Invited review summarizing our recent modeling of subcellular to multicellular systems

Modeling and Simulating Single and Collective Cell Motility

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We survey a combination of classical and recent experimental and modeling developments in eukaryotic cell migration, from the subcellular processes and regulation, to single and collective cell dynamics. We showcase several examples that demonstrate simulations at several hierarchies: subcellular actin waves, corresponding migratory cell behavior, and collective behavior of several multicellular systems. We argue that the use of shared open-source software packages (such as Morpheus, in our case) to simulate multiscale models would be a boon to the community, allowing us to recreate, investigate, and build on existing work. A brief summary of currently available software is provided.

Technological progress in microscopy, in new methods of observing, measuring, manipulating, and controlling cells has resulted in large data sets rich in content that encode subcellular, cellular, and tissue dynamics. Given that ample data are available, a remaining hurdle is making sense of that data, and bringing our understanding to a level that synthesizes information at multiple scales, and in multiple types of cells and conditions. The methods of physics, mathematical modeling, and simulations can supplement biological tools to help with this goal and to link events at one scale with those at smaller and larger scales.

Many recent reviews have summarized the current state of experiments and models of single and collective cell migration. Some examples include Camley and Rappel (2017), Alert and Trepat (2020), Buttenschön and Edelstein-Keshet (2020), Stehbens et al. (2024), and others. Many of these reviews highlight recent experimental findings and modeling efforts, with discussion of how various approaches contribute to the field, and what challenges remain. In this work, we provide a few updates, some examples of case studies, and a perspective of a personal journey from the subcellular cytoskeleton dynamics and its regulation, to how these intracellular systems play out in single and group cell migration.

SUBCELLULAR DYNAMICS

Early research on molecular aspects of cell migration focused on the dynamics of the actin cytoskeleton and forces generated by filamentous actin (F-actin). The biochemistry of many proteins that affect and bind to actin was studied experimentally and dynamically (Pollard et al. 2001; Pollard 2016). Physicists contributed tools

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from soft matter physics to study the "active gels" that represent the actin cytoskeleton (Jülicher et al. 2007), and links between in vitro and in silico experiments helped to understand how actin can produce mechanical and turning responses (Manhart et al. 2019).

In the past two decades, some of the attention has shifted to the complex regulatory pathways that control actin assembly by formins, actin filament branching and nucleation (via WASP/WAVE and Arp2/3), and association of actin with myosin to produce contractile forces (Svitkina and Borisy 1999; Skruber and Mullins 2024). Signaling networks that regulate these processes play a vital role (for review, see Devreotes and Horwitz 2015). The small GTPases (Rac, Rho, and Cdc42), their mutual connectivity, and their activators (guanine nucleotide exchange factors [GEFs]) and inactivators (GTPase activating proteins [GAPs]) have been studied experimentally with new fluorescent constructs (Bishop and Hall 2000; Itoh et al. 2002), modeled mathematically using reaction–diffusion equations (Jilkine et al. 2007; Otsuji et al. 2007), and manipulated in various cell types using optogenetics (Hadjitheodorou et al. 2021; Town and Weiner 2023). The effect of mechanical stimuli (such as membrane tension, and pulling forces, including those exerted by cells) on the regulatory circuits has also been of great interest (Houk et al. 2012).

Mathematical models have contributed various insights to the mix, including the following: (1) Some of the inherent biology of GTPases, namely, cycling between active and inactive forms (see Fig. 1A,B), membrane residence of the active form (low diffusion rate) and the approximately constant pool of GTPase (mass conservation) can, under the appropriate assumptions, lead to self-organized polar distributions that define a clear front and back of a cell. This was denoted "wave-pinning" by Mori et al. (2008), since it amounts to a stalling wave of GTPase activity. This could then explain spontaneous cell polarization given an appropriate stimulus. (2) Cell geometry contributes to the polarization, with curved portions of the lamellipod stabilizing hotspots of GTPase activity (Marée et al. 2012; Camley et al. 2017). This is an outcome inherent to the reaction–diffusion physics, and independent of membrane-curvature protein affinity. (3) A single GTPase has such properties, but in a small and relatively sensitive parameter regime. Networks of GTPases, including the mutually antagonistic Rac and Rho make the system more robust by increasing the appropriate parameter regimes (Holmes and Edelstein-Keshet 2015). (4) Linking together GTPases via mutual antagonism, autocatalysis, or positive feedback greatly increases the variety of possible cell shapes and behaviors, even for a single parameter setting (Zmurchok and Holmes 2020). Thus, variable cell behavior could be an outcome of cell history, rather than genetic or physiological state. Restated, we can explain cell heterogeneity mathematically by invoking various "initial conditions" or distinct stimuli from the environment, aside from the genetic disparity that is associated with distinct parameter sets.

While GTPases such as Rac and Cdc42 promote the nucleation of F-actin, it has also been shown that F-actin can recruit GAPs that inactivate GTPases, creating a negative feedback loop as shown in Figure 1C. These interactions, explored both experimentally and theoretically, can lead to a variety of spatiotemporal patterns of "actin waves" (Weiner et al. 2007; Holmes et al. 2012; Allard and Mogilner 2013; Bement et al. 2015; Barnhart et al. 2017; Moldenhawer et al. 2022), including static polar states, traveling waves, and spiral waves in 2D (see Fig. 1D–F).

Models of the reaction–diffusion type (parabolic partial differential equations [PDEs]) have provided valuable insight into how these patterns are generated and how they interact with one another inside cells. For example, Holmes et al. (2012) showed that the strength of the negative feedback from F-actin to its nucleation-promoting factor (e.g., Rac) controls the types of solution observed: low versus high negative feedback leads to polar versus traveling wave solutions (Hughes et al. 2024). The significance is that such distinct dynamical patterns of F-actin in the cell can create distinct cellular motility behavior, including directed motion, turning, or other dynamics (see Fig. 2). Experi-

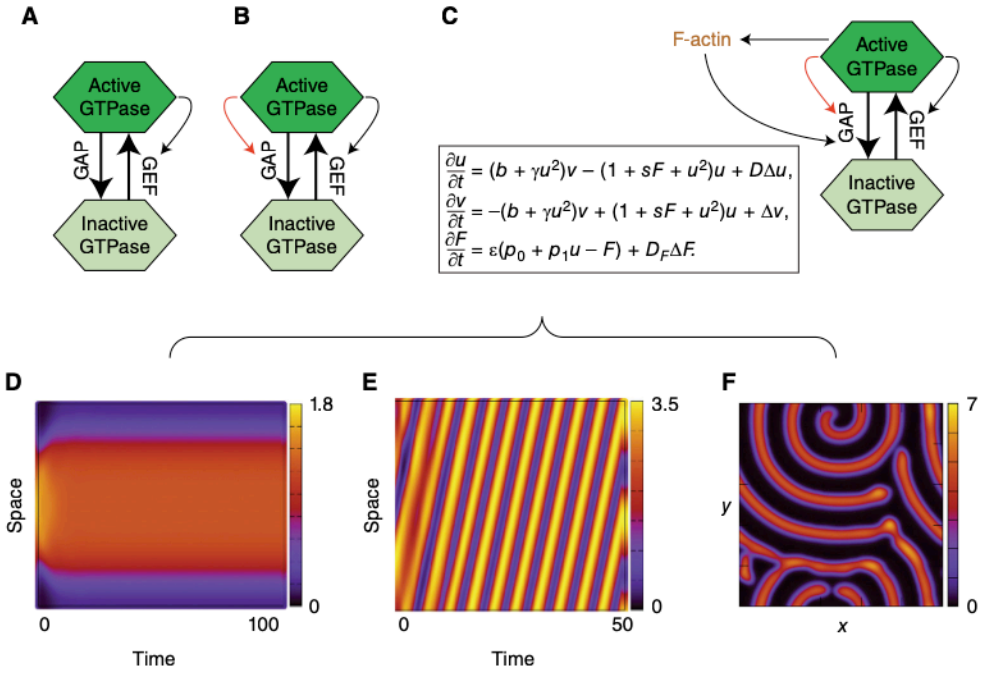

Figure 1. (*Top*) Schematic diagrams for a reaction–diffusion model of GTPase with and without interactions with filamentous actin (F-actin). (Equations: model reaction–diffusion equations for the active and inactive GTPase (u, v) and F-actin, F as in the partial differential equation [PDE] bifurcation analysis by Hughes et al. [2024]). (*Bottom*) Some of the polar and wave dynamics predicted by such models. (*A*,*B*) Two variants of the "wave-pinning" model (Mori et al. 2008) that produce polar patterns. Positive feedback (activation by GEFs) and negative feedback (inactivation by GAPS, red arrow in *B*) are shown. (*C*) A model with negative feedback from F-actin that produces more exotic patterns of the active GTPase, as shown in the kymographs (*D*,*E*), and the 2D heatmap in *F*. (*D*) A wave-pinning pattern in 1D that represents robust cell polarity—a static region of active GTPase creates the cell "front" (*E*) traveling waves of active GTPase in 1D (*F*) spiral waves in 2D (*E*). See Figure 2 for corresponding 2D cell shape dynamics based on the patterns (*C*) and (*D*) along the edge of a "motile cell" (see Supplemental Material for more information). (Reprinted from Hughes et al. 2024 under the CC BY 4.0 license (*top*); reprinted from https://identifiers.org/morpheus/M2071, Morpheus.Lab @ TUD Dresden University of Technology, under the CC BY 4.0 license (*bottom*).

ments have led to the discovery of details of the feedback (e.g., Bement et al. 2024), and this was followed by physical and mathematical modeling (Goryachev et al. 2016). A recent paper attempts to unify some of the proposed mechanisms (Beta et al. 2023). Bifurcation analysis to map out the universe of possible behaviors is ongoing (Liu et al. 2021; Buttenschön and Edelstein-Keshet 2022; Hughes et al. 2024). Some of this is largely of mathematical interest. This kind of treatment also sheds light on how cells can switch between one mode of behavior and another, for example, polarized directed migration, and random turning and searching.

Understanding such PDE models (beyond repetitive simulations and parameter sweeps) is technically challenging. Full PDE bifurcation analysis is needed to determine behavior for broad parameter regimes and values that mark critical transitions (bifurcation points), but this is much more complicated for PDEs than for time-dependent ordinary differential equations (ODEs). PDEs have additional symmetries that require more constraints on the problem (Beyn and Thümmler 2007). Numerically, PDE systems require careful spatial discretization and appropriate approximation of derivatives. These also result in much longer computational run-

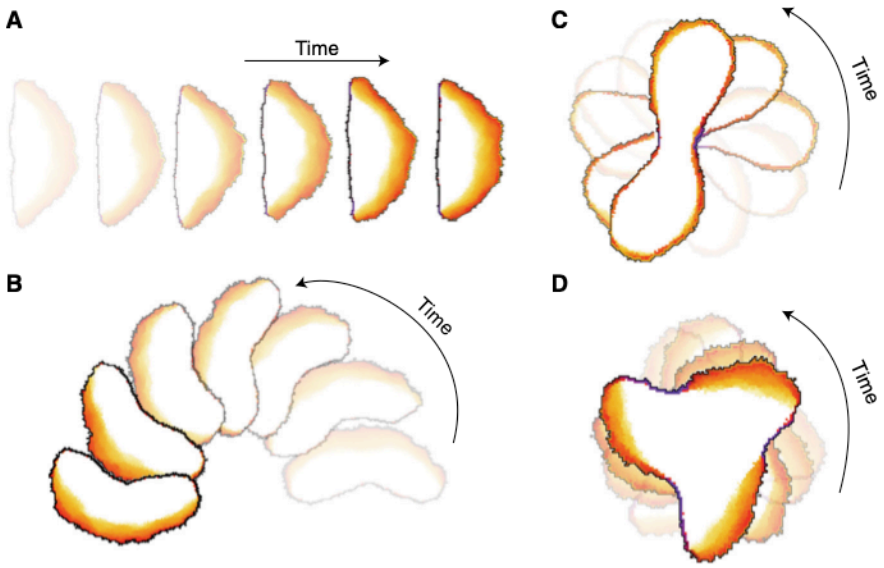

Figure 2. Shapes and motion of 2D "motile cell" corresponding to intracellular dynamics as described in Figure 1C. Implementing the partial differential equation (PDE) model of Figure 1C along the edge of a 2D cellular Potts model (CPM) cell (in Morpheus) together with protrusion (due to F-actin) results in distinct cell motility behaviors. (*A*) A polar cell results from a static zone of Rac activity, corresponding to the model and parameter values used for the kymograph in Figure 1D. (*B*) For the same model with parameters as in Figure 1E, a single zone of active Rac moves around the cell perimeter, causing the protrusion to shift clockwise. This makes the cell turn and move a long a circular trajectory. (*C*,*D*) If the traveling wave has two or more "lobes," multiple protrusions circulate around the perimeter. This cell does not move directionally: it appears to "ruffle" as lamellipodial waves circumnavigate its edge. (*B–D*) Traveling waves of various frequencies moving along the edge of the cell, corresponding to the same model, with parameter values that produced Figure 1D. Regions of high F-actin are assumed to create protrusion in the CPM cell (see Supplemental Material for more information). (Figure reprinted from https://identifiers.org/morpheus/M2072, Morpheus.Lab @ TUD Dresden University of Technology, under the CC BY 4.0 license.)

time compared to ODE systems. Local perturbation analysis (LPA) (Holmes et al. 2015) is a shortcut tool for detecting bifurcations of homogeneous steady states, but no information is gained on solution behavior away from the bifurcation point.

SINGLE-CELL MIGRATION

For migration, a eukaryotic cell needs a balance between protrusive (traction) forces, adhesion to its surrounding scaffold (substrate or extracellular matrix [ECM]), and contractile forces to break adhesion bonds and detach its rear (Cramer 2010). Inside the cell, the front assembly of actin and rear contraction of actomyosin have emerged as a dominant paradigm in the migration of keratocytes and neutrophils (see, e.g., Allen et al. 2020). But other modes of migration have been quantified, including blebbing (rupture of the cell cortex) (see Paluch and Raz 2013). Stimuli that lead to migration include chemotaxis, topotaxis, durotaxis, haptotaxis, and galvanotaxis (that are directed up gradients of a chemical, environmental topography, stiffness, adhesion, or electrical potential) are all well studied (Allen et al. 2013; Ricoult et al. 2015; Park et al. 2018; Sunyer and Trepat 2020). Most of these influences are sensed by cell-surface receptors and ultimately funneled to the actin-regulatory GTPase circuits.

The various components involved in cell motility have been studied experimentally. These include the turnover and flow of actin

(Pollard and Borisy 2003). Originally, cell observations in vitro were relegated to motion on flat surfaces. The importance of interactions between cells and the ECM (a fibrous 3D meshwork produced, degraded, and remodeled by cells) has been recognized. This resulted in experiments that aim to replicate aspects of 3D cell motility (e.g., Park et al. 2017). The investigations were extended to mechanical forces due to actomyosin (Keren et al. 2008), adhesion dynamics (Kaverina et al. 2022), and their interplay with biophysical effects such as substrate stiffness (Lo et al. 2000), 2D versus 3D motion (Pawluchin and Galic 2022), motion through the ECM (Friedl and Bröcker 2000), and curvature-dependent motion along nanofibers (Mukherjee et al. 2019), among many other topics. In a few cases, a link is made to the regulatory GTPases (e.g., Wittmann and Waterman-Storer 2001; Ridley 2015; Byrne et al. 2016).

We have seen a proliferation of inventive ways to deconstruct cell decisions. Experimental novelties in recent years included complex stimuli such as conflicting gradients (Shi et al. 2013; Lin et al. 2015), mazes (Um et al. 2019), adhesive micropatterns (Brock et al. 2003), and many more. Of recent developments, optogenetics has proven to be a remarkable tool for presenting accurate, carefully guided cues from which cell responses can be quantified (e.g., Valon et al. 2017; Hadjitheodorou et al. 2021).

The mathematical modeling approach to cell motility is reviewed in Mogilner (2009, 2019). Models and simulations of single-cell motility have been carried out in 1D, 2D, and 3D spatial coordinates (for review, see Buttenschön and Edelstein-Keshet 2022). The simplicity of 1D spatial models means that they can often be formulated in terms of PDEs for the contents of a cell, with boundary conditions for its opposed edges, or a periodic domain along the cell perimeter, with or without stochasticity (e.g., Xiong et al. 2010). Applied mathematics and scientific computing has developed many tools for understanding problems formulated in this way. Computational platforms such as Morpheus (Starruß et al. 2014), reviewed below, are starting to make PDE-based cell motility simulations available. For example, the PDE model of Figure 1C, simulated along the periodic edge of a "cell" results in directed motility, ruffling, and/or protrusions, as shown in Figure 2. While this is a great oversimplification of cell geometry, simple models can provide insights into how various effects (chemical, mechanical, size, noise) combine to generate cell protrusion, contraction, polarity, gradient sensing, directed migration, and reversal. Simulations in 2D and 3D can then be more directly targeted to exploring interesting details.

As far as a link from intracellular states to the cell's migration, one challenge has been to allow for cell shape deformation while solving equations for the reacting and diffusing components, for actin, and for mechanical forces. The methods for capturing cell shapes in 2D and 3D include phase field (Camley et al. 2013), cellular Potts model (CPM) (Marée et al. 2006; Rens and Edelstein-Keshet 2021), finite-element method (FEM) (Rubinstein et al. 2005; Cusseddu et al. 2019), and others. Currently, most simulations are custom built by a given investigator or laboratory and designed to answer specific questions studied by that laboratory. The development of software code requires expertise, troubleshooting, and fine-tuning. Typically, the code migrates with expert postdocs that developed it, having to be recreated from scratch when they depart. Unfortunately, there is no multipurpose, easily shareable suite of consensus software for cell motility modeling that is widely available to the community and adopted by many laboratories. Hence, aside from having to reinvent the wheel, the reproducibility of published results is still relatively poor, even when the code in a paper is published; documentation of decisions about how the models are built is rarely sufficient to reproduce the published results.

Several challenges are still to be addressed in modeling single-cell behavior. A partial list includes (1) understanding the essential core components and their interconnectivity, and fine-tuning due to a host of effectors and feedbacks; (2) refining the appropriate description of cell material properties, suitable for formulating the classical active-matter and solid mechanics modeling frameworks; (3) refining the model description of diffusion inside the crowded cell

interior and on its membrane, as well as the association–dissociation of molecules with the cell membrane ("bulk-surface" models); (4) representing the dynamically deforming cell shape in 3D and its effect on internal reaction-diffusion dynamics; and (5) creating a curated consensus platform for cell shape dynamics that is versatile enough to be used for multiple distinct investigations, while preserving the core cell characteristics.

SINGLE-CELL DECISIONS

To bridge to the next step of multicellular systems, it is helpful to explore how cells make decisions and change their migratory pattern. For example, how does a polarized cell react to a reversal or side stimulus that impinges on the polarity mechanism (Hadjitheodorou et al. 2021)? Until recently, this topic has had relatively little attention. Experimentally, cells migrating in one direction have been restimulated to reverse their polarity by chemical (Lin et al. 2015) or optogenetic (Hadjitheodorou et al. 2021; Town and Weiner 2023) methods, but a full systematic exploration with various cell types and conditions is yet to be done. Mathematically, the problem of cell repolarization was investigated by PDE bifurcation analysis (Buttenschön and Edelstein-Keshet 2022) to show how the intensity and width of an "optogenetic stimulus" could repolarize a minimal GTPase circuit. Theoretically, the dynamics of cell turning was shown to depend on the interaction of diffusible regulators with the curvature of a deforming cell's edge (Marée et al. 2012; Camley et al. 2017).

DYNAMICS OF CELL PAIRS

To understand the collective behavior of cells in tissues, we need to understand how cells influence one another, and how their internal dynamics affect their interactions with neighboring cells. When a cell encounters another, will it synchronize and adapt its polarity to match? Will it retract due to contact inhibition of locomotion (CIL)? Or will it lose polarity? Such questions were investigated in experiments (Desai et al. 2013; Jain et al. 2020) for cell–cell collisions and cell trains. More broadly, the idea of exploring cell pairs, cell trains, and small cell groups as intermediate steps to understanding collective behavior was eloquently proposed in De Pascalis and Etienne-Manneville (2017).

It is clear that addressing the above questions is best accomplished by targeted and controlled experiments on cell pairs that amass sufficient data to allow for statistically significant measurement of effective forces. A recent paper by physicists, Brückner et al. (2021), does precisely this, with an elegant setup for observing many ($N = 90$–150) collisions of cell pairs confined to micropatterns. Machine learning (ML) was used to "learn" and quantify attraction, repulsion, and frictional interactions, demonstrating differences between cancerous and normal cells. Such experiments hold great promise for future multicellular understanding and should be extended to a variety of cell types and conditions. While cells probably interact with multiple neighbors in nontrivial ways, understanding the simplified pairwise interactions can be illuminating.

The in vivo migration of cell pairs was studied experimentally in the tunicate Ciona and modeled using CPM simulations in 2D and 3D (Bernadskaya et al. 2021). The authors could thereby dissect the influences of proposed cell–cell versus cell–substrate adhesion, internal pressure, protrusion/retraction forces, and surface tension. They showed that the shape of the cell pair is consistent with the leading cell being mainly protrusive, and the follower cell being mainly contractile.

Simulations of cell pairs were carried out by Kulawiak et al. (2016) using the phase-field method for cell shape, a minimal cell polarity module (modified wave-pinning), and a mechanism for CIL based on the production of a local inhibitor. This minimal set of ingredients could already account for static and motile cell chains, CIL, and cells walking past one another. The advantage of such simple models is that they can be queried to investigate how cell properties (such as strength of propulsion, adhesion) and environment (confinement region size), in combination, affect the outcome.

COLLECTIVE BEHAVIOR OF MULTIPLE CELLS

Collective cell migration became a hot topic when it was observed that tumors can spread through invasion by large cell groups, clusters, or even tissue protrusion (Friedl and Gilmour 2009). A central question at the collective cell migration level is what new features (emergent properties) arise at this scale, and how do they depend on the single-cell repertoires and on the cell–cell interactions (Bi et al. 2015). At this level, many new events enter the picture, including cell heterogeneity, differential cell–cell adhesion, cell division and death, directed cell migration, and mechanical pulling by myriad cells. The subject is rich in possibilities, only a few of which have been explored so far.

On the experimental side, observing collective behavior in vivo is challenged by tissue opaqueness, difficulties of observing the tissue at both the cell scale (to resolve and track individual cells) and the macroscopic scale (to see the emergent behavior). For this reason, in vitro experiments with cell cultures, primary stem cells, tumor spheroids, and other groups of cells have been an important focus so far. Here, one can control the geometry, the initial state, the environment, and specific perturbations to probe the response of the system. In some cases, this is carried out with cell lines (normal vs. cancerous), with transformed cells (synthetic biology), or with primary cells (human pluripotent stem cells [hPSCs]). Some of the central questions driving the research are (1) what combinations of cell motility, division, and death account for observed growth and/or spread of a tissue (e.g., in wound healing or tumor growth), (2) what are minimal requirements that could account for tissue self-organization into specific layers or structures of specialized cells, and (3) how do cell clusters stay together, and how do they manage to collectively navigate to a target site? A combination of experiments and models have addressed such questions.

Models that link internal cell states to the interactions of cells in a tissue and forces between those cells have included minimal models (e.g., one or two regulators and cell size). An impressive example of the effect of extracellular signal-regulated kinase (ERK) signaling on cell-surface tension and cell size is the mechanochemical work in Boocock et al. (2021). The authors quantified ERK activity in a Madin–Darby Canine Kidney (MDCK) cell monolayer in 2D and showed that waves of activity transmitted in the epithelium could be explained by a minimal (three variable) continuum model. The model depicts feedback between ERK and local tissue stretching. This beautiful experiment-modeling work has a similar flavor, but goes well beyond the purely theoretical model by Zmurchok et al. (2018).

The problem of macroscopic spread has been addressed by continuum PDE models for cell population growth under a variety of assumptions. At the most basic level, cell proliferation is described by density-dependent (logistic) growth, coupled to random cell motility (essentially, a diffusion operator) in the classic Fisher equation (Simpson et al. 2007; Nardini and Bortz 2018). The attractive aspect of continuum approaches is the availability of PDE tools for characterizing solutions (traveling waves, steady-state distributions, fingering, etc.) and regimes of behavior (using stability and bifurcation analyses) (see Stepien and Swigon 2014), for example. PDEs can be derived from first principles based on cell-level assumptions (e.g., Othmer and Hillen 2000). Furthermore, tools for parameter reduction based on dimensional analysis and efficient methods for parameter estimation from experimental data are available. A disadvantage is that the level of detail that can be captured is limited, there is a tendency to discount cell heterogeneity, and the interpretation of parameters is not typically directly related to underlying molecular mechanisms inside the cells.

At the opposite extreme are computational models for simulating very large and possibly heterogenous cell populations with internal cell states and external nutrients, stimuli, or drugs that affect cellular growth, division, apoptosis, and so forth. These are predominately studied by simulations. Examples of this type of software include PhysiCell (Ghaffarizadeh et al. 2018) and its extensions, described briefly below. At the same time, there remain challeng-

ing aspects of collective cell migration that have not been fully addressed. One of these is the long-range cell guidance and cell–cell interactions mediated by the ECM. Cells migrate along and transmit long-ranged pulling forces via the ECM (Crossley et al. 2024).

Collective cell simulations have been designed recently to encompass multiscale models, where internal cell states are implemented (generally by bulk ODEs or Boolean regulatory networks, rather than the more costly spatially explicit PDEs for the internal cell states) (see, for example, Scianna and Preziosi 2012). The internal states then affect cell shape, division, death, cell–cell adhesion, migration speed, and other aspects. Out of many tools that facilitate simulations of such behaviors, a personal favorite of this group of authors is Morpheus, described in greater detail in the following section. Here, we highlight case studies of such multiscale collective cell behavior.

Example 1. Neural Crest Cell Swarms

Merchant et al. (2018) modeled neural crest cell (NCC) migration in a confined population of between 2 and 50 nonadherent cells. This paper employed a cell-edge-based chemical system (a Rac-Rho GTPase system diffusing on the periodic cell perimeter), with assumptions for cell–cell coattraction and CIL to show a link between internal regulatory dynamics and collective cell behavior. The model, simulated in custom-built code (Merchant et al. 2018), demonstrated that an additional assumption was needed, the persistence of cell polarity, to obtain robust collective migration. NCC migration has also been modeled by agent-based (ABM), PDEs, and nonlocal PDE models (for review, see Giniūnaitė et al. 2020).

Example 2. Self-Organizing Synthetic Cell Aggregates

Synthetic biology has provided useful experimental examples of self-organization in vitro. Toda et al. (2018) engineered cell lines in which Delta-ligand—Notch-receptor association of neighboring cells would activate genes for E-cadherin, an adhesion molecule that makes cells stick together. Suitable fine-tuning of the in vitro synthetic gene circuitry resulted in symmetry breaking, with cells differentiating into two states, "sender" and "receiver." The former has Delta dominating, and the latter has Notch dominating. Hence, by virtue of the gene circuit, the system is bistable (i.e., has two distinct eventual fates). The cells are driven to one of two states as a result of cell–cell interactions.

Delta–Notch signaling is well known and a number of models for this system are available in the form of ODEs with ABM patterning in 1D and 2D "tissue" (Collier et al. 1996), or as sets of ODEs (Boareto et al. 2015) with bifurcation analysis. By combining the known cell gene dynamics within multiscale simulations of interacting cells, Mulberry and Edelstein-Keshet (2020) recapitulate in silico the in vitro observations of Toda et al. (2018) using CPM cells in Morpheus. Starting from either two cell types (sender and receiver) or a single-cell type, the aggregation and contact of cells induce Notch activity. Neighboring cells exert lateral inhibition and there is a spontaneous symmetry breaking that results in two dynamic cell fates. The cell's genetic state dictates its adhesion to other cells, and relative adhesion results in local cell sorting that shuffles neighbors, breaking and making new contacts. This, in turn, engages Delta–Notch binding on new partners, and causes changes in cell states. As shown in Figure 3, the aggregate develops distinct cell fates—with the more adhesive (E-cad cells, shown in green) engulfed by the less adhesive (red) cells.

It is interesting to note that the Delta–Notch genetic circuit has the inherent property of bistability. Nevertheless, the capacity of this circuit to form a layered structure emerges not only from its bistable circuit, but also from the spatially distributed cell–cell interactions. Cells that converge to a position inside the core have to be more adhesive to one another than to those enveloping them, and even less prone to contact with the surrounding medium. At the same time, cell sorting acting on its own is notoriously slow, and tends to get trapped in various undesirable configurations. Hence, the combination of cell state influencing affinity to

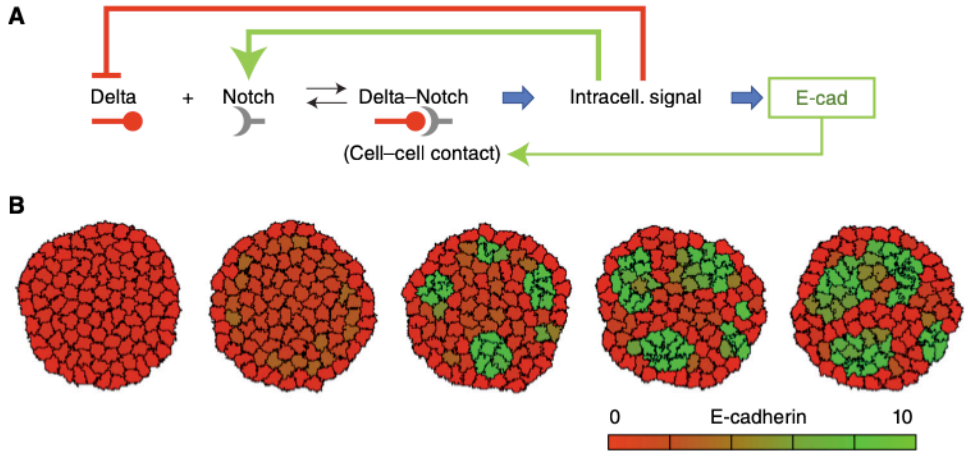

Figure 3. A multiscale model of synthetic Delta–Notch experiments by Toda et al. (2018), created in the Morpheus cellular Potts model (CPM) platform. (*A*) Schematic diagram of the signaling system (based on a model in Mulberry and Edelstein-Keshet 2020). (*B*) Starting with a single-cell type, a cluster of 100 cells develops internal self-organization, showing a time sequence (*left* to *right*) at $t = 10, 30, 60, 120, 180$ Monte Carlo time steps (MCS). (Panel reprinted, with permission, from Mulberry and Edelstein-Keshet 2020.) Delta–Notch-receptor binding on neighboring cells activates internal signaling that amplifies Notch and suppresses Delta receptor expression, as well as increasing E-cadherin. The latter adhesive protein enhances cell–cell contact (see Supplemental Material for more detail).

neighbors (adhesion) and cell contact influencing genetic state (Delta–Notch signaling) greatly speeds up a process of self-organization. Cell-based simulations can reveal emergent properties that are not apparent from the cell wiring on its own.

Example 3. Wound-Healing and YAP-Regulated Metastasis

Cells are sensitive to many features of their environment, including topography and mechanical cues. Park et al. (2019) observed the moving front in epithelial sheets of MDCK cells. The epithelia were tracked on both flat and nanoridge arrays (NRAs) substrates. On NRA topography, there was the enhanced activity of the mechanosensitive transcription factor YAP (Yes-associated protein), which resulted in uneven fronts, fingering (i.e., initiation and growth of multicellular protrusions from the edge of the tissue), and dissemination of ameboid cell clusters that broke away. The authors demonstrated that YAP is in a mutually inhibitory pathway with effectors of E-cadherin, and a mutually reinforcing pathway with the GTPase Rac (Fig. 4). Hence, up-regulation of YAP near the sheet front reduces cell adhesion and accelerates migration.

Mukhtar et al. (2022) adapted and implemented an ODE model of Park et al. (2019) in a CPM Morpheus simulation. The simple YAP-Rac-E cadherin ODE model that governs the state (and hence behavior) of each cell is bistable, and stimulation by the edge-sensing topography is able to change the outcome from low to high YAP activity. The emergence of fingering and dissemination of clusters then follows once adhesion and motility are in suitable state-dependent ranges. The model predicts an abrupt switch in YAP activity at some distance away from the front edge. Figure 4 demonstrates the time sequence and breakage of cell clusters, as well as the speed of migrating cells.

Example 4. Early Mammalian Embryogenesis

A multiscale model for mammalian embryo development (from zygote to 128 cells) is showcased in Figure 5. A crucial aspect of this devel-

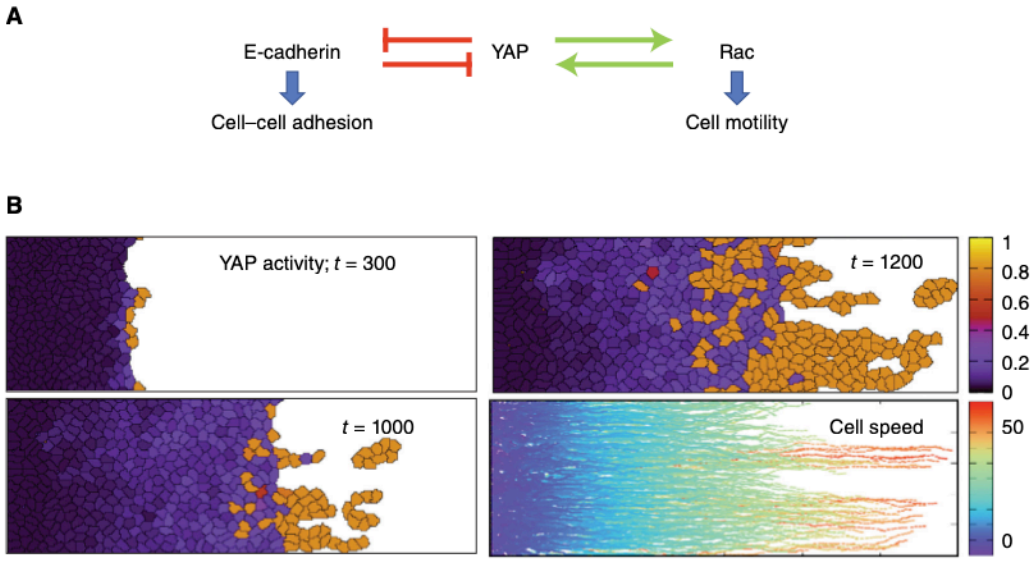

Figure 4. A multiscale model for Yes-associated protein (YAP) signaling in an epithelial sheet, demonstrating fingering behavior. (*A*) A schematic diagram of the simplified signaling circuit proposed in Park et al. (2018) and implemented in Mukhtar et al. (2022). (*B*) A time sequence of the sheet edge (at $t = 300$, 1000, 1200 Monte Carlo steps) with YAP activity indicated on a heatmap. YAP is in a mutual negative (positive) circuit with E-cadherin (Rac). Hence, active YAP loosens cell adhesion and accelerates cell motility. The tissue edge forms multicellular protrusions that resemble "fingers," some of which break away (see Supplemental Material for more information). (*B* reprinted from https://identifiers.org/morpheus/M7121, Morpheus.Lab @ TUD Dresden University of Technology, under the CC BY 4.0 license.)

opment is the self-organization of differentiated cells into a proper configuration. This model represents two stages: (1) First, from the zygote state, cell–cell contact signaling drives differentiation into two cell types, trophectoderm (TE) and inner cell mass (ICM). (2) Second, diffusible FGF4 ligand secreted by ICM cells into the medium regulates the transition from ICM to epiblast (Epi) and primitive endoderm (PrE) cells.

Preexisting models (De Mot et al. 2016; Cang et al. 2021) are highly detailed, making them excellent starting points for new users to implement and explore the system. These are medium-large models. For stage 1, cell states satisfy two ODEs with eight parameters. For stage 2, there are five ODEs with 39 parameters. Due to the careful detail presented in the above papers, assembling a multiscale CPM Morpheus simulation (where each cell has dynamic internal states) proved to be feasible for an undergraduate summer research project. Even so, translating previous models into a spatial multiscale model in a new platform always poses a challenge, requiring adjustment of the spatial assumptions, framework, and parameter sets. The user-friendly GUI (graphical user interface) of Morpheus made these challenges less daunting. Results shown in Figure 5 are consistent with those of the two cited papers and with known human embryo development.

EXISTING CHALLENGES

Assembling multiscale tissue simulations can help to further our understanding of collective cell behavior. But some challenges still make this a nontrivial undertaking, even when quantitative information or models of the single cell are available. One recurring question is which aspects of cell behavior should be included in scaling up from one cell to many. It is generally required to reduce the level of detail in such

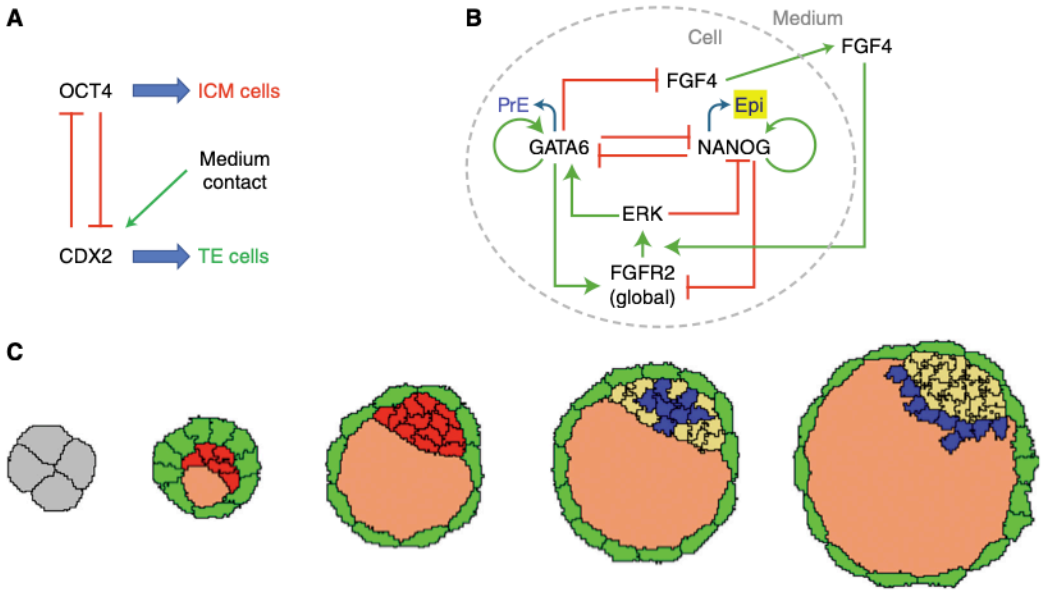

Figure 5. Stages of cell differentiation in mammalian blastocyst development. (*A*) A zygote (not shown) divides into four undifferentiated cells (gray) that divide further and differentiate. An intracellular Oct4-CDX4 transcription factor circuit regulates differentiation into trophectoderm (TE, green, exterior) and an inner cell mass (ICM, red, interior). A cell-free lumen (orange) also forms and grows. (*B*) GATA6, NANOG, and ERK signaling in ICM cells further lead to specialization into epiblast (Epi, yellow) and primitive endoderm (PrE, blue) cells. FGF4 is a diffusible ligand secreted by ICM cells that influences the differentiation of nearby cells. (*C*) Morpheus cellular Potts model (CPM) simulation time sequence (*left* to *right*, $t = 40, 210, 570, 830, 1400$ Monte Carlo time steps [MCS]) shows the dynamics of the self-organized structure. Difference in cell–cell adhesion between cell types affects the evolving embryo (see Supplemental Material for more information). (Figure based on models in Cang et al. 2021 and De Mot et al. 2016; see these publications for details about the original models.)

scale-ups to keep a coarse-grained model tractable, and to allow the cell–cell interactions to be explored efficiently. A second issue is how to build on current knowledge (rather than reinventing the wheel). Too often, existing models in scientific papers suffer from missing information and undocumented assumptions buried in proprietary code. Free software platforms reviewed below are a great boon to the community and considerably increase the efficiency of research. But what is still needed is a curated, updated archive of biomodules where model equations, values of parameters, initial conditions, boundary conditions, and other specific details are (1) documented and explained, and (2) available not only in print (or buried in code) but also in text files that are easily imported and used. Overall challenges for modeling multicellular tissues are described in the recent work of Fletcher and Osborne (2022).

SIMULATION PLATFORMS

Depending on the modeling of interest, a variety of software solutions exist. To simulate ODE models and perform necessary analyses (linear stability analysis, bifurcation analysis, sensitivity analysis, model reduction, etc.), a wide variety of tools have been developed. A longstanding free package, XPPAUTO (sites.pitt.edu/~phase/bard/bardware/xpp/xpp.html) (Ermentrout and Mahajan 2003) has been popular in the math-bio community since the late 1980s (see Klipp et al. 2016 for a comprehensive list of about 57 open-source/commercial software solutions and their capabilities). Also, the blog Stochastic

Lifestyle (www.stochasticlifestyle.com/comparison-differential-equation-solver-suites-matlab-r-julia-python-c-fortran) provides a useful comparison of available software suites categorized by programming language (Rackauckas 2017).

Due to the complexities of PDE models, there are fewer integrated software solutions. The web-based VisualPDE (visualpde.com) (Walker et al. 2023) is a great recent tool to simulate and visualize fairly complex PDE systems. It offers an interesting and growing set of examples, along with a complete explanation of each model, serving as both a great learning tool and a useful starting point for adapting existing models. Limitations include nonscalability for more complex simulations, lack of scripting, and restricted available domain shapes. Other options to simulate and visualize PDE models include FeNiCsX (jsdokken.com/dolfinx-tutorial), Matlab "PDE modeler" (www.mathworks.com/help/pde/ug/pdemodeler-app.html), freeFEM (Hecht 2012), FlexPDE (www.pdesolutions.com), and COMSOL Multiphysics (www.comsol.com). Easy-to-use libraries based on finite difference methods, such as PyPDE (Zwicker 2020) and Scikit-diff (Cellier and Ruyer-Quil 2019) often come in handy for simulations of intermediate complexity. Unlike software tools for simulating PDEs, bifurcation analysis of PDE models is still not "out of the box." Often, more dedicated libraries are needed, such as BifurcationKit.jl (Rankin et al. 2014), MATCONT (Dhooge et al. 2003), and pde2path (pde2path.uol.de) (Uecker et al. 2014; see Blyth et al. 2020 for a more detailed discussion of such software tools, along with a tutorial).

For discrete ABM, Abar et al. (2017) provide an extensive list of available software. Hybrid modeling that integrates discrete and continuous models harnesses the best of both worlds. This has become a popular approach recently and has been very successful in mathematical biology research. Various tools such as Morpheus (Box 1) (morpheus.gitlab.io) (Starruß et al. 2014)—demonstrated in our examples above—as well as CHASTE (chaste.github.io) (Mirams et al. 2013), CompuCell3D (compucell3d.org) (Swat et al. 2012), Artistoo (Wortel and Textor 2021), and CellularPotts.jl (Gregg and Benos 2024) exist that integrate the cellular Potts models with continuous ODE/PDE models. Each comes with its limitations and strengths. Comparing these software solutions can be done by considering many different aspects, such as scalability, performance, user-friendliness, GUI support for rapid model development, and usability with scripting to harness its full power (for example, see Fig. 2; Fletcher and Osborne 2022 for such a comparison).

Although the working principle is the same for various CPM software, differences in implementation and structure can make it challenging to reproduce results obtained with one software using another software package, even with the "same set of parameters." Nonetheless, slight modification generally leads to the same qualitative results (see Fig. 6). To put these software tools on the spectrum of ease of use for pedagogical purposes, Artistoo has demonstrated significant power. Developed purely with JavaScript, it runs natively in browsers, making it accessible for students. Additionally, the detailed repository of predesigned models serves

BOX 1. MORPHEUS, A USER-FRIENDLY MULTISCALE SIMULATION PLATFORM

Morpheus (Starruß et al. 2014) is an open-source freely available software platform (downloadable from morpheus.gitlab.io) for simulating models based on ordinary and partial differential equations (of the reaction–diffusion type), single and multiple cell shapes (via the cellular Potts formalism), directed cell motility, chemotaxis, division, death, and differentiation, cell–cell interactions, adhesion, and dynamic internal cell states. The software comes with many built-in examples that can be run and adapted by the user directly on a convenient graphical user interface (GUI). Each simulation run is automatically documented (xml file) for reproducibility. Morpheus is relatively easy to learn and serves as a great research and teaching tool.

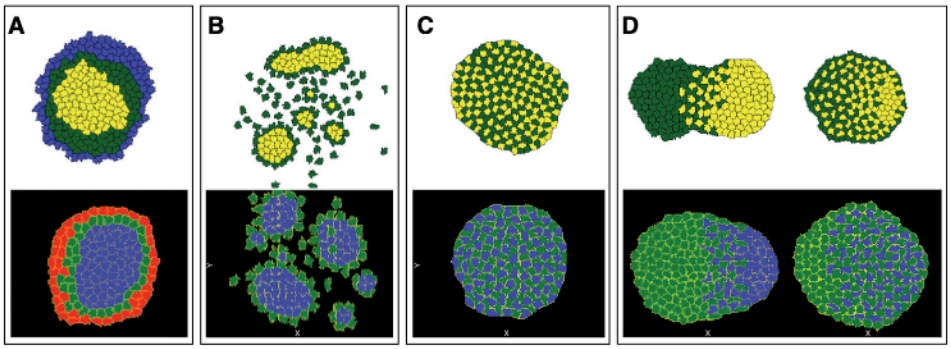

Figure 6. A comparison of cellular Potts model (CPM) results in Morpheus (*top*) and CompuCell3D (*bottom*) using four "benchmark" cell-sorting simulations in 2D. (*A*) Three-layer structure with core, intermediate, and peripheral cell types. Adhesion energy "cost" (J_{ij}) for cell–cell contact between cells of types (i,j) were in ranges $J_{cm} > J_{ci}$, $J_{im} > J_{ip}$, J_{pm}, and $J_{pi} < J_{pc}$, where c, i, and p represent core, inner, and peripheral cells and m is medium. (*B*) Fragmentation: Cell type 1 forms clusters due to low cell–cell contact energy J_{11}, while type 2 forms a single layer around the clusters due to high cell-medium contact energy, J_{1m}. (*C*) Checkerboard pattern: Two cell types have $J_{1m} \sim J_{2m}$ and $J_{12} < J_{11}, J_{22}$. (*D*) Cluster fusion: Two clusters of cells merge and create a checkerboard pattern (see Supplemental Material for more information).

as an excellent starting point. However, ease of use and learning often come at the cost of non-scalability for more complex tasks and scripts.

Compared to the software suites that support ODE/PDE models natively, CPM/hybrid software is often limited in its capability to handle the continuous part of the model (i.e., ODEs, PDEs). Essential tools like sensitivity analysis, steady-state solution analysis, bifurcation analyses, numerical schemes to support stiff models, and support for more complicated PDE models and deforming domains are either not implemented or are very basic and need expansion.

The trend in modern software ecosystems is integrated software suites that are extendable through various plugins. Some software solutions like CHASTE use this paradigm. For instance, the microvessel CHASTE plugin (Grogan et al. 2017) is designed to be used for angiogenesis models. This framework can be promising for more scalable software solutions where various modules can be developed as plugins by a dedicated user.

PhysiCell, an open-source software (Ghaffarizadeh et al. 2018) simulates cell growth dynamics, migration, and interaction with secreted chemicals in 3D. This software has become a popular tool for tumor modeling. An extension (PhysiBoss; see, e.g., Ponce-de-Leon et al. 2023) implements internal cell regulatory networks and preserves the 3D visualization capability of thousands of spherical cell agents. A benefit of open-source platforms like PhysiCell is that distinct groups can help to extend the capabilities of the system. For example, PhysiCOOL is an extension that facilitates parameter space exploration (Gonçalves et al. 2023).

DISCUSSION

Huge progress has been made over the last decade in our ability to experimentally observe, measure, model, and simulate cell biology systems, from the intracellular to the multicellular levels. The interconnections between micro-, meso-, and macroscopic levels make this into a rich and diverse field of study.

Some key challenges remain to be addressed. How should we each document and keep track of many decisions made while modeling to make our models shareable and reproducible? The description of models in most scientific papers (see Supplemental Information) is still usually deficient, as any student attempting to learn from previous work finds out rapidly. This means that while the cell biology field is reason-

ably collegial in sharing cell types, media, or probes, the modeling of cell biology lags behind this generous spirit. This deficiency means that it is hard to build on work by others. It would be especially useful to agree on and/or standardize some basic modeling guidelines and icons or nomenclature to account for those, and to document the stepwise process that went into constructing a complex model, not just the final version. We often learn more from the failure of early variants that could not account for some observed behavior than from the full complexity that could.

We are yet to converge on the best ways to describe the material properties of cells, links between stimuli and response, and how cells make decisions in the context of competing stimuli. A unified model that allows us to manipulate cell properties and include or exclude aspects of interest could be very influential. Another challenge is to decipher what aspects of the implementation (CPM, vertex-based, ABM, etc.) affect the types of results obtained in a given simulation. Benchmark comparisons similar to those of Osborne et al. (2017) would be particularly useful, not only within a given platform (CHASTE in the latter case), but across computational platforms. This would help to point to software that is particularly useful for a given context—for example, vertex-based collective cell simulations are great for epithelia, but perhaps less ideal for describing breakage of clusters and metastasis than CPM or cell-centered models.

Like the "human atlas" for tissue and for biomolecular interactions (Börner et al. 2022; Jain et al. 2023), tissue banks, and repositories of seeds, it would be highly useful to have a community-wide resource like a searchable archive of models that run across software programs. Ideally, entries would account for cell-type-dependent and environment-dependent parameters of a given model. One would be able to "upload" a model for the migration of neutrophils and compare it against *Dictyostelium* or other cell types merely with a few mouse clicks. We have much existing knowledge to draw upon, but not yet an organized way to easily share it.

More generally, the field of cell motility modeling would benefit from a more closely collaborative research community, akin to communities that arose around model organisms (Dietrich et al. 2014; Ankeny and Leonelli 2016). A process of organic self-selection would then lead to overall standardization of the types of information, data, computational methods, and metrics commonly (if not universally) adopted. The success of such a community depends on willingness to organize joint conferences, train young scientists to use and contribute to common resources, and develop databases of models and software. It also depends on stakeholder buy-in that emphasizes and rewards these collaborative needs at funding agencies (Dietrich et al. 2014; Ankeny and Leonelli 2016).

The interrelationships of distinct model types, and formal ways of deriving the PDEs and integro-PDEs from underlying cell behaviors are an important way of extending mathematical analysis to a given setting. But we still need to work on linking cell-based simulations to stochastic differential equations (SDEs) and/or PDEs in a rational way. Simulations in and of themselves can fail to address important questions: Is the simulated behavior "generic" or a coincidence in some specific corner of parameter space? What other behaviors are possible in other corners of that space? How does one regime (say "normal") behavior transition into pathological dynamics (say, "metastatic"), or how do cells migrating in one direction decide to turn or reverse? Such questions are not easy to address by parameter sweeps or trial and error in simulations that have numerous free parameters (for additional information, see Supplemental Material).

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