Chapter 1

Introduction

Cells are wonderfully complex microcosms and also the building blocks that allow living organisms to reproduce and survive. Ultimately, the cellular machinery supports the evolution of species of different organisms. Cells divide and grow by actively changing shape, thus allowing organisms to thrive. These shape changes are supported by the cytoskeleton, a mechanical scaffold of filamentous proteins in the cytoplasm. The actin cortex is the principal cytoskeletal component which provides cells with the drive for active shape changes. It consists of actin filaments and myosin II motor filaments which form a network with contractile properties. Myosin motors generate forces that propagate through the network structure and locally remodel it so as to fulfill functions as diverse as cell division or cellular rearrangement in tissues. This thesis aims at understanding how contractility on a molecular scale leads to cell-scale active behavior. To this end, a bottom-up quantitative approach is used based on the assembly of a minimal model of the actin cortex. This approach allows for a systematic study of the physical phenomena that contribute, in concert with biochemical regulatory networks, to cell contractility and opens the way for modeling of such out-of-equilibrium materials. Additionally, minimal model systems of the membrane-actin cortex interaction provide insights in the effect of confinement on the structure of the cortex. In this chapter I introduce the cell as the basic unit of life and describe how it is able to carry out contractility-driven functions. I focus on the actin cortex, the cell component which drives changes in cellular shape leading both to intracellular pattern formation and to rearrangements at the tissue/body level. Finally, I briefly review biophysical experimental studies and theories on the structure and dynamics of the actin cortex and provide an outline of the thesis.
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1.1 The cell: life in a nutshell

Life on Earth relies on an essential building block called the cell. This basic functional unit represents the beginning of the life cycle for most organisms, with the rare exception of entities such as viruses. The latter are difficult to place on a defined position on the axis of living versus non-living matter. The cell represents the smallest minimal operational component for life to be maintained. Some organisms, such as bacteria or unicellular eukaryotes, are in fact restricted to a single cell as a form of body during their entire life. However, as the amount of genetic information increases so does the possibility to explore more complex developmental programs and to translate them into highly structured bodies. As this happens communication and cooperation between cells becomes relevant. The epitome of such cellular cooperation is the complexity found in higher eukaryotes such as animals and plants. These multicellular organisms embody a number of different types of tissues where cells execute different functions and operate as part of specialized organs.

This intricate complexity has long been a powerful source of curiosity to the scientific community. Amidst complex body forms we have learned to recognize, categorize and reproduce the patterns that living beings, ourselves included, display. Organs, tissues and cells are the motifs that organize along various sorts of axes of symmetry to give rise to fully formed and functional bodies. Perhaps the most beautiful snapshots of such patterns and symmetries have been captured in the 19th century by the hand of German naturalist and comparative zoology professor Ernst Haeckel (Fig. 1.1). With much detail but at the same time allowing room for his own artistic creativity he produced numerous aquarelles, later turned to lithographs, of animals, plants and microbes emphasizing their underlying body plan symmetries [1]. His nature-inspired illustrations remain an intuitive summary of the extreme diversity and staggering degrees of complexity that emerge in living organisms. In this manner, he captured the patterns emerging from the continuous and dynamic interaction of genetics and the environment. These are the interactions that lead to the organization of single cells and of cells into complex organisms.

Common to all these organisms are their basic cellular mechanisms and type of cellular structures. Irrespective of the obvious differences in complexity and specificity of functions, all cells are essentially compartments carrying genetic information. While in prokaryotes like bacteria this information is not separated into any sub-compartments inside the cell body, eukaryotes contain a nucleus, bounded by an internal membrane that separates the genetic information from the rest of the cell medium, the cytoplasm (Fig. 1.2). Both prokaryotic and eukaryotic cells have a boundary with the external environment called the plasma membrane. This boundary confines cellular components but it allows exchanges with the surroundings or
Figure 1.1: Art forms in Nature or how patterns catch the beholder’s eye. Facsimiles of Ernst Haeckel’s lithographs of various eukaryote groups of kingdoms Plantae (A.), Protozoa (B.) and Animalia (C-H) (in Kunstformen der Natur, 1904 REF). A. Conifer pines (Coniferae). B. Amoeboid radiolarians (sub-phylum Radiolaria). C. Sea squirts (class Asciidae) D. Arachnids (class Arachnida). E. Cowfishes (Ostraciidae). F. Lizards (sub-order Lacertilia). G. Hummingbirds (family Trochilidae) H. Antelopes (Antilopina).
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with neighboring cells in a tissue. Furthermore, cells perform functions that are at the basis of the growth and reproduction of living organisms, which include cell division, cell locomotion, and tissue morphogenesis.

![Figure 1.2: Schematic drawing of different cells. A. Prokaryotic cell of an *Escherichia coli* bacterium. B. Plant cell. C. Animal cell.](image)

1.2 The cell as an active and dynamic material

The descriptive works of many naturalists, including Haeckel's [1] have generated an immense wealth of information and helped us understand the anatomy of living organisms. These works have also paved the way for the birth of genetics, as well as for Darwin's studies on evolution. However, with the discovery of Brownian motion [2] and Einstein's work describing its implication for the statistical physics of matter [3] coupled with the dawn of molecular biology describing the structure and function of cellular components, cells began to be perceived as more than simple static small bag of things.

Many biophysical techniques and microscopic technologies were developed to explore the structure and dynamics of the living cell, which as any other soft material is constantly subject to thermal fluctuations, but simultaneously operates outside the laws of thermodynamical equilibrium [4]. In cells, fluctuations emerge as a result not only of random thermal motion, but also as a consequence of active processes. These include material exchanges across the cellular membrane that require chemical energy to counteract unfavorable diffusion gradients. Other examples of active cellular processes are those where the cell must drastically change shape like division, loco-
motion and morphogenetic movements leading to tissue organization. This thesis focuses on identifying the origin of the latter contractile processes. These are undertaken by the cytoskeleton, a scaffold of proteins that confers mechanical integrity to cells while also enabling their changes in shape.

1.2.1 The cytoskeleton and the actin cortex

The cytoskeleton is composed of a set of semiflexible biopolymers with different mechanical properties and functions (Fig. 1.3A top). These biopolymers can be characterized by their resistance to bend under the influence of thermal fluctuations. Their bending rigidity determines the length scale over which their backbones remain straight when thermally fluctuating, known as the persistence length (Fig. 1.3A bottom). Microtubules are filaments that are rather stiff on the length scale of the cell, with a persistence length of millimeters [5] (Fig. 1.3B). Among other functions microtubules are responsible for the mechanical integrity of most living eukaryotic cells (except in plants in which a cellulose wall confers mechanical support) [6]. The actin cortex in its turn is composed of filaments that form a network that can actively contract and thus enables the cell to change its shape. This network comes into action when newly duplicated cells separate during division or at the leading edge of a crawling cell. Actin filaments (F-actin) are orders of magnitude more flexible than microtubules and have a 17 \( \mu m \) persistence length [5] (Fig. 1.3C). Finally, intermediate filaments are tissue specific biopolymers with an alpha-helical central rod conserved domain, with no known enzymatic activity and whose subunits belong to a heterogeneous group of proteins. A rather well conserved intermediate filament is lamin, which organizes into a meshwork that lines the inner side of the nuclear membrane (Fig. 1.3D) [7]. Intermediate filaments are the cytoskeletal filaments most sensitive to thermal motion, with a persistence length as small as 0.5 – 1 \( \mu m \) [8].

Actin-based structures display a diversity of architectures that reflect their different functions. These structures are composed of actin filaments, polar semiflexible biopolymers that assemble actively from globular actin monomers in an ATP-dependent manner (the structure and F-actin assembly dynamics are described in detail in chapter 2). The actin cortex is a randomly organized network of F-actin that exists as a thin layer underneath the cell membrane and provides mechanical stability (Fig. 1.3A). Additionally, actin-filled membrane protrusions form in cells that must move or probe their surroundings [9]. For example, the lamellipodium that emerges at the front of a migrating cell is a quasi-2D sheet of polymerizing filaments of actin that push the membrane in the direction of motion [10, 11]. Thin membrane protrusions filled with actin known as filopodia act as mechanochemical probes for the cell surroundings [12].
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Figure 1.3: A. Top Schematic drawing of the cell cytoskeleton and its main components. On the right Schematic drawing of the persistence length of polymers in response to thermal fluctuations. The persistence length \( l_p \) quantifies the decay length of the correlation between tangent vectors \( \vec{t} \) to points \( s \) and \( s' \) along the polymer contour length \( l \). Bottom Zoom in on each of the main filamentous components: B. Microtubules, polar polymers of tubulin monomers with a plus or growing end and a slower growing minus end. These are stiff polymers of the length scale of the cell with a millimeter persistence length \( l_p \). C. Intermediate filaments forming the nuclear lamina. D. Filamentous actin (F-actin) polymerized into a polar filament from globular actin monomers (F-actin forms a double helix of globular monomers, here simplified to one strand for representation purposes). On the scale of the cell F-actin is rather flexible with a persistence length on the order of micrometers.
The organization of actin in these structures is extensively regulated by a myriad of actin binding proteins which include filament crosslinkers, filament-nucleating enzymes and motor proteins like myosins [13]. Actin crosslinkers like fascin or filamin organize the network structure into either bundled or isotropic networks [14]. Nucleators of actin like the ARP 2/3 complex lead to the formation of branched networks* in vitro [15] and have been reported to organize the actin in the lamellipodium of migrating fish keratocytes into branches [11]. While gelsolin and coflin restrict filament polymerization, profilin favors it [13]. Finally, myosins represent a multifunctional group of actin binding motors with different functions that are divided into 15 classes [16].

Myosin motor functions are organism- and tissue-specific and include the transport of cargo material by myosin V [17] and the mechanical connection of actin bundles in the ear stereocilia of vertebrates by myosin I [17]. The first myosin to be described was myosin II, a dimer with a N-terminal head domain and C-terminal tail domain [16] (the structure of myosin molecules is described in detail in chapter 2). The myosin II head domain binds directly to F-actin and it exerts a power stroke as it moves towards the plus-end of actin and consumes ATP. The tail domains of myosin II attach to each other to form bipolar structures with heads pointing outwards called myosin thick filaments. The latter, extensively described in skeletal muscle cells, are an assembly of hundreds of motors able to slide antiparallel actin filaments of the sarcomere (Fig. 1.4A) towards each other as they walk to the actin plus end. This results in an effective contraction of the sarcomere [18]. In non-muscle cells, myosin II minifilaments are composed of only up to tens of motors molecules and they are responsible for a myriad of processes that result in an active change of cell shape [19].

1.2.2 Myosin-II dependent contractile processes in the actin cytoskeleton

The tension generated by myosin II motor filaments is the driving force of contractile processes both at the cell and at the tissue level (Fig. 1.4B). During cell division actin organizes into a ring-like structure at the center of the dividing cell. Myosin minifilaments colocalize with this actin structure [20] and produce a force able to constrict the cell and divide it into two daughter cells each carrying their genetic information in the chromosomes (Fig. 1.5A). Another example of myosin contractility takes place in the cortex during cell locomotion. While at the leading edge of the migrating cell actin polymerization propels the cell forward as the filaments grow against the membrane, at the back myosin filaments contract the network so as to detach the rear end of the cell from the substrate and propel it in the same direction of movement (Fig.
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Figure 1.4: Schematics of contractility generated by myosin II assemblies. A. Schematic of a sarcomere, the muscle fiber contractile unit. In physiological conditions, myosin molecules assemble tail to tail into bipolar structures with heads pointing outwards (myosin filaments) and thus become effectively processive. At each instant they have a number of their heads attached to actin filaments and as they walk to their barbed or plus end (plus sign) they contract the symmetrical parts of the sarcomere towards each other. B. In isotropic networks like the actin cortex, myosin filaments slide actin filaments past each other in the network and generate contractile tension when the actin filaments are crosslinked to each other.
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1.5B).

Figure 1.5: Schematic drawings of different contractile phenomena driven by actin-myosin. A. Myosin II motors generate force that leads to contractile ring constriction and ultimately results in cell separation during cytokinesis. B. Cell locomotion is driven at the lamellipodium by polymerizing forces of F-actin assembling, and the back of the cell the cortex is contracted by myosin filaments so as to carry the rear of the cell in the same direction of motion. C. During germ band extension actin-myosin cables form along the antero-posterior interfaces between neighboring cells. Later these collapse due to actin contractility into a vertex which leads to the formation of rosettes of cells that are responsible for the net extension of the germ band and elongation of the embryo body. D. Morphogenetic events during embryo development are driven by the constriction of a supracellular actin cable that is pulled upon by myosin II.

Actin-myosin has also been reported to regulate various contractile processes in developing tissues [21]. Among these morphogenesis-related phenomena is the movement of the germ layers in early embryos. During elongation of the body axis (a few hours after eggs are layed) in Drosophila melanogaster (fruit fly) early embryos, a tissue called the germ band (Fig. 1.5C, top) narrows along the dorso-ventral axis.
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of the embryo (vertical axis in the schematic, bottom left in Fig. 1.5 C) while it elongates along the antero-posterior axis (horizontal axis in the schematic, bottom left in Fig. 1.5C) [22]. This is a myosin motor-driven process where actin and myosin form cables along the antero-posterior axis of neighboring cells that also contract along it [23, 24]. This leads to the rearrangement of interfaces of neighboring cells, culminating in formation of a vertex and organization of cells into rosettes (bottom right, Fig. 1.5C) [25]. The latter are the functional units that drive the extension of the embryo germ band along the antero-posterior axis to generate body elongation [25]. After the embryo has undergone body elongation, cells of the amnioserosa on the dorsal surface of the embryo decrease the gap between the cephalic region and the dorsal part of the body [26]. This is a process coordinated by the formation of a supracellular actin-myosin ring-like structure that contracts to close the gap on the amnioserosa (Fig. 1.5D) [26].

1.3 Cellular microrheology reflects the out-of-equilibrium behavior of the cytoskeleton

During the past two decades, several studies sought to study in vivo the origin of the mechanical properties of the cell [27, 28, 29, 30, 31]. To this end many microrheological approaches have been developed so as to probe different cellular compartments such as the actin cortex or the microtubule- and intermediate filament-rich center of the cytoplasm. These methods are based on the time-resolved tracking of the spatial coordinates of particles applied to the surface of [27, 28, 32], inserted in [30, 33, 34] or endogenous to the cell [31, 35, 36]. The variations in particle positions reflect the fluctuations of the probed structure, which in turn are tightly connected to its mechanical properties.

The cell is a viscoelastic material whose mechanical properties are in great part defined by the susceptibility of its cytoskeletal filaments to fluctuate. Like any other semiflexible polymer, cytoskeletal biopolymers are prone to thermally fluctuate. However, recent work on the microrheology of different types of cells has shown that such cells exhibit a clear departure from thermal (equilibrium) behavior [30, 37, 38]. This deviation is attributed to active phenomena in the cytoplasm such as the transport of cellular material by microtubule binding motors [33, 35] or the contractile fluctuations induced by myosin motors in the actin cortex [34, 38, 39]. However, it is not straightforward to disentangle the contribution of thermal and non-random out-of-equilibrium fluctuations to the mechanical properties of the cell in vivo. For that purpose bottom-up biophysics has focused on the investigation of minimal models of cellular components that mimic in vivo behavior [40].
1.4 Minimal model systems to decipher the origins of cytoskeletal non-thermal dynamics

Cellular complexity is simultaneously the source of many interesting physical puzzles as well as an obstacle to understanding them. To overcome the barrier of complexity and yet to form a complete understanding of cells, minimal model systems mimicking specific cellular components have been devised. These pave the way for an integrated thorough study of cells. Many biophysicists have focused on the structure and dynamics of the actin cytoskeleton using such bottom-up approaches [15, 41, 42, 43, 44, 45, 46]. The minimal model principle provides tools to study the single molecule interactions of cytoskeletal filaments and associated binding motors [47], but also tools to understand how collections of motor molecules interacting with actin filaments can lead to meso-scale active network behavior. Studies of bulk actin networks have been largely used as minimal model systems. Originally, such systems have been used mostly as a basis to understand the underlying mechanical properties of actin networks [44, 41, 43, 46, 45], and more recently several studies moved on to the out-of-equilibrium behavior of actin networks containing active myosin motors (chapter 4, [48, 49, 50, 51]).

1.4.1 In vitro reconstituted actin networks

The mechanical properties of actin networks confer to cells the ability to swiftly adapt their shape to perform different functions. Many researchers have focused efforts on understanding what underlying factors tune actin structure and mechanics. Such work includes a vast number of experiments on how crosslinkers of actin lead to network strain stiffening at high strains [52, 53] or how they tune structure [54]. Other studies investigated the influence of actin density on structure [41, 55, 56, 57] and dynamics [44] of the network. These studies were based on assays using bulk actin networks, whose composition and structure was modulated by the controlled addition of crosslinkers. Complementary to this, research on active actin-myosin gels has focused on understanding how the myosin II motor remodels the network structure and mechanical properties by generating internal stresses [49, 58, 59, 60] and identifying the underlying dynamics of these out-of-equilibrium materials [48, 49, 50, 51] (Fig. 1.6A). Nonetheless, a clear connection between the emergence of patterns and its relation with active local contractile fluctuations remains to be elucidated.

In contrast with bulk approaches which focus on presupposed continuum, isotropic and boundary-insensitive actin gels, confinement studies investigate the influence of container size and boundaries on the network structure and dynamics by polymerizing actin networks inside vesicles [61] or liposomes [62] (Fig. 1.6B). These works
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Figure 1.6: Minimal models to study structure and dynamics of actin networks. **A.** Schematic drawing of an active bulk composite actin network. Actin filaments are crosslinked and pulled upon (in crosslinked networks) by myosin filaments which modulate the network structure and mechanics. **B.** The effect of the cell membrane on actin organization has been studied in soft confinement (using artificial liposomes or vesicles).

have reported the spontaneous formation of a thin cortical actin shell on the inside of these spherical soft containers [61]. This arrangement results from alignment of the filament with the boundary to minimize their bending energy whenever their contour length is comparable to the container diameter [63]. In soft confinement, however, a systematic variation of container size is not easy. In addition to systematically studying the effect of container size, it would be interesting to also test the effect of geometries other than spherical so as to assess the influence of the confining cellular shape on cytoskeletal biopolymers.

1.4.2 Active gel models

Active materials have captivated the interest of physicists as they at once provide a means to elucidate the mechanics and dynamics of living materials and exhibit thus far underexplored out-of-equilibrium physical phenomena. The structure of active gels has been intensively investigated with in vitro minimal systems [63, 59, 60, 64, 65, 66], and several hydrodynamic theories have been able to describe pattern formation of 2D actively assembled topologies assuming that the gel is a homogenous continuum [64, 67, 68, 69]. These coarse-grained mesoscale models of active polar gels successfully predict patterns of self-organization in systems of small motor aggregates that sort polar rigid-like filaments. Microtubules and their kinesin motors are a system well understood under these assumptions of medium continuity and filament polarity essential for pattern formation [64, 69].

Actin and myosin are a different system, since actin filaments are rather flexi-
1.5 Scope of this thesis

In this thesis, I present experimental research on the structure and dynamics of actin networks under the influence of myosin motors or spatial confinement. I use an *in vitro* approach where I study minimal model systems composed of purified actin and myosin. To test the influence of myosin on actin organization and dynamics, I use confocal microscopy and particle tracking microrheology on bulk networks. To test the influence of confinement on actin organization, I polymerize actin in cell-sized hardwall microchambers.

In *chapter 2*, I introduce the methods used to reconstitute actin-myosin networks *in vitro*. The processes of protein purification and fluorescent labeling are described in detail. Moreover, the experimental techniques used to characterize the mechanical properties of actin filaments and networks are introduced, and I describe the characterization of the structure and self-assembly of myosin into bipolar filaments.

In *chapter 3*, I study out-of-equilibrium self-organization in actin networks driven by myosin motor activity, using fluorescence confocal microscopy. I demonstrate that myosin causes actin network coarsening in a multi-stage condensation process. The motors initially self-organize into myosin clusters, which then condense adjacent actin into dense shells. Interestingly, these foci-like patterns closely resemble *in vivo* structures observed in various organisms and cell stages, which are involved in myosin-dependent developmental processes and cell division and migration. We propose a new physical mechanism that explains how coarsening and permanent coalescence can emerge in weakly crosslinked actin networks based on the fact that actin filaments have an asymmetric force-extension behavior.
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In chapter 4, I investigate the consequences of motor activity for dynamics in actin networks. A microrheological approach is used to study non-equilibrium fluctuations caused by myosin contractile activity. I embedded micro-sized probe spheres in actin-myosin networks and measured their displacements with bright field microscopy. I find markedly non-thermal particle trajectories, with periods of nondirected (random) motion interspersed with episodes of active, directed motion. We develop an automated segmentation analysis of particle trajectories to quantify the spatiotemporal frequency of this directed motion. We demonstrate that the frequency of directed motion is highest in early stages of sample aging, when the sample is still coarsening due to motor-driven coalescence, as reported in chapter 3. From ensemble averaged analysis, we retrieve signatures of out-of-equilibrium dynamics activity, such as a diffusive-like mean squared displacement and an enhanced non-Gaussianity of the displacement distribution functions. We have evidence of infrequent correlated motions of multiple particles, which occur only in early stages of sample aging. This work indicates that physical models of nonequilibrium dynamics in active gels should take into account the time-dependent, heterogeneous network structure.

Finally, in chapter 5, I investigate how steric confinement, which mimics cell boundaries, influences the spatial organization of dense solutions of actin filaments. I polymerized fluorescently labeled actin networks inside hardwall microchambers with inert surfaces, manufactured with photolithographic techniques. I demonstrate that the actin filaments spontaneously organize into bundle-like structures above a threshold concentration. These bundles align with the longest axis of anisometric chambers and with the diagonal in isometric (square or circular) chambers. We propose that bundling occurs due to the concerted effect of quasi-2D confinement of the longest filaments in the polydisperse length distribution, and depletion interactions induced by the shortest filaments. This mechanism differs from the isotropic-nematic transition previously observed in bulk networks, which occurs at much higher actin concentrations. We propose that the steady-state orientation of the bundles results from a competing liquid-crystalline ordering in the chamber center and alignment along the boundaries. This ordering effect of confinement might influence organization of the in vivo actin cytoskeleton, especially in confining spaces such as the plant cell cortex or thin membrane protrusions, in conjunction with biochemical regulation and actin-membrane adhesion.
References


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