Chapter 20
Modeling Morphodynamic Phenotypes and Dynamic Regimes of Cell Motion

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Abstract Many cellular processes and signaling pathways converge onto cell morphology and cell motion, which share important components. The mechanisms used for propulsion could also be responsible for shape changes, if they are capable of generating the rich observed variety of dynamic regimes. Additionally, the analysis of cell shape changes in space and time promises insight into the state of the cytoskeleton and signaling pathways controlling it. While this has been obvious for some time by now, little effort has been made to systematically and quantitatively explore this source of information. First pioneering experimental work revealed morphodynamic phenotypes which can be associated with dynamic regimes like oscillations and excitability. Here, we review the current state of modeling of morphodynamic phenotypes, the experimental results and discuss the ideas on the mechanisms driving shape changes which are suggested by modeling.

1 Introduction

Cell motility plays a key role in tumor cell migration and enables the directed movement of embryonic cells to the appropriate locations in the body [141]. Understanding the mechanisms of cell motility might be a basic tool to inhibit cancer spread or prevent cardiac malfunctions [48].

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The goal of this review is to critically discuss and classify mathematical models which capture the essential biological dependencies found experimentally. Such models show by reduction the most important interactions of a very complex biomechanical system. Also, models can predict how the migration pattern changes by perturbing different mechanisms, and offer therefore further insight into the biological phenomena.

Mathematical models for cell motility cover several levels of description – from single actin filaments to cell fragments, whole cells, and tissues [26]. They also focus on the description of different aspects of cell motility: initiation of actin-assembly and pre-merging conditions, perpetuation mechanisms after movement has started, adhesion and the interaction with the extra-cellular matrix, morphodynamics, or cell-to-cell communication and group dynamics of migrating cells. Here, we concentrate on the morphodynamics of single crawling cells.

The cell shape is mainly determined by the cell cytoskeleton, which is one of the main players in cell crawling. Hence, understanding the external cell shape deformations can indirectly provide information about the state of the motility machinery of the cell. Cell crawling occurs by the interplay of leading edge protrusion, adhesion of the front, deadhesion of the back, and contraction of the cell body [3, 57]. Membrane protrusion, that also determines the cell shape dynamics, occurs by the extension of a thin flat cytoskeletal structure, the lamellipodium, in the direction of motion. Inside the lamellipodium, a network of cross-linked actin filaments grows by polymerization in the direction of movement. This actin network, attached to the extra-cellular matrix and to the rest of the cell body, can be viewed as the motor of the cell. The main mechanisms that drive it are briefly reviewed in the following.

2 Basic Ideas on the Motile Machinery of Cells

The cytoskeleton of the cell contains several biopolymers that differ in stiffness and polarity. They can grow and shrink, rearrange, cross-link, and form bundles. This determines the form of the cell and can also generate movement. The force of protrusion in the lamellipodium is believed to arise from the polymerization of actin [92, 112]. Actin polymers are found in bundles in the interior of the lamellipodium, where myosin motor molecules can move along them to create contractions. Toward the leading edge membrane, actin forms a polar network with the fast polymerizing ends directed toward the membrane. At the opposite end, filaments depolymerize, actin monomers are recycled and diffuse to the front, where they are consumed by the growing tips [110]. This process of treadmilling is regulated by a number of proteins [14, 34, 55, 66, 87, 128]. Arp2/3 (actin related protein 2/3) binds to an existing actin filament and nucleates a new branch. Arp2/3 is activated by regulatory proteins, like the membrane associated WASP. Capping proteins bind to the end of a filament and prevent polymerization and depolymerization. Cofilin binds to filaments, enhances depolymerization, and severs them. Profilin binds to actin

monomers and favors the recycling of actin monomers into filaments. Thymosin β_4 binds actin monomers preventing their polymerization and acts as a buffer for monomeric actin. Different kinds of cross-linking proteins connect filaments and provide mechanical stability to the network. Other proteins are believed to bind actin polymers to the membrane.

Several studies have found differences in the actin network region just behind the leading edge and the network further in the bulk of the lamellipodium [111, 121, 133]. This leads to a picture where two different actin arrays – the wide lamella and a narrow lamellipodium in front or on top of the lamella – are pushing the membrane. While the lamellipodium is rich in branched polymerizing and depolymerizing actin filament ends, the lamella consists of more strongly cross-linked or bundled filaments. Earlier studies suggested the lamellipodium network to be highly branched and cross-linked very close to the leading edge membrane already [131, 139]. More recent studies showed that the branch point density in the lamellipodium may be rather low and the lamellipodium-like structures may extend several hundred nanometers into the cell [137]. The studies also differ in their results on filament length. While some conclude that filaments in the lamellipodium have a length of a few hundred nanometers [131, 139], others find a few micrometers [75,122,123,137]. In the dual picture, protrusion and retraction of the leading edge is due to the lamellipodium, while the lamella plays the main role in cell translocation, by integrating contractions due to myosin motors with adhesions to the substrate [13, 29, 30, 75]. Other studies however question the existence of two different actin networks in migrating cells [123] and a lamella beneath the lamellipodium [138].

3 A Short Review of the Scientific Discussion on Actin Filament Attachment to the Leading Edge Membrane of Lamellipodia and the Evidence for the Presence and Functioning of F-Actin-Membrane-Linking Proteins There

Attachment of filaments to the surface of the object which is moved by actin polymerization is found in many reconstituted systems and biomimetic systems. That observation led to the formulation of the tethered ratchet model [98]. Attached filaments may fundamentally change the force balance at the obstacle surface since they can exert pulling forces. Indeed, pushing and pulling forces exerted by attached and polymerizing filaments respectively, may both be much stronger than the resulting difference, which is then equal to the force actually moving the object [43, 98]. Hence, it is worth discussing whether filaments also attach to the leading edge membrane of lamellipodia and whether models should take that into account.

While there is no direct proof of attachment of filaments to the lamellipodium leading edge membrane, Carlier and Pantaloni state “Biomimetic assays of propulsion of N-WASP-functionalized microspheres or vesicles have demonstrated that the actin tail is attached to the particle surface…, suggesting that similar bonds

exist between the filaments and the membrane during protrusion" [14]. Attachment was also observed with oil droplets used as biomimetic system [135]. Binding of filaments to leading edge membrane is discussed as a possibility, suggestion, or even necessity for directed motion by several groups and labs [14, 15, 27, 75]. Keren and Theriot also point out "the high concentration of protein complexes at the leading edge and their extensive connections to the actin cytoskeleton" [72]. Co et al. demonstrate that actin filaments can bind to WH2 domains also independently of the branching process [27, 130]. Hence, even without considering the major F-actin-membrane linker ezrin, radixin, and moesin (ERM proteins), membrane binding of F-actin is suggested.

Only activated ERM proteins link F-actin to membrane proteins. They are activated by first binding PIP2 and subsequent phosphorylation at a threonine residue (T576 ezrin, T558 moesin, and T564 radixin) [46, 47]. ERMs are phosphorylated by myotonic dystrophy kinase-related Cdc42-binding kinase in filopodia [100], protein kinase Cα in membrane protrusions [101], and the Rho-associated kinase (ROCK) in microvilli [104], although this latter finding is controversial [93]. G protein-coupled receptor kinase 2 phosphorylates radixin in epithelial cells [71]. The Nck-interacting kinase NIK phosphorylates ERM proteins in rat mammary epithelial cells and in CCL39 fibroblasts [7]. ERM proteins are phosphorylated in response to stimuli linked to motility and morphodynamics.

Active ERM proteins and their binding partners are located at the leading edge. Phosphorylated ezrin is localized in ruffles and at the leading edge of pseudopodia of fibroblasts [85]. Similarly, phosphorylated ezrin and NIK were found at the distal margins of lamellipodia in mammary epithelial cells [7]. Baumgartner et al. mention the interesting idea that localization of kinases may sharpen the localization of pERM at the distal margins of lamellipodia beyond the localization of ERM, which is already restricted to lamellipodia [7]. The Na^+–H^+-exchanger NHE1 is one of the ERM binding partners in the plasma membrane [36]. NHE1 is enriched in lamellipodia and membrane tufts of fibroblasts [36, 60, 114] (and other cell types [74, 83]) and the membrane pool of ezrin is predominantly bound to NHE1 [36]. NHE1 can also be found along the smooth edge of the cell [36]. Ezrin localization showed a striking overlap with NHE1, but radixin was only found in lamellipodia and membrane tufts [36]. It is interesting to note in this context that radixin was originally identified as a barbed end capping protein [136].

Activation of ERM proteins may cause lamellipodium formation and ezrin–NHE1 binding is required for normal lamellipodium shape. Radixin is involved in lamellipodia stability of nerve growth cones [25]. ERM are also involved in lamellipodium formation. Phosphorylation at T567 causes formation of lamellipodia in LLC-PK cells [50]. F-actin networks extended to the peripheral edge of membrane protrusions in fibroblasts expressing NHE1, which was able to bind ezrin, but not in fibroblasts deficient of NHE1 or expressing NHE1 not able to bind ezrin [36]. Loss of NHE1-dependent cytoskeletal anchoring impairs directionality of cell migration [35]. Migrating fibroblasts expressing ezrin-binding NHE1 form a broad lamellipodium, by contrast with migrating cells expressing NHE1 unable to bind ezrin which form many small protrusions [35].

Another actin- and membrane-binding protein – myristoylated alanine-rich C kinase substrate (MARCKS) – is involved in lamellipodia formation [117]. It translocates to the membrane upon dephosphorylation. MARCKS is phosphorylated at Ser 159 by Rho-kinase as well as PKC [67, 99, 132]. In SH-SY5Y cells, stimulation with insulin-like growth factor-I (IGF-I) causes dephosphorylation of MARCKS. PI3-K has been reported to be involved in the dephosphorylation via activation of the PI3-K/Akt pathway [106,118,140]. PI3-K inhibitors attenuated the IGF-I-induced dephosphorylation of MARCKS, MARCKS translocation to lipid rafts and lamellipodia formation. These results support the idea that the transient dephosphorylation of MARCKS induced by IGF-I triggers the translocation of MARCKS to lipid rafts and lamellipodia formation [144]. IGF-I stimulation of SH-SY5Y cells caused the translocation of MARCKS to lipid rafts in the edge of lamellipodia, where it forms a complex with PIP2 [144]. Knockdown of MARCKS with siRNA technology abolished lamellipodia and neurite formation induced by IGF-I [144]. Cells exhibited a small number of tiny lamellipodia-like structures at the cell edge instead but not widely spread F-actin structures. That is evocative of the small protrusions reported from migrating fibroblasts expressing NHE1 unable to bind ezrin [35].

IGF-I stimulation also transiently decreases RhoA–GTP content in SH-SY5Y cells [118]. The RhoA/Rho-kinase pathway is considered to be a major target of the PI3-K/Akt signaling pathway, and PI3-K negatively controls RhoA activity [106, 140]. Hence, a link from MARCKS to ERM proteins via RhoA might exist.

Gelsolin is an actin severing and barbed end capping protein [129, 145, 146]. Gelsolin can bind actin filaments and membrane at the same time [61, 94]. Gelsolin interacts with PIP2, which inhibits capping [68]. Whether PIP2 also uncaps filaments [38, 115] or not [79] is a matter of debate. Gelsolin can also bind polyphosphoinositide-free lipid vesicles and simultaneously to actin microfilaments [94]. CP (called CapZ in muscle) also caps F-actin barbed ends. It also interacts with PIP2 [62, 79]. It has also been suggested that CapZ can link F-actin and membrane independently of PIP2 [125]. Both gelsolin [124] and CapZ [28] are present in the lamellipodium. Hence, gelsolin and CP are further potential F-actin-membrane linkers.

Actin binding membrane proteins can stay at the leading edge despite the retrograde flow of the actin network. References [7,85] suggest pERM to be located directly at the leading edge. This is supported by another simple consideration. Actin binding proteins in the membrane are carried away by F-actin retrograde flow in the lamellipodium, if there is no counteracting force. Hence, actin binding proteins staying in the lamellipodium must either be anchored or transported retrogradely. Proteins in the leading edge membrane experience a force orthogonal to the membrane when they bind to actin in the lamellipodium. The force keeping them in the lipid bilayer provides the force counteracting retrograde transport and they are therefore not swept away by retrograde flow. Keren and Theriot remark on the observation that actin binding proteins at the leading edge do not flow rearward "The lack of lipid flow, together with the presence of a diffusion barrier at the

leading edge, imply that physical trapping may be sufficient for maintaining the localization of various essential membrane-bound components there" [72].

In summary, it has been shown that F-actin-membrane-binding is necessary for the formation of lamellipodia and that activated linker proteins are at the leading edge.

The effect of binding of F-actin to the membrane on the shape of lamellipodia favors larger coherent structures, as mentioned above [35, 144]. This suggests that the occurrence of pushing and pulling filaments at the leading edge does not strongly distort the membrane on the length scale of typical filament distances. This is supported by another estimate. We can obtain an idea about the scale on which cellular forces cause membrane distortion from an estimate of the critical radius for bleb formation. Blebbing occurs at patches of membrane not bound to the actin cortex. The pressure difference across the membrane drives blebbing. Membrane tension and resistance to bending counteract deformation and cause a minimal critical radius of the unattached membrane patch. Sheetz et al. estimated it to be about 470 nm [117]. Hence, the critical diameter is at least by a factor of nine larger than typical distances of filaments in lamellipodia, if calculated from filament density measurements (100/μm, lamellipodium height 200 nm). More recently, filament distance in lamellipodia was estimated to be even 30 nm only [137]. In summary, there are good reasons to assume that membrane distortion is negligible on the length scale of filament distances. Modeling methods for dealing with membrane shape on larger length scales have been published [44, 73].

Modeling has shown that transient binding is compatible with protrusion [43, 43, 98, 147]. Based on these considerations, we conclude that the experimental evidence strongly suggests inclusion of F-actin-membrane binding into lamellipodium leading edge models.

4 Dynamic Regimes of Actin-Based Motion

When placed on a substrate, cells spread and eventually start moving spontaneously or as a result of mechanical or chemical stimulation. Sometimes cells are found to be testing the substrate, the topology of which influence the behavior [109]. The movement of the cell boundary can occur continuously or in cycles of protrusion and retraction. Mouse embryonic fibroblasts spreading on a fibronectin-coated glass show phase transitions from a resting state to a state of fast and continuous spreading and further to periodic membrane retractions [39]. Lateral membrane waves with a lateral speed of about 100 nm/s have been observed in a variety of spreading cells, including mouse embryonic fibroblasts, T cells, as well as wing disk cells from fruit flies [40]. For keratocytes, the leading edge morphology seems to be coupled to the motile behavior – coherent, smooth cells migrate significantly faster than decoherent, rough cells [82]. Epithelial cells show three different protrusion phenotypes: A state where long cell edge sectors are synchronized in cycles of protrusion and retraction, a state where random bursts of protrusion initiate protrusion waves propagating transversally in both directions, and a state where

continuous protrusion is occasionally interrupted by self-propagating ruffles [91]. Cells switch between states depending on the Rac1 activation level and the PAK and Arp2/3 concentrations. Increased Rac1 levels lead to increased activation of Arp2/3 and inhibition of cofilin via PAK [41, 65]. Arp2/3 nucleates filaments on existing filaments [64,127], and cofilin severs filaments and promotes their depolymerization [6,15,84]. Changing the activities of Rac1, PAK, and Arp2/3 results therefore into a change of the most important parameters of the actin network – density, length, and growth velocity. Thus, experiments show that in principle, the structure and function of the actin network inside the cell can be mapped into the external shape dynamics, which can be observed without interfering directly with the cell.

Experiments on model systems, such as protein-coated beads or fluid droplets placed in a motility medium, are helpful in understanding the motile machinery inside a cell. The motion of protein-coated plastic beads can be smooth or saltatory, depending on the bead radius and the surface concentration of the protein [9, 105]. Also, deformable lipid vesicles show both regimes of motion, and can reach up to 10 μm/min, compared to 3–4 μm/min for beads. A comparative study comes to the conclusion that hard and fluid actin propelled objects rely on different mechanisms to establish and maintain directed movement: Stress relaxation within the actin gel prevents the accumulation of filaments at the front of moving beads, while segregation of nucleators reduces actin polymerization at the front of moving vesicles [33]. Similarly, oil droplets can show continuous or hopping motion in a motility assay [12, 135]. The probability for oscillatory movement is higher for smaller droplets, and the oscillatory mechanism seems to be based on diffusion and convection of the surface protein activating actin polymerization.

5 Modeling Concepts

We distinguish in the following between continuum and filament models. This classification is not based on the mathematical form of the model, but rather on the primary treatment of the actin cytoskeleton. Continuum models start from the theory for visco-elastic gels and the filament properties enter via constitutive equations and material constants. Filament models start from the properties of single filaments and investigate how a population or network composed of them behaves.

5.1 Continuum Models

Part of the theoretical work on cell motility has been done within the framework of continuum models. Such models treat the cytoskeleton as a continuum medium and do not consider the microscopic details of the force generation process. Existing continuum models are based on various physical theories and differ in the choice of the state variables used to describe the cytoskeleton.

Several approaches focus on the biochemical processes inside the cell. The dynamics of the cytoskeleton are thereby described by the concentration of

actin filaments as well as of the regulatory proteins controlling their growth, leading to coupled systems of differential equations. Mogilner et al. establish reaction–diffusion equations for the actin monomers in their different forms (ADP–G-actin–ADF/cofilin, ADP–G-actin–profilin, ATP–G-actin–profilin, and ATP–G-actin–thymosin β_4 complexes) and include growth by polymerization of the barbed ends of actin filaments, capping and depolymerization [96]. The study calculates stationary velocities as stationary solution of the set of reaction diffusion equations in dependence on concentrations of capping proteins, thymosin β, profilin, and other biochemical parameters. Its force balance at the leading edge includes pushing forces from polymerizing filaments and a constant force as membrane resistance. Force dependence of polymerization and the limitations by G-Actin flux toward the front lead to an optimal filament density for a given membrane resistance.

A study by Grimm et al. aims at predicting the shape of the leading edge [59]. It models the dynamics of the density of right and left oriented barbed ends by considering growth, branching, and capping but not retrograde flow or filament attachment. The resistance of the membrane to motion is a constant force. Consequently, leading edge velocity increases with filament density in that model. The feedback for the shape of the leading edge to the actin density increases densities at local protrusions. This positive feedback loop may cause shape instability at high capping rate. The model predicts well the leading edge shape of fish keratocytes at low capping rates. The theory was supplemented by G-actin consumption by growth and membrane tension in the stability analysis in [73, 82].

Dawes et al. [31] consider the spatial distribution of actin filaments and their barbed ends in a simplified 1D geometry. The model includes diffusion of the Arp2/3 complex, force-dependent polymerization, retrograde flow, spontaneous nucleation, tip and side branching as well as capping and depolymerization. As in many models of this type, the protrusion rate is proportional or equal to the polymerization rate. Increasing the rate of nucleation of filaments (by the actin related protein Arp2/3) or the rate of actin polymerization leads to faster cell speed, whereas increasing the rate of capping or the membrane resistance reduces cell speed in this study. A simple model [49] considers the densities of barbed and pointed ends, coupled to a reaction–diffusion equation for the concentration of actin monomers and allows for the description of the polarization of an initially symmetric cytoskeleton and the initiation of motion.

A very extensive model has been developed in [10, 11]. It provides a method to solve the complete nucleotide profile within filaments by considering the cycle of actin-assembly and disassembly, including many details such as ATP hydrolysis and the role of profilin in the nucleotide exchange.

Other models focus on the mechanics of the cytoskeleton, which is treated as viscous or visco-elastic fluid. References [2, 81] consider two dynamic components: the cytosol, treated as a Newtonian fluid, and the polymerized actin filaments, treated as an elastic medium. Adhesion kinetics is considered here through a frictional force on the filamentous phase. The idea of a two-phase network has been elaborated further in [103], where a nonlocal pressure term modeling long-range network compaction was included. A variety of models consider one-dimensional

visco-elastic strips as a model for a radial cross-section through the lamellipodium [58, 77, 86]. Gracheva and Othmer consider a one-dimensional visco-elastic cell in contact with a viscous substrate [58]. The inclusion of graded adhesion (strong at the front, weak at the rear) allows for reproduction of the bell-shaped dependence of the cell velocity on adhesion strength [37, 58].

In [51], the actin network around a bacteria is treated by an elastic approach. Filament growth on the bacterial surface produces here elastic stresses that propel the bacterium forward. The same idea is used in [70] for the study of the symmetry break at the formation of the actin tail around a propelled bead in a biomimetic assay. Reference [126] treats the cell as an incompressible, visco-elastic solid and uses classical mass balance and equilibrium equations to describe its motion. This model allows to make predictions about the traction patterns on the substrate.

Based on a generic theory for active polar gels [76, 77], a model for the lamellipodium motion was developed in [78]. Here, the cytoskeleton is treated as a viscous polar gel. Myosin contraction in the cytoskeleton is included through an additional intrinsic anisotropic stress.

A model coupling membrane elasticity with actin polymerization has been proposed in [119] to explain membrane waves driven by actin and myosin. The wave mechanism is based on the presence of freely diffusing membrane proteins, the curvature of which influences the morphodynamic pattern of the cell.

5.2 Filament Models

A first model aiming to explain how polymerization of actin filaments can produce the force of protrusion in migrating cells was proposed in [108]. This "Brownian ratchet" model considers the polymerization of a stiff filament against a barrier, upon which a load acts. The barrier is able to diffuse, and the ratchet mechanism is based on the intercalation of monomers between the barrier and polymer tip. This model has been extended to an "elastic Brownian ratchet" model in [97], by including the thermal motions of the polymerizing actin filaments. It was further extended in [98] by including transient attachment to the obstacle ("tethered ratchet").

The entropic force exerted by a grafted semiflexible polymer on a rigid obstacle has been calculated both analytically and by Monte Carlo simulations in [53]. Explicit scaling functions as well as analytical results for certain asymptotic regimes were found. These results were used in [43, 54] in a model for the actin-based propulsion of flat rigid obstacles. Polymerization, attachment to and detachment from the obstacle as well as cross-linking between filaments were considered. The model is used to find the dynamics of the length for attached and detached filaments, which is required for the computation of the total force on the obstacle. This approach has been extended to the propulsion of soft membranes under tension in [44] as well as of rigid spherical beads in [42]. The actin network is described here also by continuous state variables reflecting the densities and lengths of the actin filaments. However, in contrast to the models discussed in the previous section, the microscopic form of the force exerted by single actin filaments is taken into account here.

Several studies model the microscopic growth of the actin network explicitly. In [16], a stochastic simulation frame for an actin network growing against an obstacle is proposed, where single filaments and single subunits in each filament are considered. Growth, depolymerization, capping, and branching are included, allowing the prediction of the network growth velocity. Based on this model, the structure of branched actin networks has been analyzed in [18]. In [4], actin filaments are treated as rigid rods under volume exclusion. Polymerization, depolymerization, branching, and capping are simulated using a continuous-time Markov algorithm, allowing for the prediction of the angular distribution of the filaments with respect to the leading edge.

A mesoscopic network approach to the cross-linked actin network has been proposed in [32]. Here, an Accumulative Particle-Spring model that builds on the elastic gel model [51] is used. Network links have no direct correspondence to actin filaments, but the bulk visco-elastic properties of the chains of nodes and springs are intended to capture the bulk visco-elastic properties of the actin network.

5.3 Coupling of Membrane and Cytoskeletal Dynamics

The common goal of most modeling approaches is finding the dynamics of the considered obstacles, e.g., the regime of motion of a bead or bacteria, or morphology of the leading edge. To this end, the dynamics of the cytoskeleton has to be coupled to the mechanics of the membrane. Most models do not include directly this interaction, but assume that the membrane moves at the growth velocity of the network. A model focusing on the membrane–cytoskeleton coupling has been proposed in [147]. It combines a filament model [54] for the filament tips that reach to the leading edge with and a continuum description of the cross-linked part of the actin network farther in terms of the active polar gel model [78]. Thereby, the filament model provides the force boundary condition for the visco-elastic part of the network. In return, the flow of this network provides the grafting points of the filament tips described by the filament model. This allows for the calculation of the total force exerted on the membrane that is used to find its dynamics.

6 Mechanisms Suggested by Models

6.1 Comparison of Model Assumptions

One of the main differences between continuum and filament models lies in the way the interaction between the actin network inside the cell and the cell membrane is included. For continuum models, the interaction force is assumed to be either a given constant [78] or to depend mainly on the membrane geometry, e.g., on the curvature [2, 119, 120]. Filament models include often the length, position, and orientation of actin filaments, which allow for a more accurate calculation of the entropic force exerted on the membrane [4, 5, 16, 17, 43, 44, 53, 54, 147].

Some continuum models require the knowledge of force boundary conditions, that have to be included artificially, by assuming for example a constant external force. In [78], such a boundary condition is needed to determine the force profile in a gel strip across the leading edge and critically influences the resulting leading edge velocity. Different other models calculate the interaction force in various ways. In the two-phase flow model [2], the pressure exerted by both filamentous phase and solvent phase are included explicitly. The analysis of *Listeria* propulsion in [51] and the study of symmetry breaking leading to actin tail formation [70] assume the interaction between the actin network and the obstacle relies on the formation of an elastic stress at the obstacles surface, due to the creation of a new layers of gel through polymerization. In [119, 120], membrane waves based on the competition between protrusive forces due to actin, and contractile forces due to myosin are studied. Here, the protrusion force is assumed to be proportional to the local concentration of membrane proteins driving actin polymerization. Additionally, membrane tension and elastic force are included. A common feature of these continuum models is that the assumed interaction between the actin network and membrane/obstacle involves almost exclusively properties of the membrane or obstacle, and not of the actin filaments, that are not modeled explicitly. However, it is known that the entropic force exerted by single actin filaments on an obstacle depends strongly on their fluctuating length and their position and orientation with respect to the obstacle being pushed [53, 97]. Additionally, in most continuum models and many filament models describing filaments as stiff rods, the sum of protrusion velocity and retrograde flow velocity equals the (effective, projected) polymerization velocity. However, experiments showed that this is not always the case [69, 89]. In order to include these observations, the properties of the filaments close to the leading edge have to be modeled explicitly like, e.g., in [43, 44, 147].

The growth of an actin network against an obstacle has been simulated in [16]. The approach includes the position of single filament tips, which is considered for the calculation of the total force on the obstacle. The dependence of this force on the filament orientation has been included in [17]. The response of filaments to force depends sensitively on the freely fluctuating length between the graft point closest to the leading edge and the filament tip experiencing the force [53]. That dependence is crucial in understanding different dynamic regimes of cell motion. Long free lengths yield slow edge velocities because filaments are too floppy to exert a strong pushing force and cell motion may even pause or stop if filaments become too long and floppy [43, 75]. Short free lengths yield slow velocities due to the polymerization rate limitation by strong force [8, 43, 75].

Explicit consideration of the length dynamics of actin filaments [43, 44, 54] allows to include the dependence of the interaction force on the free fluctuation length of the filaments. Initially, models made simplifying assumptions on the dynamics of the cross-linking points of the filaments that are critical ingredients in determining the force. Recently, a model combining a gel description of the actin network, a cross-linking dynamics accounting for diffusion of free cross-linkers and a filament description of the boundary has been studied [147]. The filament model provides here an accurate force boundary condition for the gel model, that, in turn, allows for the proper calculation of the filament position and length dynamics.

Another controversy between several models concerns the relation between local membrane velocity and local growth velocity of the actin network. Several models assume for simplicity that the membrane moves with the mean polymerization velocity of the actin network [31, 78, 113, 119, 120]. This is a strong constraint, meaning that the relative position of the filament tips with respect to the membrane is assumed to be constant. Such a constraint is realistic only during steady motion of a cell, for example, a crawling fish keratocyte. Experimentally, it has been shown that time shifts up to 20 s between the maxima of protrusion and polymerization velocity at the leading edge are possible in dynamic regimes with oscillatory motion [69].

Most models do not reproduce this phase shift between polymerization and protrusion, and that has to be seen in connection with the force balance at the leading edge, the way retrograde flow is included, the force–velocity relation and the relation between polymerization and leading edge motion. If the leading edge velocity is equated with the polymerization velocity (in some models subtracting a constant retrograde flow), there is no phase shift between protrusion and polymerization and the force–velocity relation will reflect the force dependence of the polymerization rate. However, measurements with fish keratocytes showed that the force–velocity relation is different from the force dependence of polymerization [63, 112].

Several models do not couple actin network and boundary motion by the same velocity, but by the same interaction force, according to Newton's third law. The processes contributing to the force balance and the relation between force and gel flow as well as force and membrane velocity then decide whether the measured force–velocity relation for the whole cell and the measured phase behavior are explained by the model. Many studies assume a linear relation between the total force exerted on the membrane and the resulting membrane velocity [16, 43, 44, 53, 119, 120, 147]. That relation results from the assumption of a viscous drag to over-damp membrane velocity dynamics. This drag comprises viscous drag from the external medium and the transport of membrane to the protruding parts of the cell. As mentioned above, some models describe membrane resistance as a constant force.

The force driving protrusion is due to polymerizing filaments in lamellipodial motion [1, 14, 73, 110]. The force with which these filaments push against the membrane determines the polymerization rate [53, 98]. Models for the motion of protein-coated beads include also the force exerted by attached filaments on the obstacle surface [9, 42, 98]. Groswasser et al. derive a bi-phasic friction force–velocity relation from this transient attachment of filaments [9]. It causes an additional friction force proportional to the velocity at small velocities. At high velocities, this additional friction force vanishes, since the time during which filaments are attached drops at a certain velocity. Thus, for high velocities, the proportionality constant between force and velocity is reduced. This bi-phasic friction may lead to bead velocity oscillations [9]. Interestingly, velocity oscillations are possible also when the friction force–velocity relation is assumed to be linear, but attachment to the obstacle is considered in the computation of the total force on the obstacle by separating the dynamics of attached and detached filaments and tracking their mean length [43, 54]. This explicit consideration of attached filaments

comprises contributions of each of them to the force balance at the leading edge, which again may change the phase relation between protrusion and polymerization.

If there are only viscous or constant forces resisting motion, each change in the polymerization force necessarily entails immediately a change of velocity without phase shift. Other forces resisting motion – like the one from attached filaments – can modulate this temporal relation such that an increase in polymerization may first increase retrograde flow or compress filaments close to the leading edge and only slowly or later protrusion. That can be investigated by models like introduced in [147].

Mathematical models for cell motility vary further in the complexity of the biomechanical processes considered. Creation of new filaments by nucleation has been explicitly included in some filament models, either by assuming creation on existing filaments (autocatalytic model) or free creation on subsequent attachment to existing filaments (nucleation model) [4, 17–19, 22, 24, 90]. Similarly, filament capping and severing has been included explicitly in [21,23,90,95,96]. By contrast, other filament models assume implicitly that the processes involving creation and severing of filaments are balanced, such that a steady state with a constant number of active filaments is reached [43,44,53,97,147].

Several models include contraction of myosin motors explicitly, e.g., [20, 78, 86, 102, 113, 119, 120, 147]. Similarly, adhesion to the substrate might be explicitly included [78,81,102]. Other models neglect these processes, under the tacit assumption that protrusion at the leading edge is decoupled from attachment/detachment to the substrate and contraction of the cell body. Attachment to the membrane or the surface of propelled artificial objects is explicitly included in several models [44,53,90,98], motivated by different attachment mechanisms found experimentally [27, 52, 80]. The whole actin cycle including the major regulatory mechanisms has been modeled in [10, 11].

6.2 Comparison of Sets of Experiments Explained by Models

The morphodynamics of crawling cells has been analyzed in several experimental conditions and with different cell types. Using various analysis techniques and computational tools, high-resolution membrane velocity maps along the leading edge can be obtained from processing experimental images.

Velocity maps of crawling cells show distinguishable morphodynamic patterns, some of which seem to be characteristic to the cell type under the given experimental conditions. Experiments with spreading cells show lateral membrane waves [40], periodic lamellipodial contractions [56, 143], and phase transitions between different morphodynamic pattern during the spreading process [39]. Observation of different types of migrating cells has shown traveling waves with different profiles, like protrusions spreading laterally from one point of the membrane in both directions, traveling retractions, and slightly spatially modulated velocity oscillations [91].

Early models for cell migration aim to explain the global movement characteristics of the cell, e.g., traveling speed and steady height profile [31, 78, 86, 96, 97], shape determination of motile cells [73, 113] or the structure of the actin network [4, 16]. Later models include the morphology of the leading edge. The existence of periodic traveling waves along the lamellipodium can be explained by combining protrusion forces due to polymerization and contraction forces due to the presence of myosin motors [119, 120]. The wave mechanism described by Shlomovitz et al. requires molecules inducing lateral curvature of the leading edge and myosin activity [119]. However, at least some types of waves do not depend on myosin activity [40, 91]. An alternative wave formation mechanism, based on the competition between protrusive forces due to detached, polymerizing filaments and pulling forces due to attached filaments has been proposed in [44]. It reproduces the laterally traveling protrusions, the modulated velocity oscillations and the Rac-induced transition between both patterns. In agreement with experiments, the lateral velocity of protrusions is independent from cell velocity and both patterns do not depend on myosin activity [44].

The velocity oscillations of *Listeria* bacteria have been modeled with the elastic gel theory by Gerbal et al. [51]. This theory offers an explanation for oscillations due to a competition between actin gel growth from the sides and growth from the back of the bacterium, with different velocities and strengths for each. While the simulated period agrees well with experiments, velocity amplitudes are about one order of magnitude larger than measured values [51]. The filament model by Gholami et al. including dynamics of free filament length and filament attachment to the bacterium reproduces *Listeria* velocity oscillations quantitatively with respect to periods and amplitudes [54].

The validity of model predictions can be further tested with the help of biomimetic systems, where various parameters can be changed, in contrast to migrating or spreading cells. Experiments on protein-coated spherical beads [9, 33] and oil droplets [12, 33] have revealed different regimes of motion – continuous and oscillatory, and identified parameters that might induce transitions from one state of motion to the others. The same model as used by Gholami et al. for *Listeria* with slightly changed parameter values also reproduces the velocity oscillations observed with oil droplets including the onset of oscillations due to weakening of filament attachment by VASP [43, 134]. The mechanism has periodic attachment and detachment of filaments as central processes [43, 134]. There are several theoretical studies on bead motion characteristics [9, 42, 51, 90, 98]. Mogilner and Oster demonstrated the compatibility of attachment and propulsion by polymerization by the ground-breaking tethered ratchet model [98]. Elastic gel theory explains velocity oscillations of protein-coated beads [9] by a mechanism called the “soap effect”, “because it recalls the rapid motion of a wet bar of soap slipping away as it is slowly squeezed by hand”. [51]. This is mainly justified by scaling arguments for maximal velocities of the oscillations and the threshold for the onset of oscillations in dependence on the bead radius [9, 51]. The oscillation mechanism relies on a curved obstacle surface and the bi-phasic dependence of the friction force on bead velocity mentioned in the previous section. Bead motion has also been investigated

by the model used for *Listeria* motion, oil droplets, and morphodynamic phenotypes [42–44,54]. The oscillation mechanism is essentially the same as with oil droplets or *Listeria*. That model is able to simulate the velocity oscillations quantitatively with respect to periods and amplitudes except a small shift in the average velocity [42]. It also reproduces the dependence of the onset of oscillations on the bead radius and protein density on the bead surface.

While there are many models explaining very well certain aspects or systems of cell motility or morphodynamics, the appeal of the modeling concept including the dynamics of free fluctuating length of filaments, filament binding dynamics at the obstacle surface, force-dependent polymerization and – if required – nucleation and capping is for us the reproduction of experimental results with a variety of systems in a very intuitive way [42, 43, 45, 53, 147]. It also offers a natural explanation for the variety of dynamic regimes observed in cell motility, morphodynamics, and biomimetic systems.

7 Open Problems

According to our view on the field, there are three conceptually highly relevant problems the solution of which could advance the field: (1) Despite the molecular similarities between the variety of biological and biomimetic systems, there is not a unifying theory or model. (2) There is no satisfying theoretical explanation of the force–velocity relation of fish keratocytes. (3) The phase shift between protrusion and polymerization is unexplained and the function of the two functionally and structurally different regions of the lamellipodium – often described as lamellum and lamellipodium – has not been investigated theoretically.

The force–velocity relation of fish keratocytes must be shaped by the intracellular force generation mechanism. The compatibility of the ideas on force generation by actin polymerization with measured force–velocity relations has not been shown in a mathematical model yet. But this if of course required for a consistent theory on cell motility. The force–velocity relation exhibits a dramatic velocity drop upon first contact with an AFM cantilever or glass fiber followed by a concave-down relation in the slow-velocity regime [63, 112]. The discussion around it has focused on an explanation for the concave down part since this shape is in contradiction to the convex shape of the force exponential dependence of the actin polymerization rate [53, 97]. Most theoretical studies essentially neglected the initial velocity drop. Simulations of branched actin networks made of rigid rod-like filaments with excluded volume effects taken into account [116] produce a concave-down force–velocity relation. However, they predict stall forces by a factor of 20–50 too large. Brownian dynamics simulations of stiff actin filaments in a branched network [88] also give rise to a concave shaped force–velocity curve but velocities at half stall force are orders of magnitude faster than in experiments with fish keratocytes and stall forces are by a factor 200–400 too small. No retrograde flow is found in actin networks growing under an AFM cantilever [107]. The shape

of the force–velocity curve of those systems resembles that of keratocytes, though on much different scales – it takes more than 100 min to reach the stall force which is in the order of 300 nN. Weichsel and Schwarz suggested to explain this behavior by a configurational bistability of the actin network [142]. However, that bistability has recently been excluded as the mechanism of the force–velocity relation in fish keratocytes by Heinemann et al. [63]. They repeated the measurement with the same cell and a time lag of 30–40 s. The second measurement should have provided results different from the first one, if the actin network exhibited configurational bistability and a state transition shaped the force–velocity relation. However, two repetitions showed the same outcome as the first measurement. Heinemann et al. excluded the autocatalytic branching model by the same reasoning. That model explains the plateau after the initial drop by growing filament density [16]. Such an increase in density should also affect the second and third measurement, according to [63], what was not observed.

We subsume the phase shift between polymerization and protrusion and the structure of the lamellipodium under one problem, because it is likely that the phase shift depends on structural elements close to the leading edge membrane showing dynamics which has not been accounted for by mathematical models yet. Several processes may contribute to such a phase shift: Polymerization drives not only protrusion but also retrograde flow, the region close to the leading edge might be much softer than modeled until now, protrusion might not only depend on polymerization forces but also on the binding of filaments to the membrane pulling it back. Zimmermann et al. have recently suggested a model including these processes but it has not been applied to the problem yet [147].

The vision of modeling of morphodynamics is to infer at least in part the state of signaling pathways and the cytoskeleton from observing the changes of cell shape and velocity. The starting point can be a biomechanical model in terms of the elemental processes and rates like polymerization, depolymerization, capping and nucleation, elastic responses, retrograde flow, membrane tension, etc. The above-mentioned problems show that this still has to be established. Modeling of the control of the parameters of such a model by signaling pathways can then lead to a comprehensive understanding of morphodynamics and motility.

References

1. Abraham V, Krishnamurthi D, Taylor D, Lanni F (1999) The actin-based nanomachine at the leading edge of migrating cells. Biophys J 77:1721–1732
2. Alt W, Dembo M (1999) Cytoplasm dynamics and cell motion: two phase flow models. MathBiosci 156:207–228
3. Ananthakrishnan R, Ehrlicher A (2007) The forces behind cell movement. Int J Biol Sci 3(5):303–317
4. Atilgan E, Wirtz D, Sun S (2005) Morphology of the lamellipodium and organization of actin filaments at the leading edge of crawling cells. Biophys J 89:3589–3602
5. Atilgan E, Wirtz D, Sun S (2006) Mechanics and dynamics of actin-driven thin membrane protrusions. Biophys J 90:65–76

6. Bamburg J (1999) Proteins of the adf/cofilin family: essential regulators of actin dynamics. Ann Rev Cell Dev Biol 15:185–230
7. Baumgartner M, Sillman A, Blackwood E, Srivastava J, Madson N, Schilling J, Wright J, Barber D (2006) The nck-interacting kinase phosphorylates erm proteins for formation of lamellipodium by growth factors. PNAS 103:13391–13396
8. Bear JE, Svitkina TM, Krause M, Schafer DA, Loureiro JJ, Strasser GA, Maly IV, Chaga OY, Cooper JA, Borisy GG, Gertler FB (2002) Antagonism between ena/vasp proteins and actin filament capping regulates fibroblast motility. Cell 109(4):509–521
9. Bernheim-Groswasser A, Prost J, Sykes C (2005) Mechanism of actin-based motility: a dynamic state diagram. Biophys J 89:1411–1419
10. Bindschadler M, McGrath J (2007) Relationships betwenn actin regulatory mechanisms and measurable state variables. Annals Biomed Eng 35:995–1011
11. Bindschadler M, Osborn E, Dewey C, McGrath J (2004) A mechanistic model of the actin cycle. Biophys J 86:2720–2739
12. Boukellal H, Campas O, Joanny J, Prost J, Sykes C (2004) Soft listeria: actin-based propulsion of liquid drops. Phys Rev E 69:061906
13. Bugyi B, Didry D, Carlier M (2010) How tropomyosin regulates lamellipodial actin-based motility: a combined biochemical and reconstituted motility approach. EMBO J 29:14–26
14. Carlier M, Pantaloni D (2007) Control of actin assembly dynamics in cell motility. J Biol Chem 282(32):23005–23009
15. Carlier M, Ressad F, Pantaloni D (1999) Control of actin dynamics in cell motility – role of adf/cofilin. J Biol Chem 274:33827–33830
16. Carlsson A (2001) Growth of branched actin networks against obstacles. Biophys J 81: 1907–1923
17. Carlsson A (2003) Growth velocities of branched actin networks. Biophys J 84:2907–2918
18. Carlsson A (2004) Structure of autocatalitically branched actin solutions. Phys Rev Lett 92(23):239102
19. Carlsson A (2005) The effect of branching on the critical concentration and average filament length of actin. Biophys J 89(1):130–140
20. Carlsson A (2006) Contractile stress generation by actomyosin gels. Phys Rev E 74:051912
21. Carlsson A (2006) Stimulation of actin polymerization by filament severing. Biophys J 90:413–422
22. Carlsson A (2010) Dendritic actin filament nucleation causes traveling waves and patches. Phys Rev Lett 104:228102
23. Carlsson A, Sept D (2008) Mathematical modeling of cell migration. Biophys Tools Biol: Vol 1 In Vitro Techniques 84:911
24. Carlsson A, Wear M, Cooper J (2004) End versus side branching by arp2/3 complex. Biophys J 86:1074–1081
25. Casteo L, Jay D (1999) Radixin is involved in lamellipodial stability during nerve growth cone motility. Mol Biol Cell 5:1511–1520
26. Chauvière A, Preziosi L, Verdier C (eds) (2010) Cell mechanics. From single scale-based models to multiscale modeling. Taylor & Francis Group, Chapman & Hall/CRC Mathematical and Computational Biology Series, Boca Raton
27. Co C, Wong D, Gierke S, Chang V, Taunton J (2007) Mechanism of actin network attachment to moving membranes: barbed end capture by n-wasp wh2 domains. Cell 128:901–913
28. Cooper JA, Sept D (2008) New insights into mechanism and regulation of actin capping protein. Int Rev Cell Mol Biol, 267:183–206
29. Danuser G (2005) Coupling the dynamics of two actin networks – new views on the mechanics of cell protrusion. Biochem Soc Trans 33:1250–1253
30. Danuser G (2009) Testing the lamella hypothesis: the next steps on the agenda. J Cell Sci 122:1950–1962
31. Dawes A, Ermentrout G, Cytrynbaum E, Edelstein-Keshet L (2006) Actin filament branching and protrusion velocity in a simple 1d model of a motile cell. J Theor Biol 242:265–279

32. Dayel M, Akin O, Landeryou M, Risca V, Mogilner A, Mullins R (2009) In silico reconstitution of actin-based symmetry breaking and motility. PLoS Biol 7:e1000201
33. Delatour V, Shekhar S, Reymann AC, Didry D, Lê KHD, Romet-Lemonne G, Helfer E, Carlier MF (2008) Actin-based propulsion of functionalized hard versus fluid spherical objects. New J Phys 10(2):025001
34. Delorme V, Machacek M, DerMardirossian C, Anderson K, Wittmann T, Hanein D, Waterman-Storer C, Danuser G, Bokoch G (2007) Cofilin activity downstream of pak1 regulates cell protrusion efficiency by organizing lamellipodium and lamella actin networks. Dev Cell 13:646–662
35. Denker S, Barber D (2002) Cell migration requires both ion translocation and cytoskeletal anchoring by the Na–H exchanger NHE1. J Cell Biol 159:1087–1096
36. Denker S, Huang D, Orlowski J, Furthmayr H, Barber D (2000) Direct Binding of the Na H Exchanger NHE1 to ERM Proteins Regulates the Cortical Cytoskeleton and Cell Shape Independently of H^+ Translocation. Mol Cell 6:1425–1436
37. DiMilla PA, Barbee K, Lauffenburger DA (1991) Mathematical model for the effects of adhesion and mechanics on cell migration speed. Biophys J 60:15–37
38. DiNubile MJ, Huang S (1997) High concentrations of phosphatidylinositol-4,5-bisphosphate may promote actin filament growth by three potential mechanisms: inhibiting capping by neutrophil lysates, severing actin filaments and removing capping protein-[beta]2 from barbed ends. Biochim Biophys Acta (BBA) – Mol Cell Res 1358(3):261–278
39. Doebereiner HG, Dubin-Thaler B, Giannone G, Xenias H, Sheetz M (2004) Dynamic phase transitions in cell spreading. Phys Rev Lett 93(10):108105
40. Doebereiner HG, Dubin-Thaler B, Hofman J, Xenias H, Sims T, Giannone G, Dustin M, Wiggins C, Sheetz M (2006) Lateral membrane waves constitute a universal dynamic pattern of motile cells. Phys Rev Lett 97(3):038102
41. Eden S, Rohatgi R, Pdtelejnikov A, Mann M, Kirschner M (2002) Mechaniscm of regulation of wave1-induced actin nucleation by rac1 and nck. Nature 418:790–793
42. Enculescu M, Falcke M (2011) Actin-based propusion of spatially extended objects. New J Phys 13:053040
43. Enculescu M, Gholami A, Falcke M (2008) Dynamic regimes and bifurcation in a model of actin-based motility. Phys Rev E 78:031915
44. Enculescu M, Sabouri-Ghomi M, Danuser G, Falcke M (2010) Modeling of protrusion phenotypes driven by the actin-membrane interaction. Biophys J 98:1–11
45. Faber, M, Enculescu, M, Falcke, M (2010) Filament capping and nucleation in actin-based motility. Eur Phys J Special Topics 191:147–158, DOI 10.1140/epjst/e2010-01347-3, URL http://dx.doi.org/10.1140/epjst/e2010-01347-3
46. Fievet B, Gautreau A, Roy C, Del Maestro L, Mangeat P, Louvard D, Arpin M (2004) Phosphoionositide binding and phosphorylation act sequentially in the activation mechanism of ezrin. J Cell Biol 164:653–659
47. Fievet B, Louvard D, Arpin M (2007) Erm proteins in epithelial cell organization and functions. BBA – Mol Cell Res 1773:653–660
48. Friedel P, Hegerfeld Y, Tusch M (2004) Collective cell migration in morphogenesis and cancer. Int J Dev Biol 48:441–449
49. Fuhrmann J, Ks J, Stevens A (2007) Initiation of cytoskeletal asymmetry for cell polymerization and movement. J Theor Biol 249:278–288
50. Gautreau A, Louvard D, Arpin M (2000) Morphogenic effects of ezrin require a phosphorylation-induced transition from oligomers to monomers at the plasma membrane. J Cell Biol 150:193–203
51. Gerbal F, Chaikin P, Rabin Y, Prost J (2000) An elastic analysis of listeria monocytogenes propulsion. Biophys J 79:2259–2275
52. Gerbal F, Laurent V, Ott A, Carlier M, Chaikin P, Prost J (2000) Measurement of the elasticity of the actin tail of listeria monocytogenes. Eur Biophys J with Biophys Lett 29:134–140
53. Gholami A, Wilhelm J, Frey E (2006) Entropic forces generated by grafted semiflexible polymers. Phys Rev E 74(4):041803

54. Gholami A, Falcke M, Frey E (2008) Velocity oscillations in actin-based motility. New J of Phys 10:033022
55. Ghosh M, Sonx X, Mouneimne G, Sidani M, Lawrence D, Condeelis J (2004) Cofilin promotes actin polymerization and defines the direction of cell motility. Science 304: 743–746
56. Giannone G, Dubin-Thaler J, Doebereiner HG, Kieffer N, Bresnick A, Sheetz M (2004) Periodic lamellipodial contractions correlate with rearward actin waves. Cell 116: 431–443
57. Giannone G, Dubin-Thaler B, Rossier O, Cai Y, Chaga O, Jiang G, Beaver W, Dobereiner H, Freund Y, Borisy G, Sheetz M (2007) Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell 128:561–575
58. Gracheva MA, Othmer HG (2004) A continuum model of motility in ameboid cells. Bull Math Biol 66:167–193
59. Grimm H, Verkhovsky A, Mogilner A, Meister JJ (2003) Analysis of actin dynamics at the leading edge of crawling cells: implications for the shape of keratocyte lamellipodia. Eur Biophys J 32:563–577
60. Grinstien S, Woodside M, Waddell T, Downey G, Orlowski J, Pouyssegur J, Wong D, Foskett J (1993) Focal localozation of the nhe-1 isoform of the na1/h1 antiport: assesment of effects on intracellular ph. EMBO J 12:5209–5218
61. Hartwig JH, Chambers KA, Stossel TP (1989) Association of gelsolin with actin filaments and cell membranes of macrophages and platelets. J Cell Biol 108(2):467–479
62. Hartwig JH, Bokoch GM, Carpenter CL, Janmey PA, Taylor LA, Toker A, Stossel TP (1995) Thrombin receptor ligation and activated Rac uncap actin filament barbed ends through phosphoinositide synthesis in permeabilized human platelets. Cell 82(4):643–653
63. Heinemann F, Doschke H, Radmacher M (2011) Keratocyte lamellipodial protrusion is characterized by a concave force–velocity relation. Biophys J 100(6):1420–1427
64. Higgs H, Pollard T (2001) Regulation of actin filament network formation through arp2/3 complex: activation by a diverse array of proteins. Ann Rev Biochem 70:649–676
65. Huang T, DerMardirossian C, Bokoch G (2006) Cofilin phosphytases and regulation of actin dynamics. Curr Op Cell Biol 18:26–31
66. Ichetovkin I, Grant W, Condeelis J (2002) Cofilin produces newly polymerized actin filaments that are preferred for dendritic nucleation by the arp2/3 complex. Curr Biol 12:79–84
67. Ikenoya M, Hidaka H, Hosoya T, Suzuki M, Yamamoto N, Sasaki Y (2002) Inhibition of rhokinase-induced myristoylated alanine-rich c kinase substrate (marcks) phosphorylation in human neuronal cells by h-1152, a novel and specific rho-kinase inhibitor. J Neurochem 81: 9–16
68. Janmey PA, Stossel TPS (1987) Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate. Nature 325:362–364
69. Ji L, Lim J, Danuser G (2008) Fluctuations of intracellular forces during cell protrusion. Nat Cell Biol 10(12):1393–1400
70. John K, Peyla P, Kassner K, Prost J, Misbah C (2008) Nonlinear study of symmetry breaking in actin gels: implications for cellular motility. Phys Rev Lett 100(6):068101
71. Kahsai A, Zhu S, Fenteany G (2010) G protein-coupled receptor kinase 2 activates radixin, regulating membrane protrusion and motility in epithelial cells. BBA – Mol Cell Res 1803:300–310
72. Keren K, Theriot J (2008) Biophysical Aspects of actin-based cell motility in fish epithelial keratocytes. In: Cell motility, Springer New York, pp 31–58
73. Keren K, Pincus Z, Allen G, Barnhart E, Marriott G, Mogilner A, Theriot A (2008) Mechanism of shape determination in motile cells. Nature 453:485–U1
74. Klein M, Seeger P, Schuricht B, Alper S, Schwab A (2000) Polarization of Na^+/H^+ and Cl^-/HCO_3^- exchangers in migrating renal epithelial cells. J Gen Physiol 115:599–608
75. Koester S, Auinger S, Vinzenz M, Rottner K, Small J (2008) Differentially oriented populations of actin filaments generated in lamellipodia collaborate in pushing and pausing at the cell front. Nat Cell Biol 10:306–313

76. Kruse K, Joanny J, Jlicher F, Prost J, Sekimoto K (2004) Asters, vortices, and rotating spirals in actove gels of polar filaments. Phys Rev Lett 92(7):078101
77. Kruse K, Joanny J, Jlicher F, Prost J, Sekimoto K (2005) Generic theory of active polar gels: a paradigm for cytoskeletal dynamics. Eur Phys J E 16:5–16
78. Kruse K, Joanny J, Jlicher F, Prost J (2006) Contractility and retrograde flow in lamellipodium motion. Phys Biol 3:130–137
79. Kuhn JR, Pollard TD (2007) Single molecule kinetic analysis of actin filament capping. J Biol Chem 282(38):28014–28024
80. Kuo S, McGrath J (2000) Steps and fluctuations of listeria monocytogenes during actin-based motility. Nature 407:1026–1029
81. Kuusela E, Alt W (2009) Continuum model of cell adhesion and migration. J Math Biol 58:135–161
82. Lacayo C, Pincus Z, VanDujin M, Wilson C, Fletcher D, Gertler F, Mogilner A, Theriot J (2007) Emergence of large-scale cell morphology and movement from local actin filament growth dynamics. PLoS Biol 5(9):2035–2052
83. Lagana A, Vadnais J, Le P, Nguyen T, Laprade R, Nabi I, Noel J (2000) Regulation of the formation of tumor cell pseudopodia by the Na^+/H^+ exchanger NHE1. J Cell Sci 113: 3649–3662
84. Lai F, Bosse T, Szczodrak M, Benesch S, Auinger S, Faix J, Small J, Stradel T, Rottner K (2008) Arp2/3-complex regulation in motility and host–pathogen interaction. FEBS J 275:39
85. Lamb R, Ozanne B, Roy C, McGarry L, Stipp C, Mangeat P, Jay D (1997) Essential functions of ezrin in maintenance of cell shape and lamellipodial extension in normal and transformed fibroblasts. Curr Biol 7:682–688
86. Larripa K, Mogilner A (2006) Transport of a 1d viscoelastic actin–myosin strip of gel as a model of a crawling cell. Phys A 372:113–123
87. Le Clainche C, Carlier M (2008) Regulation of actin assembly associated with protrusion and adhesion in cell migration. Physiol Rev 88:89–513
88. Lee KC, Liu AJ (2009) Force–velocity relation for actin-polymerization-driven motility from brownian dynamics simulations. Biophys J 97(5):1295–1304
89. Lim JI, Sabouri-Ghomi M, Machacek M, Waterman CM, Danuser G (2010) Protrusion and actin assembly are coupled to the organization of lamellar contractile structures. Exp Cell Res 316(13):2027–2041
90. Lin Y (2009) Mechanics model for actin-based motility. Phys Rev E 79:021916
91. Machacek M, Danuser G (2006) Morphodynamic profiling of protrusion phenotypes. Biophys J 90:1439–1442
92. Marcy Y, Prost J, Carlier MF, Sykes C (2004) Forces generated during actin-based propulsion: a direct measurement by micromanipulation. PNAS 101(16):5992–5997
93. Matsui T, Yonemura S, Tsukita S, Tsukita S (1999) Activation of erm proteins in vivo by rho involves phosphatidyl-inositol 4-phosphate 5-kinase and not rock kinases. Curr Biol 9: 1259–1262
94. Méré J, Chahinian A, Maciver SK, Fattoum A, Bettache N, Benyamin Y, Roustan C (2005) Gelsolin binds to polyphosphoinositide-free lipid vesicles and simultaneously to actin microfilaments. Biochem J 386:47–56
95. Michalsky P, Carlsson A (2010) The effects of filament aging and annealing on a model lamellipodium undergoing disassembly by severing. Phys Biol 7:026004
96. Mogilner A, Edelstein-Keshet L (2002) Regulation of actin dynamics in rapidly moving cells: a quantitative analysis. Biophys J 83:1237–1258
97. Mogilner A, Oster G (1996) Cell motility drivern by actin polymerization. Biophys J 71:3030–3045
98. Mogilner A, Oster G (2003) Force generation by actin polymerization ii: the elastic ratchet and tethered filaments. Biophys J 84:1591–1605
99. Nagumo H, Ikenoya M, Sakurada K, Furuya K, Ikuhara T, Hiraoka H, Sasaki Y (2001) Rho-associated kinase phosphorylates marcks in human neuronal cells. Biochem Biophys Res Commun 280:605–609

100. Nakamura N, Pshiro N, Fukata Y, Amoano M, Fukata M, Kuroda S, Matsuura Y, Leung T, Lim K, Kaibuchi K (2000) Phosphorylation of erm proteins at filopodia induced by cdc42. Genes Cells 5:571–581
101. Ng T, Parsons M, Hughes W, Monypenny J, Zicha D, Gautreau A, Arpin M, Gschmeissner S, Verveer P, Bastiaens P, Parker P (2001) Ezrin is a downstream effector of trafficking pkc-integrin complexes involved in the control of cell motility. EMBO J 20:2723–2741
102. Novak I, Slepchenko B, Mogilner A, Loew L (2004) Cooperativity between cell contractility and adhesion. Phys Rev Lett 93:268109
103. Oliver J, King J, Mckinlay K, Brown P, Grant D, Scotchford C, Wood J (2005) Thin-film theories for two-phase reactive flow models of active cell motion. Math Med Biol 22:53–98
104. Oshiro N, Fukata Y, Kaibuchi K (1998) Phosphorylation of moesin by rho-associated kinase (rho-kinase) plays a crucial role in the formation of microvilli-like structures. J Biol Chem 273:34663–34666
105. Paluch E, van der Gucht J, Joanny JF, Sykes C (2006) Deformations in actin comets from rocketing beads. Biophys J 91(8):3113–3122
106. Papakonstanti E, Ridley A, Vanhaesebroeck B (2007) The p110d isoform of pi 3-kinase negatively controls rhoa and pten. EMBO J 26:3050–3061
107. Parekh SH, Chaudhuri O, Theriot JA, Fletcher DA (2005) Loading history determines the velocity of actin-network growth. Nat Cell Biol 7(12):1219–1223
108. Paskin C, Odell G, Oster G (1993) Cellular motions and thermal fluctuations: the Brownian ratchet. Biophs J 65:316–324
109. Pierres A, Benoliel A, Touchard D, Bongard P (2008) How cells tiptoe on adhesive surfaces before sticking. Biophys J 94:4114–4122
110. Pollard T (2003) The cytoskeleton, cellular motility and the reductionist agenda. Nature 422:741–745
111. Ponti A, Machacek M, Gupton S, Waterman-Storer C, Danuser G (2004) Two distinct actin networks drive the protrusion of migrating cells. Science 207:1782–1786
112. Prass M, Jacobson K, Mogilner A, Radmacher M (2006) Direct measurement of the lamellipodial protrusive force in a migrating cell. J Cell Biol 174:767–772
113. Rubinstein B, Jacobson K, Moginer A (2005) Multiscale two-dimensional modelling of a motile simple-shaped cell. Multiscale Model Simul 3(2):413–439
114. Sardet C, Counillon L, Franchi A, Pouyssegur J (1993) Growth factors induce phosphorylation of the na1/h1 antiporter, glycoprotein of 110 kd. Science 247:723–726
115. Schafer DA, Jennings PB, Cooper JA (1996) Dynamics of capping protein and actin assembly in vitro: uncapping barbed ends by polyphosphoinositides. J Cell Biol 135(1):169–179
116. Schreiber CH, Stewart M, Duke T (2010) Simulation of cell motility that reproduces the force–velocity relationship. Proc Natl Acad Sci 107(20):9141–9146
117. Sheetz M, Sable J, Dobereiner HG (2006) Continous membrane-cytoskeleton adhesion requires continous accomodation to lipid and cytoskeleton dynamics. Annu Rev Biophys Biomol Struct 35:417–434
118. Shiraishi M, Tanabe A, Saito N, Sasaki Y (2006) Unphosphorylated marcks is involved in neurite initiation induced by insulin-like growth factor-l in sh-sy5y cells. J Cell Physiol 209:1029–1038
119. Shlomovitz R, Gov N (2007) Membrane waves driven by actin and myosin. Phys Rev Lett 98:168103
120. Shlomovitz R, Gov N (2008) Exciting cytoskeleton-membrane waves. Phys Rev E 78:041911
121. Small J, Resch G (2005) The comings and goings of actin: coupling protrusion and retraction in cell motility. Curr Op Cell Biol 17:517–523
122. Small J, Herzog M, Anderson K (1995) Actin filament organization in the fish keratocyte lamellipodium. J Cell Biol 129(5):1275–1286
123. Small J, Auinger S, Nemethova M, Koestler S, Goldie K, Hoenger A, Resch G (2008) Unravelling the structure of the lamellipodium. J Microsc-Oxford 231:479–485
124. Small JV, Stradal T, Vignal E, Rottner K (2002) The lamellipodium: where motility begins. Trends Cell Biol 12(3):112–120

125. Smith J, Diez G, Klemm A, Schewkunow V, Goldmann W (2006) Capz-lipid membrane interactions: a computer analysis. Theor Biol Med Model 3(1):30
126. Stolarska M, Y K, Othmer H (2009) Multi-scale models of cell and tissue dynamics. Phyl Trans Roy Soc A 367:3525–3553
127. Stradal T, Scita G (2006) Protein complexes regulation arp2/3-mediated actin assembly. Curr Op Cell Biol 18:4–10
128. Stradal T, Rottner K, Disanza A, Confalonieri S, Innocenti M, Scita G (2004) Regulation of actin dynamics by wasp and wave family proteins. Trends Cell Biol 14:303–311
129. Sun HQ, Yamamoto M, Mejillano M, Yin HL (1999) Gelsolin, a multifunctional actin regulatory protein. J Biol Chem 274(47):33179–33182
130. Svitkina T (2007) N-wasp generates a buzz at membranes on the move. Cell 128:828–830
131. Svitkina TM, Verkhovsky AB, McQuade KM, Borisy GG (1997) Analysis of the actin-myosin II system in fish epidermal keratocytes: mechanism of cell body translocation. J Cell Biol 139:397–415
132. Tatsumi S, Mabuchi T, Katano T, Matsumura S, Abe T, Hidaka H, Suzuki M, Sasaki Y, Minami T, Ito S (2005) Involvement of rho-kinase in inflammatory and neuropathic pain through phosphorylation of myristoylated alanine-rich c-kinase substrate (marcks). Neurosci 131:491–498
133. Timpson P, Daly R (2005) Distinction at the leading edge of the cell. Bioessays 27:349–352
134. Trichet L, Campàs O, Sykes C (2007) Vasp governs actin dynamics by modulating filament anchoring. Biophys J 92:1081–1089
135. Trichet L, Campas O, Sykes C, Palastino J (2007) Vasp governs actin dynamics by modulating filament anchoring. Biophys J 92:1081–1089
136. Tsukita S, Hieda Y, Tsukita S (1989) A new 82-kd barbed end-capping protein (radixin) localized in the cell-to-cell adherens junction: purification and characterization. J Cell Biol 108:2369–2382
137. Urban W, Jacob S, Nemethova M, Resch G, Small J (2010) Electron tomography reveals unbranched networks of actin filaments in lamellipodia. Nat Cell Biol 12:429–435
138. Vallotton P, Small JV (2009) Shifting views on the leading role of the lamellipodium in cell migration: speckle tracking revisited. J Cell Sci 122(12):1955–1958
139. Verkhovsky A, Chaga O, Schaub S, Svitkina T, J-J M, Borisy G (2003) Orientational order of the lamellipodial actin network as demonstrated in living motile cells. Mol Biol Cell 14: 4667–4675
140. Watt S, Kular G, Fleming I, Downes C, Lucocq J (2002) Subcellular localozation of phosphytidylinositol 4,5-biphosphate using the pleckstrin homology domain of phospholipase cd1. Biochem J 363:657–666
141. Wedlich D (ed) (2004) Cell Migration in Development and Disease. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
142. Weichsel J, Schwarz US (2010) Two competing orientation patterns explain experimentally observed anomalies in growing actin networks. Proc Natl Acad Sci 107(14):6304–6309
143. Wolgemuth C (2005) Lamellipodial contractions during crawling and spreading. Biophys J 89:1643–1649
144. Yamaguchi H, Shiraishi M, Fukami K, Tanabe A, Ikeda-Matsuo Y, Naito Y, Sasaki Y (2009) MARCKS regulates lamellipodia formation induced by IGF-I via association with PIP2 and β-actin at membrane microdomains. J Cell Physiol 220:748–755
145. Yin HL, Stossel TP (1979) Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein. Nature 281:583–586
146. Yin HL, Albrecht JH, Fattoum A (1981) Identification of gelsolin, a Ca^{2+}-dependent regulatory protein of actin gel–sol transformation, and its intracellular distribution in a variety of cells and tissues. J Cell Biol 91(3):901–906
147. Zimmermann J, Enculescu M, Falcke M (2010) Leading edge - gel coupling in lamellipodium motion. Phys Rev E 82(5):051925