POLITECNICO DI TORINO
Repository ISTITUZIONALE

Plasticity of Cell Migration in Vivo and in Silico

*Original*
Plasticity of Cell Migration in Vivo and in Silico / Te Boekhorst, Veronika; Preziosi, Luigi; Friedl, Peter. - In: ANNUAL REVIEW OF CELL AND DEVELOPMENTAL BIOLOGY. - ISSN 1081-0706. - STAMPA. - 32:1(2016), pp. 491-526. [10.1146/annurev-cellbio-111315-125201]

*Availability:*
This version is available at: 11583/2670891 since: 2020-02-18T22:09:39Z

*Publisher:*
Annual Reviews Inc.

*Published*
DOI:10.1146/annurev-cellbio-111315-125201

*Terms of use:*

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

*Publisher copyright*

(Article begins on next page)

12 October 2024

REVIEWS IN ADVANCE

# Plasticity of Cell Migration In Vivo and In Silico

Veronika te Boekhorst,[1] Luigi Preziosi,[2] and Peter Friedl[1,3,4]

[1]David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030; email: PFriedl@mdanderson.org

[2]Department of Mathematical Sciences, Politecnico di Torino, 10129 Torino, Italy

[3]Department of Cell Biology, Radboud University Medical Centre, 6525GA Nijmegen, The Netherlands; email: Peter.Friedl@radboudumc.nl

[4]Cancer Genomics Center, 3584 CG Utrecht, The Netherlands

Annu. Rev. Cell Dev. Biol. 2016. 32:28.1–28.36

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi: 10.1146/annurev-vision-111815-114456

Copyright © 2016 by Annual Reviews. All rights reserved

## Keywords

cell adhesion, proteolysis, amoeboid migration, mesenchymal migration, collective movement, mathematical modeling

## Abstract

Cell migration results from stepwise mechanical and chemical interactions between cells and their extracellular environment. Mechanistic principles that determine single-cell and collective migration modes and their interconversions depend upon the polarization, adhesion, deformability, contractility, and proteolytic ability of cells. Cellular determinants of cell migration respond to extracellular cues, including tissue composition, topography, alignment, and tissue-associated growth factors and cytokines. Both cellular determinants and tissue determinants are interdependent; undergo reciprocal adjustment; and jointly impact cell decision making, navigation, and migration outcome in complex environments. We here review the variability, decision making, and adaptation of cell migration approached by live-cell, in vivo, and in silico strategies, with a focus on cell movements in morphogenesis, repair, immune surveillance, and cancer metastasis.

## Contents

INTRODUCTION ..... 28.2
MODES OF CELL MIGRATION ..... 28.3
DETERMINANTS CONTROLLING MIGRATION MODES ..... 28.3
Cellular Determinants ..... 28.3
Tissue Determinants ..... 28.9
RECIPROCITY OF CELL-TISSUE INTERACTION AND MIGRATION ..... 28.11
Strain Stiffening of Substrate and Cytoskeletal Reinforcement ..... 28.11
Substrate Pushing and Space Unfolding ..... 28.13
Proteolytic Repatterning of Tissue ..... 28.13
Autocrine Stimulation ..... 28.14
Paracrine Stimulation ..... 28.14
PLASTICITY PROGRAMS OF CELL MIGRATION ..... 28.16
Decision Making While Retaining an Ongoing Migration Program ..... 28.16
Transitions Between Migration Programs ..... 28.18
MULTISCALE MATHEMATICAL MODELING OF CELL MIGRATION AND PLASTICITY ..... 28.22
Mathematical Toolbox to Model Cell Migration ..... 28.22
Cause-Consequence Relations and Decision Making ..... 28.25
Virtual Assembly of Moving Cells ..... 28.26
FUTURE DIRECTIONS ..... 28.27

## INTRODUCTION

The assembly and positioning of cells to build, reshape, defend, and repair a multicellular organism depend upon the cells' ability to migrate (Friedl & Weigelin 2008, Scarpa & Mayor 2016, Sonnemann & Bement 2011). Cell migration is consequently a multipurpose process, which allows cells to reach and change their position in a given environment to execute their function, to form or abandon assemblies with neighboring cells and move either individually or collectively, and to mechanically and chemically interact with structural tissue components and thereby alter interstitial tissue composition and organization (Friedl et al. 2012b, Rowe & Weiss 2009). These kinetic processes are controlled by molecular programs that enable cells to perceive, interact with, and (if required) remodel tissue structure while moving or anchoring the cell body (Chen et al. 2004). Despite the enormous complexity of cell migration and its potential vulnerability to mechanical and signaling assault, cell migration as a process is remarkably robust and resilient upon challenge. Due to redundancy and complementarity of signaling and execution mechanisms, cells are equipped with a plethora of adaptation strategies to adjust and secure migration and to make stop/go decisions (Friedl & Wolf 2010).

**Parameters:** represent the properties that influence the spatial distribution and temporal evolution of state variables, including chemical affinities, reaction rates, chemotactic sensitivity, cell stiffness, adhesivity, and cell traction force

In this review, we develop an inventory of molecular and physical principles underlying cell migration and its diverse forms and adaptation programs in different tissue and cell function contexts in vitro; in vivo; and, using mathematical modeling, in silico. We emphasize the need for examination and modular integration of multiple parameters to generate a framework of cell kinetics in health and disease, with a focus on physiological key processes, including embryological development, tissue homeostasis, immune defense, and cancer progression.

## MODES OF CELL MIGRATION

Migrating cells can move either individually, mediated by cytoskeletal activity without cell-cell interactions to neighboring cells (Ridley et al. 2003), or collectively, as cohesive groups that retain cell-cell junctions and coordinate cytoskeletal activity between neighboring cells as well as the surrounding tissue (Friedl & Gilmour 2009). Single-cell migration is prototypic for moving leukocytes to transit through and between tissues as part of their surveillance function (Friedl & Weigelin 2008); for stromal cells producing, depositing, and resorbing extracellular matrix (ECM) (Grinnell & Petroll 2010); and for stem cells populating tissue niches before terminal integration and anchorage (Paksa & Raz 2015). In vivo, on the basis of morphology, kinetics, and function, two operational types of single-cell migration are amoeboid movement and mesenchymal movement (**Figure 1*a***). Collective movement occurs when cells maintain cell-cell junctions to their neighbor cells and move as a coordinated group (Friedl & Gilmour 2009). Their collective morphology, dynamics, and outcome are consequently determined by the type and stability of intercellular junctions and extracellular tissue conditions (**Figure 1*b***). These types of single-cell and collective movement underlie distinct molecular programs, which define the specificity, mechanical strength and turnover, and consequences of cell-cell and cell-matrix interactions.

**Actin focalization:** local enrichment of actin filaments, typically in contact with solid substrate mediated by engaged and clustered adhesion receptors and connecting adaptor and signaling proteins. Focalized actin enables regions of increased adhesion, signaling, and mechanocoupling to cell and tissue substrates

## DETERMINANTS CONTROLLING MIGRATION MODES

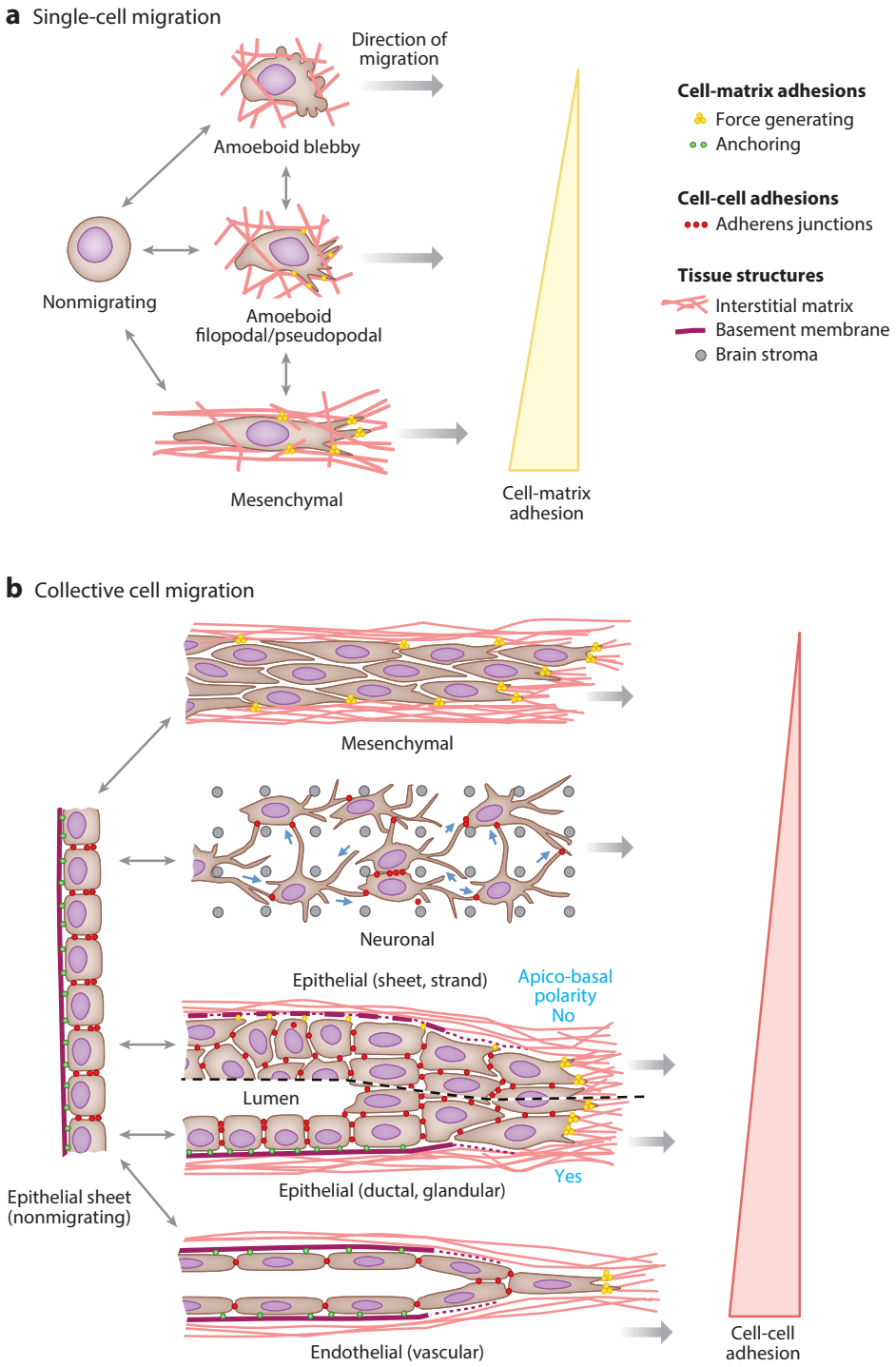
The efficiency, purpose, and type of migration mode that a cell adapts to or maintains in a particular tissue context are determined by cellular and tissue-intrinsic properties, here termed modules. Each module is adaptive, cooperates with other modules, and responds to local and global mechanical and molecular signals.

### Cellular Determinants

Cell-intrinsic modules include the organization and dynamics of the cytoskeleton, its connection with cell-matrix and cell-cell adhesion sites, and the deformability of the cell body and nucleus. These modules are cell type specific and adaptive, as they respond to cell activation, differentiation, and environmental signaling.

**Cytoskeletal organization.** The cytoskeleton, including actin filaments, microtubules, and intermediate filaments, defines how, and how efficiently, cells move. Cytoskeletal dynamics include actin network kinetics to generate membrane protrusions and regulate adhesion to ECM and/or other cells and actomyosin contractility to define cell shape, create cell tension, and pull on extracellular cues (Gardel et al. 2008, Pollard & Borisy 2003, Roca-Cusachs et al. 2013). Thus, cytoskeletal functions define cell adhesion and vice versa. At light-microscopical resolution, at least three organization types of cortical actin networks support cell migration: networks without actin focalization (**Figure 2*a***, ①); networks with short-lived foci (**Figure 2*a***, ②); and networks with longer-living, larger, actin-rich foci and inserting stress fibers (**Figure 2*a***, ③).

Cortical actin is a sheetlike network that is composed of branched actin filaments in parallel with the plasma membrane, which forms and dissolves locally (**Figure 2*a***, ①) (Bergert et al. 2015, Renkawitz et al. 2009, Roubinet et al. 2012). Cortical actin networks either are involved in poorly adhesive cell-ECM interactions with surrounding substrates, such as in nonadherent stationary cells (Andrade et al. 2015), or align along cell-cell junctions, supporting both stable and dynamic contacts in stationary epithelia and during collective cell migration (Wu et al. 2014). Actin


a Single-cell migration
Direction of migration
Amoeboid blebby
Nonmigrating
Amoeboid filopodal/pseudopodal
Mesenchymal
Cell-matrix adhesion
Cell-matrix adhesions
Force generating
Anchoring
Cell-cell adhesions
Adherens junctions
Tissue structures
Interstitial matrix
Basement membrane
Brain stroma
b Collective cell migration
Mesenchymal
Neuronal
Epithelial (sheet, strand)
Apico-basal polarity
No
Lumen
Yes
Epithelial (ductal, glandular)
Epithelial sheet (nonmigrating)
Endothelial (vascular)
Cell-cell adhesion

focalization typically correlates with the strength of local adhesion. Short-lived, small, actin-rich adhesions connect with transient clusters of adhesion receptors and adaptor proteins and provide weak traction force (**Figure 2*a***, ②) (Balcioglu et al. 2015, Case et al. 2015, Steinwachs et al. 2016, Swaminathan et al. 2016). Larger, focalized cell-matrix adhesions connect to contractile stress fibers consisting of actin bundles and myosin-II and transmit focally high force toward the substrate (**Figure 2*a***, ③) (Balcioglu et al. 2015, Case et al. 2015, Chrzanowska-Wodnicka & Burridge 1996).

**Hydrostatic pressure:** intracellular pressure jointly controlled by contraction and relaxation of the cortical actin network; water and ion influx and efflux controlled by transmembrane channels; and, likely, the available plasma membrane surface, which is regulated by endo- and exocytosis

Depending on cortical actin assembly, disassembly, and actin flow, different cell protrusions provide distinct types of contact with extracellular structures. Local weakening of cortical actin as a consequence of the small GTPase RhoA, which induces myosin-mediated actin network contraction and/or increased intracellular hydrostatic pressure, leads to local formation of bleb-like membrane protrusions that become stabilized by a newly assembled cortical actin network (**Figure 2*a***, ①) (Paluch & Raz 2013). Blebs typically form toward the leading edge; engage with surrounding tissue structures; move laterally and rearward; and resolve within minutes by cortical actomyosin–mediated retraction, which drives single cells forward (Goudarzi et al. 2012, Paluch & Raz 2013). Bleb-like protrusions mediate amoeboid migration in *Dictyostelium discoideum*, leukocytes, certain cancer cells (Bergert et al. 2015, Friedl et al. 1998, Liu et al. 2015), and germ cells in the zebrafish embryo (Goudarzi et al. 2012). Cortical actin flow and bleb-based protrusions arguably represent the most primordial and least complex cytoskeletal kinetics involved in cell migration. The organization of other specialized and more-complex-structured protrusions, including lamellipodia, filopodia, lobopodia, podosomes, and invadopodia, depends upon regulators of actin polymerization (e.g., the small GTPases Rac and Cdc42), cross-linking proteins (e.g., fascin,

**Figure 1**

Single-cell and collective migration modes. (*a*) Transition from nonmigrating to single-cell migration states. Characteristics of amoeboid-moving cells include a roundish or ellipsoid morphology with a relatively short trailing edge but a plastic, highly dynamic front edge with bleb-like protrusions (e.g., in primordial germ cells) or leading dendrites, filopodia, or pseudopodia (e.g., in dendritic cells, monocytes); prominent deformability of the cell body; weak adhesion toward the substrate; and limited ability to remodel tissue while these cells move (Renkawitz et al. 2009). Amoeboid-moving cells apply adaptive adhesive and nonadhesive interactions for force generation (Schmidt & Friedl 2010) and can readily cross epithelial, endothelial, and basement membrane barriers. Mesenchymal movement generates an elongated cell shape, with long extensions in the forward and rearward directions, strong adhesion and traction followed by tissue realignment, and tissue remodeling during migration. Mesenchymal migration fulfills complex functions of position change together with tissue remodeling and deposition of extracellular matrix and cytokines during interstitial tissue formation, maintenance, and repair (Grinnell & Petroll 2010, Rhee 2009). (*b*) Collective cell migration modes, determined by the morphology and strength of cell-cell interactions. Mesenchymal collective movement is mediated by relatively weak cell-cell junctions and is supported by high cell density and tissue confinement (Shih & Yamada 2012, Alexander et al. 2008, Wolf et al. 2007). The neuronal type of collective movement is used by migrating astrocytes or glioma cells moving through complex brain stroma while retaining filamentous cell-cell junctions (Osswald et al. 2015). Collective migration of epithelial cells is mediated by relatively sustained cell-cell junctions that discourage single cells to detach. Depending on the cell type and environmental context, epithelia move as a sheet across 2D surfaces, such as epithelial cells in scratch-wound assays (Bazellieres et al. 2015, Reffay et al. 2014); as solid, three-dimensional strands, clusters, or multilayered masses, such as the ectodermal sheet during gastrulation or border cells moving through the *Drosophila* ovary (Cai et al. 2014, Collins & Nelson 2015); or as moving monolayered or stratified epithelium that develops apico-basal polarity, with an inner lumen and basement membrane deposition toward the basal side (Cheung & Ewald 2014). Endothelial collective movement leads to vascular sprouting with multicomponent cell-cell junctions that support both front-rear polarity toward a leader cell and apico-basal polarity for lumen formation (Eilken & Adams 2010, Tornavaca et al. 2015).

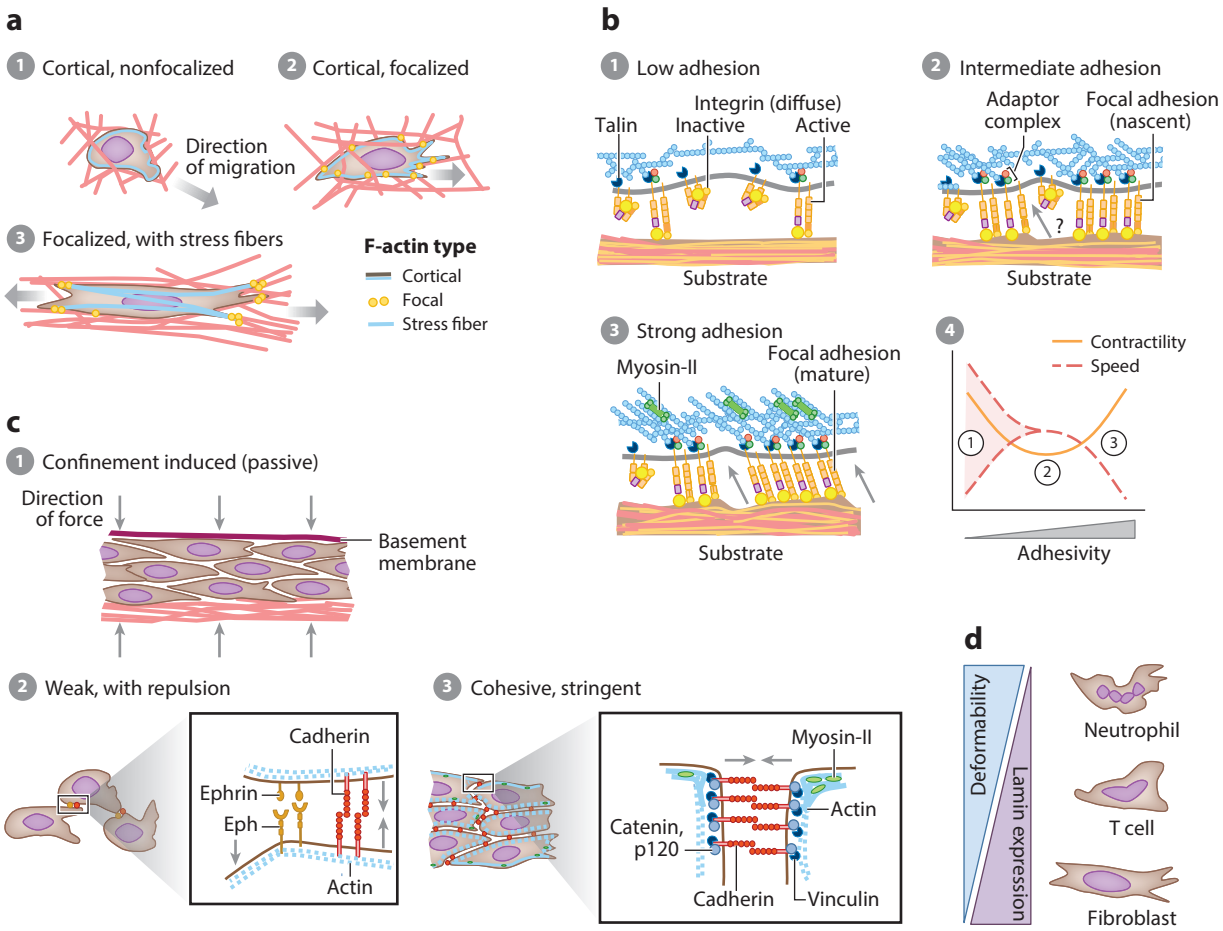



**Figure 2**

Physical and molecular cellular modules determining migration modes. (*a*) Organization of the actin cytoskeleton, including ① cortical-diffuse, ② cortical-focal, and ③ focal with stress fibers. (*b*) Cell-substrate adhesion regulation. ① Low adhesion exerted by integrins diffusely distributed in nonclustered adhesion domains. ② Intermediate adhesion resulting from clustered integrins and locally focalized cortical actin cytoskeleton (focal contacts). ③ Strongly adherent, mature focal adhesions with focalized actin filaments and insertion of contractile stress fibers containing myosin-II. ④ Migration speed as a function of adhesion strength in different 2D and 3D environments. Numbers in subpanel ④ denote ① low, ② intermediate, and ③ strong adhesion types. (*c*) Molecular type and strength of cell-cell interaction. ① Passive to low-adhesive interactions favored by high cell density and confinement. ② Transient, weak interactions composed of both adhesive and repulsive signals mediated by cadherins and ephrin/Eph receptors, respectively. ③ High cohesion mediated by cadherin-based adherens junctions. Thin gray arrows denote protrusion and retraction forces. (*d*) Shape, lamin content, and deformability of the nucleus in different cell types. The physical limits of cell migration in 3D environments are $<2\ \mu m^2$ pore cross section for neutrophils and T lymphocytes and $>4$–$6\ \mu m^2$ for stromal and tumor cells (Wolf et al. 2013).

filamin, spectrins), membrane-microfilament-binding proteins (e.g., ERM proteins, ankyrin, dystrophin, spectrin), and proteins defining membrane curvature (e.g., I-BAR domain proteins Bin, amphiphysin, Rvs), which jointly define protrusion shape, lifetime, and function (**Figure 2*a***, ①) (Blanchoin et al. 2014, Parsons et al. 2010, Petrie & Yamada 2012, Wolf & Friedl 2009).

By forming and resolving these and other morphologically and functionally distinct actin-rich substructures, moving cells rely upon a portfolio of mechanically and molecularly distinct

strategies to polarize and interact with the environment (Plotnikov et al. 2012, Renkawitz et al. 2009). Filopodia and lobopodia transmit moderate force, whereas bleb-like interactions are largely nonadhesive and generate little traction force but, due to their stiffness, provide intercalation and friction to surrounding tissue (Paluch & Raz 2013, Petrie & Yamada 2012). Because of their defined shape and function, these principal actin organizations, together with cell shape, are useful classifiers in inferring the mode and mechanics of individually migrating cells (Friedl & Wolf 2010, Petrie & Yamada 2012, Starke et al. 2014).

**Integrins:** cell surface receptors composed of α and β integrin chains with differential substrate-binding strength. α2β1 and α3β1 integrins preferentially engage with fibrillar type I and III collagens; αVβ3, αVβ1, and α5β1 prioritize fibronectin; and α3β1 and α6β1 connect with laminins

**Cell-ECM adhesion.** The actin cytoskeleton is coregulated with transmembrane adhesion receptors, which form nonfocalized, poorly focalized, or strongly focalized adhesions of different molecular composition and stability (**Figure 2*b***, ①②③) (Bergert et al. 2015, Chrzanowska-Wodnicka & Burridge 1996, Gad et al. 2012, Liu et al. 2015, Renkawitz et al. 2009). In moving cells, adhesion to substrates is provided predominantly by integrins (Geiger et al. 2009, Maaser et al. 1999, Schmidt & Friedl 2010) and is modulated by substrate-binding signaling molecules, including cell surface proteoglycans (Couchman 2010, Geiger et al. 2009), CD44 (Kim & Kumar 2014), and discoidin domain receptors (DDRs) (Shintani et al. 2008, Xu et al. 2012). On the basis of the types and amount of available adhesion receptors, cells prioritize the substrates for migration versus anchorage, with intermediate interaction strength enabling the highest migration rates (**Figure 2*b***, ④).

Integrins and coengaged signaling and adaptor molecules regulate the type and size of adhesions, as well as their molecular complexity, mechanotransduction capability, and life span (**Figure 2*b***, ①②③) (Balcioglu et al. 2015). Nonadherent or weakly adherent cell-matrix interactions provide a physical interface between a cell body and substrate to support mechanical friction and cell intercalation in 3D environments (**Figure 2*b***, ①) (Bergert et al. 2015, Renkawitz et al. 2009). Nascent integrin-containing adhesions or focal complexes at the leading edge generate small transient forces mediating initial substrate grab of forward-moving cells (**Figure 2*b***, ②) (Changede et al. 2015, Swaminathan et al. 2016). Nascent adhesions can grow and stabilize further by engaging the adaptor protein talin, followed by recruitment of additional cytoskeletal adaptors (kindlin, paxillin) and mechanosensing modulators (vinculin, p130Cas) (Bachir et al. 2014, Beningo et al. 2001). With additional engagement of myosin-IIA, nascent adhesions maturate into focal adhesions and support cell contractility (Kubow et al. 2013). At the high end of size and strength, mature focal adhesions interact with contractile stress fibers, provide stable anchorage to the surrounding substratum, transmit traction force, and maintain integrin activation and focal adhesion signaling (**Figure 2*b***, ③) (Beningo et al. 2001, Geiger et al. 2009). High force reinforces the downstream intracellular signaling through focal adhesion kinase (FAK), Src, and Rac and RhoA, which jointly define the size, duration, and strength of adhesions (Geiger et al. 2009, Grashoff et al. 2010). Consequently, high cell contractility is particularly relevant during movement with low adhesion, to control cortical actin and hydrostatic pressure, as well as during movement with high adhesion, to generate sufficient tension between focal adhesions and achieve rear retraction (**Figure 2*b***, ④).

Besides integrin-mediated mechanotransduction, weaker and less well defined adhesion mechanisms are provided by cell surface proteoglycans, including syndecans, glypicans, and neuropilin, which interact with ECM substrates through sugar moieties (Mythreye & Blobe 2009, Schmidt & Friedl 2010). When coengaged in parallel, adhesion systems and growth factor receptor signaling cooperate by converging signaling through PKC and Src and thereby support integrin-mediated mechanocoupling (Couchman 2010, Moon et al. 2005).

**Cadherins:** cell surface receptors that mediate cell-cell interactions by homophilic or heterophilic binding. Interactions between classical cadherins (e.g., E-, N-, or P-cadherin and cadherins 7, 11, 13) provide relatively stable interactions. Junctions between desmosomal and atypical cadherins provide less well defined and probably weaker adhesions

**IgCAMs:** mediate hemophilic and heterophilic interactions between cells by connecting to the actin cytoskeleton via adaptor proteins (e.g., α-actinin, ankyrin, ezrin). Family members include NCAMs, VCAMs, ICAM, ALCAM, L1CAM, and EpCAM

**Cell-cell adhesions.** Cell-cell contacts determine whether cells migrate individually or as a cohesive group (Friedl et al. 2012a). Cell-cell interactions are supported by several receptor systems, including cadherins, immunoglobulin family members of adhesion molecules (IgCAMs), connexins, ephrins, and erythropoietin-producing hepatocellular (Eph) receptors (Batlle & Wilkinson 2012, Ilina & Friedl 2009). Cadherins connect to the actin and microtubule cytoskeleton through the adaptor molecules α-catenin, β-catenin, and p120-catenin under the signaling control of Src, RhoA, and Rac1 (Harris & Tepass 2010, Meng & Takeichi 2009, Pokutta & Weis 2007). With particular relevance when classical cadherin function is low, IgCAMs support transient cell-cell binding between moving cells and toward cells encountered in tissues (Wai Wong et al. 2012), cooperate with integrins, and support migration through cell-cell adhesion as well as cell-substrate interaction (Cavallaro & Christofori 2004). In cooperation with cadherins, Eph receptors and their respective ephrin ligands provide bidirectional signaling between cells, which modulates actomyosin contractility and locally delivers pro- or antiadhesive signaling to cell protrusions and cell-cell junctions (Halloran & Wolman 2006, Kania & Klein 2016, Rohani et al. 2014).

At least two functionally distinct types of intercellular junctions are formed between moving cells (**Figure 2*c***). Weak cell-cell adhesions can be mediated by IgCAMs, which allow for transient cell attachment and trigger intracellular signaling (**Figure 2*c***, ①) (Haeger et al. 2014, Wai Wong et al. 2012). These junctions support neuronal and leukocyte cell-cell interactions and individually moving cells under confluence (Cayrol et al. 2008, Haeger et al. 2014). Another type of weak, transient junction consists of adhesion-promoting cadherins and adhesion-repelling ephrin/Eph receptors, which delivers combined proadhesive intercellular forces and repulsion signals, respectively (Halloran & Wolman 2006). This contact mode contributes to moderately cohesive migration and multicellular streaming, in which cells can oscillate between individual and collective behaviors (Scarpa & Mayor 2016, Theveneau et al. 2010). For example, moving neural crest cells migrate as a cell network by alternating intercellular adhesion with local contact inhibition of locomotion and retraction (the so-called kiss-and-run mechanism) (**Figure 2*c***, ②) (Theveneau et al. 2010). In stable adherens junctions, classical cadherins connect to contractile cortical actin filaments, often in cooperation with desmosomal and tight junctions and in the absence of repulsion signals (**Figure 2*c***, ③) (Peglion et al. 2014, Tornavaca et al. 2015, Wu et al. 2014), as in moving epithelial and endothelial cells during cohesive collective migration (Bazellieres et al. 2015, Friedl et al. 2012a).

**Cell deformability.** Cell movement through 3D tissue requires deformation of the cell body, including the plasma membrane, the cytoplasm, and the nucleus, which is the largest and stiffest organelle (Friedl et al. 2011, Liu et al. 2014). Whereas the membrane and cytoskeleton are strongly adaptive and can flow through very small pores ($<1\ \mu m^2$ in cross section), the deformability of the nucleus in mononuclear cells is limited to 10% of the relaxed cross section (**Figure 2*d***) (Wolf et al. 2013). The mechanical integrity and deformability of the nucleus are controlled by the nuclear lamina, which is composed of A/C- and B-type lamin intermediate filaments (Friedl et al. 2011). Through adaptor proteins, including nesprins and SUN proteins, the nuclear lamina further interacts with the actin cytoskeleton and thereby participates in mechanical responses of the whole cell (Razafsky & Hodzic 2009). Adaptation in deformability of the nucleus is achieved by two complementary mechanisms. Nuclear deformability is supported by morphological lobulation or segmentation in granulocytes (**Figure 2*d***), which permits particularly flexible adaptation of the nuclear shape during passage through very small pores, such as dense interstitial tissue and the basement membrane (Carvalho et al. 2015, Wolf et al. 2013). In addition, downregulation of A/C-type lamin, which occurs in neutrophils during terminal maturation, supports effective cell deformation, circulation, and immigration into tissues (Wolf et al. 2013). Thus, moving cells can

generate varying types and degrees of cell-cell and cell-matrix interactions and deformation of the cell body in response to encountered substrates.

## Tissue Determinants

To accommodate various physical and chemical environments, moving cells adjust their mechanical and signaling strategies to control morphology, migration mode, and speed.

**Physical determinants: dimensions, topography, space, and organization of tissues.** Moving cells can cope with distinct substrate patterns and geometries present in tissue and organ contexts (Weigelin et al. 2012, Wolf & Friedl 2011).

As a minimum ligand requirement, cells can move along a thin line of protein ligand, such as fibronectin, termed 1D migration (**Figure 3*a***, ①) (Doyle et al. 2009). 1D migration is adhesion dependent, with integrin-ligand interactions focused toward the line, and mediates precise path alignment along the ligand (Doyle et al. 2009). In vivo, 1D structures include long, singular collagen fibers, which provide guidance for moving cells (S. Alexander & P. Friedl, unpublished observation).

Cell migration across 2D surfaces occurs via adhesion-dependent engagement with the underlying substrate, whereby the leading edge protrudes and the cell rear slides along the continuous substrate (**Figure 3*a***, ②) (Ridley et al. 2003). Because of infinite lateral space, cells can spread, can form a broad leading lamellipod gliding along the substrate, and can freely change direction (Starke et al. 2014). Nonconfined 2D interfaces are provided by the inner surface of blood and lymph vessels, serous epithelia (e.g., peritoneum), and the surface of wound tissue during epithelial wound closure (Alexander et al. 2013, Carlin et al. 2013) and in experimental 2D liquid culture environments (e.g., petri dish, culture flask). For example, intravascular macrophages utilize αLβ2 integrin (LFA-1) engaging with endothelial ICAM-1 to migrate along the inner vessel wall (Carlin et al. 2013).

Confined linear 3D geometries consist of apposing 2D surfaces close enough for cells to interact with both interfaces (**Figure 3*a***, ③). Because the cell touches both contact surfaces, unilateral adhesion is dispensable, and depending on the level of integrin-ligand interactions, both nonadhesive interactions and adhesive interactions can support cell movement (Bergert et al. 2015, Malawista & de Boisfleury Chevance 1997). Experimentally, confined 3D geometries are obtained in underagar assays and microfluidic devices (Bergert et al. 2015, Hung et al. 2013, Liu et al. 2015). In vivo, interstitial tissues provide confined channels and tubelike spaces (tissue tracks) between collagen bundles, along the surfaces of myofibers or nerves, and along perivascular space (Weigelin et al. 2012, 2016).

Discontinuous 3D meshworks consist predominantly of structural ECM proteins, including fibrillar collagen, fibronectin, and elastin (**Figure 3*a***, ④). The network topology of such meshworks may be irregular, with variably sized gaps and trails (**Figure 3*b***, ①), or aligned with bundled collagen bordering aligned gaps (tracks) (**Figure 3*b***, ②). Moving cells typically follow the orientation of preexisting gaps and/or patterned tracks, resulting in random or persistent migration, depending on the tissue pattern (Salmon et al. 2012, Weigelin et al. 2012, Wolf et al. 2003b).

Beyond topology, each substrate displays viscoelastic material properties (stiffness) that directly, by mechanosensing, and indirectly, by inducing regulation of gene transcription, control cell migration programs (**Figure 3*c***). In multicellular organisms, moving cells are confronted with diverse material properties ranging from liquid to crystalline. In body fluids, including blood, lymph, and mucinous fluids contained in body and tissue cavities, cells detach and adopt spherical shape, lack cell-matrix adhesions, and adopt a nonfocalized softened cortical actomyosin

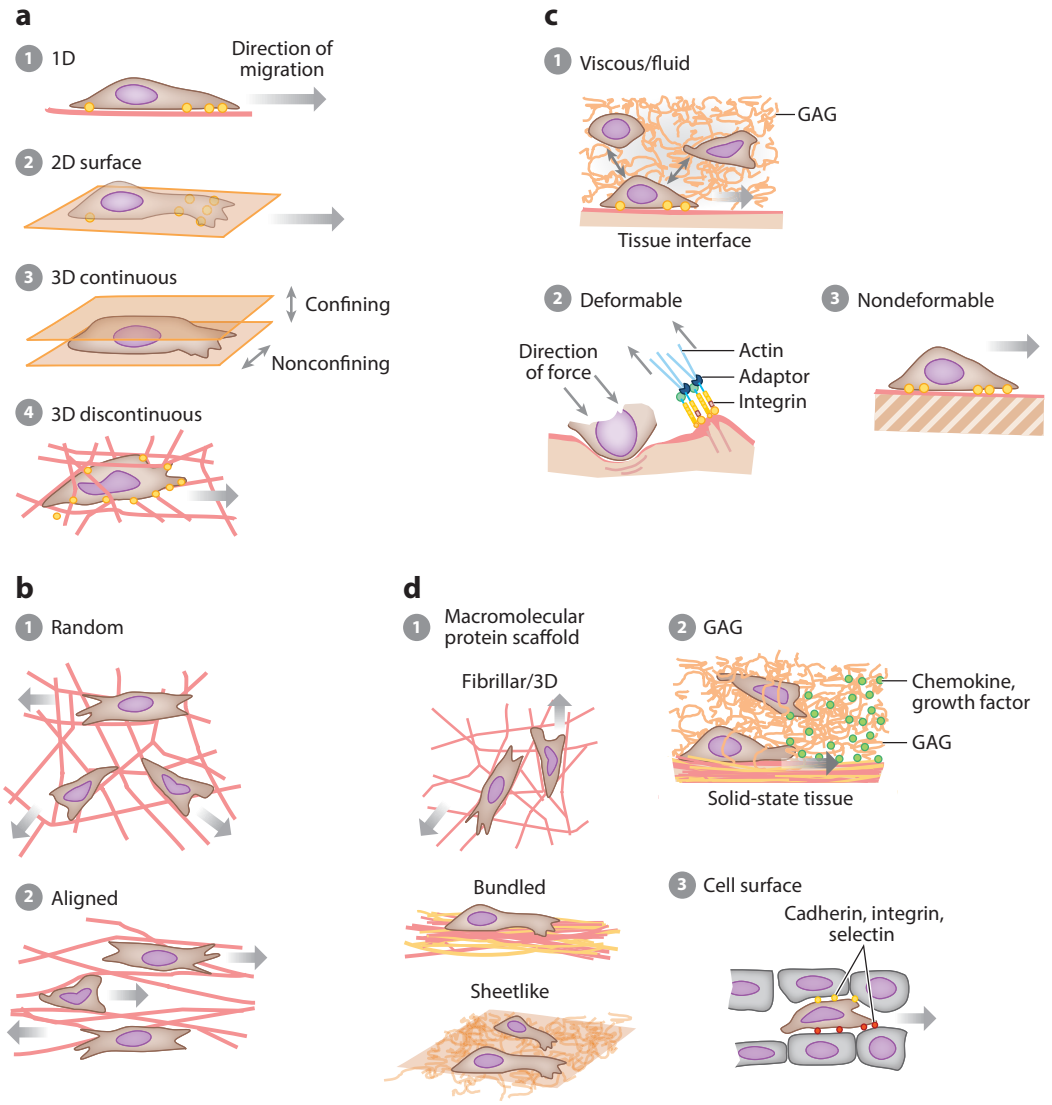



**Figure 3**

Properties of tissue substrates. (*a*) Substrate topography and dimension, including ① 1D, ② 2D, ③ 3D continuous, and ④ 3D discontinuous. (*b*) Substrate geometry: ① random or ② aligned orientation. (*c*) Physical properties of substrates (pliability). ① Near-fluid substrate rich in glycosaminoglycans (GAGs). ② Deformable solid-state substrate undergoing compression upon pushing or extension upon pulling. ③ Nondeformable solid-state substrate (e.g., bone, petri dish). (*d*) Molecular substrate properties, including ① macromolecules forming bundles and surfaces, ② GAGs binding growth factors, and ③ surface receptors and glycans present on counterpart cell surfaces.

network while undergoing passive drift (Chan et al. 2015). Alternatively, when triggered by stimuli, detached cells polarize and engage the cytoskeleton for movement propelled by shape change (**Figure 3*c***, ①) (Li & Gundersen 2008, Xu et al. 2003). Passage through fluids typically enables long-range cell transport within or between tissues and organs.

Sufficiently soft and reactive substrates, including soft fibrillar matrix and stiff but reactive collagen bundles, can be deformed by cells; i.e., such substrates can be compressed when pushed and can be extended when pulled (**Figure 3*c***, ②) (Chen et al. 2004, Koch et al. 2012). During migration, substrate pushing is in equilibrium with cell deformation and depends upon the cell volume and cytoskeletal dynamics. Substrate pushing and compression are observed when cells move along soft microchannels, e.g., dissected 3D microtracks in fibrillar collagen (Ilina et al. 2011), reversible deformation of embryonic basement membrane during cell passage (Morrissey & Sherwood 2015), and propulsive migration of germ cells pushing between multilayered epithelial cells in the zebrafish embryo (Paksa & Raz 2015). Pulling caused by adhesion and traction results in the realignment of matrix along the tension line (Beningo et al. 2001, Hegerfeldt et al. 2002, Steinwachs et al. 2016). Very stiff substrates, such as mineralized bone, cross-linked collagen bundles, or glass/plastic material used for experimental cell culture, lack such deformation responses to moving cells (**Figure 3*c***, ③).

**Molecular organization: spectrum of ligands.** Elastic properties coincide with the particular macromolecular composition of substrates engaged by migrating cells, including protein arrays, glycan-rich scaffolds, and surfaces of encountered cells.

Protein scaffolds consist of fibrillar or reticular 3D networks, including interstitial collagen and fibronectin networks, which provide an array of ligands for mechanosensing and guidance (**Figure 3*d***, ①) (Wolf et al. 2009). Sheetlike microfibrillar meshworks, composed of collagen type IV, laminins, fibrillin, versican, and perlecan, form basement membranes, which underlie all epithelia and surrounding vessels, myofibers, adipocytes, and nerves and lead to integrin engagement (Glentis et al. 2014, Proebstl et al. 2012). Protein scaffolds often interface with proteoglycan-rich matrix, predominantly glycosaminoglycans (heparin sulfate, hyaluronic acid, keratin sulfate), which provide concurrent signaling input via CD44, syndecans, and other surface proteoglycans (**Figure 3*d***, ②) (Couchman 2010, Schaefer & Schaefer 2010). Besides retaining high water content in tissues, glycosaminoglycans act as matrix for immobilizing soluble proteins, such as chemokines and growth factors, which may provide additional directional cues to moving cells (Monneau et al. 2016). An even more complex spectrum of ligands is present in lipid membranes of cell-rich tissues provided by stromal cells and epithelial and endothelial cells, all of which can serve as direct migration substrates. In particular, moving leukocytes, germ cells, and metastasizing tumor cells move across cell surfaces by engaging cadherins, integrins, and/or selectins (**Figure 3*d***, ③) (Carlin et al. 2013, Kardash et al. 2010, Proebstl et al. 2012).

Thus, distinct physical and molecular substrates are interpreted by moving cells and regulate adhesion strategy, shape change, and direction of migration.

## RECIPROCITY OF CELL-TISSUE INTERACTION AND MIGRATION

In morphogenesis, tissue repair, and cancer invasion, moving cells impact tissue organization reversibly by deformation and/or irreversibly by structural modification. In turn, altered tissue organization reactively feeds back to the migrating cell. Both processes are in reciprocal exchange and thus support a continuum and coevolution between dynamic cell organization and dynamic tissue organization (Friedl & Alexander 2011, Rozario & DeSimone 2010).

### Strain Stiffening of Substrate and Cytoskeletal Reinforcement

Cells moving with moderate to high adhesion force and contractility pull and deform ECM meshworks, including fibrillar fibrin and collagen (Friedl et al. 1997, Steinwachs et al. 2016). When

pulled, ECM biopolymers undergo a nonlinear conformational change, including unfolding and unmasking of functional epitopes as well as elasticity change, termed strain stiffening (Jansen et al. 2013, Smith et al. 2007, Storm et al. 2005).

Epitope unmasking occurs when cells pull on molecules that contain force-sensitive domains, which unfold when stressed and refold when reentering a relaxed state. When strained, otherwise hidden epitopes of fibronectin become exposed and provide additional binding sites for cell attachment and network alignment (**Figure 4*a***, ①) (Klotzsch et al. 2009, Zhong et al. 1998).

Strain stiffening is caused by moving cells via integrin- and actomyosin-mediated pulling, which reversibly aligns fibers and increases ligand density and rigidity in the direction of migration (**Figure 4*a***, ②) (Helvert & Friedl 2016, Jansen et al. 2013). Moving cells thereby undergo mechanosensory autotuning of their own focal adhesion strength via strain-sensitive adaptor proteins (e.g., talin and vinculin) (Grashoff et al. 2010) and create their own "traveling wave" of aligned and stiffened matrix (Helvert & Friedl 2016, Steinwachs et al. 2016).

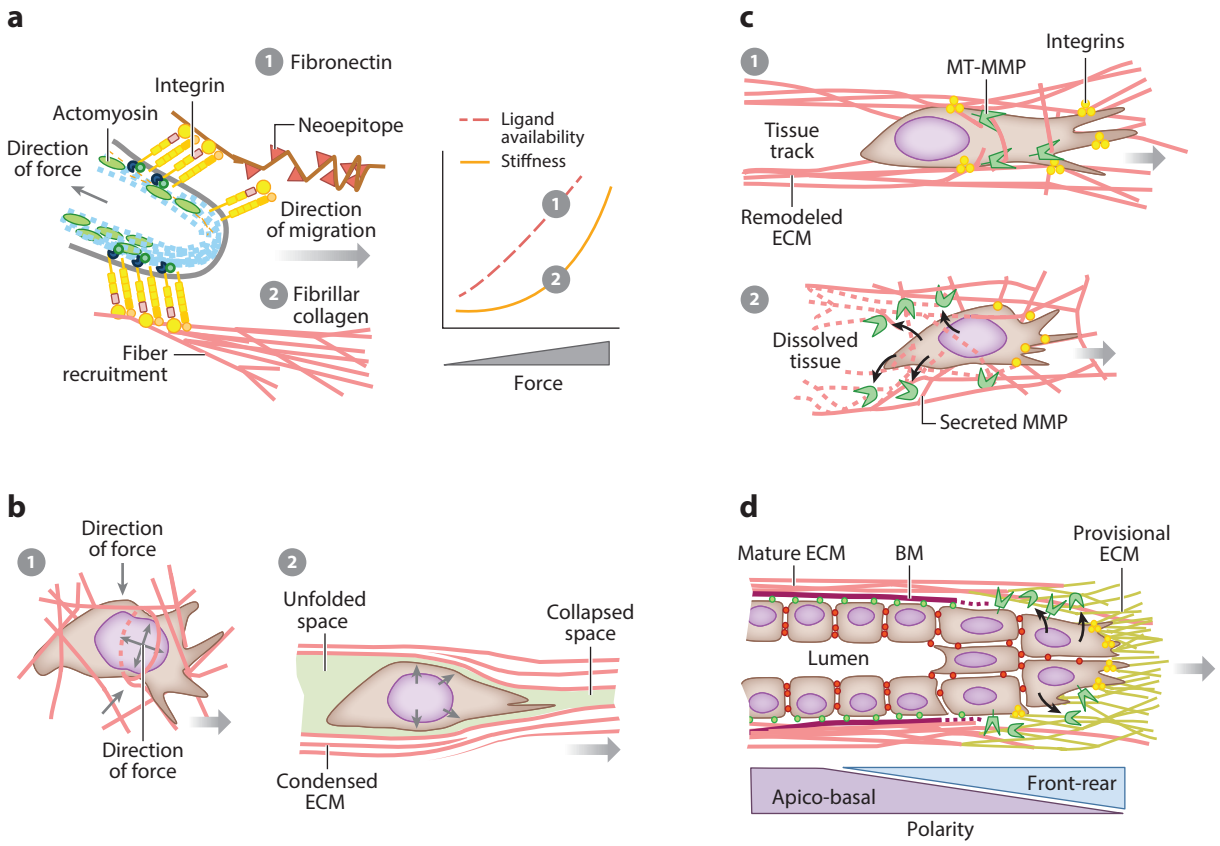



**Figure 4**

Interdependence of cell-tissue interactions during cell migration. (*a*) Traction and ① neoepitope unmasking (occurring in fibronectin) or ② strain stiffening of fibrillar collagen-based extracellular matrix (ECM) (*left panel*) as a linear or exponential function in response to force (*right panel*). (*b*) Unfolding of tissue space, either ① by opening of circular, belt-like fibrillar pores or ② by unfolding adjacent tissue layers. (*c*) Pericellular proteolysis through cell contact–dependent targeting of cell surface proteases ① and secreted proteases ② results in confined ECM reorganization ① and diffuse ECM lysis ②, respectively. MT-MMP denotes membrane-type matrix metalloproteinase. (*d*) Deposition of ECM components, in cooperation with proteolytic ECM processing to convert provisional ECM to mature ECM with a basement membrane (BM) toward epithelial cells and reactive apico-basal cell polarization.

Epitope unmasking and strain stiffening are reciprocal processes. Cell contraction mediates local tissue compaction and alters both ligand density and stiffness perceived by the leading edge; this process arguably reinforces a positive feedback loop of adhesion maturation, the formation of new protrusions nearby, and steers migration in an already established direction (Jiang et al. 2006, Roca-Cusachs et al. 2013). Tissue tension extending beyond the cells' immediate surrounding also impacts the mechanosensing and directional migration of more distant cells (Jansen et al. 2013).

## Substrate Pushing and Space Unfolding

Moving cells represent viscoelastic units that deform themselves to match available space and, simultaneously, deform the tissue. When moving through discontinuous, 3D, fibrillar ECM, cells unfold pores until a force equilibrium is reached between cell deformability and tissue force, resulting in belt-like cell compression and deformation during forward movement (**Figure 4*b***, ①) (Wolf et al. 2003a, 2013). Conversely, in linear tissue tracks, moving cells laterally push and unfold entire ECM layers in vitro (Ilina et al. 2011) or interstitial tissue in vivo (**Figure 4*b***, ②) (Weigelin et al. 2012, 2016). When available tissue space exhausts cell deformability, the nucleus, despite strong deformation, stalls and migration stops (Wolf et al. 2013) until the cell retracts the leading edge and explores alternative routes (Friedl et al. 2001). Similar to pulling, tissue unfolding is purely mechanical and reversible; however, it often occurs in the context of molecular tissue remodeling.

## Proteolytic Repatterning of Tissue

Structural tissue remodeling by migrating cells occurs through both proteolytic degradation and deposition of ECM; cells thereby generate irreversibly restructured de novo space (Friedl & Wolf 2008), which facilitates the movement of follower cells (Haeger et al. 2014). Tissue remodeling is executed through cell-derived proteases, including soluble and membrane-anchored matrix metalloproteinases (MMPs), disintegrin and metalloproteinases (ADAMs), cathepsins, and serine proteases (e.g., serpins, urokinase plasminogen activator) (Moali & Hulmes 2009, Sabeh et al. 2009, Sternlicht & Werb 2001, Wolf & Friedl 2011). Expressed protease systems equip moving cells with two types of pericellular tissue-processing capability (Wolf & Friedl 2011).

Cell surface contact–dependent pericellular proteolysis occurs at focal cell–ECM interaction sites at the leading edge and along the cell body of moving cells (**Figure 4*c***, ①) (Wolf & Friedl 2009). Membrane-bound MMPs, notably MT1-MMP (membrane-type 1 matrix metalloproteinase), and ADAMs proteolytically cleave space confining and restricting structural ECM molecules, including collagens, laminins, and fibronectins (Friedl & Wolf 2008, Sabeh et al. 2009, Wolf et al. 2007). By clearing encountered tissue barriers during migration, cells create their own path irrespective of tissue density and reduce the need for shape adaptation (Friedl et al. 1997, Wolf et al. 2013).

When cells release soluble proteases, diffuse tissue remodeling can mediate ECM restructuring and support migration (**Figure 4*c***, ②). Soluble MMPs and plasmin cleave virtually all interstitial and basement membrane components, including collagens, fibronectin, and laminins (Sternlicht & Werb 2001). As a consequence of poor spatial control, soluble proteases not only generate physical space along the cell-tissue interface, but disrupt wider tissue regions and thereby enable migration of other cells independently of their proteolytic ability (Orgaz et al. 2014).

In mesenchymal movement as well as epithelial and endothelial tissue invasion, local tissue degradation is associated with the deposition of ECM components. Fibroblasts deposit collagens, fibronectin, and proteoglycans onto partially degraded matrix (Rhee 2009). Epithelial sprouting and endothelial sprouting lead to the deposition of basement membrane proteins, including laminins, type IV collagen, and fibronectin, along the region of remodeled tissue with engagement

of altered sets of integrins and facilitate apico-basal cell polarization and anchorage (**Figure 4*d***) (Chaqour 2013, Haigo & Bilder 2011, Kariya et al. 2012, Larsen et al. 2006, Nguyen-Ngoc et al. 2012, Weaver et al. 2014). By defining both the physical and molecular organization of tissue, proteolytic migration forms an integrated process that provides defined interfaces for cell movement and anchorage and defines complex internal tissue shapes and compositions (Haigo & Bilder 2011).

## Autocrine Stimulation

The release and deposition of chemokines, cytokines, and growth factors during migration can feed back on moving cells as soluble factors that either directly bind to cell surface receptors or become immobilized to functionalize ECM structures where they are detected by passenger cells. Moving cells then adapt and orient in response to multiple autocrine or paracrine pro- or antimigratory cues.

Autocrine self-steering occurs when moving cells or cell groups release promigratory factors that engage with surface receptors of the same cell (**Figure 5*a***). Autocrine chemokine loops induced by, e.g., SDF-1, CXCL10, CXL12, CCL21, or CCL25 activate intracellular signaling through several pathways, including JAK, PI3K, Src family members, and RhoA and Rac, to control cytoskeletal dynamics (Griffith et al. 2014, Kroeze et al. 2012). Autocrine growth factor receptor signaling is initiated by, e.g., HGF, FGF, EGF, and TGF-β, which signal through, e.g., ERK and PI3K and engage Cdc42 and Rac to regulate cytoskeletal dynamics (Joslin et al. 2007, Miller et al. 2013). For example, autocrine EGF can be released from surface proteoglycans by sheddases (e.g., ADAMs), which via EGFR activate MAP kinase/ERK signaling and transiently enhance migration speed (**Figure 5*a***) (Joslin et al. 2007). Similarly, nucleotides are released by leader cells via connexin hemichannels and engage with adenosine receptors that induce Rac-dependent polarization and migration (**Figure 5*a***) (A. Khalil & P. Friedl, unpublished observation). Autocrine stimulation is likely a common but overlooked mechanism of the "spontaneous" cell migration observed in vitro and in vivo.

## Paracrine Stimulation

For coordination of migration between individually moving cells, the paracrine release of promigratory factors provides a mechanism for front-rear direction sensing (**Figure 5*b***, ①). Moving *Dictyostelium* amoebae release chemotactic cAMP preferentially from the cell rear, which stimulates orientation of the leading edge in follower cells (Das et al. 2011). In moving neural crest cells, SDF-1 is released between cells and mediates their coordination for multicellular streaming in vivo (Theveneau & Mayor 2010). Likewise, activated leukocytes release copious amounts of chemokines (e.g., IL-8) and lipid mediators (e.g., leukotriene B4), which amplifies the recruitment of additional cells to tissue regions of wounding or bacterial infection (Lammermann et al. 2013, Phillipson & Kubes 2011). This relay through paracrine chemotactic signal amplification supports coordination between cells moving individually and/or transit toward collective migration (Theveneau et al. 2010).

Similarly, collectively moving cells deposit chemokines and growth factors toward the ECM. Such chemokines and growth factors include SDF-1, VEGF, FGF, and TGF-β, which become immobilized by their matrix-binding domains and exert signaling toward the same and/or follower cells (Scarpa & Mayor 2016). As example, FGF released by mesenchymal cells induces tip cells and the sprouting of bronchial epithelial cells to form the primordial tracheal system (**Figure 5*b***, ②) (Lebreton & Casanova 2014).

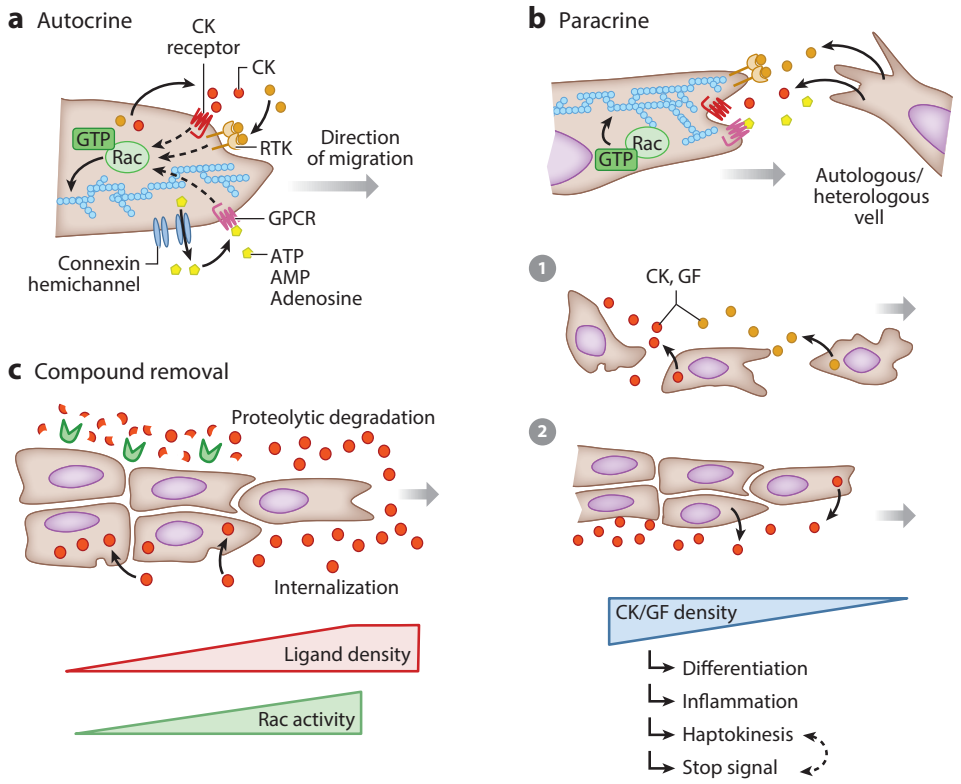



**Figure 5**

Guidance of cell-matrix interaction and migration in response to extracellular signals. (*a*) Autocrine stimulation of leading edge activity. Local release of chemokines (CK), growth factors (GF), or nucleotides followed by autocrine stimulation of G protein–coupled receptors (GPCR) and receptor tyrosine kinases (RTK), leading to Rac activation and actin polymerization. (*b*) Paracrine stimulation by adjacent cells of the same cell type or stromal cell. ① CK and GF release and relay function toward follower cells guiding single-cell migration. ② CK deposition during collective cell migration, resulting in pericellular gradient formation and modulation of cell functions distinct for leader and follower cells. (*c*) Gradient formation during collective movement by degradation of migration-inducing factors by extracellular proteases or removal by internalization, resulting in a ligand gradient toward the rearward direction.

To modulate extracellular promigratory signals, secreted proteases, including MMPs and other proteases released by moving cells, execute limited proteolysis to activate or degrade extracellular chemokines and growth factors (**Figure 5*c***) (Cox et al. 2008, Dean et al. 2008). In parallel, endocytosis of promigratory molecules lowers local chemokine availability, and this process creates a cell-generated gradient. Collective migration of the lateral line in zebrafish depends upon such endocytic removal of SDF-1 by CXCR7, which acts as a decoy receptor, decreases SDF-1 levels along the cell group, and supports collective front-rear polarity (**Figure 5*c***) (Dona et al. 2013).

As a further reciprocal mechanism, proteolytic degradation and inactivation of tissue-associated antimigratory molecules allow moving cells to overcome barrier and/or stop signals. During epithelial cell invasion, MT1-MMP polarizes toward the cell-matrix interface to degrade migration-inhibiting TGF-β and thereby enhances migration (**Figure 5*c***) (Weaver et al. 2014). Thus, rather than treating tissue as a static framework, moving cells alter molecular and physical tissue signatures and thereby reinforce their own decision making.

## PLASTICITY PROGRAMS OF CELL MIGRATION

Moving cells integrate mechanical and signaling modules to adjust their migration direction, speed, and mode of migration.

### Decision Making While Retaining an Ongoing Migration Program

By direction sensing and choosing between ligand systems, moving cells navigate through and between tissues without altering their once initiated migration program.

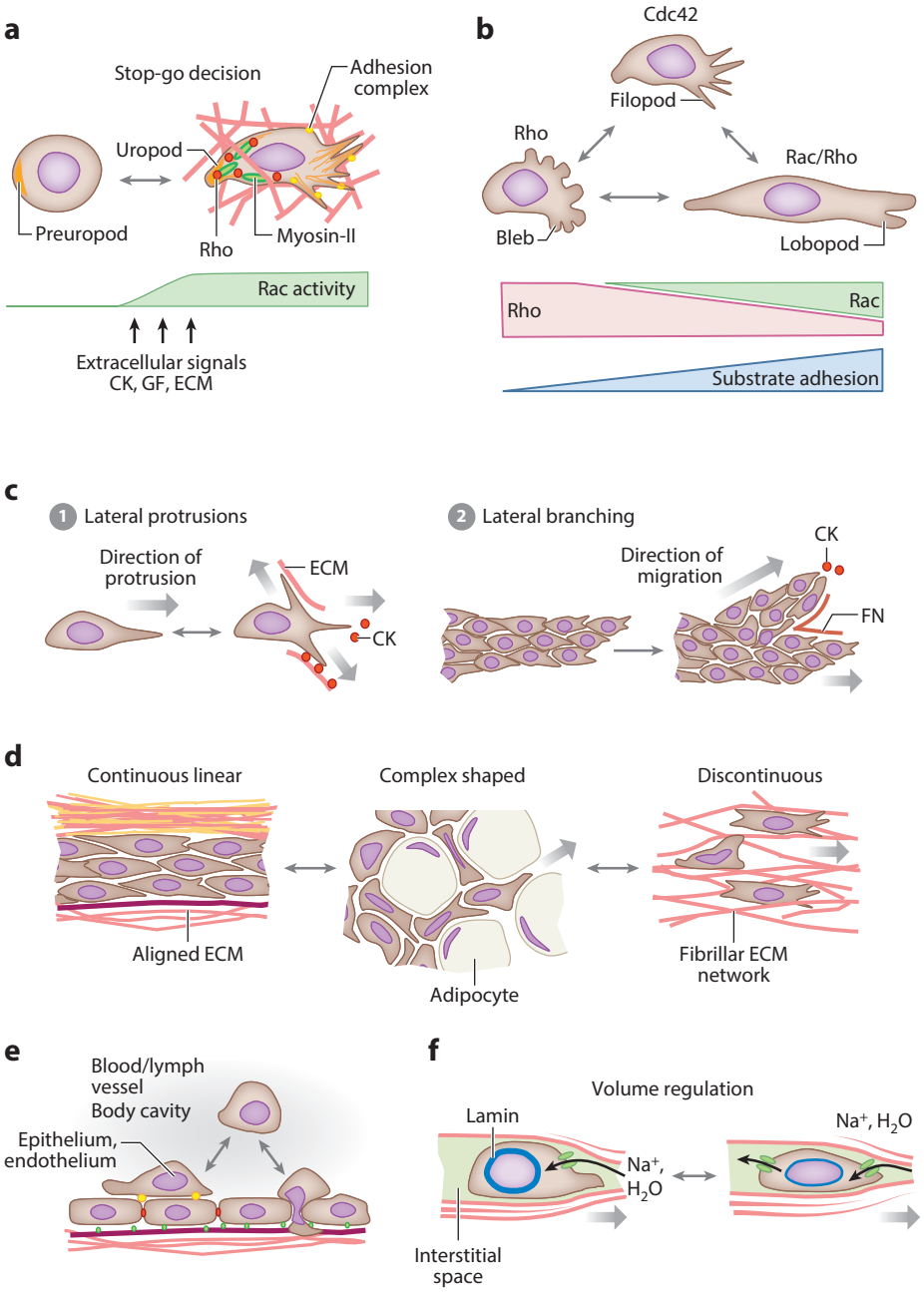
**Directional steering in single-cell migration.** Individually moving cells perceive migration stimuli and directional cues through their leading edges. Leading edge kinetics are guided by physical stimuli, e.g., structural discontinuities of the substrate that are sensed and bound by adhesion receptors, or by chemical triggers from autocrine or paracrine promigratory factors (A. Khalil & P. Friedl, unpublished observation). These signals converge toward the local generation and clustering of phospholipids at the plasma membrane, particularly PIP2 and PIP3, and local activation of Rac or Cdc42, allowing cells to switch between mobile and sessile behavior (**Figure 6*a***) (Heit et al. 2002, Kolsch et al. 2008). The location of leading cell protrusions defines the direction of movement, and a change in cell polarity is followed by a change in direction (**Figure 6*c***). By differentially protruding their leading edge, single cells decide between competing chemokine gradients (Heit et al. 2002) and between soluble and immobilized cytokines (Weber et al. 2013) and adjust the direction of migration according to adhesion ligand availability and the geometric organization of the tissue (Doyle et al. 2009, Starke et al. 2014).

By tuning Rac and Cdc42, different types of leading edge shapes can be adopted, and cells can switch between protrusion types, including lamellipodia, filopodia, lobopodia, and blebs, sequentially or simultaneously (**Figure 6*b***) (Bergert et al. 2012, Petrie & Yamada 2012, Roubinet et al. 2012, Starke et al. 2014). Because protrusion types differ in their shape, kinetics, and content of actin-cross-linking proteins (Sarmiento et al. 2008, Tseng et al. 2001), as well as in their ability to focalize actin, integrins, and surface proteases, their capacity to adhere, generate force, and degrade proteins varies (**Figure 6*b***). Whereas each protrusion type fulfills a unique function, their interconversion and coexistence in response to encountered cues result in versatility and adaptability of cell-tissue interactions during migration (Starke et al. 2014, Tyson et al. 2014).

**Steering collective migration.** Collective direction change can be achieved by two distinct but likely cooperating mechanisms: decision making by leader cells and ECM deposition. Leader cells sense and follow tissue cues and migration-enhancing factors and thereby steer the cell group (**Figure 6*c***, ②). Leader cell guidance by extracellular chemokines (e.g., SDF-1) and morphogens (e.g., FGF, HGF) defines sprouting of a lateral branch from veins to form an artery in developing tissue (Xu et al. 2014), the development of bronchial ducts (Lebreton & Casanova 2014), sprouting mammary end buds (Nguyen-Ngoc et al. 2012), and the lateral line in zebrafish embryos (Dona et al. 2013). Collective branching can be further induced by deposition of ECM proteins by moving epithelial cells, such as fibronectin, which causes bifurcation of the strand or duct and initially separates tissue compartments (Larsen et al. 2006, Sakai et al. 2003). Both leader cell steering and lateral ECM deposition may cooperate to result in collective branching to form stable branched vascular or epithelial duct patterns (Xu et al. 2014).

**Transitions between tissue types.** During movement through interstitial tissue as well as during trafficking between tissues and organs, cells transit from one molecular tissue compartment

to another; such transit leads to switching of adhesion mechanisms and to reprogramming of intracellular signals. Transitions between tissues include transit from the blood to interstitial tissue, as in circulating leukocytes during immune surveillance and circulating tumor cells undergoing metastasis to distant organs (Kienast et al. 2010). In both cases, circulating cells first interact with the endothelium and then adhere to and migrate along the vessel wall until they change polarity to a vertical orientation. At this point, they penetrate through the endothelial layer



and basement membrane and subsequently reach the interstitial tissue (Kienast et al. 2010, Nourshargh et al. 2010). Transendothelial migration consists of a complex sequence of (*a*) direction change; (*b*) molecular transition from a cell-cell interaction engaging αLβ2 integrin/ICAM and α5β1/VCAM toward a cell-matrix interaction engaging α6β1 to the basement membrane, followed by the engagement of α2β1 and α3β1 to collagen; (*c*) vigorous deformation of the cell body and nucleus; and (*d*) an optional proteolytic step for passage through the basement membrane (Nourshargh et al. 2010). Transit between the circulation and tissue represents one of the most complex and tightly controlled processes of kinetic adaptation and modular integration in rapid sequence.

**Cell detachment.** A transition from solid-state tissue to the fluid phase occurs when cells residing on endothelium detach into the bloodstream or when intraepithelial cells detach into the duct lumen. Cells circulating in the blood, including stem cells, leukocytes, and tumor cells, originate from the bone marrow or peripheral tissues and reach the vessel lumen through intravasation, a reverse transmigration process (Zijlstra et al. 2008). This conversion from migration, with adhesion to cell-ECM interfaces and cell deformation toward a cortical nonengaged and nonpolar cytoskeleton, represents an active, but poorly understood, migration detachment program to abandon adhesion and polarization in favor of a spherical floating state (**Figure 6*e***).

**Adaptation of cell volume and deformability.** To cope with confinement, cells can adjust the volume of both the cytoplasm and the nucleus. Intracellular water content is regulated by aquaporins and $Na^+/H^+$ ion channels that transport water molecules across the plasma membrane (**Figure 6*f***). Water transport contributes to regulation of volume of moving cells, thus likely supporting shape adaptation and movement through tight spaces (Watkins & Sontheimer 2011), and can further occur in a directed front-to-rear manner and thereby support cell displacement (Stroka et al. 2014). Cell volume change likely cooperates with stiffness regulation of the cytoskeleton and with the deformability of the nucleus (Greiner et al. 2015), yet the integration of these properties remains to be defined.

## Transitions Between Migration Programs

Adaptation of migration mode occurs at both the cellular and supracellular levels, which allows cells to transition between migration strategies in response to external stimuli.

**Mesenchymal-to-amoeboid transitions.** The conversion from mesenchymal movement to amoeboid movement is a multicomponent process that enables cells to transition between migra-

←

**Figure 6**

Decision making during cell migration. (*a*) Stop-go decision. Reversible front-rear polarization forms a leading edge and a cell rear in response to extracellular stimuli. In some cells, even in a nonpolar, round state, the rear is predefined by an actin-rich preuropod. (*b*) Conversion of protrusion type, depending on the balance and location of active Rac, Cdc42, and Rho and the related strength of cell-substrate adhesion. (*c*) Directional decision making by lateral branching of cell protrusions ① at the single-cell level and ② of multicellular strands in collective migration. (*d*) Adaptation of migration modes in response to different tissue geometries, including confined tubelike tissue (*left*), complex-shaped tissue (*middle*), and discontinuous fibrillar tissue (*right*). (*e*) Cell attachment and detachment between inner body walls and fluid compartments, including blood and lymph vessels and serous cavities. (*f*) Regulation of cell volume and/or deformability by hydrostatic volume regulation and stiffness adaptation of the nucleus. Abbreviations: CK, chemokine; ECM, extracellular matrix; FN, fibronectin; GF, growth factor.

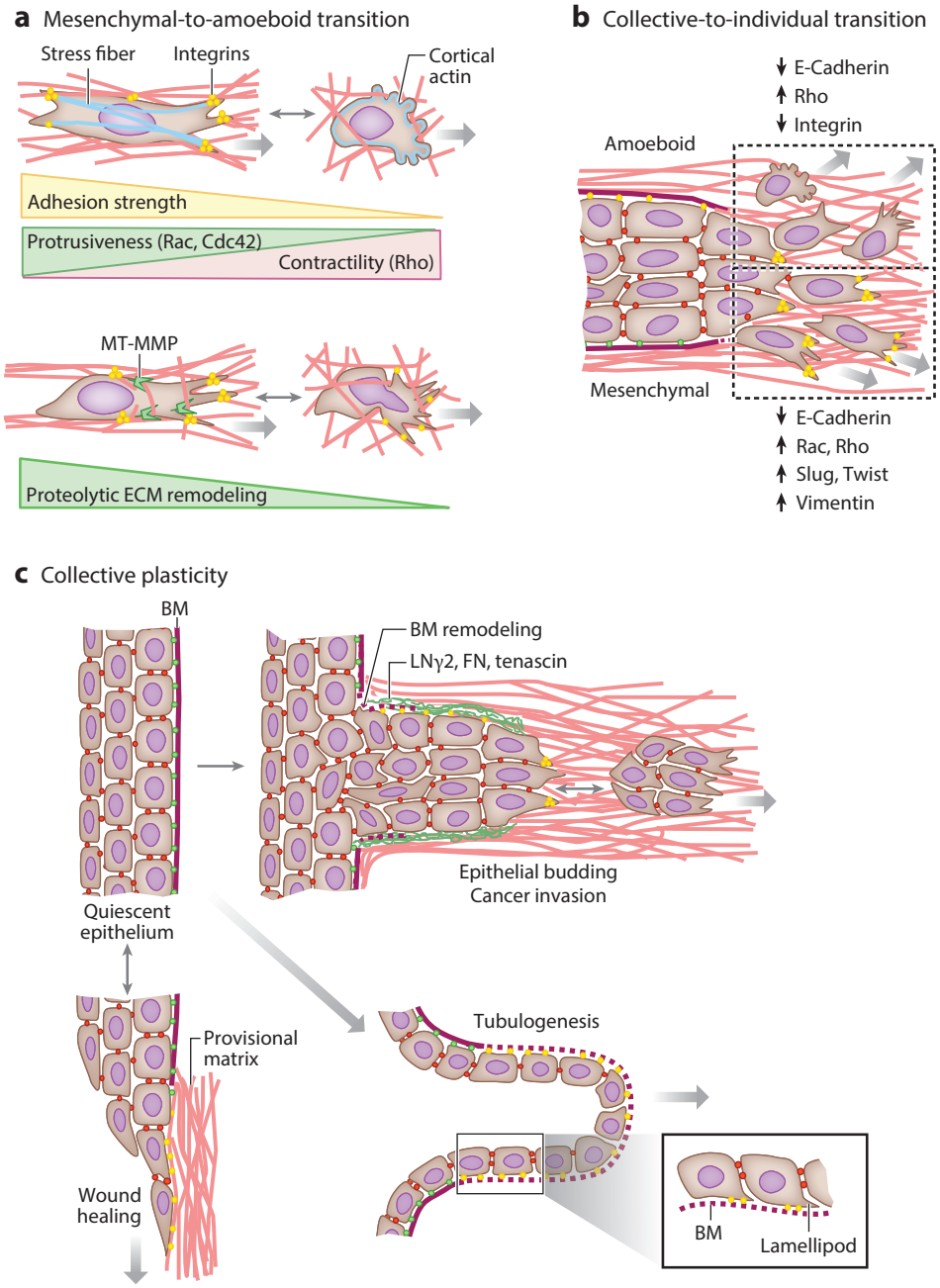
tion strategies and, depending on cell model and protrusion type, to develop a range of subtypes and intermediate states (Cooper et al. 2015). Mesenchymal-to-amoeboid transition can be experimentally induced (*a*) by lowering cell-matrix adhesion strength by either limiting adhesion receptor expression or inducing repulsion signals (Parri et al. 2009, Taddei et al. 2011); (*b*) by increasing Rho-mediated actomyosin contractility by activating the Rho/myosin-II axis and/or limiting focal adhesion maturity and cell spreading (Sahai & Marshall 2003, Sanz-Moreno et al. 2008, Taddei et al. 2014); (*c*) by reducing pericellular proteolysis; and, as a consequence, (*d*) by increasing cell deformation while bypassing tissue barriers by shape change (**Figure 7*a***) (Wolf et al. 2003a). The alternative routes of lowering ligand density and limiting cell protrusion formation, e.g., by inhibiting Rac (Sanz-Moreno et al. 2008, Taddei et al. 2014), favor amoeboid movement (**Figure 7*a***). In the reverse process, amoeboid cells can develop mesenchymal movement (*a*) by activating Rac-mediated protrusion formation, (*b*) by integrin-mediated adhesion, and/or (*c*) by protease functions (Sanz-Moreno et al. 2008). Interconversions between mesenchymal and amoeboid behaviors are most prominently observed in tumor cells, which adapt their migration strategy and thereby tune their ability to cope with different tissue environments during metastasis. Such reprogramming of migration mode is likely associated with an altered molecular signature (Vaskovicova et al. 2015) and with increased stemness and metastatic ability associated with amoeboid mobility (Taddei et al. 2014, Vaskovicova et al. 2015).

**Collective-to-individual transitions.** Conversion from collective migration to single-cell migration results from cell detachment from a moving group by either mesenchymal or amoeboid movement. At least two mechanistic individualization routes, including unjamming and the downregulation of adherens junctions, support single-cell detachment (**Figure 7*b***) (Haeger et al. 2015).

Before unjamming, moving cells retain weak cell-cell junctions while coordinating their movement in confined space, such as when mesenchymal cells in high numbers move through tubelike tracks in dense collagen (Haeger et al. 2014, Ilina et al. 2011) or interstitial space in vivo (Weigelin et al. 2012). Liberation from such cell-cell interactions is driven by the ability of detaching cells to move into free space and to overcome low constitutive retention force to neighbor cells (Haeger et al. 2014). When moving along confined space, such as continuous linear tissue interfaces along vessels, nerves, and myofibers, or when moving along complex-shaped interfaces between fat cells, moving melanoma tumor cells adopt a collective migration mode (**Figure 6*d***) (Weigelin et al. 2012), consistent with cell jamming by space confinement (Sadati et al. 2014). Conversely, when mesenchymal cells move through loose fibrillar tissue, they individualize and move predominantly by single-cell migration (Haeger et al. 2014, Weigelin et al. 2012), consistent with cells downregulating cell-cell junctions and unjamming in response to tissue space (Park et al. 2015). Thus, unjamming likely supports the decision making of tumor cells in vivo, according to encountered tissue geometries.

The transcriptional downregulation of adherens junctions as well as of tight junctions and desmosomes occurs when cells become activated and undergo an epithelial-to-mesenchymal transition (EMT). EMT is induced by extracellular triggers, including cytokines, growth factors, and metabolic stress, leading to the internalization of the neural tube and E-cadherin, conversion to expression of N-cadherin, and the induction of migration (Nieto 2011, Theveneau & Mayor 2012). Cells thereby lower intercellular attachment; detach from the epithelium and move individually; and, depending on the retained level of intercellular interactions and spatial confinement, alternate between single-cell and collective behaviors (Theveneau & Mayor 2012, Wong et al. 2014). This combination of collective and single-cell behaviors upon EMT is present in neural crest cells delaminating from somites, giving rise to relatively loose collective and multicellular streaming and single-cell movements to reach peripheral tissues (Scarpa & Mayor 2016, Theveneau et al.

2010). Likewise, during gastrulation, EMT induces the sheetlike invagination of epithelial cells to form the primordial mesoderm, the movement of which is due to both individual and collective dynamics (Chuai et al. 2012). Thus, lowering cell-cell junctions during EMT involves a significant probabilistic component in transitions between migration modes (discussed in Friedl et al. 2012a).



In quiescent epithelial cells anchored on a basement membrane, contact to collagen leads to reprogramming by outside-in signaling and to induction of migration plasticity. Contact with collagen induces signaling mediated by α2β1 integrins and DDR1, which, through protein tyrosine kinases FAK and Pyk2, upregulate N-cadherin and convert collective sheets to scattering individual cells (Shintani et al. 2008). Likewise, interaction with 3D collagen induces the downregulation of P-cadherin, followed by both single-cell migration and collective migration in otherwise nonmoving mammary epithelial mammospheres growing in reconstituted basement membrane (Nguyen-Ngoc et al. 2012).

**Plasticity of collective cell migration.** Collective plasticity is the adaptation of cell groups to change position and simultaneously reshape their organization, a prominent process in morphogenesis and tissue repair (**Figure 7*c***). In border cells that comprise primordial stem cells in the developing *Drosophila* ovary, detachment of a cell group from the primordial ovary epithelium is induced by Rac-mediated induction of one or a few leader cells that guide the collective unit by E-cadherin-mediated cell-cell junctions through the organ (Wang et al. 2010). Likewise, detachment of multicellular groups from tumor lesions is followed by intravasation and circulation as a multicellular cluster, which in sequence constitute important and efficient steps toward metastatic organ colonization (Aceto et al. 2014, Cheung et al. 2016, Friedl et al. 1995). The mechanisms underlying collective detachment remain unclear but likely comprise a combination of lowering cadherin-based cell-cell adhesion and passive detachment through drag force generated by the group, which jointly support dissolution of otherwise unperturbed cell-cell junctions (Casares et al. 2015).

As a morphological and functional variant, collective sheet migration, which depends upon particularly stable cell-cell junctions, is induced in quiescent epithelia, such as the epidermis or vascular sprouting, after wounding (Eilken & Adams 2010, Tornavaca et al. 2015). At the free edge, a multicellular sheet connected by cadherin-based junctions is induced to move across the wound; this process is supported by autocrine activation of the epithelium by chemokines (Kroeze et al. 2012), the use of both pushing activity and pulling activity, and coordination by apical cell-cell junctions (Bazellieres et al. 2015, Kim et al. 2013).

In tubulogenesis, collective sheet migration and apico-basal polarity are combined in 3D environments. A group of leader cells paves the way through the ECM, followed by an epithelial monolayer that moves forward by front-rear polarity while retaining a lumen due to apico-basal polarity and the deposition of a basement membrane (**Figure 7*c***). Thus, proteolytic tissue remodeling at the leading edge is combined with tube generation and deposition of a basement membrane as a contextual basis for tubular structures in epithelial organs (Haigo & Bilder 2011, Kariya et al. 2012, Nguyen-Ngoc et al. 2012, Weaver et al. 2014, Wolf et al. 2007).

**Figure 7**

Plasticity of cell migration programs. (*a*) Mesenchymal-to-amoeboid transition resulting from lowering adhesion to substrate, pericellular proteolysis, and leading edge protrusion or from increasing Rho-mediated actomyosin contractility either independently or jointly. (*b*) Collective-to-amoeboid or collective-to-mesenchymal single-cell transition, mediated by molecular programs that lower cell-cell adhesions or strengthen cell-matrix interactions and modulate cytoskeletal organization. (*c*) Collective plasticity. Transition from a quiescent epithelium to a collectively invading strand or detached cluster, to tubulogenesis for duct/gland formation, or to epithelial sheet migration for epithelial regeneration during wound healing. Abbreviations: BM, basement membrane; FN, fibronectin; LNγ2, laminin gamma2; MT-MMP, membrane-type matrix metalloproteinase.

Plasticity programs in biological contexts, and the wealth of underlying molecular and physical mechanisms, provide a fascinating range for multiparameter stimulation and data analysis; these contexts also create significant challenges due to their ever-increasing complexity, with often multiple levels of control signals and feedback loops (Friedl & Wolf 2010). Thus, to deepen the understanding of such complex cause-consequence relationships, cell-based in vitro and in vivo analysis requires additional processing by computational analysis and mathematical modeling.

**Input-output:** given that a cell or a subcellular element is an information-processing unit, input-output is the communication between an information-processing system (cell) and the outside world (tissue, other cells). Input is the ensemble of signals to which the system is exposed, and output is the product

**Mathematical module:** a set of mathematical equations and terms that define a molecular or physical parameter, such as the strength of cell-cell or cell-ECM bonds. Mathematical modules describe how biological modules may interact with, cooperate with, or counteract each other to maintain or modify a given cell function

**Complex system:** consists of multiple connected parameters and variables nonlinearly interacting with each other. Its overall behavior exceeds the sum of effects from each individual parameter and depends upon their mutual interactions (emergent behavior), including positive, negative, and reciprocal inhibitory feedback loops

## MULTISCALE MATHEMATICAL MODELING OF CELL MIGRATION AND PLASTICITY

### Mathematical Toolbox to Model Cell Migration

Cell-based analysis of plasticity of cell migration in vitro and in animal models is limited by the number of physical and chemical parameters that can be probed simultaneously and in context. To enrich wet-lab analysis, many cooperating modules, including cell adhesion, cytoskeletal function, and cell-tissue interaction, can be probed simultaneously by mathematical modeling (DiMilla et al. 1993, Palsson & Othmer 2000). Starting from parameters and response patterns identified by wet-lab analyses, simulation tools and algorithms are used to mechanistically link multiple inputs by intra- and intercellular signal processing and gene activation to migration outputs to predict how signals control a migration mode and a cell's adaptation responses (Danuser et al. 2013). As a first step, mathematical modeling aims to repeat known behaviors established by wet-lab research. Then modeling is used to extract mechanisms and identify which migration modules cooperate with each other and are critical for the response of an individual cell and cell ensembles. Modeling can further identify unexpected outlier behavior as a new phenotype; in wet-lab experimentation, outlier behavior is commonly interpreted as irreproducible and thus escapes in-depth analysis. A mathematical model thus creates a virtual reality for cell behaviors by examining ensembles of inputs and their connectivity over ranges that exceed experimental wet-lab possibilities. This aspect gains relevance, given the high speed and moderate cost of computational approaches relative to wet-lab experimental analyses.

The execution of a set of interconnected mathematical modules depicts a migrating cell and its environment as a complex system that reproduces the multiscale nature of biological features and their emergent behaviors. To combine input variables, a multiscale model defines a reception fingerprint (**Figure 8*a***) that feeds into the intracellular processing machinery (**Figure 8*b***). The relevance of particular modules, both interfacial and subcellular (molecular), can be tested by virtual expression regulation, interference, or deletion, thus recapitulating gene expression, protein expression, or signaling profiles characteristic of cell activation or disease states.

Different mathematical models allow one to simulate the phenotypes and mechanisms of single-cell and collective behaviors with cellular and/or subcellular resolution. Single-cell-based models process the behavior of single cells, including how single cells perceive extracellular signals, polarize, interact with the substrate or with other single cells, and migrate in context (**Table 1**; **Figure 8*b***). Each model has its own strengths, application range, and weaknesses (**Table 1**) and addresses particular aspects of moving cells better than others. As example, Voronoi models reliably predict cohesive epithelial sheet movements, but not single-cell motions (Meineke et al. 2001); actomyosin-based models have achieved relevance for single cells or cell fragments, but not yet for collective motion (Kozlov & Mogilner 2007).

Each module that determines an aspect of cell migration consists of one or more state variables and their associated parameters, which jointly determine cell or tissue properties and thereby determine migration.

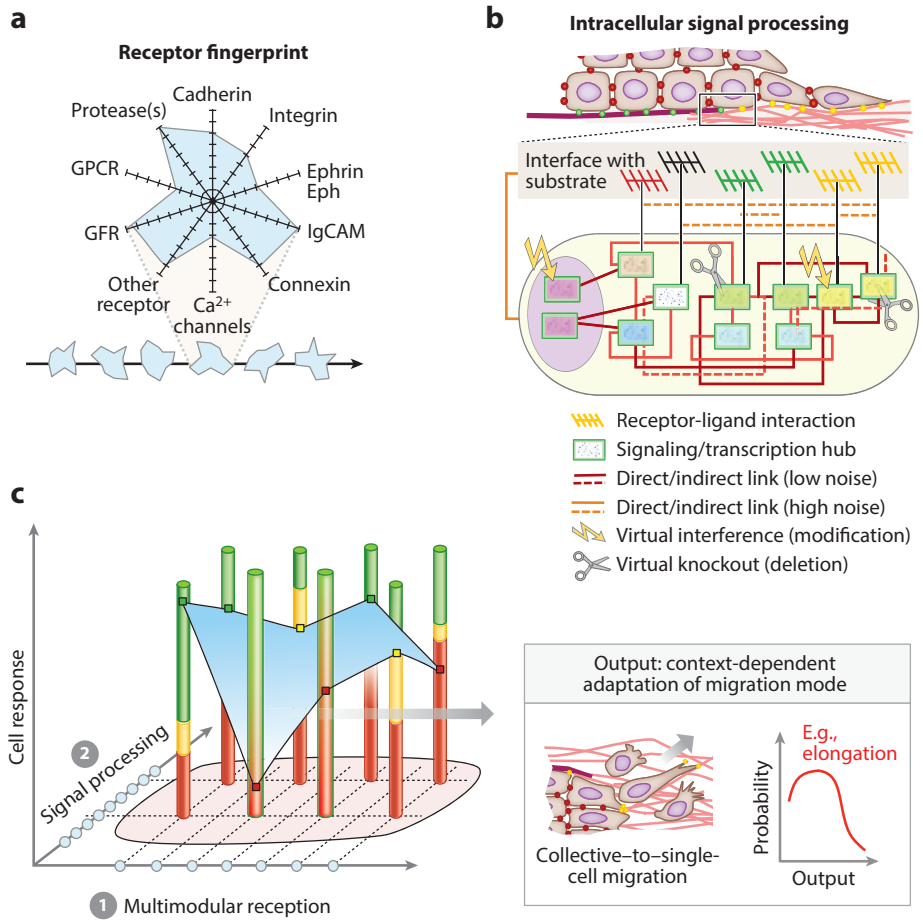



**Figure 8**

Mathematical modeling of cell migration modules and modes. (*a*) Modeling of input parameters of adhesion and growth factor signaling (receptor fingerprint). Abbreviations: GFR, growth factor receptor; GPCR, G protein–coupled receptor; IgCAM, immunoglobulin family member of adhesion molecules. (*b*) Modeling of intracellular signal transduction and gene expression, including positive and negative feedback loops and interference approaches, such as knockdown and ablation strategies. The cartoon represents mathematical connections between modules. (*c*) Multiparametric integration of multiple input parameters and intracellular processing. The output is represented as a 3D landscape (*blue surface*) defined by the state of each function module. The kinetic evolution of both reception and signal processing determines adaptation of migration mode (*inset at right*). Due to stochasticity, output is probabilistic, with a range of possible responses in cell ensembles (*inset*, *red line*). The cartoon graphically represents how multiple inputs are combined by linear and nonlinear mathematical operations to reach a complex output, which is delivered by computational analysis (summarized in **Table 1**).

Parameters are included in equations and can be defined as constant and autonomous or as adaptive and influenced by other state variables via connecting functions. Physical modules describe a morphological quality (e.g., tissue stiffness, speed). Molecular modules describe the interaction dynamics of protein networks, ranging from strong to negligible or absent interactivity.

Physical and molecular modules are interconnected, vary in expression level and strength of signal response, and respond to input received from the environment through signaling systems

**Table 1 Mathematical models for individual and collective migration modes**

| Mathematical model | General variables and features of the model | Key parameters (modules) | Strengths and limitations | Reference(s) |
|---|---|---|---|---|
| Individual-based models | Location of cell center and cell size | Adhesivity, stiffness, random migration | Near-spherical cell shape, lack of nucleus; recapitulates cell aggregates | Drasdo & Hohme (2005) |
| Cellular Potts models | Generalized energetic cost of the state of the cell ensemble to be minimized | Adhesivity, target volume and cell surface, random migration | Parameters cannot be directly obtained by measurements; shape evolves on a fixed grid | Scianna et al. (2013), Swat et al. (2012) |
| Voronoi models | Location of centers of polyhedral cells | Deformation energy, membrane surface tension energy, cell-cell adhesion energy | Mainly for cohesive epithelial tissues; not suited for single-cell motion or cell detachment | Bi et al. (2016), Dunn et al. (2013), Meineke et al. (2001) |
| Vertex element models | Location of vertices of polyhedral cells | | | Fletcher et al. (2014), Honda et al. (2004) |
| Subcellular element models | Meshwork of points (elements) discretizing the cell surface | Interactions between subcellular elements or between a cell and surrounding cells and their environment, random migration | Computational costs increase with the number of subelements per cell | Frascoli et al. (2013), Milde et al. (2014), Sandersius & Newman (2008), Zaman et al. (2006) |
| Tensegrity methods | Meshwork of connected struts and cables discretizing the cell body | Stresses between elements | Computationally heavy | Ingber (2003) |
| Actomyosin-based models | Concentration and speed of cytoskeletal components treated as a continuum | Traction force, cytosol viscosity, adhesive strength | Strength in predicting cell mechanics in smoothly shaped cells and steady motion | Kozlov & Mogilner (2007), Manhart et al. (2015), Oelz & Schmeiser (2012), Schmeiser & Winkler (2015) |
| Cytoskeleton models | Cells as deformable ellipsoids, cell centers and axis lengths | Cytoskeleton-ECM adhesiveness, motility, membrane stiffness, cytosol viscosity, traction force | Simplified cytoskeletal dynamics and cell shape | Dallon & Othmer (2004), Palsson & Othmer (2000) |

(**Figure 8*a*,*b***). Interface modules connecting the cell and environment are interlinked and translate extracellular signals into the cell by multiscale cooperation (**Figure 8*b***). For example, cell velocity during chemotaxis represents a state variable that depends on a chemotactic parameter (or term) describing the distribution of a chemoattractant, whereby the concentration of chemoattractant represents a second state variable that changes in time and space (Palsson & Othmer 2000). Adhesiveness and traction ability (parameters) depend on the expression of adhesion receptors (state variables) defined by molecular modules (state variable) and their reaction rates (parameters) (**Figure 8*b***) (Frascoli et al. 2013). Hence, each state variable is dynamic and responds to the activity of connected subcellular protein networks (**Figure 8*a***). Assembling such multiple variables

and parameters involved in cell migration and adaptive behavior requires multiparametric data acquisition and intuitive graphical display of results (**Figure 8*c***).

Key challenges for mathematical modeling are to control varying interactivity between modules, their sequence, and hierarchies and to simulate a meaningful response. For example, nested models (or matryoshka models) integrate and prioritize multiple signals (i.e., mathematical submodels) operating at different spatial scales. The output of one or more molecular models is thereby used as input for other physical modules to combine protein network models and interface models and to predict individual cell function (**Figure 8*b***). As an example of interactivity, in a cellular Potts model, VEGF-induced motility also depends upon a calcium signaling pathway that fulfills a dual role, inducing migration at free edges but migration arrest in inner regions of the capillary plexus (Scianna et al. 2011).

**Emergent behavior:** an autoorganized behavior deriving from a set of (usually simple) rules that individually cannot explain the result; in cell motility, e.g., collective migration can be described as emergent behavior resulting from the motion of individual cells and from their interaction with each other and with the environment

**Multiscale model:** describes cell behaviors occurring at the cell and tissue scales (macroscopic behavior) with subcellular and molecular events (microscopic processes), and their forward and/or backward feedbacks. Multiple inputs deliver a range of possible outputs

**State variables:** space- and time-dependent quantities that describe the model configurations, including concentration of chemicals, expression of receptors, cell position and velocity, and geometrical characteristics of the cell and the environment

## Cause-Consequence Relations and Decision Making

To reflect biological behavior, response functions of a mathematical module to a single, well-defined stimulus can define different cause-consequence relationships, including continuous linear, nonlinear (e.g., nonmonotonic), and discontinuous dependency (**Figure 9*a***). For example, biopolymers can display nonlinear force-stiffness relationships (**Figure 4*a***), with an exponential stiffness increase in response to applied traction force (Licup et al. 2015). Likewise, cell speed on 2D substrates depends on adhesiveness in a bimodal fashion (**Figure 2*a***) (DiMilla et al. 1993, Verkhovsky et al. 1999). Criteria for a threshold are fulfilled for cells moving in 3D discontinuous confinement, for which, below a certain ECM pore size, migration is suddenly arrested (Wolf et al. 2013). When analyzed using a cellular Potts model, such threshold behavior is reproduced and an additional bimodal behavior identified, with peak speed reached at a pore size larger than the nucleus and a gradual decline when pores are much larger or smaller (Scianna et al. 2013).

A sigmoidal dependence is characterized by many phase transition phenomena that typically underlie transitions in migration modes. For example, in the glass jamming transition, cell ensembles transit between liquid-like to crystalline solid-like behavior (Park et al. 2015), which also applies to transitions between single-cell and collective behaviors (Haeger et al. 2014). This phase transition depends upon motility, adhesion, density, persistence, and a morphological parameter relating cell area to cell volume (Pegoraro et al. 2016). In a modified vertex model named the self-propelled Voronoi model, higher cell speed is associated with a sharper transition between moving and nonmoving behaviors, not unlike an on-off response (Bi et al. 2016).

Bistability represents another important principle of phase transition and is often associated with feedback loops (Angeli et al. 2004). Here, the stimulus response function has an S shape comprising zones with a low response and a high response region that are separated by a functionally important region of coexistence of both phases (**Figure 9*a*,*b***). Thus, a jump response may result from a gradual change of stimulus (**Figure 9*b***). Examples of bistability include (*a*) the transition between under- and overexpression of a protein (**Figure 8*b***) (Byrne et al. 2016); (*b*) the transition from one migration mode to another, whereby an intermediate number of cell-cell junctions may allow cells to adhere to, detach from, and reattach to their neighbors, thus flipping between collective and individual behaviors (Wong et al. 2014); and (*c*) intermediate adhesion strength leading to bistability between amoeboid and mesenchymal motion, with cells switching between elongated and rounded movements in an oscillatory manner and thus rendering classification and statistical analysis difficult (**Figure 7*a***) (Shafqat-Abbasi et al. 2016). Such rather unpredictable transitions from one state to another depend on the strength and temporal history of the stimulus and can give rise to hysteretic behavior, the modeling of which requires dynamic simulations and yields statistical uncertainty in the region where equilibrium states coexist.

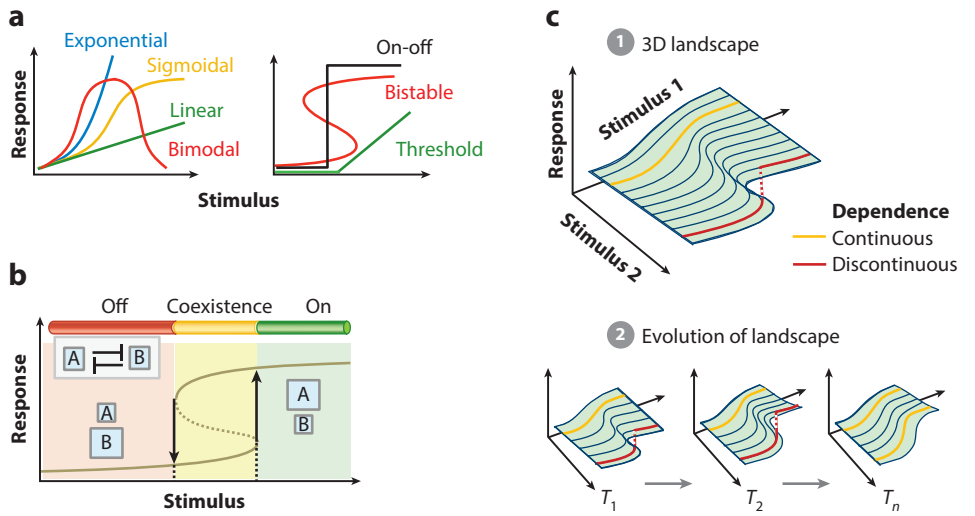



**Figure 9**

Mathematical functions describing input-output relationships. (*a*) Cause-consequence relations defining different types of cell or signaling responses to stimuli of increasing strength. (*b*) Bistability. A and B denote dominant function states; *red* and *green regions* represent only one equilibrium, and the *yellow region* contains two (or more) equilibrium states. The horizontal bar at the top represents the response range used in **Figure 8*c*** to illustrate bistable responses. (*c*) ① Two-parametric landscape changing from a weakly sigmoidal dependence of Stimulus 1 when Stimulus 2 is low (*orange line*) gradually toward a bistable dependence of Stimulus 1 on high Stimulus 2 (*red line*). As a consequence, the same value of the stimulus may yield two or more very different behaviors. ② Kinetic integration of the landscape over time.

**Nonmonotonic dependence:** nonlinear response to increasing strength of input, e.g., an extracellular signal, which may include both increasing and decreasing behaviors and one or several maxima and minima. Examples are oscillatory or multiphasic dependencies (see **Figure 9*a***, bimodal curve)

**Bistability:** a system with two stable equilibrium states in response to the same set of parameters and level of stimulus. Periodicity in the level of stimulus may lead to hysteretic behavior, with the system jumping between the local minimum and maximum of energy

On-off responses trigger decision making, allowing the cell to adopt behavior A or behavior B in a mutually exclusive manner (**Figure 9*a*,*b***). For example, an immobile nonpolar cell, when mechanically perturbed with a micropipette, spontaneously polarizes and transitions to migration with keratocyte-like shape and speed (Tozluoglu et al. 2013), representing a go decision in response to mechanical stimulation (**Figure 6*a***). Another example is the initial cell polarization by a shallow chemoattractant gradient, which is amplified within the cell to polarize PI3K and PIP3 production toward the leading edge, near the highest chemoattractant concentration, whereas PTEN and PIP2 localize to the cell rear (Semplice et al. 2012).

## Virtual Assembly of Moving Cells

Any physical or molecular input module is connected with one or several molecular modules, with linear or complex dependence in response to the reception fingerprint, which represents multiple stimuli that vary in strength and duration (**Figure 8*c***). The interlinked activation of several protein cascades downstream of the sensory apparatus then mediates an integrated cell response generating a behavioral landscape. Because each parameter generally depends on state variables that are not constant but are time dependent, landscapes change over time as a representation of complex cell behavior with, e.g., dynamic cell-tissue interactions or variable cell response to alternating signal strength. Multidimensional landscapes thus reflect the evolution of parameter ensembles, which typically require display as a series of 3D diagrams or multiparametric heat maps (Lomakin et al. 2015, Shafqat-Abbasi et al. 2016, Tozluoglu et al. 2013).

For example, migration efficacy of tumor cells in 3D space depends upon EGF stimulation, fibronectin and matrigel concentration and stiffness, and available integrin receptors; peak speed

is thereby determined by intermediate matrigel concentration but maximum integrin availability (Zaman et al. 2006). Bimodal behavior in the multidimensional landscape is found by using a cellular Potts model and spanning the parameter space (Scianna & Preziosi 2013, Scianna et al. 2013), which represents, in particular, varying adhesivity, fiber concentration, fiber rigidity, and pore size. Likewise, the landscape of jamming transition can be described as a function of cell density, motility, and adhesion (Sadati et al. 2014). As a further example, adaptation of migration mode in response to matrix geometry engages a multiparametric receptor fingerprint, which is affected by stochasticity and the related signaling machinery (Huang et al. 2015) (see **Supplemental Text**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**).

The availability of such landscapes allows for predictions on critical steps of decision making to guide biologists to conditions of interest, to adjust biological hypotheses, and to refine wet-lab experiments accordingly. Thus, mathematical models support the understanding of how complex reception fingerprints and the individual responsiveness repertoire cooperate and generate *n*-dimensional migration footprints.

**Hysteresis:** a history-based dependence of the cell response that depends on the past evolution, determined by, e.g., internal molecular variables, combined with the input from external stimuli. The response curve to repeated increasing followed by decreasing stimulus results in a response deviation (a hysteresis loop)

**Landscape:** array-type representation of a complex cell response established by multiparametric analysis from wet-lab experiments or predicted by modeling. A landscape expresses multivariate relationships, either from multiscale modeling or as an extrapolation of bivariate cause-consequence relationships over time

**Stochasticity:** a stochastic system contains states that are not deterministic but random because of uncertainties and effects that cannot be precisely quantified and therefore requires reproduction and statistical analysis. Stochasticity particularly impacts systems containing phase transitions or states of coexisting equilibria

## FUTURE DIRECTIONS

A combined strategy linking mechanistic knowledge on the mechanical and molecular modules that define cell migration with in vivo observation and perturbation and mathematical modeling is required to further delineate different types of cell migration and their environmental contexts. The range of potential outcomes and the probabilistic component in migratory adaptations may be beneficial for integrating multicellular responses in higher organisms to a robust outcome, such as when cells form an organ or reach and reliably repair damaged tissue in due time; however, the resulting range of outcomes precludes simplified schemes of data analysis and requires a diversified multicomponent analysis of cell-based assays (Ruprecht et al. 2015, Shafqat-Abbasi et al. 2016). Achieving large numbers of events, in contrast, is a challenge when rare material or spatially limited imaging window approaches are applied, such as intravital microscopy (Alexander et al. 2013, Osswald et al. 2015), or when rare events are observed, such as stem cell behaviors and fate decisions (Ritsma et al. 2014). Likewise, approaches to value and verify stochastic events and outlier behaviors from wet-lab experiments need to be developed and flanked by mathematical analysis. Concepts such as bistability can be easily generalized to multistability when multiple equilibrium configurations need to be considered for interpreting what is perceived as inconsistent results that, despite consistent input parameters, deliver an inconclusive range of outputs instead of a definitive behavior (**Figure 8*c***). The resulting biological variability of cell functions calls for standardized approaches of image analysis and annotation, as well as multiparametric analysis beyond current possibilities, which will depend upon the availability of curated public databases and terminology for cell migration data sets and analyses (Masuzzo et al. 2016, Shafqat-Abbasi et al. 2016).

### SUMMARY POINTS

1. Cell migration in vivo is complex and multiscale but can be dissected by defining multiparametric physicochemical modules.
2. Modules include cell adhesion, cytoskeletal function, and molecular and mechanical types of cell-tissue interaction.

3. Each module contributes to the type and kinetics of cell-tissue interaction, which coevolves by reciprocal adaptation and jointly determine migration outcome (mode).
4. Basic cell migration modes include single-cell amoeboid, mesenchymal, and collective movements that interconvert in response to molecular and physical stimuli.

### FUTURE ISSUES

1. Key principles are typically established in well-defined but reductionist in vitro models and require validation in vivo using model organisms and small animal models
2. To cope with complexity, strategies are required to translate plasticity of cell migration in vitro and in vivo to computational multiscale analysis and mathematical modeling to inform wet-lab analysis in a reciprocal manner.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

We thank Mirjam Zegers for helpful discussions on epithelial collective invasion and John Lock for insightful comments on multiscale analysis and modeling of cell migration. V.t.B. is a recipient of the Rosali B. Hite fellowship. L.P. is partially supported by the National Institute of High Mathematics. P.F. is supported by the European Research Council (617430-DEEPINSIGHT), NWO-Vici (918.11.626), Horizon 2020 consortium MULTIMOT (634107-2), the Cancer Genomics Center, and the MD Anderson Cancer Center Moon Shot program.

## LITERATURE CITED

Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, et al. 2014. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* 158:1110–22

Alexander S, Koehl GE, Hirschberg M, Geissler EK, Friedl P. 2008. Dynamic imaging of cancer growth and invasion: a modified skin-fold chamber model. *Histochem. Cell Biol.* 130:1147–54

Alexander S, Weigelin B, Winkler F, Friedl P. 2013. Preclinical intravital microscopy of the tumour-stroma interface: invasion, metastasis, and therapy response. *Curr. Opin. Cell Biol.* 25:659–71

Andrade DM, Clausen MP, Keller J, Mueller V, Wu C, et al. 2015. Cortical actin networks induce spatiotemporal confinement of phospholipids in the plasma membrane—a minimally invasive investigation by STED-FCS. *Sci. Rep.* 5:11454

Angeli D, Ferrell JE Jr., Sontag ED. 2004. Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems. *PNAS* 101:1822–27

Bachir AI, Zareno J, Moissoglu K, Plow EF, Gratton E, Horwitz AR. 2014. Integrin-associated complexes form hierarchically with variable stoichiometry in nascent adhesions. *Curr. Biol.* 24:1845–53

Balcioglu HE, Van Hoorn H, Donato DM, Schmidt T, Danen EH. 2015. The integrin expression profile modulates orientation and dynamics of force transmission at cell-matrix adhesions. *J. Cell Sci.* 128:1316–26

Batlle E, Wilkinson DG. 2012. Molecular mechanisms of cell segregation and boundary formation in development and tumorigenesis. *Cold Spring Harb. Perspect. Biol.* 4:a008227

Bazellieres E, Conte V, Elosegui-Artola A, Serra-Picamal X, Bintanel-Morcillo M, et al. 2015. Control of cell-cell forces and collective cell dynamics by the intercellular adhesome. *Nat. Cell Biol.* 17:409–20

Beningo KA, Dembo M, Kaverina I, Small JV, Wang YL. 2001. Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. *J. Cell Biol.* 153:881–88

Bergert M, Chandradoss SD, Desai RA, Paluch E. 2012. Cell mechanics control rapid transitions between blebs and lamellipodia during migration. *PNAS* 109:14434–39

Bergert M, Erzberger A, Desai RA, Aspalter IM, Oates AC, et al. 2015. Force transmission during adhesion-independent migration. *Nat. Cell Biol.* 17:524–29

Bi D, Yang X, Marchetti MC, Manning ML. 2016. Motility-driven glass and jamming transitions in biological tissues. *Phys. Rev. X* 6:021011

Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J. 2014. Actin dynamics, architecture, and mechanics in cell motility. *Physiol. Rev.* 94:235–63

Byrne KM, Monsefi N, Dawson JC, Degasperi A, Bukowski-Wills J-C, et al. 2016. Bistability in the Rac1, PAK, and RhoA signaling network drives actin cytoskeleton dynamics and cell motility switches. *Cell Syst.* 1:38–48

Cai D, Chen SC, Prasad M, He L, Wang X, et al. 2014. Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. *Cell* 157:1146–59

Carlin LM, Stamatiades EG, Auffray C, Hanna RN, Glover L, et al. 2013. *Nr4a1*-dependent Ly6C$^{low}$ monocytes monitor endothelial cells and orchestrate their disposal. *Cell* 153:362–75

Carvalho LO, Aquino EN, Neves AC, Fontes W. 2015. The neutrophil nucleus and its role in neutrophilic function. *J. Cell. Biochem.* 116:1831–36

Casares L, Vincent R, Zalvidea D, Campillo N, Navajas D, et al. 2015. Hydraulic fracture during epithelial stretching. *Nat. Mater.* 14:343–51

Case LB, Baird MA, Shtengel G, Campbell SL, Hess HF, et al. 2015. Molecular mechanism of vinculin activation and nanoscale spatial organization in focal adhesions. *Nat. Cell Biol.* 17:880–92

Cavallaro U, Christofori G. 2004. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat. Rev. Cancer* 4:118–32

Cayrol R, Wosik K, Berard JL, Dodelet-Devillers A, Ifergan I, et al. 2008. Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. *Nat. Immunol.* 9:137–45

Chan CJ, Ekpenyong AE, Golfier S, Li W, Chalut KJ, et al. 2015. Myosin II activity softens cells in suspension. *Biophys. J.* 108:1856–69

Changede R, Xu X, Margadant F, Sheetz MP. 2015. Nascent integrin adhesions form on all matrix rigidities after integrin activation. *Dev. Cell* 35:614–21

Chaqour B. 2013. Molecular control of vascular development by the matricellular proteins CCN1 (Cyr61) and CCN2 (CTGF). *Trends Dev. Biol.* 7:59–72

Chen CS, Tan J, Tien J. 2004. Mechanotransduction at cell-matrix and cell-cell contacts. *Annu. Rev. Biomed. Eng.* 6:275–302

Cheung KJ, Ewald AJ. 2014. Illuminating breast cancer invasion: diverse roles for cell-cell interactions. *Curr. Opin. Cell Biol.* 30:99–111

Cheung KJ, Padmanaban V, Silvestri V, Schipper K, Cohen JD, et al. 2016. Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. *PNAS* 113:E854–63

Chrzanowska-Wodnicka M, Burridge K. 1996. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J. Cell Biol.* 133:1403–15

Chuai M, Hughes D, Weijer CJ. 2012. Collective epithelial and mesenchymal cell migration during gastrulation. *Curr. Genom.* 13:267–77

Collins C, Nelson WJ. 2015. Running with neighbors: coordinating cell migration and cell-cell adhesion. *Curr. Opin. Cell Biol.* 36:62–70

Cooper S, Sadok A, Bousgouni V, Bakal C. 2015. Apolar and polar transitions drive the conversion between amoeboid and mesenchymal shapes in melanoma cells. *Mol. Biol. Cell* 26:4163–70

Couchman JR. 2010. Transmembrane signaling proteoglycans. *Annu. Rev. Cell Dev. Biol.* 26:89–114

Cox JH, Dean RA, Roberts CR, Overall CM. 2008. Matrix metalloproteinase processing of CXCL11/I-TAC results in loss of chemoattractant activity and altered glycosaminoglycan binding. *J. Biol. Chem.* 283:19389–99

Dallon JC, Othmer HG. 2004. How cellular movement determines the collective force generated by the *Dictyostelium discoideum* slug. *J. Theor. Biol.* 231:203–22

Danuser G, Allard J, Mogilner A. 2013. Mathematical modeling of eukaryotic cell migration: insights beyond experiments. *Annu. Rev. Cell Dev. Biol.* 29:501–28

Das S, Rericha EC, Bagorda A, Parent CA. 2011. Direct biochemical measurements of signal relay during *Dictyostelium* development. *J. Biol. Chem.* 286:38649–58

Dean RA, Cox JH, Bellac CL, Doucet A, Starr AE, Overall CM. 2008. Macrophage-specific metalloelastase (MMP-12) truncates and inactivates $ELR^+$ CXC chemokines and generates CCL2, -7, -8, and -13 antagonists: potential role of the macrophage in terminating polymorphonuclear leukocyte influx. *Blood* 112:3455–64

DiMilla PA, Stone JA, Quinn JA, Albelda SM, Lauffenburger DA. 1993. Maximal migration of human smooth muscle cells on fibronectin and type IV collagen occurs at an intermediate attachment strength. *J. Cell Biol.* 122:729–37

Dona E, Barry JD, Valentin G, Quirin C, Khmelinskii A, et al. 2013. Directional tissue migration through a self-generated chemokine gradient. *Nature* 503:285–89

Doyle AD, Wang FW, Matsumoto K, Yamada KM. 2009. One-dimensional topography underlies three-dimensional fibrillar cell migration. *J. Cell Biol.* 184:481–90

Drasdo D, Hohme S. 2005. A single-cell-based model of tumor growth in vitro: monolayers and spheroids. *Phys. Biol.* 2:133–47

Dunn SJ, Nathke IS, Osborne JM. 2013. Computational models reveal a passive mechanism for cell migration in the crypt. *PLOS ONE* 8:e80516

Eilken HM, Adams RH. 2010. Dynamics of endothelial cell behavior in sprouting angiogenesis. *Curr. Opin. Cell Biol.* 22:617–25

Fletcher AG, Osterfield M, Baker RE, Shvartsman SY. 2014. Vertex models of epithelial morphogenesis. *Biophys. J.* 106:2291–304

Frascoli F, Hughes BD, Zaman MH, Landman KA. 2013. A computational model for collective cellular motion in three dimensions: general framework and case study for cell pair dynamics. *PLOS ONE* 8:e59249

Friedl P, Alexander S. 2011. Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell* 147:992–1009

Friedl P, Borgmann S, Brocker EB. 2001. Amoeboid leukocyte crawling through extracellular matrix: lessons from the *Dictyostelium* paradigm of cell movement. *J. Leukoc. Biol.* 70:491–509

Friedl P, Entschladen F, Conrad C, Niggemann B, Zanker KS. 1998. $CD4^+$ T lymphocytes migrating in three-dimensional collagen lattices lack focal adhesions and utilize β1 integrin–independent strategies for polarization, interaction with collagen fibers and locomotion. *Eur. J. Immunol.* 28:2331–43

Friedl P, Gilmour D. 2009. Collective cell migration in morphogenesis, regeneration and cancer. *Nat. Rev. Mol. Cell Biol.* 10:445–57

Friedl P, Locker J, Sahai E, Segall JE. 2012a. Classifying collective cancer cell invasion. *Nat. Cell Biol.* 14:777–83

Friedl P, Maaser K, Klein CE, Niggemann B, Krohne G, Zanker KS. 1997. Migration of highly aggressive MV3 melanoma cells in 3-dimensional collagen lattices results in local matrix reorganization and shedding of α2 and β1 integrins and CD44. *Cancer Res.* 57:2061–70

Friedl P, Noble PB, Walton PA, Laird DW, Chauvin PJ, et al. 1995. Migration of coordinated cell clusters in mesenchymal and epithelial cancer explants in vitro. *Cancer Res.* 55:4557–60

Friedl P, Sahai E, Weiss S, Yamada KM. 2012b. New dimensions in cell migration. *Nat. Rev. Mol. Cell Biol.* 13:743–47

Friedl P, Weigelin B. 2008. Interstitial leukocyte migration and immune function. *Nat. Immunol.* 9:960–69

Friedl P, Wolf K. 2008. Tube travel: the role of proteases in individual and collective cancer cell invasion. *Cancer Res.* 68:7247–49

Friedl P, Wolf K. 2010. Plasticity of cell migration: a multiscale tuning model. *J. Cell Biol.* 188:11–19

Friedl P, Wolf K, Lammerding J. 2011. Nuclear mechanics during cell migration. *Curr. Opin. Cell Biol.* 23:55–64

Gad AK, Ronnlund D, Spaar A, Savchenko AA, Petranyi G, et al. 2012. Rho GTPases link cellular contractile force to the density and distribution of nanoscale adhesions. *FASEB J.* 26:2374–82

Gardel ML, Sabass B, Ji L, Danuser G, Schwarz US, Waterman CM. 2008. Traction stress in focal adhesions correlates biphasically with actin retrograde flow speed. *J. Cell Biol.* 183:999–1005

Geiger B, Spatz JP, Bershadsky AD. 2009. Environmental sensing through focal adhesions. *Nat. Rev. Mol. Cell Biol.* 10:21–33

Glentis A, Gurchenkov V, Matic Vignjevic D. 2014. Assembly, heterogeneity, and breaching of the basement membranes. *Cell Adhes. Migr.* 8:236–45

Goudarzi M, Banisch TU, Mobin MB, Maghelli N, Tarbashevich K, et al. 2012. Identification and regulation of a molecular module for bleb-based cell motility. *Dev. Cell* 23:210–18

Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, et al. 2010. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature* 466:263–66

Greiner AM, Klein F, Gudzenko T, Richter B, Striebel T, et al. 2015. Cell type–specific adaptation of cellular and nuclear volume in micro-engineered 3D environments. *Biomaterials* 69:121–32

Griffith JW, Sokol CL, Luster AD. 2014. Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu. Rev. Immunol.* 32:659–702

Grinnell F, Petroll WM. 2010. Cell motility and mechanics in three-dimensional collagen matrices. *Annu. Rev. Cell Dev. Biol.* 26:335–61

Haeger A, Krause M, Wolf K, Friedl P. 2014. Cell jamming: collective invasion of mesenchymal tumor cells imposed by tissue confinement. *Biochim. Biophys. Acta* 1840:2386–95

Haeger A, Wolf K, Zegers MM, Friedl P. 2015. Collective cell migration: guidance principles and hierarchies. *Trends Cell Biol.* 25:556–66

Haigo SL, Bilder D. 2011. Global tissue revolutions in a morphogenetic movement controlling elongation. *Science* 331:1071–74

Halloran MC, Wolman MA. 2006. Repulsion or adhesion: Receptors make the call. *Curr. Opin. Cell Biol.* 18:533–40

Harris TJ, Tepass U. 2010. Adherens junctions: from molecules to morphogenesis. *Nat. Rev. Mol. Cell Biol.* 11:502–14

Hegerfeldt Y, Tusch M, Brocker EB, Friedl P. 2002. Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, β1-integrin function, and migration strategies. *Cancer Res.* 62:2125–30

Heit B, Tavener S, Raharjo E, Kubes P. 2002. An intracellular signaling hierarchy determines direction of migration in opposing chemotactic gradients. *J. Cell Biol.* 159:91–102

Helvert VS, Friedl P. 2016. Strain stiffening of fibrillar collagen during individual and collective cell migration identified by AFM force spectroscopy. *ACS Appl. Mater. Interfaces.* In press; doi: 10.1021/acsami.6b01755

Honda H, Tanemura M, Nagai T. 2004. A three-dimensional vertex dynamics cell model of space-filling polyhedra simulating cell behavior in a cell aggregate. *J. Theor. Biol.* 226:439–53

Huang B, Jolly MK, Lu M, Tsarfaty I, Ben-Jacob E, Onuchic JN. 2015. Modeling the transitions between collective and solitary migration phenotypes in cancer metastasis. *Sci. Rep.* 5:17379

Hung WC, Chen SH, Paul CD, Stroka KM, Lo YC, et al. 2013. Distinct signaling mechanisms regulate migration in unconfined versus confined spaces. *J. Cell Biol.* 202:807–24

Ilina O, Bakker GJ, Vasaturo A, Hofmann RM, Friedl P. 2011. Two-photon laser-generated microtracks in 3D collagen lattices: principles of MMP-dependent and -independent collective cancer cell invasion. *Phys. Biol.* 8:015010

Ilina O, Friedl P. 2009. Mechanisms of collective cell migration at a glance. *J. Cell Sci.* 122:3203–8

Ingber DE. 2003. Tensegrity. I. Cell structure and hierarchical systems biology. *J. Cell Sci.* 116:1157–73

Jansen KA, Bacabac RG, Piechocka IK, Koenderink GH. 2013. Cells actively stiffen fibrin networks by generating contractile stress. *Biophys. J.* 105:2240–51

Jiang G, Huang AH, Cai Y, Tanase M, Sheetz MP. 2006. Rigidity sensing at the leading edge through $\alpha v\beta 3$ integrins and RPTPα;. *Biophys. J.* 90:1804–9

Joslin EJ, Opresko LK, Wells A, Wiley HS, Lauffenburger DA. 2007. EGF-receptor-mediated mammary epithelial cell migration is driven by sustained ERK signaling from autocrine stimulation. *J. Cell Sci.* 120:3688–99

Kania A, Klein R. 2016. Mechanisms of ephrin-Eph signalling in development, physiology and disease. *Nat. Rev. Mol. Cell Biol.* 170:240–56

Kardash E, Reichman-Fried M, Maitre JL, Boldajipour B, Papusheva E, et al. 2010. A role for Rho GTPases and cell-cell adhesion in single-cell motility in vivo. *Nat. Cell Biol.* 12:47–53; suppl. 1–11

Kariya Y, Sato H, Katou N, Kariya Y, Miyazaki K. 2012. Polymerized laminin-332 matrix supports rapid and tight adhesion of keratinocytes, suppressing cell migration. *PLOS ONE* 7:e35546

Kienast Y, Von Baumgarten L, Fuhrmann M, Klinkert WE, Goldbrunner R, et al. 2010. Real-time imaging reveals the single steps of brain metastasis formation. *Nat. Med.* 16:116–22

Kim JH, Serra-Picamal X, Tambe DT, Zhou EH, Park CY, et al. 2013. Propulsion and navigation within the advancing monolayer sheet. *Nat. Mater.* 12:856–63

Kim Y, Kumar S. 2014. CD44-mediated adhesion to hyaluronic acid contributes to mechanosensing and invasive motility. *Mol. Cancer Res.* 12:1416–29

Klotzsch E, Smith ML, Kubow KE, Muntwyler S, Little WC, et al. 2009. Fibronectin forms the most extensible biological fibers displaying switchable force-exposed cryptic binding sites. *PNAS* 106:18267–72

Koch TM, Munster S, Bonakdar N, Butler JP, Fabry B. 2012. 3D traction forces in cancer cell invasion. *PLOS ONE* 7:e33476

Kolsch V, Charest PG, Firtel RA. 2008. The regulation of cell motility and chemotaxis by phospholipid signaling. *J. Cell Sci.* 121:551–59

Kozlov MM, Mogilner A. 2007. Model of polarization and bistability of cell fragments. *Biophys. J.* 93:3811–19

Kroeze KL, Boink MA, Sampat-Sardjoepersad SC, Waaijman T, Scheper RJ, Gibbs S. 2012. Autocrine regulation of re-epithelialization after wounding by chemokine receptors CCR1, CCR10, CXCR1, CXCR2, and CXCR3. *J. Investig. Dermatol.* 132:216–25

Kubow KE, Conrad SK, Horwitz AR. 2013. Matrix microarchitecture and myosin II determine adhesion in 3D matrices. *Curr. Biol.* 23:1607–19

Lammermann T, Afonso PV, Angermann BR, Wang JM, Kastenmuller W, et al. 2013. Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. *Nature* 498:371–75

Larsen M, Wei C, Yamada KM. 2006. Cell and fibronectin dynamics during branching morphogenesis. *J. Cell Sci.* 119:3376–84

Lebreton G, Casanova J. 2014. Specification of leading and trailing cell features during collective migration in the *Drosophila* trachea. *J. Cell Sci.* 127:465–74

Li R, Gundersen GG. 2008. Beyond polymer polarity: how the cytoskeleton builds a polarized cell. *Nat. Rev. Mol. Cell Biol.* 9:860–73

Licup AJ, Munster S, Sharma A, Sheinman M, Jawerth LM, et al. 2015. Stress controls the mechanics of collagen networks. *PNAS* 112:9573–78

Liu H, Wen J, Xiao Y, Liu J, Hopyan S, et al. 2014. In situ mechanical characterization of the cell nucleus by atomic force microscopy. *ACS Nano* 8:3821–28

Liu YJ, Le Berre M, Lautenschlaeger F, Maiuri P, Callan-Jones A, et al. 2015. Confinement and low adhesion induce fast amoeboid migration of slow mesenchymal cells. *Cell* 160:659–72

Lomakin AJ, Lee KC, Han SJ, Bui DA, Davidson M, et al. 2015. Competition for actin between two distinct F-actin networks defines a bistable switch for cell polarization. *Nat. Cell Biol.* 17:1435–45

Maaser K, Wolf K, Klein CE, Niggemann B, Zanker KS, et al. 1999. Functional hierarchy of simultaneously expressed adhesion receptors: Integrin $\alpha 2\beta 1$ but not CD44 mediates MV3 melanoma cell migration and matrix reorganization within three-dimensional hyaluronan-containing collagen matrices. *Mol. Biol. Cell* 10:3067–79

Malawista SE, De Boisfleury Chevance A. 1997. Random locomotion and chemotaxis of human blood polymorphonuclear leukocytes (PMN) in the presence of EDTA: PMN in close quarters require neither leukocyte integrins nor external divalent cations. *PNAS* 94:11577–82

Manhart A, Oelz D, Schmeiser C, Sfakianakis N. 2015. An extended filament based lamellipodium model produces various moving cell shapes in the presence of chemotactic signals. *J. Theor. Biol.* 382:244–58

Masuzzo P, Van Troys M, Ampe C, Martens L. 2016. Taking aim at moving targets in computational cell migration. *Trends Cell Biol.* 26:88–110

Meineke FA, Potten CS, Loeffler M. 2001. Cell migration and organization in the intestinal crypt using a lattice-free model. *Cell Prolif.* 34:253–66

Meng W, Takeichi M. 2009. Adherens junction: molecular architecture and regulation. *Cold Spring Harb. Perspect. Biol.* 1:a002899

Milde F, Tauriello G, Haberkern H, Koumoutsakos P. 2014. SEM++: a particle model of cellular growth, signaling and migration. *Comput. Part. Mech.* 1:211–27

Miller MA, Meyer AS, Beste MT, Lasisi Z, Reddy S, et al. 2013. ADAM-10 and -17 regulate endometriotic cell migration via concerted ligand and receptor shedding feedback on kinase signaling. *PNAS* 110:E2074–83

Moali C, Hulmes DJ. 2009. Extracellular and cell surface proteases in wound healing: New players are still emerging. *Eur. J. Dermatol.* 19:552–64

Monneau Y, Arenzana-Seisdedos F, Lortat-Jacob H. 2016. The sweet spot: how GAGs help chemokines guide migrating cells. *J. Leukoc. Biol.* 99:935–53

Moon JJ, Matsumoto M, Patel S, Lee L, Guan JL, Li S. 2005. Role of cell surface heparan sulfate proteoglycans in endothelial cell migration and mechanotransduction. *J. Cell Physiol.* 203:166–76

Morrissey MA, Sherwood DR. 2015. An active role for basement membrane assembly and modification in tissue sculpting. *J. Cell Sci.* 128:1661–68

Mythreye K, Blobe GC. 2009. Proteoglycan signaling co-receptors: roles in cell adhesion, migration and invasion. *Cell. Signal.* 21:1548–58

Nguyen-Ngoc KV, Cheung KJ, Brenot A, Shamir ER, Gray RS, et al. 2012. ECM microenvironment regulates collective migration and local dissemination in normal and malignant mammary epithelium. *PNAS* 109:E2595–604

Nieto MA. 2011. The ins and outs of the epithelial to mesenchymal transition in health and disease. *Annu. Rev. Cell Dev. Biol.* 27:347–76

Nourshargh S, Hordijk PL, Sixt M. 2010. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat. Rev. Mol. Cell Biol.* 11:366–78

Oelz D, Schmeiser C. 2012. Simulation of lamellipodial fragments. *J. Math. Biol.* 64:513–28

Orgaz JL, Pandya P, Dalmeida R, Karagiannis P, Sanchez-Laorden B, et al. 2014. Diverse matrix metalloproteinase functions regulate cancer amoeboid migration. *Nat. Commun.* 5:4255

Osswald M, Jung E, Sahm F, Solecki G, Venkataramani V, et al. 2015. Brain tumour cells interconnect to a functional and resistant network. *Nature* 528:93–98

Paksa A, Raz E. 2015. Zebrafish germ cells: motility and guided migration. *Curr. Opin. Cell Biol.* 36:80–85

Palsson E, Othmer HG. 2000. A model for individual and collective cell movement in *Dictyostelium discoideum*. *PNAS* 97:10448–53

Paluch EK, Raz E. 2013. The role and regulation of blebs in cell migration. *Curr. Opin. Cell Biol.* 25:582–90

Park JA, Kim JH, Bi D, Mitchel JA, Qazvini NT, et al. 2015. Unjamming and cell shape in the asthmatic airway epithelium. *Nat. Mater.* 14:1040–48

Parri M, Taddei ML, Bianchini F, Calorini L, Chiarugi P. 2009. EphA2 reexpression prompts invasion of melanoma cells shifting from mesenchymal to amoeboid-like motility style. *Cancer Res.* 69:2072–81

Parsons JT, Horwitz AR, Schwartz MA. 2010. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat. Rev. Mol. Cell Biol.* 11:633–43

Peglion F, Llense F, Etienne-Manneville S. 2014. Adherens junction treadmilling during collective migration. *Nat. Cell Biol.* 16:639–51

Pegoraro AF, Fredberg JJ, Park JA. 2016. Problems in biology with many scales of length: cell-cell adhesion and cell jamming in collective cellular migration. *Exp. Cell Res.* 343:54–59

Petrie RJ, Yamada KM. 2012. At the leading edge of three-dimensional cell migration. *J. Cell Sci.* 125:5917–26

Phillipson M, Kubes P. 2011. The neutrophil in vascular inflammation. *Nat. Med.* 17:1381–90

Plotnikov SV, Pasapera AM, Sabass B, Waterman CM. 2012. Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. *Cell* 151:1513–27

Pokutta S, Weis WI. 2007. Structure and mechanism of cadherins and catenins in cell-cell contacts. *Annu. Rev. Cell Dev. Biol.* 23:237–61

Pollard TD, Borisy GG. 2003. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112:453–65

Proebstl D, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, et al. 2012. Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. *J. Exp. Med.* 209:1219–34

Razafsky D, Hodzic D. 2009. Bringing KASH under the SUN: the many faces of nucleo-cytoskeletal connections. *J. Cell Biol.* 186:461–72

Reffay M, Parrini MC, Cochet-Escartin O, Ladoux B, Buguin A, et al. 2014. Interplay of RhoA and mechanical forces in collective cell migration driven by leader cells. *Nat. Cell Biol.* 16:217–23

Renkawitz J, Schumann K, Weber M, Lammermann T, Pflicke H, et al. 2009. Adaptive force transmission in amoeboid cell migration. *Nat. Cell Biol.* 11:1438–43

Rhee S. 2009. Fibroblasts in three dimensional matrices: cell migration and matrix remodeling. *Exp. Mol. Med.* 41:858–65

Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, et al. 2003. Cell migration: integrating signals from front to back. *Science* 302:1704–9

Ritsma L, Ellenbroek SI, Zomer A, Snippert HJ, de Sauvage FJ, et al. 2014. Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging. *Nature* 507:362–65

Roca-Cusachs P, Del Rio A, Puklin-Faucher E, Gauthier NC, Biais N, Sheetz MP. 2013. Integrin-dependent force transmission to the extracellular matrix by alpha-actinin triggers adhesion maturation. *PNAS* 110:E1361–70

Rohani N, Parmeggiani A, Winklbauer R, Fagotto F. 2014. Variable combinations of specific ephrin ligand/Eph receptor pairs control embryonic tissue separation. *PLOS Biol.* 12:e1001955

Roubinet C, Tran PT, Piel M. 2012. Common mechanisms regulating cell cortex properties during cell division and cell migration. *Cytoskeleton* 69:957–72

Rowe RG, Weiss SJ. 2009. Navigating ECM barriers at the invasive front: the cancer cell–stroma interface. *Annu. Rev. Cell Dev. Biol.* 25:567–95

Rozario T, Desimone DW. 2010. The extracellular matrix in development and morphogenesis: a dynamic view. *Dev. Biol.* 341:126–40

Ruprecht V, Wieser S, Callan-Jones A, Smutny M, Morita H, et al. 2015. Cortical contractility triggers a stochastic switch to fast amoeboid cell motility. *Cell* 160:673–85

Sabeh F, Shimizu-Hirota R, Weiss SJ. 2009. Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. *J. Cell Biol.* 185:11–19

Sadati M, Nourhani A, Fredberg JJ, Taheri Qazvini N. 2014. Glass-like dynamics in the cell and in cellular collectives. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 6:137–49

Sahai E, Marshall CJ. 2003. Differing modes of tumour cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nat. Cell Biol.* 5:711–19

Sakai T, Larsen M, Yamada KM. 2003. Fibronectin requirement in branching morphogenesis. *Nature* 423:876–81

Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, et al. 2012. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J. Clin. Investig.* 122:899–910

Sanz-Moreno V, Gadea G, Ahn J, Paterson H, Marra P, et al. 2008. Rac activation and inactivation control plasticity of tumor cell movement. *Cell* 135:510–23

Sandersius SA, Newman TJ. 2008. Modeling cell rheology with the Subcellular Element Model. *Phys. Biol.* 5:015002

Sarmiento C, Wang W, Dovas A, Yamaguchi H, Sidani M, et al. 2008. WASP family members and formin proteins coordinate regulation of cell protrusions in carcinoma cells. *J. Cell Biol.* 180:1245–60

Scarpa E, Mayor R. 2016. Collective cell migration in development. *J. Cell Biol.* 212:143–55

Schaefer L, Schaefer RM. 2010. Proteoglycans: from structural compounds to signaling molecules. *Cell Tissue Res.* 339:237–46

Schmeiser C, Winkler C. 2015. The flatness of lamellipodia explained by the interaction between actin dynamics and membrane deformation. *J. Theor. Biol.* 380:144–55

Schmidt S, Friedl P. 2010. Interstitial cell migration: integrin-dependent and alternative adhesion mechanisms. *Cell Tissue Res.* 339:83–92

Scianna M, Munaron L, Preziosi L. 2011. A multiscale hybrid approach for vasculogenesis and related potential blocking therapies. *Prog. Biophys. Mol. Biol.* 106:450–62

Scianna M, Preziosi L. 2013. Modeling the influence of nucleus elasticity on cell invasion in fiber networks and microchannels. *J. Theor. Biol.* 317:394–406

Scianna M, Preziosi L, Wolf K. 2013. A cellular Potts model simulating cell migration on and in matrix environments. *Math Biosci. Eng.* 10:235–61

Semplice M, Veglio A, Naldi G, Serini G, Gamba A. 2012. A bistable model of cell polarity. *PLOS ONE* 7:e30977

Shafqat-Abbasi H, Kowalewski JM, Kiss A, Gong X, Hernandez-Varas P, et al. 2016. An analysis toolbox to explore mesenchymal migration heterogeneity reveals adaptive switching between distinct modes. *eLife* 5:e11384

Shih W, Yamada S. 2012. N-cadherin-mediated cell-cell adhesion promotes cell migration in a three-dimensional matrix. *J. Cell Sci.* 125:3661–70

Shintani Y, Fukumoto Y, Chaika N, Svoboda R, Wheelock MJ, Johnson KR. 2008. Collagen I–mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. *J. Cell Biol.* 180:1277–89

Smith ML, Gourdon D, Little WC, Kubow KE, Eguiluz RA, et al. 2007. Force-induced unfolding of fibronectin in the extracellular matrix of living cells. *PLOS Biol.* 5:e268

Sonnemann KJ, Bement WM. 2011. Wound repair: toward understanding and integration of single-cell and multicellular wound responses. *Annu. Rev. Cell Dev. Biol.* 27:237–63

Starke J, Wehrle-Haller B, Friedl P. 2014. Plasticity of the actin cytoskeleton in response to extracellular matrix nanostructure and dimensionality. *Biochem. Soc. Trans.* 42:1356–66

Steinwachs J, Metzner C, Skodzek K, Lang N, Thievessen I, et al. 2016. Three-dimensional force microscopy of cells in biopolymer networks. *Nat. Methods* 13:171–76

Sternlicht MD, Werb Z. 2001. How matrix metalloproteinases regulate cell behavior. *Annu. Rev. Cell Dev. Biol.* 17:463–516

Storm C, Pastore JJ, Mackintosh FC, Lubensky TC, Janmey PA. 2005. Nonlinear elasticity in biological gels. *Nature* 435:191–94

Stroka KM, Jiang H, Chen SH, Tong Z, Wirtz D, et al. 2014. Water permeation drives tumor cell migration in confined microenvironments. *Cell* 157:611–23

Swaminathan V, Fischer RS, Waterman CM. 2016. The FAK-Arp2/3 interaction promotes leading edge advance and haptosensing by coupling nascent adhesions to lamellipodia actin. *Mol. Biol. Cell* 27:1085–100

Swat MH, Thomas GL, Belmonte JM, Shirinifard A, Hmeljak D, Glazier JA. 2012. Multi-scale modeling of tissues using CompuCell3D. *Methods Cell Biol.* 110:325–66

Taddei ML, Giannoni E, Morandi A, Ippolito L, Ramazzotti M, et al. 2014. Mesenchymal to amoeboid transition is associated with stem-like features of melanoma cells. *Cell Commun. Signal.* 12:24

Taddei ML, Parri M, Angelucci A, Bianchini F, Marconi C, et al. 2011. EphA2 induces metastatic growth regulating amoeboid motility and clonogenic potential in prostate carcinoma cells. *Mol. Cancer Res.* 9:149–60

Theveneau E, Marchant L, Kuriyama S, Gull M, Moepps B, et al. 2010. Collective chemotaxis requires contact-dependent cell polarity. *Dev. Cell* 19:39–53

Theveneau E, Mayor R. 2010. Integrating chemotaxis and contact-inhibition during collective cell migration: small GTPases at work. *Small GTPases* 1:113–17

Theveneau E, Mayor R. 2012. Cadherins in collective cell migration of mesenchymal cells. *Curr. Opin. Cell Biol.* 24:677–84

Tornavaca O, Chia M, Dufton N, Almagro LO, Conway DE, et al. 2015. ZO-1 controls endothelial adherens junctions, cell-cell tension, angiogenesis, and barrier formation. *J. Cell Biol.* 208:821–38

Tozluoglu M, Tournier AL, Jenkins RP, Hooper S, Bates PA, Sahai E. 2013. Matrix geometry determines optimal cancer cell migration strategy and modulates response to interventions. *Nat. Cell Biol.* 15:751–62

Tseng Y, Fedorov E, McCaffery JM, Almo SC, Wirtz D. 2001. Micromechanics and ultrastructure of actin filament networks crosslinked by human fascin: a comparison with $\alpha$-actinin. *J. Mol. Biol.* 310:351–66

Tyson RA, Zatulovskiy E, Kay RR, Bretschneider T. 2014. How blebs and pseudopods cooperate during chemotaxis. *PNAS* 111:11703–8

Vaskovicova K, Szabadosova E, Cermak V, Gandalovicova A, Kasalova L, et al. 2015. PKC$\alpha$ promotes the mesenchymal to amoeboid transition and increases cancer cell invasiveness. *BMC Cancer* 15:326

Verkhovsky AB, Svitkina TM, Borisy GG. 1999. Self-polarization and directional motility of cytoplasm. *Curr. Biol.* 9:11–20

Wai Wong C, Dye DE, Coombe DR. 2012. The role of immunoglobulin superfamily cell adhesion molecules in cancer metastasis. *Int J. Cell Biol.* 2012:340296

Wang X, He L, Wu YI, Hahn KM, Montell DJ. 2010. Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. *Nat. Cell Biol.* 12:591–97

Watkins S, Sontheimer H. 2011. Hydrodynamic cellular volume changes enable glioma cell invasion. *J. Neurosci.* 31:17250–59

Weaver SA, Wolters B, Ito N, Woskowicz AM, Kaneko K, et al. 2014. Basal localization of MT1-MMP is essential for epithelial cell morphogenesis in 3D collagen matrix. *J. Cell Sci.* 127:1203–13

Weber M, Hauschild R, Schwarz J, Moussion C, de Vries I, et al. 2013. Interstitial dendritic cell guidance by haptotactic chemokine gradients. *Science* 339:328–32

Weigelin B, Bakker G-J, Friedl P. 2012. Intravital third harmonic generation microscopy of collective melanoma cell invasion: principles of interface guidance and microvesicle dynamics. *Intravital* 1:32–43

Weigelin B, Bakker GJ, Friedl P. 2016. Third harmonic generation microscopy of cells and tissue organization. *J. Cell Sci.* 129:245–55

Wolf K, Alexander S, Schacht V, Coussens LM, Von Andrian UH, et al. 2009. Collagen-based cell migration models in vitro and in vivo. *Semin. Cell Dev. Biol.* 20:931–41

Wolf K, Friedl P. 2009. Mapping proteolytic cancer cell–extracellular matrix interfaces. *Clin. Exp. Metastasis* 26:289–98

Wolf K, Friedl P. 2011. Extracellular matrix determinants of proteolytic and non-proteolytic cell migration. *Trends Cell Biol.* 21:736–44

Wolf K, Mazo I, Leung H, Engelke K, Von Andrian UH, et al. 2003a. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J. Cell Biol.* 160:267–77

Wolf K, Muller R, Borgmann S, Brocker EB, Friedl P. 2003b. Amoeboid shape change and contact guidance: T-lymphocyte crawling through fibrillar collagen is independent of matrix remodeling by MMPs and other proteases. *Blood* 102:3262–69

Wolf K, Te Lindert M, Krause M, Alexander S, Te Riet J, et al. 2013. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *J. Cell Biol.* 201:1069–84

Wolf K, Wu YI, Liu Y, Geiger J, Tam E, et al. 2007. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. *Nat. Cell Biol.* 9:893–904

Wong IY, Javaid S, Wong EA, Perk S, Haber DA, et al. 2014. Collective and individual migration following the epithelial-mesenchymal transition. *Nat. Mater.* 13:1063–71

Wu SK, Gomez GA, Michael M, Verma S, Cox HL, et al. 2014. Cortical F-actin stabilization generates apical-lateral patterns of junctional contractility that integrate cells into epithelia. *Nat. Cell Biol.* 16:167–78

Xu C, Hasan SS, Schmidt I, Rocha SF, Pitulescu ME, et al. 2014. Arteries are formed by vein-derived endothelial tip cells. *Nat. Commun.* 5:5758

Xu H, Bihan D, Chang F, Huang PH, Farndale RW, Leitinger B. 2012. Discoidin domain receptors promote $\alpha 1\beta 1$- and $\alpha 2\beta 1$-integrin mediated cell adhesion to collagen by enhancing integrin activation. *PLOS ONE* 7:e52209

Xu J, Wang F, Van Keymeulen A, Herzmark P, Straight A, et al. 2003. Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell* 114:201–14

Zaman MH, Trapani LM, Sieminski AL, MacKellar D, Gong H, et al. 2006. Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *PNAS* 103:10889–94

Zhong C, Chrzanowska-Wodnicka M, Brown J, Shaub A, Belkin AM, Burridge K. 1998. Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly. *J. Cell Biol.* 141:539–51

Zijlstra A, Lewis J, Degryse B, Stuhlmann H, Quigley JP. 2008. The inhibition of tumor cell intravasation and subsequent metastasis via regulation of in vivo tumor cell motility by the tetraspanin CD151. *Cancer Cell* 13:221–34