought to be more explorative in sensing chemoattractants[34] or matrix rigidity[35,36] rather than generating forces during migration.[37,38] The formation of filopodia depends on actin bundlers like Fascin, α-integrin and elongators like the formin, Dia or Ena/VASP downstream of Rho-GTPases like Rac and Cdc42.[39] Thus, filopodia may probe local environmental cues, control directionality but also maintain persistence of migrating cells by promoting cell-matrix adhesiveness at the leading edge.[18,40] Biophysical measurements and mathematical modelling were used to dissect the capacity of filopodia to exert force in detail.[41,42] Thus, the question is whether filopodia could play an important role—similar to the lamellipodium—in advancing the cell front during migration. Previous experiments in which the Arp2/3 complex was depleted in different cell types, demonstrated that cells still migrate without a lamellipodium. Instead, numerous filopodia are formed.[43–45] Recent observations in vitro and in vivo have further challenged our current view of the function of filopodia in migrating cells.[4,46] Adebowale and colleagues found that filopodial protrusions mediate migration of cultured human cancer cells on soft, fast-relaxing substrates suggesting that filopodia-based migration may play a key role in cancer metastasis in physiologically relevant 3D environments.[46] (Figure 1A). Recent live-cell-imaging experiments further showed that filopodia play not only an important role in cancer progression, but also in collective migration of testis nascent myotubes during Drosophila development (Figure 1D).[4] Shortly after myoblast fusion in the Drosophila pupa, nascent myotubes start to migrate beneath the pigment cell layer and along the testes towards the apical end, and then cover the whole pupal testis as a thin
**FIGURE 1**  Actin dependent modes of single cell migration. F-actin is depicted in blue, integrin-based cell adhesions in green and the direction of cell migration in red. (A, B) There are many modes of motility employed by single cells in 2D and 3D. (A) In 2D, besides lamellipodial migration, blebbing can also be employed. In some cases, both processes are used next to each other in the same cell. Depending on the substrate, single cells can also use filopodia-based migration mode. (B) In 3D, there are varieties of both, lamellipodia-based migration and bleb-based motility. Lobopodia are protrusions that share traits of both. They are fueled by hydrostatic pressure but contain cell-matrix adhesions. (C) Lamellipodial cell migration is well characterized. In the lamellipodium, an Arp2/3 complex dependent dendritic network of actin fibers pushes against the membrane, creating a strong retrograde flow. Filopodia anchored in the lamellipodium sense the environment. Nascent adhesions—that anchor the actin network to the substrate—are assembled at the front of the lamellipodium. The lamella region is characterized by contractile bundled actin and Myosin II and a slower retrograde actin flow. Here, nascent adhesions mature into focal complexes. At the trailing edge, contractile actomyosin-fibers are employed to retract. Focal adhesions can mature into focal adhesions. (D) Filopodial testis myotube migration is employed by testis nascent myotubes. It relies on bundled actin fibers pushing the membrane. Force is transmitted by integrin-based adhesions assembled inside the shaft of a filopodium and entering the cell body, following the retrograde flow. Here, they are quickly disassembled.

Muscular sheet (see also Figure 2C). Migrating myotubes completely lack lamellipodial protrusions, but instead use filopodia to move as a cohesive cluster in a formin-dependent manner. Interestingly, pharmacologic and genetic of Arp2/3 complex function revealed that the Arp2/3 complex it is not completely negligible for filopodia-based myotube migration. Upon Arp2/3 suppression branched filopodia were absent and the migration distance was shortened. Consistently, an Arp3-GFP fusion protein localized to filopodial branches. These data suggest that Arp2/3 is responsible for filopodia branching and thereby might increase the total amount of filopodial tips pushing against the...
FIGURE 2 Model systems for collective cell migration. Cells and tissues of interest are depicted in dark grey, surrounding cells and tissues in light grey and the direction of migration in red. (A) In Drosophila egg-chambers—depending on stage—two different types of collective cell migration can be studied. Follicle cells rotate while creating an ECM and actin-based scaffold for the subsequent elongation of the egg chamber. In later stages, motile border cells enwrap immotile polar cells. The cluster migrates from its point of origin, the anterior pole of the complex, towards the oocyte in an invasive fashion in-between the nurse cells. (B) During Xenopus embryogenesis, neural crest cells (NC) emerge during neurulation from cells positioned between the epidermis and the neural plate (presumptive neural tube). These cells start to migrate along multiple distinct streams beneath the epidermis to their different destinations. In doing so, they remain mesenchymal and self-regulate their behavior. Cells often exchange their neighbors and thereby move in a fluidic fashion. Explants in culture build clusters with an intricate behavior elucidated in Figure 4. (C) During Drosophila pupal testis development, multinuclear testis myotubes migrate in a gap between large pigment cells and cyst cells after they entered the testis between ~30 h and ~33 h APF. Single cells remain in a mesenchymal state, independent of their position. Still, the position of single cells within the sheet remains unchanged. The sheet moves towards the apex of the testis and reaches it ~40 h APF. Subsequently, the myotubes start to stretch. Between 40 h and 72 h APF the testis develops its 2.5 x coiled structure.

membrane. How exactly the Arp2/3 complex can branch entire actin bundles remains unknown. A similar function of the Arp2/3 complex might be required in dendrite-branchlet formation in Drosophila larva sensory neurons.[48] Growing dendrites in principle resemble filopodia, hence similar mechanics can be assumed to take place during filopodial cell migration. Dynamic actin-based filopodia are also very important in indirect flight muscles (IFM) myotubes. Myotube filopodia seem to facilitate adhesion with myoblasts promoting myoblast-myotube fusion and muscle-tendon cell attachment. It remains to be elucidated if they provide mechanical forces for myotube migration.[49–51]

Thus, cells show an astonishing migratory plasticity[52] the mode of cell motility and membrane protrusions highly depend on various factors, such as tissue topography, tissue composition and environmental cues. Filopodia-based migration is part of the repertoire of muscle cells and probably also other cell types, including cancer cells with an extreme cellular plasticity that aids cancer spread by metastasis. Such
migratory plasticity also involves the ability to switch the migration mode from single-cell to collective cell migration. Collective migration of cohesive cell clusters is not only a prominent driver for metastasis but also for many developmental processes, tissue and organ formation that require coordinated changes in cell shape and in cell behavior as discussed in some prominent examples below.

**Model Systems for Collective Cell Migration**

Cells can migrate collectively in tightly or loosely associated groups. Live-cell imaging combined with in vivo/organ culture techniques enabled researchers to determine many of the basic principles driving collective motility. Recently, the testis myotube migration system was introduced. To put it into context, some well-established models will be summarized in this section followed by a description of the myotube system highlighting new concepts of self-regulation in collective cell migration.

**Follicle Cell Rotation in the Drosophila Egg Chamber**

Ex-vivo culture of *Drosophila* egg chambers, for example, opened up the possibility to study two different types of collective cell migration: follicle rotation and border cell migration (Figure 2A). Follicle cells are organized in a planar polarized epithelium that revolves around egg chambers in early stages. During this collective motion—and probably caused by it—a “molecular corset” of bundled actin fibers inside the cells and polarized ECM is build all-around the egg chamber. This structure—resembling lines of latitude on a globe—defines the axis and the poles of the subsequent egg chamber elongation. Follicle cell rotation provides an excellent model for epithelial migration, steered by planar polarity. Follicle cell rotation inherently differs from some other modes of epithelial migration—like wound closure—as there is no free edge which dictates a superordinate polarization with leader cells. Instead, every cell has the same part in the collective process and is individually polarized. The fundamental question is, what causes the break of symmetry in each cell. Every single cell is front-rear polarized, containing a WRC-dependent lamellipodium that drives rotation. In addition, there are WRC-dependent atypical whip-like protrusions at tricellular junctions that seem crucial for migration. Lar—a receptor tyrosine phosphatase—is present at the front of each cell, where it appears to signal the cells in front to retract. Conversely, Fat2—an atypical cadherin—is localized in the rear, seemingly stimulating the follower cells to protrude by stabilizing Lar in their front-edge. In an alternative model, Lar and Fat2 work cell-autonomously to activate WRC at tricellular junctions to build whip-like protrusions. Similar to the first model, Semaphorin/Plexin signaling seems to be employed for planar cell polarity. It could be shown, that Sema-5c at the leading edge activates Plexin A at the trailing edge in the adjacent cells, causing retraction.

**Border Cell Migration in the Drosophila Egg Chamber**

The migration of the border cell cluster provides an example of invasive collective migration in 3D. Border cells delaminate from the follicle epithelium and migrate in-between the germ-line cells called nurse cells towards the oocyte where they later build the sperm entry point—the micropyle. Before, the two polar-most cells of the follicle epithelium—the so-called polar cells—secrete Unpaired (Upd), activating the JAK/STAT pathway in their direct neighbors, the so-called border cells. This causes the expression of the transcription factor Slow border cells (Slbo) which in turn activated target genes as the *Drosophila* fascin singed, the *Drosophila* beta-catenin armadillo and E-cadherin. Several factors promote epithelial-to-mesenchymal transition (EMT) in six to eight border cells that in turn enwrap the polar cells which remain immotile throughout migration. Border cells build long protrusions pointing in the direction in which the cluster migrates and constantly change their position around the polar cell pair (Figure 2A). There are multiple redundant systems of chemotraction at work, guiding the cluster posterior by activating two receptor tyrosine kinases. The oocyte secretes the ligand PVF1 (PDGF and VEGF related factor 1) that binds to the receptor PVR (PDGF- and VEGF-receptor related) and the EGFR ligands Keren and Spitz. Both build a posterior gradient reaching throughout the extracellular environment between nurse cells. In the final stage of migration, the cluster undergoes a small dorsal turn, due to a third EGFR ligand—the TGAlpha ligand Gurken—that is dorsally higher concentrated. In border cell migration, there is an externally dictated distinction between leader cells and follower cells, that becomes clear when suppressing PVR or EGFR in the cluster. In both situations, all border cells start to build protrusions in all directions, demonstrating that the highest amount of receptor tyrosine kinase mediated Rac-1 activation defines the transient “leader” stage of the cell currently at the front. Conversely, photoactivation of Rac1 in single cells caused them to adapt a leader-state. The notion that differences in receptor tyrosine kinase activation between cells is more important than cell-autonomous directionality, could be supported by designing experiments in which receptor activation was decoupled from ligand-binding. Expression of constitutive-active RTK fusion proteins or photoactivation of Rac1 in a ligand-free environment in single cells, allowed for effective migration in which the activated cell took the role of a leader.

There is an inherent difference between border cell migration and most other modes of 3D cell migration. Border cells do not migrate through an ECM but in-between the nurse cells. The actin polymerization-based force is applied via E-Cadherin analogous to integrin “canonical” 3D migration. But not only the border cell/nurse cell interface has an impact on migration, but also the adhesion between nurse cells themselves. Recently, the role of tissue-topography in keeping the cluster restricted to the medial cleft was...
addressed in a fascinating study combining 3D-live-cell-imaging and agent-based computer simulations. Dai and colleagues demonstrated that the cluster always choses the path with the highest amount of contacting nurse cells which builds—due to simple geometry—the widest gap. Due to the tissue architecture, this path always leads through the center of the complex. If cell-cell adhesion between nurse cell is affected by tissue-specific suppression of E-Cadherin, the cluster instead uses non-medial trajectories adjacent to the follicle cells.  

Neural crest collective cell migration—insights from the frog

Unlike border cells neural crest cells (NC) migrate collectively in a loose cohort using contact inhibition of migration (CIL, explained in more detail in the following section). This can be studied and is best-characterized in Xenopus. During gastrulation, the cells of the neural crest delaminate from the neural tube epithelium and migrate in several distinct streams between beneath the epidermis (Figure 2B).  

During Xenopus development, the migration of NC can be imaged well enough, to track single cells and observe their behavior. To address junctional and cytoskeletal dynamics, NC can be analyzed in culture in high-resolution, where they mostly recapitulate their in vivo behavior. Xenopus NC migrate in a fluidic fashion, in which every single cell appears mesenchymal and bears the potential to migrate on its own, employing mechanisms of self-regulation.

Testis myotube collective cell migration—common and unique aspects

Detailed observation employing live-cell 4D imaging of cultured pupal Drosophila testis revealed that myotubes seem likewise mesenchymal and homogeneous. Before migration starts, until 28–30 h after pupae formation (APF), the anlagen of the testis and the rest of the genital tract lie separately inside the body cavity. The so-called genital imaginal disc contains myoblasts that later build the musculature of the entire system. At 24–28 h APF, the presumptive testis myoblasts start to fuse and build 2–6 nuclei syncytia, called nascent myotubes, here simply referred to as myotubes. At 30 h APF the fusion is completed. Subsequently, the testis and the genital imaginal disc coalesce.  

Immediately, the testis myotubes start to migrate towards the apex of the testis. During migration, gaps open inside the sheet, that are actively sealed either through protrusion or by employing actomyosin-based purse string dynamics (explained in more detail in the final section). Myotubes do not move over the testis surface, but between an outer layer of large pigment cells and cyst cells of the testis (see magnification in 2C). This makes the system self-contained and resilient to outside influences, similar to the Drosophila egg-chamber; a feature that can be exploited by live-cell imaging. Defective coverage of the testis in musculature—for example due to migration defects—results in characteristic shape defects of the adult testis. Instead of a 2.5 x coiled tube, the testis resembles a club with a dilated tip and less coils. Such defects could be observed, when the FGF-receptor Heartless (Htl) or its downstream factor Stumps is suppressed in myotubes and in a hypomorphic mutant of the Htl-ligand thisbe (ths). At first, it seems like the logical conclusion to assume that this might be due to a chemo-attractive function of Ths, guiding myotubes towards the apex. However, close examination of the migrating myotubes employing the newly established 4D imaging methods revealed that all myotubes resembled each other in terms of cell protrusion.  

Unlike border cell migration, no subset of cells seemed to exclusively react to an outside gradient. Instead, myotubes likely self-regulate their behavior. In this regard, they resemble NC.  

In the following section Xenopus neural crest cell migration and Drosophila testis myotube migration will be compared to find common principles and new features in self-regulation in mesenchymal collective cell migration.  

SELF-REGULATION IN MESENCHYMAL COLLECTIVE CELL MIGRATION

In 1995, the theoretical physicist Tamás Vicsek presented a simple ruleset that explains the complex motions observed in animal swarms, liquid crystals and also groups of cells without the need of any external control. He could demonstrate, that every agent in such a system must simply adapt its motion angle to its closest neighbors with a degree of uncertainty. Vicsek-Dynamics constitutes one typical example of self-regulation. More recent studies further showed that different cell and tissue types use variations of self-regulation, rendering the notion that mostly external control steers collective migration outdated. Another typical variation of self-regulation is contact inhibition of locomotion (CIL). During CIL, two cells either cease migration upon contact or repel each other, causing a deflective motion. This behavior was observed and described for the first time in cultured fibroblasts by Michael Abercrombie. It is used by many cell-types as a type of mesenchymal collective migration.

The social behavior of testis myotubes reveals new principles in self-regulation

The first in vivo evidence for CIL was found examining Xenopus NC. Here, N-cadherin is transiently localized at cell-cell contact sites, followed by an immediate repolarization of the partaking cells. Traction force is built-up by the newly formed lamellipodia, resulting in divergent migration. Due to its advantages in live-cell imaging and experimental accessibility, Xenopus neural crest cell migration became the gold-standard for CIL in the last years. The migration of Drosophila testis nascent myotubes appears to be another example for mesenchymal collective cell migration, in which cells autonomously
regulate their behavior. In this section, common features and key differences between *Xenopus* NC and *Drosophila* testis nascent myotubes will be outlined. By comparing cellular behavior and its regulation, it will become clear that myotubes undergo contact stimulation of migration (CSM), a phenomenon observed by Thomas and Yamada in cultured cells long time ago.\[80\]

Looking at the morphology of a single cell, there is a striking difference between *Xenopus* NC and testis nascent myotubes. The former have a classical front-rear fibroblast-like polarity (Figure 3A).\[71\] Testis myotubes completely lack lamellipodia. Instead, they project filopodia in all directions, lacking a directional protrusive component (Figure 3B).\[4\] Consistently, analysis of RhoA activity using a biosensor...
revealed that a classical "trailing edge" was lacking. Instead, RhoA becomes active in short pulses in single filopodia at cell-free edges. Therefore, myotubes can be described as radial symmetric with respect to protrusions.

In NC during the transient phase of cell-cell contact, Wnt/PCP-signaling activates RhoA at the contact-site, inducing "rear-end" dynamics.[71] This is promoted by N-Cadherin/Par3 signaling, inhibiting Rac activity locally (Figure 3A').[78,81,82] In testis myotubes only contact-dependent local changes in substrate-adhesion could be observed. At contact sites, integrin-based adhesions appeared to be disassembled faster, enabling a break of symmetry (Figure 3B').[14] A similar observation could also be made during Xenopus neural crest migration, as additional safeguard for CIL downstream of Src and Fak.[79]

Master regulators of this behavior in Drosophila testis myotubes seem to be Rac2 and Cdc42. Rac2 seemed to positively affect matrix adhesion longevity. Cdc42 in contrast, surprisingly seemed to take an antagonistic role by negatively regulating Integrin-based adhesion stability. RNAi-mediated suppression of cdc42 led to a characteristic phenotype with elongated static rod-like filopodia, that could be explained by a prominent lack of substrate adhesion-disassembly. In some cases, adhesions that were assembled in a filopodium, persisted throughout migration, reaching the opposite edge of the cell, causing filopodia-like retraction fibers. Such tail-retraction defects were never before observed upon loss of Cdc42, but as a consequence of RhoA depletion.[83]

The most striking difference setting apart Drosophila testis myotube behavior from CIL, is the absence of any cell-cell repulsion. Testis myotubes keep their neighbors relatively constant, except if N-Cadherin gets suppressed. A cell even keeps contact to its neighbor when both are surrounded by free edge, as all other adjacent cells have been artificially removed (Figure 3B''). This self-regulation scheme of testis myotubes, resembles a process initially observed by Thomas and Yamada in cultured quail NC and termed contact stimulation of migration (CSM).[80] Cells separated from clusters ceased directional migration. A similar observation could be made in zebrafish prechordal plate cells based on transplantation experiments in vivo.[84] Likewise, ablation of single migrating myotubes by artificially removing their neighbors, showed that the absence of cell-cell contacts resulted in a loss of directional locomotion, only to be regained after contact to the sheet is established (Figure 3B'').[4] In contrast, Xenopus neural crest quickly exchange neighbors, in a process that depends on N-Cadherin internalization regulated by LPar2.[85] NC in isolation that are surrounded by free edge induce a deflection in motion towards each other (Figure 3A').[71] Testis myotube CSM results in cells evenly covering all available space—which is limited by the 3D tissue architecture—while moving in a coherent stream (Figure 3B''). Similarly, space is covered by NC but in a more fluidic fashion with cells quickly changing their position (Figure 3A''). This fluidity is also an important factor in the "rear-wheel drive", summarized in the last section.[86] Nevertheless, cells are migrating in spatially clearly circumscribed streams. This is only possible, as there is additional chemo-repulsive lateral confinement and chemotaxis-based co-attraction, ensuring cohesion.[87] The latter is not necessary for testis myotubes, as they are constantly physically adhered due to quickly renewing filopodial contacts.[4]

CSM is a CIL-related process employed by different cell types

The term "stimulation" falsely implies CSM to be the opposite of CIL. However, both seem to be closely related processes. In both, CIL and CSM, local contact-dependent inhibition of protrusion or substrate adhesion gives a cell automatically a free-edge-based directionality. If subsequently the cell loses contact to its neighbors and is able to maintain the asymmetry for a while, it undergoes CIL. If the cell is either constantly adhered or not able to maintain asymmetry after losing contact, the result is CSM. CSM-like processes also play a role in collective cancer cell migration. It has been demonstrated long time ago that fibroblasts can stimulate migration in fibrosarcoma cells, which are non-migratory without stimulation.[88] Similarly, it was shown more recently that fibroblasts—subsequently called cancer associated fibroblasts (CAFs)—contact squamous cell carcinoma cells to promote and lead collective invasive migration.[89] During this type of CSM, a division of labor between the populations seemed to be maintained, as different GTPases have different cell type-specific functions. RhoA is crucial for CAF migration whereas cancer cells rely on Cdc42 activity.[89] Recently, it was shown that the CAF-led cancer-clusters need N-Cadherin/E-Cadherin heterotypic adhesion for invasion.[90] Furthermore, a higher amount of N-Cadherin in cancer tissues of patients is correlated with a higher amount of metastasis and lethality.[91]

A self-regulated contact-dependent behavior—not directly related to CSM—is contact following locomotion (CFL), also termed contact activation of migration.[92–94] CFL is a co-attractive cue, discovered in cultured cells on micropatterned stripes. When a cell contacts the trailing edge of another cell, it gets chemically attracted.[92] In Dictyostelium, a similar process has been observed. Upon cell-cell contact, the heterophilic adhesion molecules TgrB1 and TgrC1 interact to recruit the WRC to the contact site inducing local lamellipodia formation.[93] In principle, the contact dependent regulation via Fat2/Lar[57] and Semaphorin/Plexin[58] exhibited by Drosophila follicle cells during rotation could also be described as an example for CFL.

NON-AUTONOMOUS "PURSE STRING"—DYNAMICS COMPLEMENT SELF-REGULATION

Cell cohesion is crucial in collective cell migration. In particular in migrating cell sheets, mechanical stress can result in ruptures, that need to be continuously sealed. Detailed observation of the testis myoblast migration revealed that cells seem to close gaps in a collective effort, beyond self-regulation. Such gaps, which always open during migration, mostly contain supracellular actin-cables parallel to the edge, surrounding the opening (Figure 4A, B). Therefore, it seemed as purse string dynamics are employed for cohesion. Sometimes, the
Supracellular actin-cables in collective cellular motion. The direction of protrusions is symbolized by a red arrow, retraction/constriction are highlighted by blue arrows. Actin cables are depicted as blue lines. Thin arrows represent the direction of motion. (A) Gaps in the testis myotube sheet close by employing both, protrusion and actin-cable derived purse string dynamics. (B) Schematic representation of the gaps depicted in A. (C) Graphic of mechanisms used in myotube gap closure. Protrusion and purse string dynamics can be utilized independently or together. (D) During Drosophila dorsal closure, force derived by amnioserosa ingression and purse-string dynamics are used to seal the epidermis in a zip-like process. Supra-cellular actin cables reach from cell to cell in the leading edge of the epidermis. Protrusive dynamics are not used to slide over the amnioserosa. (E) During border cell migration, supracellular actin cables are used to ensure that follower cells do not build competing protrusions. (F) Tissue explants of Xenopus NC use supracellular actin cables to steer intercalation of cells at the rear of the cluster. Thereby, cells are pushed forward where they can advance the cluster by means of protrusions.
whereas protrusive lamellipodia were observed at the tip of positive curvature regions.\cite{95} It is known for a long time, that wound closure relies on both mechanisms.\cite{96,97} An extensive study further revealed—by employing traction force microscopy and laser ablation—that in Madin Darby canine kidney (MDCK) cells, supracellular actin-cables do not work exclusively in a purge string fashion. In early stages, protrusion activity seemed to prevail. In a later stages, supracellular actin cables transmitted force to the substratum via focal adhesions, supporting a closure process beyond purge string dynamics.\cite{98}

Drosophila dorsal closure is commonly used as an in vivo model for wound closure. During late embryogenesis, the dorsal part of the embryo is covered in a flat epithelium called amnioserosa. This tissue—which eventually undergoes apoptosis—gets replaced by the surrounding epidermis that undergoes a wound-like closure (Figure 4D).\cite{99,100} The amnioserosa is in contact with the epidermis via adherens junctions mediated by E-cadherin and supports its motion, employing apical constriction,\cite{99,100} a process that surprisingly appeared strong enough to ensure closure alone.\cite{101} However, it remains a fascinating process how the epidermis closes in a zip-like collective motion using constriction of supracellular E-cadherin-linked actin cables.\cite{102,103} Decades of research elucidated the regulation of actomyosin contractility by modulating RhoA dynamics and the workings of the E-cadherin-actin interface—the core component of mechanotransduction—that must react to tensile stress.\cite{100} Filopodial and lamellipodial protrusions support purse string dynamics, however, not as originally thought, in a way that flanking epidermal sheets migrate over a substrate that is the amnioserosa.\cite{99} Both tissues are constantly adhered to each other, while the amnioserosa ingresses through apical constriction and gradually undergoes apoptosis.\cite{99} Filopodia play a role in exploration and recognition of prospective "zipping-partners" on the opposing side.\cite{104} Detailed examination, using transmission electron microscopy, further revealed an interplay of protrusions at both sides during segment matching. Protruding lamellipodia start to overlap in a "roof-tile" fashion, to subsequently pull the "partner"-cells towards each other. Intriguingly, growing and subsequent shrinking of microtubules might contribute the energy for these processes, as actin appears absent from the cellular protrusions.\cite{105}

**Supracellular actin cable-mediated cohesion during collective migration**

Supracellular actin cables are also known to have a function in keeping cohesion in other collective migration systems. In Drosophila border cell migration, myosin activity at the periphery of the cluster was shown to create the cortical tension that is important to withstand the outside pressure of the nurse cells.\cite{106} A network of supracellular cables, surrounding the cluster and enforcing cohesion, stops follower cells from building protrusions (Figure 4E).\cite{107} Furthermore, proper localization of Cdc42, moesin as well as E-Cadherin and normal myosin function and localization at the cables are interdependent, revealing a role of myosin in cell-cell signaling during border cell migration.\cite{107}

Border cell migration and dorsal/wound closure are examples for epithelial/partially epithelial cells, linked via E-Cadherin. Testis myotubes are linked via N-Cadherin.\cite{14,47} There is also another intriguing example for N-Cadherin linked supracellular cables in mesenchymal collective migrating cells. In Xenopus neural crest tissue explants, rear-positioned supracellular cables take an unexpected role as the main engine of migration, building a so called "rear-wheel drive" (Figure 4F). The cables depend on N-Cadherin, and myosin is internalized upon E-Cadherin overexpression.\cite{86} Sheillard and colleagues were able to demonstrate that the contraction-based intercalation at the rear end, causes single cells to move inside the cluster in a way that creates vortex-like fluid motion. This motion—which resembles Viscell-like milling—\cite{108}—propels cells towards the leading edge where absence of supracellular contractility allows them to expand, advancing the entire cluster and enabling chemotaxis.\cite{86}

In both, Drosophila border cell clusters and Xenopus neural crest clusters, peripheral cables enforce cohesion. In contrast, in migrating testis myotubes internal gaps are closed by actin cables in a much more wound/dorsal closure-like fashion. Hence, in this system mesenchymal cells coordinate like epithelia which require constant cell-cell adhesion. Future studies will have to elucidate how myosin and the actin-cadherin interface are specifically modulated by factors also known from dorsal closure like different GAPS and GEFs targeting RhoA, Rac and Arf, catenins, Canoe, Polychaetoid and Girdin.\cite{100} The exciting question remains, how autonomously self-regulating cells can spontaneously turn on this cooperative mechanism to enable cohesion.

**CONCLUSION**

Collective cell migration plays an important role in numerous processes that shapes cells into tissues and organs with high efficiency. Over the years, many model systems have been successfully established to dissect collective cell migration in diverse physiological and pathophysiological contexts. These studies have highlighted emerging concepts of self-organization of collectively migrating cells. This also includes contact-dependent polarization of cell clusters that allows a high migratory efficiency with a surprising regulatory simplicity of underlying molecular system. The testis myotube system is versatile new tool in the toolbox of collective cell migration model systems. High-resolution 4D imaging combined with mechanical, chemical and genetic manipulation allows researchers to identify new unexplored cellular mechanisms of collective cell migration. The system also allows to address a wide range of exciting unique aspects of myotube migration, including filopodia-driven motility, contact stimulation and purge string closure of gaps. Future work on testis nascent myotubes could provide exciting new insights into the invasive mechanics how these cells migrate through the ECM between cell layers. These studies might also provide answers to long-standing questions such as the exact role of filopodia during 3D cancer cell invasion in metastasis.
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