Collective cell migration is emerging as a significant component of many biological processes including metazoan development, tissue maintenance and repair and tumor progression. Different contexts dictate different mechanisms by which migration is guided and maintained. In vascular endothelia subjected to significant shear stress, fluid flow is utilized to properly orient a migrating group of cells. Recently, we discovered that the developing zebrafish pronephric epithelium undergoes a similar response to luminal fluid flow, which guides pronephric epithelial migration towards the glomerulus. Intratubular migration leads to significant changes in kidney morphology. This novel process provides a powerful in vivo model for further exploration of the mechanisms underlying mechanotransduction and collective migration.

The term “collective cell migration” (collective motion) was first introduced to describe the behavior of starved Dictyostelium discoideum. The term has rapidly gained general acceptance as encompassing a wide variety of coordinated cell migratory behaviors. A number of definitions have been proposed to unify the various collective migratory behaviors. Friedl et al. defined it as “the movement of cell groups, sheets or strands consisting of multiple cells that are mobile yet simultaneously connected by cell-cell junctions.” This definition implies a number of features setting collective migration apart from other migratory behaviors. First, it points to the spatial restrictions on the individual cells within the migrating groups. The cells cannot leave the group and continue on their own. Therefore, they must respect the behavior of their neighbors and the overall migration occurs through the integration of individual cell activities across the collective. Second, it implies that different cells within the migrating group may play different roles. Some of them may not be migratory at all and simply “ride” the rest of the group, as indeed seen in border cell migration. Other cells within the group may further specialize into leaders and followers as can be seen in most current models of collective migration.

A variety of biological processes satisfy this definition. They include, among others, closure of wounded epithelial sheaths, physiological maintenance of intestinal epithelium, cancer invasion, developmental processes of branching morphogenesis, vascular sprouting, gastrulation, dorsal embryo closure, as well as movements of some basal metazoan organisms such as sponges. Over the years, a number of models emerged to study the process of collective migration. When starved, thousands of single cells of Dictyostelium discoideum aggregate and form a “slug” that migrates to the soil surface to form a fruiting body. This process has two general stages: the stage of aggregation, where individual migrating cells respond to cAMP concentration to form a multicellular aggregate and the stage of collective migration. In the latter stage, the leading (pre-stalk) cells of the slug secrete cAMP. In addition, they produce slime sheath that provides traction support for the aggregate. The slime sheath allows outermost cells of the aggregate to develop necessary traction for the entire slug to propel itself towards guidance cues. A number of molecular and cellular components have been recently

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mechanisms of collective cell migration, the most developed in vivo vertebrate model comes from the studies of the zebrafish lateral line formation. In this process, the lateral line primordium cells move as a group in the anterior-posterior direction. The migration is dependent on the interaction of stromal factor Cxcl12 along the guidance path and its receptor Cxcr4b. The direction of migration is defined by the interplay between Fgf and Wnt signaling (rear and front of the migrating group, respectively). Wnt signaling in the front of the migrating lateral line inhibits Cxcr7b expression and promotes Cxcr4b expression. It also results in the secretion of Fgf ligands. Expression of sef at the front (also under control of Wnt) prevents Fgf from acting in this front domain. Fgf ligands interact with their receptors in the trailing end of the migrating group. As a result, the cells at the trailing end express dkk1 (to limit Wnt signaling) and Cxcr7b while downregulating Cxcr4b. Thus, Cxcl12-Cxcr4b interaction is limited to the migration front. Cxcr7b expressed in the back of the migrating collective is believed to further interfere with Cxcl12-Cxcr4b interaction by sequestering Cxcl12. The net result of the differential signaling is the establishment of a distinct migratory front at the posterior aspect of the precursor population. At the same time, groups of cells at the back stop migrating and give rise to individual lateral line organs.

The existence of a distinct migratory front is a unifying feature of all the models of collective migration described above. The migratory front defines the interface between the migratory collective and the tissues into which the migratory group advances. The front may be maintained by a stable pattern of signaling within the migrating group, as seen in lateral line migration where Wnt signaling at the front and FGF signaling at the back are maintained through mutual exclusion. Alternatively, the front may be maintained through spatial differences in concentrations of chemotactants rendering the front of the group more migratory, as seen in the Drosophila border cell migration. In other systems, the migratory front may be maintained through cell-to-cell direct signaling, such as Notch signaling in determining the tips of vascular sprouts. Furthermore, migrating epithelial cultures in wound assays are inherently polarized by the presence of a free margin. Interestingly, the presence of the margin, which becomes the migratory front, is sufficient even in the absence of the wound to initiate a directed migration. However, several new studies revealed that the existence of a distinct migratory front is not a universal or required feature of collective migration.

Recently we discovered a novel form of collective migration that guides the morphogenesis and maturation of pronephric kidney. The zebrafish pronephros is a simple bilaterally symmetrical structure consisting of two fused glomeruli, each connected to a pronephric tubule that runs posteriorly, eventually exiting at the level of the cloaca. The pronephros begins to function shortly after 1 dpf. After the onset of its function, a significant maturation of the pronephros takes place, manifested at the structural level by the development of proximal convolution and re-positioning of nephron segment boundaries (Fig. 1). We demonstrated that both of these structural changes are a direct consequence of the collective epithelial migration that starts at about 30 hpf and lasts for the next three days. This proximal migration is governed by the onset of luminal fluid flow. The cells of the pronephric epithelium move en-masse towards the glomerulus and against the flow of urine. As a result, the proximal segment becomes compressed, shortened and convoluted. In contrast, the distal segment straightens and becomes longer (Fig. 1 and Suppl. Movie 1). This lengthening of the distal kidney is accompanied by cell proliferation that compensates for the proximal shift of kidney segments and allows for the en-masse migration to continue for three days.

As mentioned above, this novel developmental process differs from most models of collective cell migration in at least one aspect; it lacks a distinct migratory front. In the absence of such front, the polarity of the migrating pronephric epithelium is established by using fluid flow as the guiding cue. When directed fluid flow is eliminated by obstructing the pronephros, the proximal migration is disrupted. Instead,
the cells of the pronephric epithelium can often be seen migrating circumferentially, around the tube perimeter. This circumferential pseudo-migration correlates with the presence of local vortex currents in obstructed pronephroi due to the presence of beating cilia. Indeed, we failed to observe similar circumferential pseudo-migratory behavior in paralyzed cilia mutants (unpublished data). In addition, we were able to engineer an ectopic convolution (about 500 µm distal to its normal location next to the glomerulus) by selectively eliminating proximal, but not distal sources of fluid flow (Fig. 1 and Suppl. Movie 2). This finding further supports the conclusion that luminal fluid flow guides the epithelial migration. It is still possible that different cells within the pronephric epithelium have distinct roles in orchestrating the migration. For instance, a small subset of cells could act as functional leaders and organize the migration process. Alternatively, luminal flow could directly interact with each migrating cell. Further studies should determine which scenario is present in the pronephros.

There are at least two other systems where cell migration is governed by the mechanical forces generated by luminal fluid flow. Vascular endothelial cells respond to fluid shear stress, orient in the direction of the flow and migrate in the direction of shear force. This behavior is thought to be important in vascular remodeling. A related model was developed in macaque placental trophoblast cells which demonstrate a similar behavior. It is notable that in a wound assay, endothelial cells respond in a way similar to that in other in vitro wound models described above. Thus, more than one mode of guidance may be present in a given tissue.

Significant advances have been made in our understanding of the cellular responses to shear stress in vascular endothelium. Endothelial cells sense and respond to fluid shear by utilizing a system of adhesion molecules including PECAM and VE-cadherin, integrin activation, activation of VEGFR, calcium influx, and modulation of the cytoskeleton by Rho family GTPases. Recent evidence also suggests that sensory cilia play a role in the endothelial response to shear stress. Fluid shear first induces lamellipodial cell extensions, followed by basal protrusions and new focal adhesion formation in the direction of the flow. Subsequent migration requires remodeling of adhesions and release of cell substratum attachments at the rear of the migrating cell.

Migration of pronephric epithelial cells is likely to involve similar basic mechanisms. For instance, we have observed a strong correlation between the presence of directed lamellipodial extensions of epithelial cells on the tubule basement membranes and the basal phosphoFAK staining, suggesting that pronephric epithelial cells actively remodel their matrix attachments as they migrate. The similarities and differences between these two systems are likely to prove useful in determining how mechanical forces establish self-perpetuating cell movement. A notable difference between the pronephric cell migration and endothelial cell migration is that pronephric cells migrate against the flow as opposed to in the direction of the flow, suggesting that the exact nature of

![Figure 1. Effect of pronephric migration on tubule architecture.](image-url)
signaling have been linked to mechanotransduction. It remains to be determined which of these processes mediate the relation between pronephric flow and epithelial migration.

It is possible, however, that multiple components (focal adhesion complexes, cell junctions, sensory cilia, etc.) interact with each other, and these interactions are integrated by the cell to generate a response to a mechanical stimulus. There is evidence showing that various components are indeed linked together by cytoskeleton. The apical ciliary response to shear stress by cultured kidney cells (as measured by the cytoplasmic calcium increase) can be prevented by altering the integrity and the tensile properties of the cytoskeleton. The same result can be achieved by blocking the integrin interaction with extracellular matrix at the basal surface. Conversely, disrupting ciliary function in vascular endothelial cells significantly attenuates the overall response of the cell to fluid shear, the result that can also be achieved by disrupting cytoplasmic microtubule polymerization.

These findings suggest a model in which multiple identified components of the mechanotransduction response are linked by cytoskeletal elements, that allow events at each specific location to influence the state of a different remote cell component directly. For example, bending the cilium would have opposite mechanical effects on cell-cell junctions located in the direction of the bending, compared to those located on the opposite side. Importantly, this mechanical communication is inherently bidirectional and would allow the cell to instantly integrate signals originating at different locations and initiate a robust and coordinated response to external mechanical cues.

Concluding Remarks

Studies of collective cell migration are now performed across a variety of contexts and encompass such diverse processes as development of social ameba, metazoan embryo morphogenesis, epithelial maintenance, wound healing and cancer progression. Different contexts in which collective cell migration takes place define diverse mechanisms by which collective migration occurs. There is an accumulating body of evidence that mechanosensation plays an important role in guiding collective migration in different settings, such as pronephric development and maturation, extraembryonic placental development and blood vessel homeostasis. The zebrafish pronephric model of collective epithelial migration offers significant advantages to the study of collective migration because it allows for a direct in vivo visualization of the involved processes. Future studies should reveal similarities as well as differences in the mechanisms of mechanosensation and collective migration in diverse organism and organ settings.

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Supplementary materials can be found at: www.landesbioscience.com/supplement/VasilyevCAM4-3-Sup.mov

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