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Patterning in Motion: Cell Interfaces Guide Mesenchymal Collective Migration and Morphogenesis
Running title: Mesenchymal Collective Migration and Patterning

eTOC: Bischoff and Mayor present a framework for comparing mesenchymal collective migration modes and explain their roles in patterning and morphogenetic complexity.

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Abstract: Collective cell migration is a fundamental process in development, wound healing, and cancer. The best-characterized modes of collective migration typically involve cells that retain an epithelial architecture. However, in this review, we explore less well-understood modes of migration driven by cell with a more mesenchymal phenotype. To better understand and compare contact-dependent collective cell behaviors, we propose conceptualizing cells as architectural patchworks of morphodynamic building blocks and inferring collective dynamics from behaviors of these subunits. By examining how local cell shapes influence single-cell behaviors, we gain insight into how swarm-like behaviors emerge through cell-cell contact. Through this lens, we compare key processes such as contact inhibition of locomotion, mesenchymal cell intercalation, and more complex heterotypic swarm behaviors. Finally, we will discuss the emerging concept of contact-mediated rules that regulate motility and have the potential to encode blueprints for complex patterns and even organ shapes.

Key Words: Cell Biology, Development, Collective Cell Migration, Mesenchymal, Intercalation, Emergent Behavior, Cell shape changes, Pattern-Formation, Morphogenesis.

Introduction

Collective cell migration is a fundamental process essential for the development of multicellular animals and fungi. It plays a central role in tissue repair and serves as a hallmark of cancer progression and metastasis (Friedl and Gilmour, 2009). Even facultative multicellular organisms, such as *Dictyostelium*, utilize very similar cytoskeletal architectures, chemokine signaling pathways and even collective dynamics when compared to animals, highlighting their evolutionary conservation (Fritz-Laylin and Titus, 2023; Stuelten et al., 2018). In the 1950s, Michael Abercrombie and Joan Heaysman observed that fibroblasts repel each other, coining this phenomenon the "*social behavior of cells*" (Abercrombie and Heaysman, 1953). Since then, the definitions of collective cell migration have varied among researchers. This review adopts the broad definition proposed by Mayor and Etienne Manneville (2016), who argue that the defining feature of collectively migrating cell groups is their ability to migrate more efficiently than isolated cells (Mayor and Etienne-Manneville, 2016); thus, collective cell migration requires coordination and cooperation among migrating cells (Scarpa and Mayor, 2016). This definition not only encompasses tightly connected epithelial cells but also more individual mesenchymal cells, which can be only transiently connected and influence each other's migratory behavior (Theveneau and Mayor, 2011). Here, we introduce a framework that applies the terms "epithelial" and "mesenchymal" to local cellular properties, allowing a single cell to simultaneously exhibit both. This approach is not intended to replace current categorizations, but to offer additional resolution when considering contact-dependent swarm behaviors. For example, even when migrating epithelia display some local mesenchymal traits in this model, their overall architecture — including stereotypical apico-basal polarity — remains predominantly epithelial, making the term "epithelial collective cell migration" both accurate and useful. However, our focus lies on more "individual" collective cell behaviors such as mesenchymal collective cell migration and crawling-based intercalation. We also explore novel roles for such modes of collective cell motility as drivers of morphogenesis and pattern formation during development.

Morphodynamic Building Blocks: Diverse Architectures Drive Single Cell Shape and Behavior

In addition to their pioneering description of collective dynamics, Michael Abercrombie and Joan Heaysman and their colleagues uncovered the architectural complexity within a single cell that enables cell migration (Abercrombie et al., 1970a; Abercrombie et al., 1970b; Abercrombie et al., 1971; Abercrombie et al., 1970c). They were the first to describe key cellular structures involved in mesenchymal motility: an advancing and sometimes retracting front protrusion they named the lamellipodium, followed by a more steady-moving region called lamella, and finally, a trailing edge that undergoes retraction. This revealed that different regions of a cell must build different structures, and that their precise spatial organization determines migration speed, directionality, and overall behavior. As a result, we can think of cells as complex patchworks of morphodynamic building blocks, with internal coordination.

How exactly this coordination is achieved remains a matter of ongoing research and is described in detail elsewhere (Ghose et al., 2022; Peglion and Etienne-Manneville, 2023). At its core, small Rho family GTPases—Rac, Cdc42, and Rho—play a pivotal role (Lawson and Ridley, 2018; Ridley, 2003; Zegers and Friedl, 2014) as molecular switches that can be turned on and off. Upon activation, they regulate a wide array of effectors controlling the cytoskeleton and cell shape. An intricate interplay of GTPases and their regulators enables the self-organization of simple front-rear gradients in highly persistent and fast-migrating cell types, as well as more complex, spatially diverse architectures in

exploratory cell types that lack a single dominant protrusion (Nalbant and Dehmelt, 2018; Nanda et al., 2023). In addition, the distribution of phosphoinositides, notably phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P₃) and phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P₂), plays a crucial role. (Wu et al., 2014). These two systems and others, are interconnected in multiple ways, forming a large and complex, self-regulating system. This system can respond to diverse stimuli—such as chemoattractants, cell-cell contact, and the physical properties of the environment—as well as other key regulators of single and collective cell behaviors, to generate the appropriate cellular architectures required for processes like migration. (Llense and Etienne-Manneville, 2015). Aside from “Abercrombie”-type fibroblast-like migration, in vitro and vivo research has demonstrated that cells employ many other migration strategies depending on the dimensionality and the curvature of the substrate, and the specific microenvironment, accompanied by multiple protrusion types and different global cell shapes (Balaghi et al., 2023; Bodor et al., 2020; Chen et al., 2019; Lämmermann and Sixt, 2009; Madsen et al., 2014; Roca-Cusachs et al., 2013; SenGupta et al., 2021; Yamada and Sixt, 2019).

For this review, however, the key point is not how specific structures, like lamellipodia, blebs or actomyosin cables work, for which we refer the reviewer to excellent recent reviews (Chastney et al., 2025; Fritz-Laylin and Titus, 2023; Merino-Casallo et al., 2022; SenGupta et al., 2021; Yamada and Sixt, 2019). Instead, we aim to highlight that a single cell can be conceptualized as a patchwork of multiple local architectures, which can be sorted and grouped within a conceptual map where proximity reflects similarity. (Figure 1 A; a speculative representation). Importantly, such local architectures don’t rely on single regulators; instead, they arise from the activation of a wide array of factors. For example, Arp2/3-based actin branching comes to mind when thinking about lamellipodia. However, in melanoma cells and fibroblasts, the actin bundling Formin FMNL has been shown to increase force generation inside lamellipodia (Kage et al., 2017). This, and many other examples, show that multiple regulators in specific combinations give rise to the emergent architectures that we mean when we use words like “lamellipodium”. Thus, each such local architecture can be imagined occupying a position within our conceptual map. More accurately, our semantics probably reflect an *area* of very similar structures, not a single point on this map. This is especially true when taking many cell types or even many species into account. For example, the Arp2/3/FMNL-ratio probably differs from cell-type to cell-type, and a lamellipodium is not *exactly* the same “thing” in all model systems. The number of dimensions of this purely hypothetical map reflects the sum of all structural proteins and their regulators (with dimensions for their concentrations) but also additional dimensions, *e.g.* representing diverse activation states of different proteins. This vast range of potential architectures represents the complexity observed in cell shape.

Crowd Control: Cell-Cell Contact as a Driver of Many Emergent Behaviors

A single cell can be conceptualized as a patchwork of various morphodynamic building blocks, all coexisting and organized in a specific spatial pattern. This spatial organization dictates the cell's behavior. For instance, a highly stable keratocyte may migrate quickly and persistently, while an exploratory fibroblast with numerous protrusions may exhibit more erratic behavior, frequently changing direction (Nalbant and Dehmelt, 2018). In general, localized modifications to subcellular architecture can lead to dramatic shifts in behavior (Wang et al., 2010). In some modes of epithelial collective cell migration, leader cells exhibit a hybrid morphology, characterized by a mesenchymal/lamellipodial front and an epithelial backside, which creates a dynamic interface with the epithelial sheet, as reviewed recently (Stebens et al., 2024). This specific combination of architectures drives their characteristic behavior: migrating away from the epithelial sheet and thereby introducing a break of symmetry. In our conceptual map, each individual region of the cell occupies a distinct position,

and their combined interactions produce emergent behavior. Thus, to understand single or collective cell behavior, it can be useful to apply terms like 'mesenchymal' and 'epithelial' to specific subcellular regions rather than to the entire cell (Campbell and Casanova, 2016). Within the conceptual map presented before, these terms do not represent fixed, well-defined states but larger areas containing groups of local architectures (Figure 1A). Using our model, some cell types can be viewed as combination of mesenchymal and epithelial architectures or activate protrusive dynamics under specific conditions, such as during wound closure (Brugués et al., 2014), extrusion (Le et al., 2021), or to stabilize junctions against constriction forces (Li et al., 2020). This example highlights that viewing a cell as an architectural patchwork—and to extrapolate collective dynamics from individual components rather than from simplified single cell behavior—provides an alternative framework for understanding individual and collective cell migration.

Cell-cell contact acts as a critical leverage point, converting local architectural changes into emergent collective behaviors (Figure 1B, 2B). Typically, through cadherin-based adherens junctions, cells can influence one another's behavior by modifying exactly these localized structures. Cell-cell adhesions serve as essential hubs for signaling, physically linking the junction to the cytoskeleton and driving structural modifications. The diverse roles of cell-cell adhesions in cytoskeletal remodeling have been reviewed extensively (Campàs et al., 2024). Beyond adhesions, transmembrane receptors-ligand couples such as EGFR-cadherin (Ramírez Moreno and Bulgakova, 2022) or FGFR-cadherin (Nguyen and Mège, 2016) also detect direct cell-cell contacts and can trigger localized architectural changes. Receptors traditionally known as contact-dependent “axon guidance factors” also act in non-neuronal tissues and regulate cytoskeletal dynamics upon binding to their transmembrane ligands. Examples are the Eph/ephrin (Taylor et al., 2017) system or the Semaphorin/Plexin (Worzfeld and Offermanns, 2014) system.

The Wnt-PCP pathway is one of the most crucial regulators of collective polarity in epithelial and mesenchymal tissues (Butler and Wallingford, 2017; Devenport, 2016; Strutt, 2008). Across animal species the Wnt-PCP pathway is a common pathway to introduce planar polarity to epithelial tissues (Devenport, 2016). This could potentially influence collective cell migration by synchronizing the directionality of cryptic lamellipodia. However, Wnt/PCP is also employed during mesenchymal intercalation of frog mesoderm, ensuring cell alignment by defining the position of growth-cone like lamellipodia (Keller and Sutherland, 2020; Wallingford et al., 2000). Furthermore, it is one of the main drivers of contact inhibition of locomotion in neural crest cells (Mayor and Carmona-Fontaine, 2010). Next to cell contact-based regulation, there are diverse known and highly important ways how cell collectives can follow non-cellular cues, like chemical gradients, e.g. secreted factors or physical gradients, e.g. ECM stiffness. Such properties are not necessarily external, but can also be self-regulated (Wong and Gilmour, 2021). Famously discovered by the Gilmour lab, the Zebrafish lateral line primordium self-generates a chemokine gradient, using polarized receptor-mediated internalization (Donà et al., 2013). However, here, we want to focus on self-regulation based on cell-cell contact, rather than by protein secretion or scaffold remodeling.

At the subcellular level, contact-driven modifications influence individual cell behaviors. When scaled to the multicellular context, such interactions can give rise to specific **swarm behaviors**, exemplifying how local architecture drives collective dynamics (Figure 1B). A quintessential example to explain how local modification introduce a specific swarm behavior, is contact inhibition of locomotion (CIL) (Theveneau and Mayor, 2012a; Theveneau and Mayor, 2012b; Theveneau and Mayor, 2013). During vertebrate development, neural crest cells (NCCs) migrate in characteristic streams to later differentiate into a variety of cell types contributing to several organs; such as cartilage, bone, muscle, and connective tissues in the skull and facial structures, as well as their innervations; the aortic outflow tract of the heart and to the enteric nervous system with connective tissue and neuronal and glial cells; and dorsal root ganglia, the adrenal gland and melanocytes (Szabó and Mayor, 2018). When two

neural crest cells make contact, the initial response is a change in architecture. N-cadherin and Wnt/PCP signaling pathways reconfigure the contacting regions of the cells into a “trailing edge” architecture, leading to complete repolarization of the contacting cells, away from the point of contact (Carmona-Fontaine et al., 2008). The phenomenon of CIL enforces a behavioral rule: “*walk away when you touch another cell*”! It was first observed by Abercrombie and Heaysman (Abercrombie and Heaysman, 1953), and dissected over many years by the Mayor Group and illustrates how molecular changes in architecture underpin behavior. Zooming further out, this simple rule of contact-induced repulsion generates a specific swarm behavior, causing even cell distribution across a surface (Stramer and Mayor, 2017). This example highlights how collective emergent behaviors originate from molecular-level changes that influence cell shape and architecture. Just as there is a diversity of cellular architectures and thus architectural transitions, there is a corresponding landscape of swarm behaviors that remains to be explored and understood (Figure 2A, B).

Beyond CIL: Exploring Other Contact-Dependent Rules of Collective Migration

Cell-cell adhesions play a crucial role in many examples of self-regulation during collective cell migration. Remarkably, even cells that lack cell-cell adhesions can still alter each other's morphology, resulting in specific swarm behaviors as a purely passive physical process. For example, spindle-shaped cells in high density can passively align with one another, leading to liquid crystal-like behavior (Duclos et al., 2017) (Figure 2A). However, active restructuring near cell-cell adhesions allows for much more complexity in cell behavior. For example fibroblasts also use an active mechanism to align, that opposite to CIL (which they can also perform) involves down-regulation of actomyosin contraction at contact sites. This contact-dependent mode was termed collision guidance (Park et al., 2020).

In some epithelial modes of collective migration, the entire sheet behaves like a single unit, with a division of labor between rows of front cells phenotypically more mesenchymal cells and rear cells phenotypically more epithelial (Figure 2A). These dynamics are also partially directly regulated by cell-cell contact (Campàs et al., 2024), and have been extensively reviewed in recent literature (Gupta and Yap, 2021; Stock and Pauli, 2021; Vishwakarma et al., 2020). Our review focuses on modes of collective cell migration described by the term “mesenchymal collective cell migration”. This refers to migration modes in which each cell migrates independently, not necessarily remaining permanently adhered to each other. Sometimes, like in CIL, contact is only transient. Even in epithelial collectives, there are instances of “mesenchymal dynamics” outside the front edge. Cryptic lamellipodia from follower cells can support sheet migration from within the sheet (Gupta and Yap, 2021). Such structures are directly promoted by E-cadherin in flat adenocarcinoma-derived epithelial cells, where cryptic lamellipodia are located directly adjacent to adherens junctions (Ozawa et al., 2020), fitting well with the general notion that cellular interfaces directly influence emergent cell behaviors via local restructuring. However, it remains unclear whether this mechanism would also function in more columnar epithelia, where cryptic lamellipodia are located spatially much farther from adherens junctions. Meanwhile, in mammary epithelia, cryptic lamellipodia formation requires the MYO6-DOCK7 axis to spatially restrict Rac activation (Menin et al., 2023). The idea of a transition between epithelial and mesenchymal modes of collective migration or “hybrid/partial EMT” has been discussed in the field for some time (Campbell and Casanova, 2016; Peglion and Etienne-Manneville, 2023; Revenu and Gilmour, 2009; Theveneau and Mayor, 2013; Weijer, 2009). In our opinion, the existence of cryptic lamellipodia nicely demonstrates this notion, especially as it allows modes of migration in which every cell within the sheet can be motile itself.

Mesenchymal testis nascent myotubes (TNMs), muscle precursors needed to cover and shape the *Drosophila* testis during pupal development, undergo contact-mesenchymalization (Figure 2A). To do

this, they use Plexin A signaling upon contact to maintain protrusions even at cell-cell interfaces, allowing each cell to spread (Bischoff et al., 2025a). This is crucial, as cells must remain flat to migrate under confinement, sandwiched between two cell layers on top of an ECM, while also restricting growth of the underlying testis. Even though cells project filopodial protrusions in all directions, protrusions near contacts have integrin-adhesions with a shorter lifetime than at the free edges. This causes cells to move toward open space and fill the finite area of an ellipsoid testis surface—rendering it an example of contact-stimulation of migration (Figure 2A, B) (Bischoff et al., 2021; Yamada and Sixt, 2019). Like in CIL, this also causes space filling, with the critical difference that cells remain in contact to each other, which is crucial for morphogenesis.

CIL, contact-stimulation of migration and collision guidance represent symmetric interactions, as both contacting cells react with identical architecture transitions. In contrast, some cells exhibit asymmetric interactions at cell-cell interfaces, where one cell forms protrusions and the other develops a trailing-edge architecture. This asymmetry can result in contact-following behavior (Figure 2A, B), as observed in follicle cell rotation during *Drosophila* egg chamber development (Cetera and Horne-Badovinac, 2015). Interestingly, while follicle cells are part of a highly epithelial tissue, every single cell generates planar polarized cryptic lamellipodia. Unlike most migratory tissues, there is no free edge to define directionality. Instead, these cells form a closed epithelial ellipsoid that migrates collectively on a surrounding ECM layer. Lacking external open edge-derived polarity, these cells must self-regulate planar polarity to synchronize their directionality. This coordination is achieved through the action of several key molecules, including the non-conventional cadherin Fat2 together with the receptor tyrosine kinase Lar (Barlan et al., 2017), and PlexinA/Semaphorin signaling (Stedden et al., 2019). Fat2 locally promotes protrusions by activating Arp2/3-mediated actin branching through the WAVE complex (Williams et al., 2022), consistent with the broader principle that localized changes in protrusion dynamics act as drivers of self-regulation in collective migration.

Contact-following behavior is not limited to epithelial systems. For example, *Dictyostelium* cells exhibit contact-following within swirling streams during fruit body formation (Figure 2A, B). Unlike in follicle cell migration, there is a free edge, and therefore no need to synchronize directionality “everywhere at once”, but instead front-to-back (Figure 2A). The following-behavior behavior is mediated by heterophilic adhesion molecules TgrB1/TgrC1, which locally activate the SCAR/WAVE complex to drive protrusions (Fujimori et al., 2019). Similar behaviors have also been observed in macrophage-tumor cell interactions, further highlighting the conservation of this behavior across diverse biological systems (Miskolci et al., 2021).

All aforementioned examples are based on the concept of cells migrating within or on an extracellular matrix (ECM) environment, with integrins providing linkage to the substratum and cadherins serving as mediators of cell-cell adhesion and signaling hubs. In the early 1990s, Ray Keller observed in *Xenopus* mesodermal explants that during mediolateral intercalation cells exert traction forces directly on each other, resulting in cell-on-cell crawling that drives cell elongation (Shih and Keller, 1992). In *Drosophila* border cell migration, cells can also use other cells as a substrate (Montell, 2003). This represents another way to utilize cell-cell interfaces to guide migration. In this context, cadherins take on a role akin to that of integrins in mesenchymal migration, providing the necessary anchorage for the actin cytoskeleton to generate force and propel movement. Not only do other cells provide anchorage, but their topography also directs the border cell cluster during migration, as it consistently follows the path with the most cell contacts, since this path is geometrically the widest (Dai et al., 2020). Only very few in vitro studies have examined cadherin-based substrates in cell migration (Collins et al., 2017; Dehli et al., 2019; Li et al., 2019; Suh et al., 2024). Recently, the Cohen group tested this by placing MDCK cells on a split substrate: one half integrin-based and the other cadherin-based (Suh et al., 2024). The results were striking, with cells drastically slowing down on the cadherin-based substrate, showing reduced fluidity and dynamics and cell division. These experiments demonstrated significantly altered collective cell

migration dynamics, with sharp borders forming at the split substrate boundary. As cadherin-based substrates might be used by many migratory cells in vivo, such as cancer cells, it is crucial to adopt similar approaches across diverse cell types and cadherin variants to better understand the distinct architectures and behaviors at cell-cell interfaces. The fascinating phenomenon of cell-on-cell migration was reviewed recently (Le and Mayor, 2025).

Mesenchymal intercalation (Figure 2A, B) represents its own type of mesenchymal collective motility. Epithelial intercalation often relies on T1 transitions—a stereotyped sequence of cell shape changes involving junctional constriction, formation of a transient four-cell vertex, and subsequent neighbor exchange through elongation of the newly formed interface (Butler et al., 2009; Fernandez-Gonzalez and Zallen, 2011; Sawyer et al., 2011). However, mesenchymal cells can use another form of intercalation, driven by cell elongation through two opposing growth cone-like protrusions (Devenport, 2016). Interestingly, work by John Wallingford and others suggests that many processes likely employ a combination of both approaches (Huebner and Wallingford, 2018). By analyzing each individual cell’s architectural components, it becomes evident that mesenchymal intercalation shares similarities with CIL and contact-mesenchymalization. At some cellular interfaces, protrusions are symmetrically inhibited and local constriction enforced, reminiscent of CIL dynamics, while at others, protrusions are reinforced, reminiscent of contact-mesenchymalization and collision guidance, all together resulting in cell elongation. To define the two axes that specify which edges respond to cell-cell contact, polarity is established in a collective self-organized process which is often based on Wnt/PCP (Wallingford et al., 2000). Wnt/PCP as known from epithelial tissues, however, can create distinct anterior and posterior edges. The question arises, whether this plays a role in mesenchymal intercalation, too. High-resolution live cell imaging in elongating *Xenopus* mesoderm cells recently led to the identification of architectural asymmetries between the anterior and the posterior interface. PCP signaling, mediated by Vangl2 and Prickle2, not only establishes mediolateral symmetry but also generates a cryptic anterobasal polarity, characterized by a denser contractile actin cytoskeleton at the anterior edge. This is mediated by Septins downstream of Wnt/PCP (Devitt et al., 2024).

This combination of contact-based interactions in mesenchymal cell migration underscores the versatility of collective migration mechanisms, where cells can adapt their behavior based on context. This enables mesenchymal cells to exhibit a broad range of migration strategies, similar in complexity to epithelia, but often employing more individual and fluid strategies when it comes to emergent behaviors. Next, we will explore how such dynamics can shape tissue patterning and morphogenesis.

Dynamic Blueprints: Mesenchymal Collective Behaviors Can Encode Complex Patterns

In his pioneering work, Eric Davidson distinguished between two fundamental modes of cell specification: *autonomous* and *conditional* (Davidson, 1990). Autonomous specification depends on intrinsic, lineage-based factors, while conditional specification relies on extrinsic cues such as morphogens or the surrounding cellular environment. Thus, in conditional specification the logic is “location first, identity second”, with a cell’s identity determined by its position relative to its neighbors. In tissues with minimal cell rearrangement, it is helpful to think of them as static grids of cellular automata, where information flows between cells—for example via morphogenetic gradients—in a manner similar to *Conway’s Game of Life*. These concepts build on the foundational ideas of Alan Turing and Lewis Wolpert. In the groundbreaking Heidelberg screen 40 years ago, Christiane Nüsslein-Volhard, Eric Wieschaus, and colleagues discovered that similar complex interactions in an epithelial grid drive patterning in fly development, and many of the basic principles they identified held true in vertebrates as well (Jürgens et al., 1984; Nüsslein-Volhard et al., 1984; Peifer, 2024; Wieschaus et al., 1984).

However, in some systems, a cell's identity is specified first—whether by autonomous or conditional mechanisms—and its relative position within the tissue emerges from this identity through guided motility. It is well established that complex epithelial rearrangements can drive morphogenetic processes, with portions of an epithelial sheet moving relative to others—as seen, for example, in kidney development (Vasilyev et al., 2009), or during *Drosophila* dorsal closure (Kiehart et al., 2017). However, such rearrangements can also occur at the level of individual cells, giving rise to more complex and individual swarm behaviors in which each cell acts as an autonomous agent, responding to local cues through context-dependent rules.

An example for this is the ability of cells to self-sort according to the specific cadherins they express (Moscona and Moscona, 1952). Recently, cadherin-mediated self-sorting has been proposed as a robust mechanism that operates in concert with morphogen gradient-based patterning (Tsai et al., 2020). Differential adhesion can organize not only epithelial sheets by cadherin type but also drive more complex tissue rearrangements, particularly through the interplay between cell-cell and cell-matrix adhesion. For example, during budding morphogenesis in the stratified epithelium of the developing mouse salivary gland, cells with low E-cadherin expression—regardless of their initial position—reliably sort into the outer, basement membrane-contacting layer. This directed sorting behavior promotes tissue buckling and exemplifies how adhesive differences can be harnessed to coordinate patterning and morphogenesis. (Wang et al., 2021).

Active protrusive dynamics are well known to change rheological properties in tissues (Hannezo and Heisenberg, 2022). This notion is based on the finding that cell behavior can resemble atoms in different states of matter – solid, fluid or gas-like. Protrusive/mesenchymal dynamics typically cause cells to behave like particles in a fluid or, when interactions are minimal, like a gas (Lenne and Trivedi, 2022). Such dynamic tissues can cause collectives to respond passively in a stereotypical way to physical cues – which can be used to direct morphogenesis and form patterns. Jamming and unjamming transitions—shifts between fluid-like and solid-like states—have thus been proposed as key mechanisms in shaping tissues (Angelini et al., 2011; Hannezo and Heisenberg, 2022). We propose the term **mesenchymal patterning** to describe the process by which mesenchymal cells use motility to self-regulate cell rearrangement and generate spatial patterns. This complements the diverse and essential ways in which epithelia can self-organize, reshape, and rearrange. Mesenchymal patterning can occur either through general fluid- or gas-like behaviors—driven by random single-cell motility and shaped by environmental cues—or through directed interactions mediated by cell-cell contact, as discussed next.

Phenomena like CIL, contact following, or contact-stimulated migration and many more demonstrate, cells—typically those on the mesenchymal end of the spectrum—can display individual highly **directed** movements governed by complex, rule-based behaviors. These often contact-dependent dynamics allow for intricate, self-organized rearrangements that generate patterns on their own. During active, contact-dependent changes in cell motility driving patterning, there are only a limited number of ways in which two cells can alter each other's behavior. We began assembling a list of known and potential modes of contact-dependent behavior changes either affecting migration directionality or cell shape (Fig 3a). In tissues composed of multiple cell types heterotypic interactions can generate the complexity needed for more intricate patterns to emerge from contact-dependent changes in cell behavior. Rules such as CIL or contact following or co-attraction can be specific to particular combinations of cell types—meaning a given cell might respond one way to one cell type and differently to another (Fig. 3b, a hypothetical illustration of this concept). These rules can be symmetric (both cells reacting the same) or asymmetric (each cell reacting individually), depending on the cell-type pairing. Expanding the landscape of possible interactions even further, some rules may shift based on context—such as the physical environment—or change over time. Such mechanisms could for example be used to generate pattern variations along body axes (Fig. 3b). In this view, the rules underlying mesenchymal collective cell migration themselves encode the blueprint for tissue patterning. We propose the term

directed mesenchymal patterning to describe this form of dynamic, rule-based pattern formation. Next, we will present examples of directed mesenchymal patterning as well as mesenchymal patterning more broadly.

A Ballet of Cells: Vignettes on Directed Mesenchymal Patterning

Although these phenomena have not previously been unified under a single conceptual framework, a diverse array of biological systems display features consistent with our definition of *directed* mesenchymal patterning. In this section, we highlight representative examples—pigment cells and neural crest cells—that illustrate how contact-mediated behavioral rules in mesenchymal cells can generate spatial patterns. We also briefly summarize key conceptual connections and contributing mechanisms, including parallels to axon guidance, the importance of cytonemes for long-range signaling, and insights from computational modeling that help to understand directed mesenchymal patterning.

Pigment cells in fish. The development of fish coloration has revealed a striking example of directed mesenchymal patterning. Adult fish have very diverse colorful patterns, created by different types of pigment cells (Irion and Nüsslein-Volhard, 2019). Most of them do not adopt a fixed identity based on position. Instead, they actively migrate and self-organize, and then either settle or proliferate (Irion and Nüsslein-Volhard, 2019). One might argue that such migration is guided by a morphogen pre-patterned epithelium. However, in *Danio rerio* it has been shown that while the underlying horizontal myotome establishes a primary axis, everything beyond this initial cue is a self-organized, pigment cell-autonomous process (Frohnhofer et al., 2013). During this, both homotypic and heterotypic interaction rules play essential roles. Yellow xanthophores migrate as an interconnected network, extending and filling space (Walderich et al., 2016), likely using homotypic CIL or contact-stimulated migration. Meanwhile, black melanophores also exhibit homotypic repulsion, which generates free-edge-based directionality and prevents clustering except when influenced by heterotypic interactions (Takahashi and Kondo, 2008).

Pattern formation in pigment cells relies on multiple heterotypic interaction rules: reflective iridophores attract xanthophores, while xanthophores repel melanophores (Frohnhofer et al., 2013). Xanthophores extend protrusions that repel melanophores and intriguingly, these protrusions are maintained, so that xanthophores get “dragged” by melanophores (Inaba et al., 2012; Yamanaka and Kondo, 2014)—resembling CIL followed by chase-and-run (Theveneau et al., 2013) or long range contact-following (Figure 3a). Together, these findings illustrate that cells integrate multiple forms of contact-dependent interactions to self-regulate and harness these interactions to generate complex patterns. However, one notable exception to this are the two types of iridophores that differentiate into their respective form, based on the presence or absence of migration patterned melanophores (Gur et al., 2020). This demonstrates that patterns formed by directed mesenchymal patterning can themselves be instructive for further conditional specification.

Color patterns also change over the course of evolution. *Danio rerio* displays a striped pattern, whereas other *Danio* species exhibit dotted patterns. If these patterns arise through directed mesenchymal patterning, this raises the question of how one pattern can evolve into another. Evolutionary-developmental studies using interspecies chimeric genetics have identified regulatory genes that were modified in the transition from stripes to dots. Fittingly, these genes are not signaling molecules, but proteins involved in cell-cell contact, such as tight junction and gap junction molecules (Fadeev et al., 2015; Irion et al., 2014). This suggests that evolutionary changes in patterning can arise

through the modulation of direct cell-cell interactions—consistent with the central principle of directed mesenchymal patterning.

Cephalic Neural Crest Migration. Neural crest cells in the head migrate collectively in stereotyped streams. Until recently, this stream migration was thought to be guided by a pre-existing pattern of inhibitory signals located at the borders of each stream. According to this view, migratory neural crest cells followed this pattern, avoiding the inter-stream regions due to the presence of inhibitory molecules such as Ephrins, Semaphorins, and Slit/Robo (Szabó and Mayor, 2018). However, recent findings show that no such inhibitory pattern exists before migration. Instead, cells expressing these inhibitory molecules are uniformly distributed adjacent to the neural crest. The initial patterning arises from mutual cell repulsion between neural crest cells and inhibitory cells (Szabó et al., 2019). Importantly, these inhibitory cells also express a chemoattractant for neural crest cells, which reinforces the emerging pattern. Thus, neural crest cells "push" inhibitory cells away, while inhibitory cells simultaneously attract neural crest cells, together generating the stream migration pattern. Mathematical simulations have successfully reproduced this behavior, identifying the speed difference between neural crest and inhibitory cells as a key parameter: neural crest cells migrate faster than inhibitory cells (Szabó et al., 2019).

Self-Generated Mechanical Gradients. Neural crest cells migrate directionally by integrating both chemical and mechanical cues (Shellard and Mayor, 2019). One key mechanical cue is tissue stiffness (Barriga et al., 2018), which exists in the form of a gradient that neural crest cells sense and follow—a process known as durotaxis (Shellard and Mayor, 2021). Interestingly, this stiffness gradient is not present before migration begins; instead, it is generated by the neural crest cells themselves, which actively modify the mechanical properties of their environment. Neural crest cells migrate using other cells as substrates, engaging with them via N-cadherin-mediated adhesion. This adhesion alters the actin cortex of the substrate cells, resulting in reduced mechanical stiffness. The duration of N-cadherin engagement determines the degree of stiffness modulation, creating a gradient that increases with distance from the neural crest. This self-generated mechanical gradient helps direct neural crest migration.

Modeling. One crucial way to decode mesenchymal patterning is through mathematical modeling. For example, Painter and colleagues introduced a system that allowed modelling responses such as attraction and repulsion with interactions over variable spatial ranges within in a homo- or heterogenous cell population. They used this framework to model neural crest-like dispersal driven by CIL and some homotypic pigment cells dynamics (Painter et al., 2015). Volkening and Sandstede took this further by creating stripe patterns from a two-population model that recapitulates stripe formation and regeneration (Volkening and Sandstede, 2015). They extended this model to include a third pigment cell type (Volkening and Sandstede, 2018) and even simulated fin stripes in a fin-shaped environment, providing an excellent example of how modeling can capture the complexity of biological systems (Volkening et al., 2020). These and many other advances are reviewed comprehensively elsewhere (Volkening, 2020).

Parallels to Axon Guidance and Neuronal Migration. Contact-dependent axonal pathfinding can be viewed as a type of directed mesenchymal patterning. Driven by diverse cell-cell interactions, they at least share many parallels (Raper and Mason, 2010), involving a similar challenge: coordinating the movements of individual cells or growth cones to establish a precisely organized pattern. It is therefore not surprising that many proteins traditionally classified as “axon guidance factors” are now recognized as crucial regulators of mesenchymal migration. For instance, Semaphorins, Ephrins, and Slit/Robo play essential roles in Neural Crest CIL (Szabó and Mayor, 2018); Plexins and Semaphorins are involved in follicle cell and testis myotube migration in *Drosophila* (Stedden et al., 2019), and Slit/Robo guides

myotube elongation during muscle development (Johnson, 2024; Schnorrer and Dickson, 2004). Interestingly, neuronal cells can also undergo migration with net translocation, exhibiting mesenchymal patterning dynamics. Work from the Norden lab has recently shown that during retinal growth, photoreceptors must migrate both apically and basally. These two directions of movement are governed by distinctly different cytoskeletal architectures: apical migration depends on actin dynamics, while basal migration relies on microtubule-based mechanisms (Rocha-Martins et al., 2023).

Long-range cell-cell contact. Cytonemes—long and thin cellular protrusions interconnecting cells—were originally described in *Drosophila* imaginal discs and in Mouse limb buds (Ramírez-Weber and Kornberg, 1999). In principle, these structures enable migrating cells to maintain cell-cell interfaces over long distances. This allows long-range interactions independent of diffusible gradients. (Figure 2A, Figure 3A). Co-attracting cytoneme-like structures have for example been identified in cancer cells (Muscarella et al., 2020). In development, cells might use numerous cytonemes to probe their environment, responding selectively to specific cell types with tailored reactions or vice versa causing reactions in other cells (Figure 3A). Fittingly, a high number of cytoneme-like structures have been observed protruding from fish pigment cells (Eom et al., 2015), also linking pigment cells in the aforementioned chase-and-run process (Inaba et al., 2012; Yamanaka and Kondo, 2014). Independent of migration, the importance of cytonemes in development, often replacing morphogenetic gradients, is increasingly evident. New work by the Stern Group on chick embryos revealed that cytonemes can transmit BMP signals over long distances, bypassing intermediate cells to create a kind of developmental "nervous system" (Lee et al., 2024). Similarly, the Scholpp Group elucidated Wnt signaling mediated by cytonemes (Brunt et al., 2021; Stanganello and Scholpp, 2016; Zhang et al., 2024) – a finding that might have implications for Wnt/PCP in the light of mesenchymal patterning. These results demonstrate that in the context of cell-cell interactions, "neighbors" are not necessarily defined by physical proximity.

Active Material: Mesenchymal Collectives as Organ Sculptors

Mesenchymal cells surround many organs, such as smooth muscle cells or glial cells (Jaslove and Nelson, 2018). Significant research over the years has taught us how epithelial tissues bend and reshape to give organs their characteristic shapes (Moon and Xiong, 2022). Convergent extension based on cell intercalation is the best-understood example of cell redistribution-based morphogenesis (Perez-Vale and Peifer, 2020). However, mesenchymal cells – which are able to perform many forms of directed redistribution as described above – are often overlooked as active participants of organogenesis. There is a growing body of evidence - such as work from Celeste Nelson's, Zev Gartner's, Sebastian Streichan labs and others - indicating that mesenchymal cells actively sculpt organs. For instance, pulmonary smooth muscle cells play a role in shaping vertebrate lungs (Goodwin et al., 2023; Goodwin et al., 2019) airways muscles shape the airways (Paramore et al., 2024a), while visceral musculature contributes to vilification (Hughes et al., 2018; Huycke et al., 2024; Shyer et al., 2013) and gut looping in *Drosophila* (Aghajanian et al., 2016; Mitchell et al., 2022).

Mesenchymal layers can influence organogenesis through general tissue properties such as elasticity. Seminal work in the chick gut showed that the formation of villi depends on the elastic properties of the muscle layers and the endoderm, which together lead to endodermal buckling (Palmquist et al., 2022; Shyer et al., 2013). However, a recent study by the Gartner lab demonstrated that mesenchymal patterning also contribute to gut villification in mammals (Hughes et al., 2018; Huycke et al., 2024). They showed that within the mesenchymal layer, located between the endoderm and muscle layers, cohesive aggregates form that, in turn, cause the epithelium to buckle. These aggregates arise through a process called *dewetting*, reminiscent of fluid droplets forming on a hydrophobic surface. A crucial step

in their formation is fluidization of mesenchymal cells, mediated by matrix metalloprotease activity. This work illustrates that cells with general material-like properties and random single-cell motility can nonetheless self-organize into complex structures—making it an example of mesenchymal patterning in the absence of contact-directed directed motility.

Recent work from the Nelson group revealed that mesenchymal motility of lung mesenchyme mediated by the Wnt/PCP component Vangl1/2 plays a crucial role lung sculpting. Vangl acts downstream of Wnt5 to induce a more motile and protrusive architecture in mesenchymal cells surrounding lung epithelia, leading to cell elongation and increased tissue fluidization. This transition enables proper epithelial sacculization (Paramore et al., 2024b). These findings reinforce the idea that mesenchymal motility and dynamic cell behaviors are key regulators of morphogenesis, but also highlight the recurring involvement of PCP components in cell-elongation processes - even within mesenchymal contexts (Devitt et al., 2024; Huebner and Wallingford, 2018; Wallingford et al., 2000). Remarkably, this holds true even in a 3D environment lacking clear apicobasal or dorsoventral polarity. This is more evidence that permutations of Wnt/PCP signaling appear to be a recurring feature of contact-based mesenchymal motility (Carmona-Fontaine et al., 2008; Wallingford et al., 2000). The authors of the study note that it remains unclear whether the observed motility is random or directionally guided which opens an important avenue for further exploration with regards to *directed* mesenchymal patterning.

Mesenchymal cells can also directly drive morphogenesis without relying on epithelial intermediates. Recent studies in the *Drosophila* testis highlight how flat mesenchymal cells can elongate and even bend organs (Bischoff and Bogdan, 2021; Bischoff et al., 2021; Bischoff et al., 2025a; Bischoff et al., 2025b). The *Drosophila* testis is covered and shaped by migrating mesenchymal muscle-precursor cells, or testis nascent myotubes (TNMs), that move toward the testis tip (Fig. 4) (Kozopas et al., 1998; Kuckwa et al., 2016; Rothenbusch-Fender et al., 2017; Susic-Jung et al., 2012). Testes lack a surrounding epithelium (Stern, 1941). TNMs must evenly fill the finite surface of the testis while migrating beneath a layer of large mesenchymal pigment cells (Bischoff et al., 2021; Kuckwa et al., 2016). Unlike neural crest cells, TNMs must remain continuously connected, probably to compress the underlying ellipsoid testis (Fig. 4). This demonstrates that supporting organ shape and migration can occur at the same time. When migration works, and the sheet remains integrity, the testis forms a mesmerizing spiral structure (Bischoff et al., 2021; Bischoff et al., 2025b; Rothenbusch-Fender et al., 2017), by a subsequent step of mesenchymal intercalation (Bischoff and Bogdan, 2023), in which muscle precursors probably act as a hydrostatic skeleton “directing” sperm growth. Migratory directionality is achieved by downregulating matrix adhesions at cell-cell interface protrusions, enabling a free-edge-based migration (Bischoff et al., 2021). To maintain sheet integrity, TNMs PlexA ensures that cellular interfaces remain protrusive (Fig. 3A). Disruptions in this delicate fine-tuning - such as those caused by Plexin-A knockdown - lead to more epithelial looking cell-cell interfaces and taller cells (Fig. 3A), with strongly reduced integrin adhesions, resulting in gaps that compromise collective migration and sculpting (Bischoff et al., 2025a). Having ECM-tethered filopodia even close to cell-cell contacts in wild type might also be due to the sculpting role TNMs take – parallel to migration. This demonstrates how the fine-tuning of local cell architecture directly influences morphogenesis - emphasizing the importance of focusing on “local architecture”.

Interestingly, protrusive activity at cell-cell edges observed in TNMs (characterized by bilateral protrusions rather than unilateral cryptic lamellipodia) bears some similarities to interdigitating polarized filopodia (cadherin fingers) and lamellipodia near junctions in migrating endothelia, needed for directionality and tissue integrity (Cao et al., 2017; Hayer et al., 2016). Protrusive forces can stabilize junctions, as shown by Li and colleagues in MDCK cells, where filopodia-like microspikes at cell-cell adhesions counteract contractile forces – preventing them from tearing E-cadherin junctions (Li et al., 2020). In Plexin-A knockdown, the absence of filopodia at cell-cell interfaces causes cells to elongate

and adopt highly irregular shapes, suggesting that protrusion at “all edges” is required for spread. Future work will show if local activation of protrusive activity is an ancestral function of Plexins, for example in wound closure or extrusion - which involve Plexin-activity (Yoo et al., 2016).

Conclusion

The findings and perspectives presented here underscore the intricate interplay between local cell shape and emergent cell behaviors. A conceptual map of sub-cellular architectures such as protrusions, adhesions, and contractile units scales up to a landscape of collective behaviors through interactions between cells. A key requirement for this mechanism to function is a spatially restricted cytoskeletal response to cell-cell contact. It is therefore not surprising that components of the Wnt/PCP pathway and various axon guidance factors emerge as recurring themes. Emergent collective behaviors can encode the blueprints for complex patterns. This capacity of self-organization of mesenchymal cells can also be used to drive morphogenesis. What the cell and developmental biology communities need - moving forward - are new model systems and innovative computational approaches to uncover the organizational principles and molecular underpinnings of mesenchymal collective migration, patterning, and morphogenesis.

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Author contributions

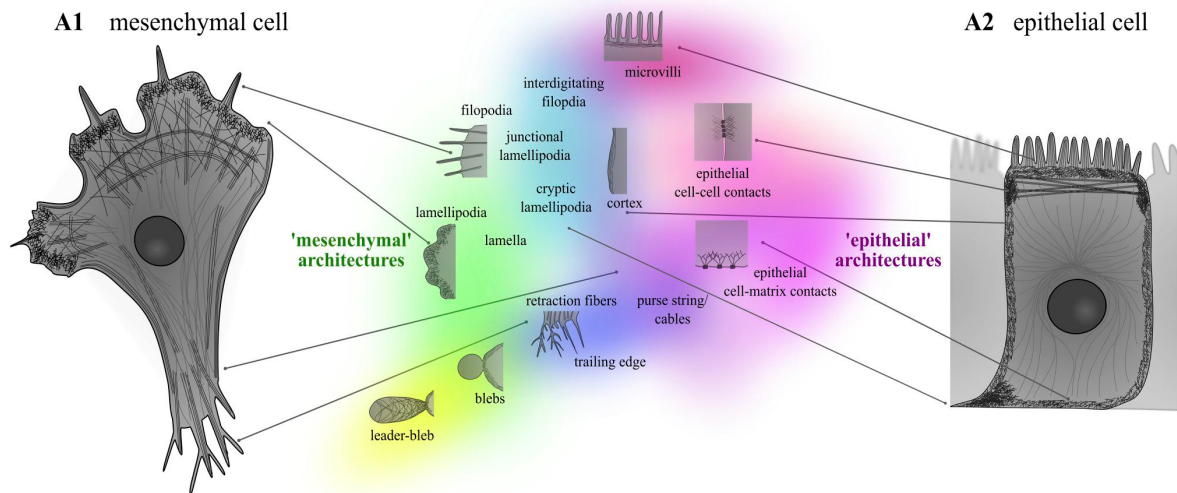
M.C.B. wrote the initial version of manuscript, excluding sections about Neural Crest Cell Migration, and drew the figures.

R.M. contributed crucial paragraphs about Neural Crest Cell Migration and critically read and corrected the manuscript.

Competing interests

The authors declare that they have no competing interests.

A. Conceptual map of morphodynamic building blocks (speculative)



B. Swarm behavior through local architecture transitions

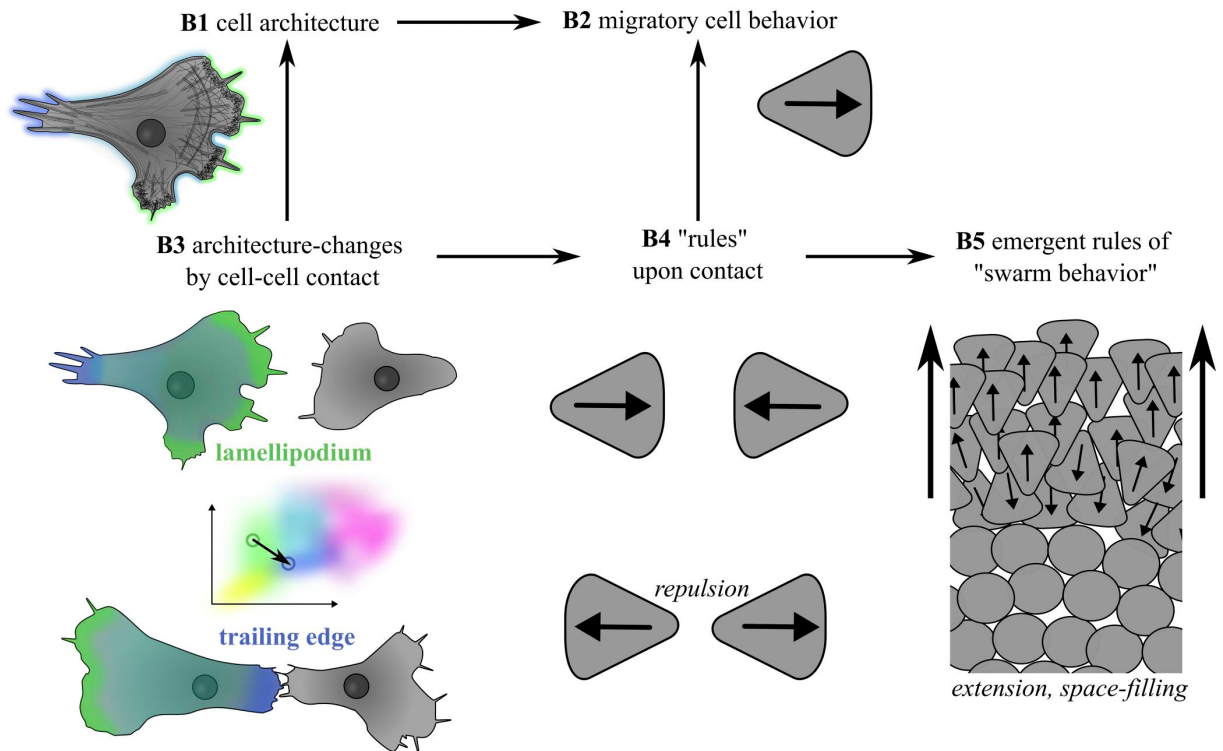
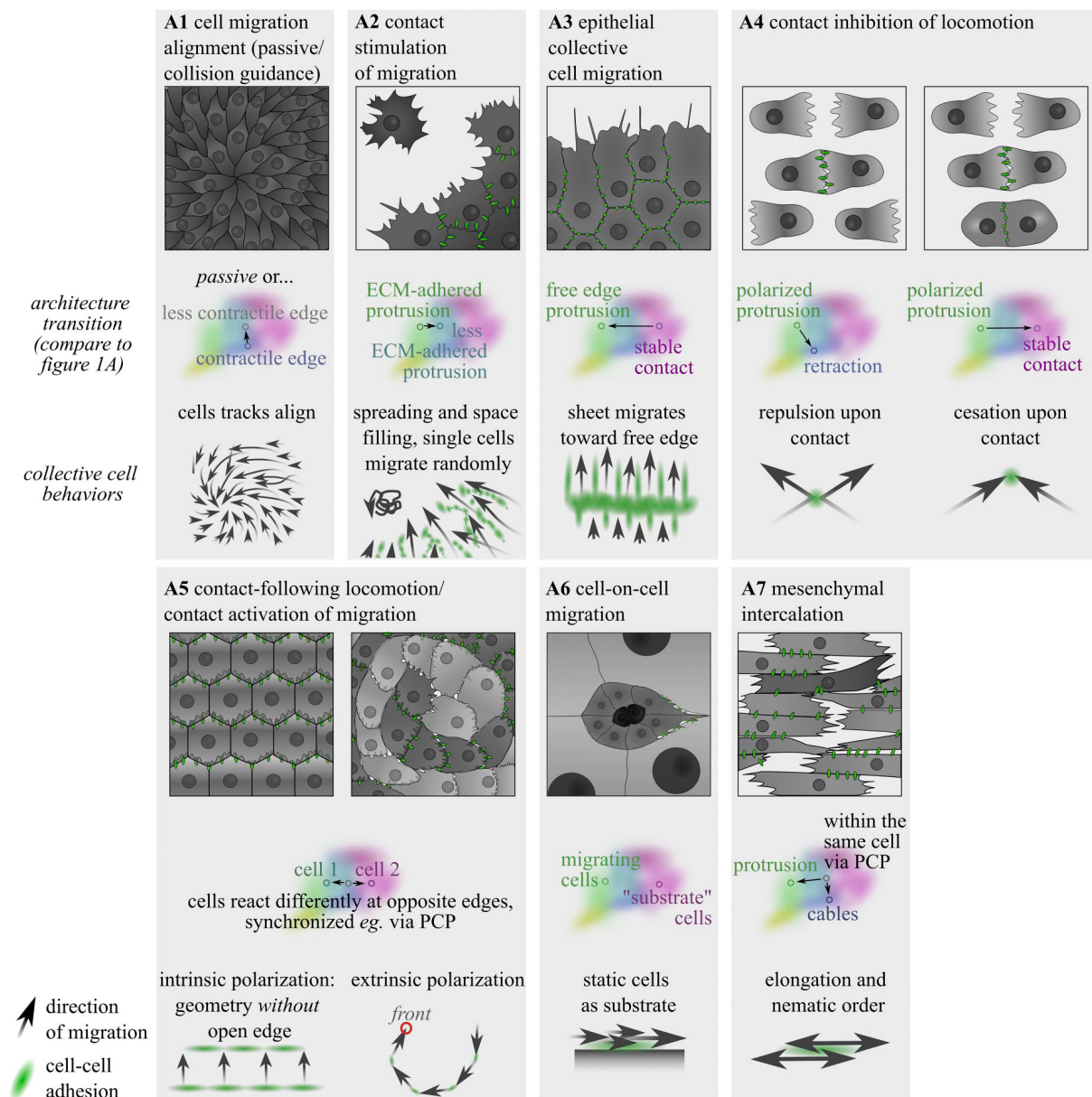


Figure 1. (A) Graphical representation of the speculative architecture space concept. Different positions represent different local cell architecture in a multidimensional space, reduced to two dimensions. (B) The composition of local architectures gives rise to cell behavior (top row). Cell-cell contact-based reorganization gives rise to behavior changes and thus to specific collective behaviors.

A. Cell contact-dependent modes of collective migration



B. Comparison of CIL, contact-following and mesenchymal intercalation

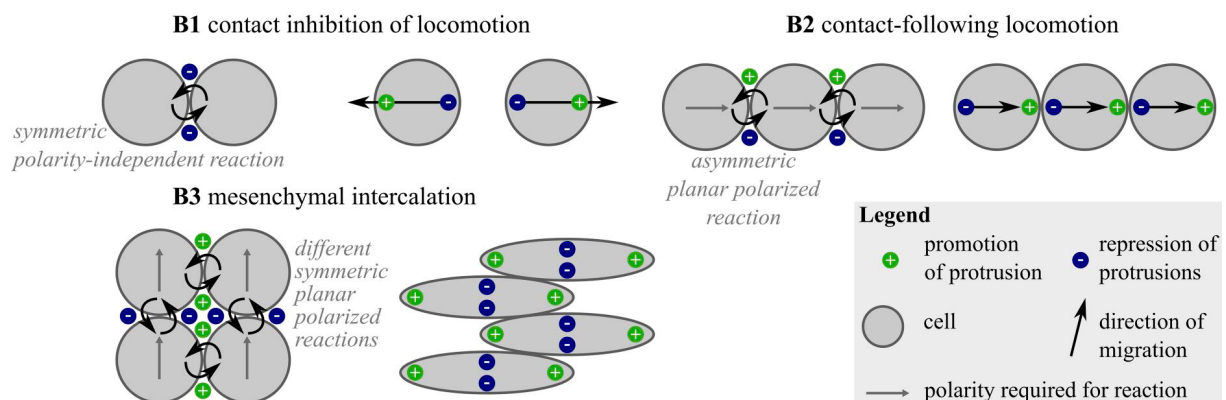
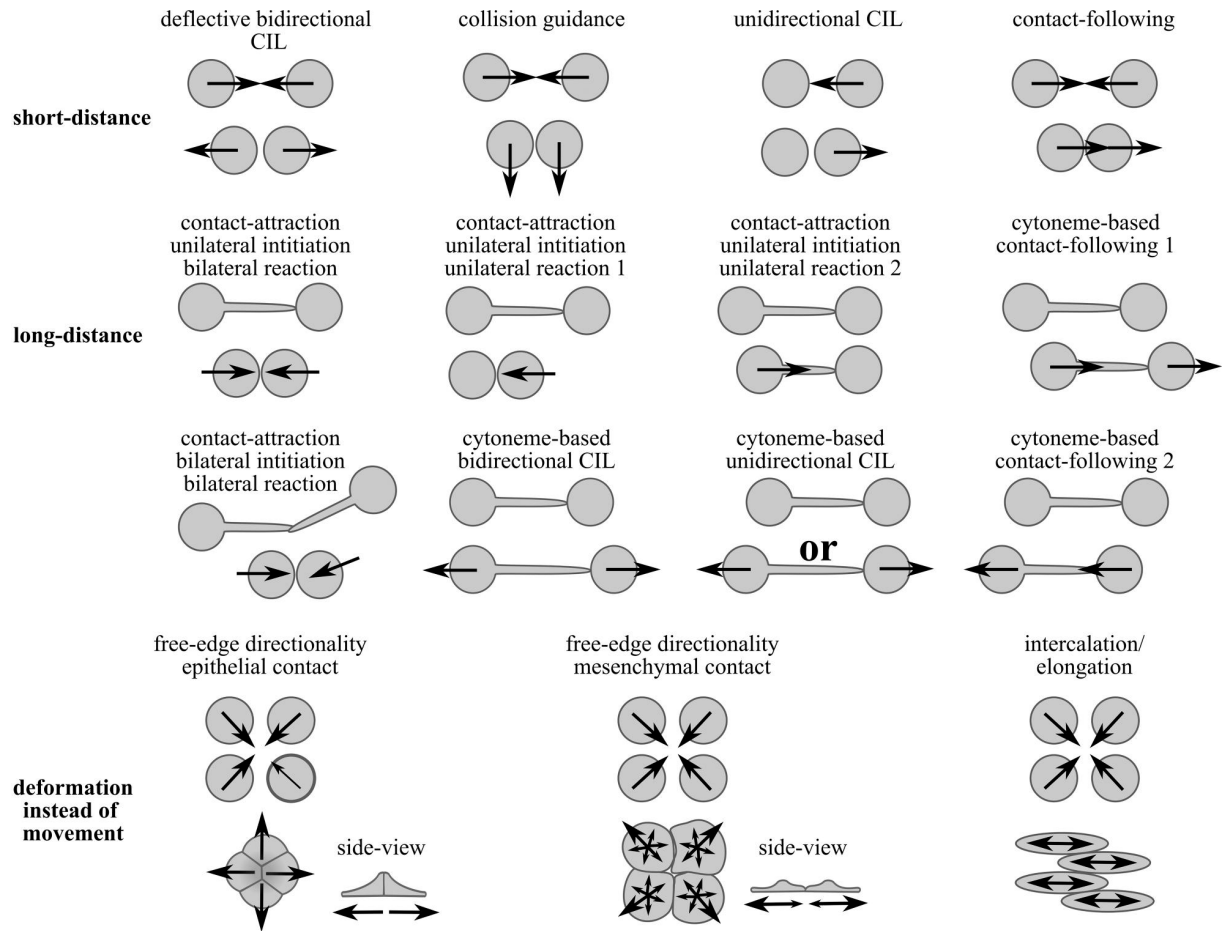


Figure 2. (A) Diverse ways of how cell-cell contact gives rise to specific swarm behaviors. (B) A comparison of contact inhibition of locomotion, contact following, contact-

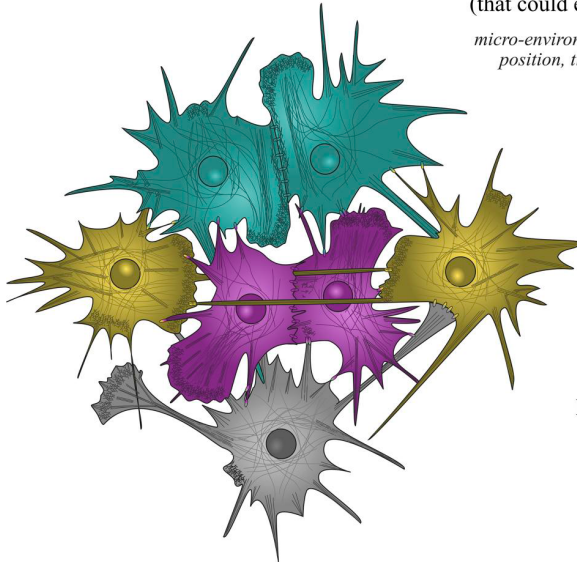
mesenchymalization and mesenchymal intercalation demonstrating how these processes are closely related, but can lead to different motile behaviors or shape changes.

A. Potential contact/protrusion-based rules guiding mesenchymal collectives

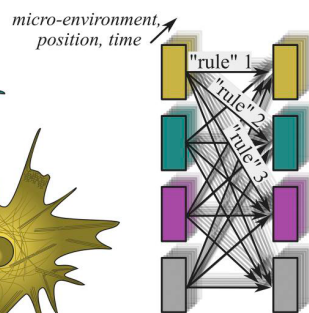


B. Directed mesenchymal self-patterning (hypothetical example)

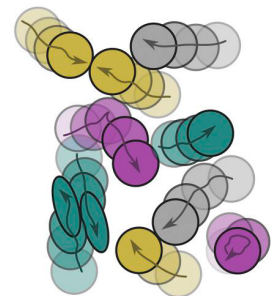
B1 In heterotypic cell populations, ...



B2 ...different heterotypic & homotypic rules (that could even vary based on context)...



B3 ...can lead to lead to complex directed cell movements and deformations...



B4 ...resulting in self-patterning

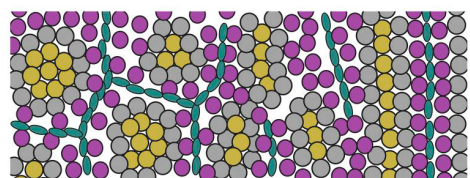
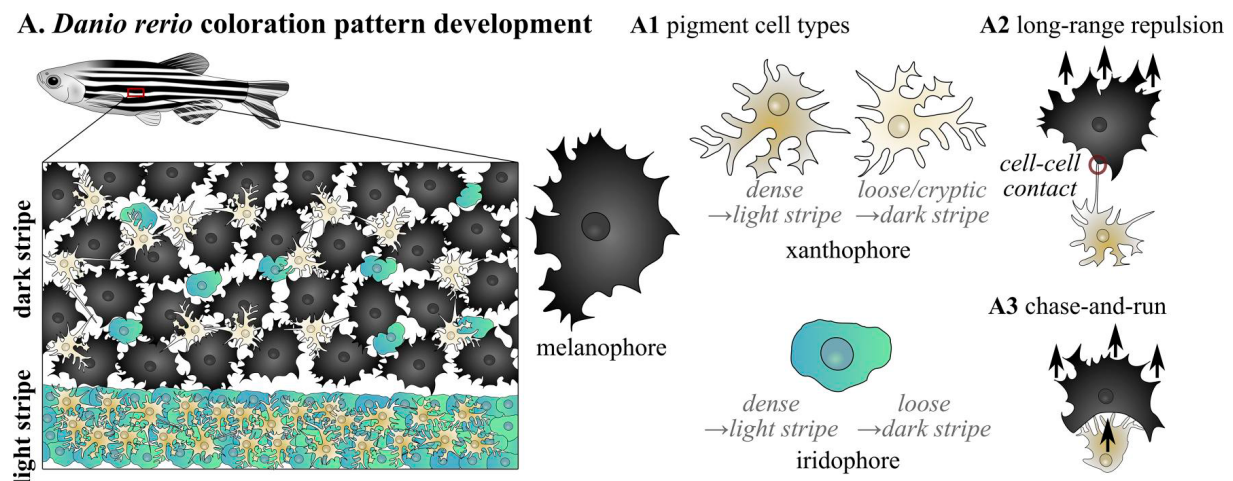
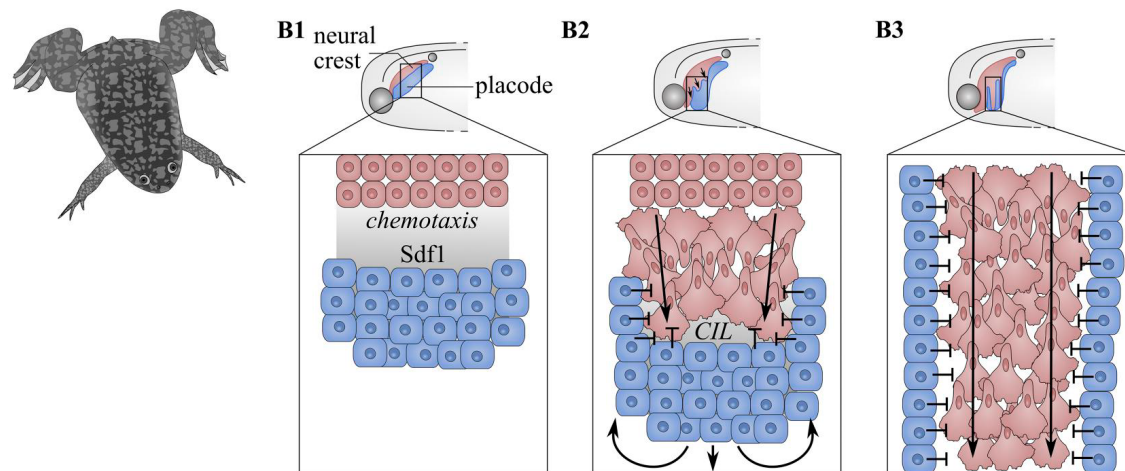


Figure 3. (A) Different rules - some of which are speculative – of how two mesenchymal cells could react to cell-cell contact based on local fine-tuning of protrusion dynamics. This is just a selection – much more potential reactions are conceivable. (B) A speculative model of how defined homo- and heterotypic rules can lead to complex patterns without external interference.

A. *Danio rerio* coloration pattern development



B. *Xenopus* cephalic neural crest migration



C. A self-generated mechanical gradient in *Xenopus* neural crest migration

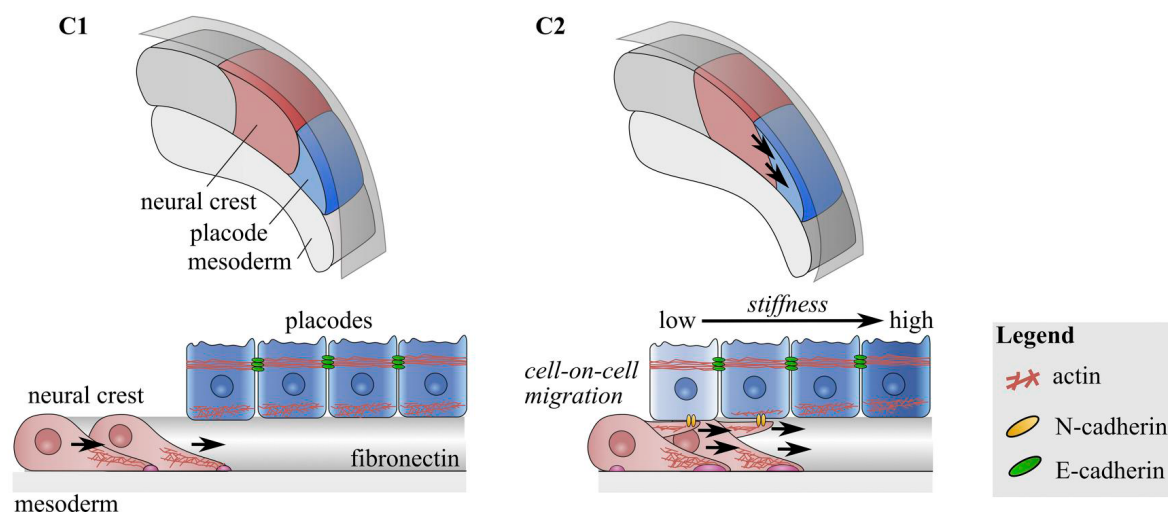


Figure 4. *Drosophila* testis myotubes as an example for mesenchymal collective cell behavior-based morphogenesis of the testis. Changes in protrusive and adhesive properties at cell-cell contacts (box) translate to a specific cell shape and collective behavior, resulting in the correct shape of the organ. Upon perturbation, the organ is not shaped correctly.