Chapter 2
From Molecules to Cells: Machines, Symmetries, and Feedbacks

Abstract The first step on the way from chemistry to morphogenesis is the chemo-mechanical transduction that is extensively retarded relaxation of the stored energy onto a small number of selected degrees of freedom. The next level to up is presented by the microtubules and microfilaments-associated supramolecular machines which transform this energy into the dipoles (tensors) of mechanical forces. As a result, the balanced system of tensional and compressive mechanical stresses is created. On the level of isolated supramolecular machines, the processes characterized by the increase of a symmetry order and the drive toward thermo-dynamical equilibrium are dominated. At the upper structural levels, the role of energy-consuming non-equilibrium structures is increased, producing sub- and super-diffusion movements of the particles and a number of temporal and spatial symmetry break—the dynamic elements of still higher levels events. Among them of the most morphogenetic importance is tensile homeostasis of cell membranes and establishment of the polar cell organization.

2.1 Introductory Remarks

It will not be an exaggeration to claim that the main link in the activity of living beings, distinguishing them from non-living ones is what is called chemo-mechanical transduction: This is the transformation of random molecular movements into much more directed ones which we call mechanical. In terms of symmetry theory, it corresponds to enormous decrease of the symmetry order on the molecular level. It is not surprising therefore that even those scientists of the past which did not know Curie principle were intuitively searching for a dissymmetrizer, capable to provide the "information" for such a symmetry break. By a strange coincidence, in the same year of 1944, at the height of WW2 two independent researchers ascribed this property to hereditary substance (which histologists of that time often called the "chromatin"). One of them, the Russian biologist Alexander Gurwitsch postulated the existence of a chromatin-associated anisotropic field which "transforms a part of excitation energy

L.V. Beloussov, *Morphomechanics of Development*,
DOI 10.1007/978-3-319-13990-6_2

of the protein molecules into kinetic energy, oriented along field vectors" (Gurwitsch 1944; reprinted in 1991). This same year Ervin Schroedinger in his famous book (Schroedinger 1944) qualified the hereditary substance as "aperiodic crystal" emphasizing thus the fundamental role of its translational dissymmetry. Subsequent development of molecular and cell biology showed, however ,that it was quite unnecessary to link chemo-mechanical transduction with a single principle, associated with the "hereditary substance": This property appeared to be rooted in the very structure of protein macromolecules and their non-covalently bound multimolecular ensembles, often defined as "molecular machines." The latter can be attributed to the lowest link in the complicated hierarchy of devices coined by the nature for providing the movements of various living structures—from molecules to multicellular formations. In this chapter, we start to review this hierarchy and commence our analysis from describing the physical foundations of chemo-mechanical transduction on the level of single macromolecules.

2.2 Chemo-Mechanical Transduction and Molecular Machines

Among few contributions discussing the physical principles of chemo-mechanical transduction, the most important are those of McClare (1971) and Bluemenfeld (1983). We shall follow mainly the latter author, who elaborated his concepts in greater details. His main idea is that the transduction should be based upon a more or less considerable *reduction of the freedom degrees* of constituent particles of the "working body" (exemplified by either a single macromolecule or an entire supramolecular ensemble) and the *increase of characteristic times* of their deformations (compared to thermal motion). Or, in other formulation, the transduction requires *extremely slow relaxation of the stored energy onto a small number of the so-called distinguished* (*selected*) *degrees of freedom*. The simplest man-made machines, the heat engines, perfectly illustrate this requirement: The rate of thermal movements of single molecules of a gas or a steam are of the order of 10^2–10^3 m/s, while that of the piston should be at least in 2–3 orders less. Protein molecules can be also regarded as machines, which provide even much greater retardations: While the characteristic times (reverse frequencies) of oscillatory, rotational, or translational movements of atoms lay in the range of 10^{-11}–10^{-12} s, the working cycle of the main device for producing movements and deformations at all scales—the actomyosin machine—lasts about 0.2 s exhibiting thus enormous delay.

Such a considerable slowing down of molecular movements can be interpreted as enormous decrease of temperature within the molecular machine. Though it was first proposed by such an outstanding person as Ervin Schroedinger, it was neglected for several decades for being revived recently by several authors (Matsuno 2006; Igamberdiev 2012). By their estimations, a slow conformational

relaxation of actin-activated myosine ATPase releases 5×10^{-13} erg of ATP energy in 10^{-2} s. This corresponds to extremely low temperature of 1.6×10^{-3} K. As a whole, molecular machines performing chemo-mechanical transduction look to be consisting of two parts: The ordinary "heat machine" acting at the room temperature and the "frozen part" performing very much delayed and refined molecular movements on few selected degrees of freedom; the second part is fueled by the energy by the first one. In this, molecular machines differ principally from more familiar to us, man-made ones: So far as the latter are working in the dimensions/timescales very much removed from molecular ones, such a drastic splitting to the heated and frozen parts does not take place (although a much smaller distinction between the heater and the refrigerator remains).

Under more detailed consideration (Chernavskii and Chernavskaia 1999), the following details can be distinguished in the protein machine:

- Reservoir of energy ("springs")—relatively soft elastic elements able to store energy in the form of elastic stresses. Such a role is played by non-spiral parts of the protein molecule and electrically charged groups. Reservoir size is about 5×10^{-9} m.
- Levers, the rigid details which transmit stresses. This role is played by α-spirals.
- Hinges, the flexible joints represented by small amino acids, for example, glycine.
- Fixing points, presented by hydrogen bonds or by S–S bonds.

The energy stored in elastic elements is estimated as ≈0.5 eV per molecule and the lifetimes of metastable (mechanically stressed) states of protein machines ranges from 10^{-6} to 10^{3} s. By comparing these values with the above-presented rates of thermal oscillations of protein molecules, one can see that the relaxation of stressed states is slowed down by about 15 orders of magnitude. That means that macromolecules are spending almost all of their lifetime in mechanically stressed states.

Bluemenfeld emphasized principal importance of such extensive slowing down of the relaxation of the stored mechanical energy. He defined this property as kinetic non-equilibricity (opposed to thermodynamic one, associated with a constant energy inflow) because it prevents a system to reach otherwise available energetic states simply by keeping "frozen" a certain selected state for a long enough time period. This is a property of mechanical, rather than statistical systems, endowing the first ones by memorizing capacities. In other words, molecular machines in Bluemenfeld's sense are *not* dissipative structures: The latter are too vulnerable to the changes in initial/border conditions and have no required amount of memory.

Another peculiar property of molecular machines is that contrary to man-made devices, the regulatory and executing parts are merged together and the amplitude of produced movements is comparable with the dimensions of the entire machine. Next, due to the presence of bound ions and electrically charged parts of protein molecules (negatively charged carbonyls and positively charged amino acids), any mechanical shift is accompanied by changes of electrical field, which contribute to maintaining mechanical stresses within macromolecules. A special role in this

process is played by charged ligands and first of all by ATP molecules, each of them containing three negatively charged phosphate groups. Accordingly, by binding ATP molecules, the proteins should pass to more stressed and rectified configurations which may play an important role in the signaling cascades associated with several rounds of the proteins phosphorylation/dephosphorylation.

Therefore, an essentially mechanical behavior characterized by the presence of long-living stressed conformations which will be shown later to play a crucial role in regulating morphogenesis, can be distinctly traced already on the level of single macromolecules. That does not mean, however, that these properties are directly translated to upper levels, as it takes place in the crystal-like bodies. Rather, they numerously disappear and appear de novo, while moving upwards along the organizational levels scale. Now, we start this way, each time focusing our interests onto morphogenetically relevant properties of the dynamic structures specific for each next level.

2.3 Structures and Actions of Supramolecular Machines, Treated in Symmetry Terms

The above-described mechanochemical transduction on the level of individual macromolecules still cannot provide properly ordered deformations on the large enough scales, required for cells functioning and, the more, morphogenesis. A next step of integration is provided by the so-called supramolecular machines held by weak van der Waals and hydrophobic forces of molecular attraction and combining extremely high efficiency with fast responses to regulatory agents. Without repeating here a detailed description of these machines (see textbooks on the molecular biology of the cell, e.g., Alberts et al. 2003), we shall concentrate ourselves on the rarely discussed topics, directly related to morphogenesis: Those are the structural and functional symmetry transformations of supramolecular machines and the associated energy profiles.

First of all, let us remind that the two main classes of supramolecular machines—microtubules and microfilaments—are self-assembled from tubulin or actin subunits correspondingly into the formations which we shall define in some cases under the common name of filaments. In water solutions under high enough concentration of subunits, the assembly is energetically favorable (directed toward diminishment of a total free energy of the subunits) due to the presence of hydrophobic forces. As mentioned in Chap. 1, the free energy decrease is usually associated with increase of the symmetry order. Just this is taking place in the case considered: Whereas the solution of randomly distributed subunits has a symmetry order 1 (worth mentioning, the symmetry of single subunits is also 1), the assembled supramolecular structure acquires several new symmetry orders, the main of them corresponding to that of a polar vector ($\infty \cdot m$). Polarity is the main property of both kinds of filaments, manifested by the presence of the pole of preferential subunits' assembly (defined as

"+" end) and the opposite pole of preferential disassembly ("−" end). On reverse, the filaments disassembly (corresponding to the decrease of symmetry order back to 1) demands energy spending which comes from splitting of nucleotides (ATP in the case of microfilaments and GTP in the case of microtubules) bound to subunits. More detailed consideration of energy-driven configurations of cytoskeleton will be given in the next sections.

In addition to polarity, actin microfilaments also have translational symmetry with the period $a \approx 13.5$ subunits length and the screw pattern consisting of two left-handed helices. The symmetry order of two latter structures taken together is written as $(a) \cdot 2_1$. Microtubules do not reveal translational symmetry but possess complicated screw patterns with 3, 5, and 8 (members of Fibonacci series) inclining the spiral rows to different angles.

What should be the morphogenetic role of these symmetries? While no data on the role of translational microfilaments symmetry are available, the significance and mutual cooperation of two others is obvious. The main manifestation of the filaments polarity is the exertion of pushing forces deforming cell membrane by "+" end directed growth of either microfilaments or microtubules. These deformations are taking place at the leading edges of migrating cells. They are the elements of the most powerful collective cell events, embraced by the name of cell intercalation which will be discussed in details in the following chapters. "+" end directed growth is accompanied by the chiral component associated, in case of microfilaments, with the activity of membrane-bound proteins, the formins. According to a model suggested (Shemesh et al. 2005), circular formin dimers are periodically rotated in opposite directions around the filament's bulk at the level of its "+" end. During the so-called stair-stepping phase, there are 12 successive turnings of the same direction, each for 14°, producing a torsion stress of the double helical microfilament. Each next turn leaves a space for another actin subunit to be inserted. On the whole, stair-stepping phase takes about a second and spends about 1 eV of energy per actin filament. This energy is taken from ATP and GTP hydrolysis (formins were shown to be associated with GTPases). The stair-stepping phase is followed by the oppositely directed rotation for ≈166° (so-called screw mode) releasing torsion stress. As a result, the forces of several hundreds nanonewtons range are produced by actin polymerization at the leading edge of the crawling cell. Interestingly, up to about 300 nN resistance force, the actin network is growing at the relatively constant rate independently of the applied load (Carey et al. 2011). Such wide-ranging load independence seems to be an inherent and morphogenetically important property of actomyosin machinery.

The chirality (handedness) of the cytoskeletal filaments creates also a molecular basis of the macroscopic handedness of whole organisms. In spite of many blank spots and controversies, there is a growing amount of evidences indicating that the handedness is arisen in early development and is expressed by the chiral organization of tubulin, actin, and actin-bound proteins (Danilchik et al. 2006). For example, in amphibian eggs, the chirality is assumed to be imprinted by the microtubules array grown out of the organizer center brought by the male centriole. Later on, the chirality is amplified up to tissue-level asymmetries by as yet

non-specified ways including asymmetric distributions of potassium channels, proton pumps, and/or some neuromediators like serotonin (Vandenberg and Levine 2013). A peculiar mechanism of establishing left–right asymmetry with the involvement of class I myosins has been discovered in Drosophila (Speder and Noselli 2007). In this case, the so-called unconventional myosin I interacts with a universal regulatory protein, β-catenin and becomes bound with adherent junctions, connecting epithelial cells. Fixed in this location, it provides chiral cell rotations leading to asymmetric looping of intestine rudiment. In general, either right- or left-handed cell rotations, asymmetric inclinations of cell division axes or even of vast cell streams (the latter ones are taking place in chicken embryos: Tsikolia et al. 2012) play a crucial role in establishing handedness and essentially precede asymmetric patterns of genes expression. Thus, the morphological symmetry of a roundworm, *Caenorhabditis elegans*, is broken during the four- to six-cell transition due to inclination of the division spindle in two sister blastomeres called ABa and Abp from the direction orthogonal to antero-posterior embryo axis (Pohl and Bao 2010). Notably, by reversing through micromanipulation the previous division spindle, the body handedness will be also reversed. "…this result suggests that asymmetry in cytoskeletal and spindle mechanics may instruct handedness choice upstream of differential gene expression" (op. cit.).

Leaving now this intriguing and largely non-explored topic, we come to the next step in self-assembly of supramolecular machines. It is the association of the filaments with the so-called motor proteins (briefly "motors"), the ability of the motors to percept the filaments' polarity and to move, by spending energy, toward one of the filaments ends, either "+" or "−". For our purpose, it is enough to remind that motors from the kinesins and dyneins families are associated with microtubules while those from a large myosins family—with microfilaments. Most of kinesins are translocated toward "+" and dyneins to "−" ends of microtubules, while most of myosins (including myosin II) toward "+" ends of microfilaments. Motors are able to transport different molecular loads or cargoes. This ability becomes morphogenetically important when the load is represented by another similar filament, oriented in the same or reverse polarity in relation to the first one. In these cases, the filaments are arranged in doublets; the doublets members may be either identical in the sense that both are pulled by motors in symmetric manner, or nonidentical, one of them (the loading filament) firmly fixing the motor, while another serving as rails for the motor's shifts (a leading filament).

Missing a lot of molecular details of the motors–filaments interactions described in many textbooks and special papers, we will emphasize two main points. The first of them is that so-called motor's working stroke which pulls a load is directed toward relaxation, that is, toward the restoration of de-energized configuration. This step is always preceded by that of energy consumption (provided by ATP or GTP splitting), associated with translocation of the motor's "head" toward one of the poles of the leading filament. The second point is related to a rarely discussed issue of the symmetry of motors–machines associations. Let us consider the following examples:

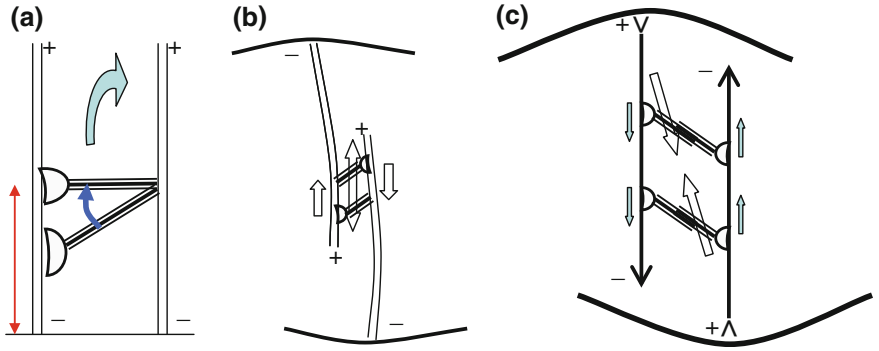

Fig. 2.1 Action symmetry of the double filaments molecular machines. Depicted are working strokes of an eukaryotic flagella (**a**), polar microtubules during cell division (**b**), and actomyosin contraction (**c**). + and − filaments ends are denoted which in the case of microfilaments are also marked as barbed and pointed arrowheads correspondingly. While in (**a**), a static structural symmetry of the microtubules arrangement is broken, in (**b**, **c**) the symmetry order of action is greater than that of the individual filaments. For detailed comments see text

1. Both microtubules creating the doublet have parallel polarities. One of the doublet members is loading and the other leading. This is what takes place in Eukaryotic flagella (recent review: Lindemann and Lesich 2010) where the function of motors is played by "−" oriented dyneins. Under these conditions, if the microtubules poles were not fixed, both microtubules from the same doublet would slide apart, the leading one in the direction of its "+" end, while the loading one in the opposite direction. However, because of the firm fixation of the both microtubules "−" ends, the result will be stretching of the leading microtubule and shrinkage of the loading one, which will make the entire machine bent (Fig. 2.1a).
2. Microtubules doublets consist of antiparallel oriented units. Their "−" ends are fixed at the opposite poles of a cell and both are loading and leading at the same time, each of them translocating kinesins toward their free "+" ends. Such is the activity of the so-called polar microtubules during cell division. In this way, they generate the force pushing apart the microtubules and polar cell areas to which they are attached (Fig. 2.1b). This is the main driving force of cell division translocated on the multicellular level in most powerful pushing forces ever existed in living tissues.
3. Actin microfilaments are oriented to the opposite cell surfaces by their "+" ends so that they are antiparallel and equivalent to each other. The motors (double head myosin II) are translocated toward "+" ends of each filament and pull the filaments in converged directions. This is the most general scheme of actomyosin contraction. It displays the universal mechanism for one-dimensional and circular contractions of cells which producing passive tensional stresses in the surrounding tissues if their outer edges are fixed (Fig. 2.1c).

Let us consider now the static and action symmetries of these machines. So far as in the case of flagella, both microtubules keep the same polarity while only one of them serves as the leading one (the other one being loaded), the action symmetry of the doublet is reduced to $1 \cdot m$ (similarly to an organism possessing both antero-posterior and dorso-ventral polarity). During the working stroke (performed in a coherent way by several dozens of dynein molecules), this dissymmetry is transformed into that of a bending keeping the same symmetry order.

This is not the case, however, for the next two examples (Fig. 2.1b, c): Because of the opposite polarity of the filaments and symmetric attachment of the motors to both of them, the symmetry order of the entire system is *increased* (compared with that of a single filament) by acquiring a reflection plane oriented perpendicularly to the filaments axes. As a result, the symmetry group for these two systems (without considering translational symmetry) becomes $m \cdot 2{:}m$, corresponding to second range *polar tensors*, rather than vectors. Accordingly, they may be called the "force dipoles" (Bishofs and Schwartz 2003). These two kinds of machines produce most substantial elements of morphogenetic forces. In the case of microtubules, they are associated with the post-division moving apart of sister cells and in the case of the microfilaments with various kinds of cell contractions.

2.4 Hierarchy of Stressed Networks and the Condition of Force Balance

By moving up from the isolated supramolecular machines, we enter the world of networks consisting of mechanically stressed (stretched or compressed) cables and the nodules where several such cables are met. Remarkably, such networks create a hierarchy embracing enormous dimensional range. The lowest level of the hierarchy is exemplified by the so-called actin gels—the networks of perplexed actin microfilaments with the nodules (fixed by gel-forming proteins, in most cases filamins) being about 10^{-7} m apart from each other. The upper scale networks belong to cellular and supracellular levels to be described in the next chapter: The greatest distances between the nodules which correspond in these cases to the multicellular clusters are extended up to 10^{-3} m. At the given moment, we have to concentrate ourselves on the most tiny networks for substantiating the important but often missed principle called the condition of the force balance (see, e.g., Odell et al. 1981; Goodwin and Trainor 1985; Forgacs 1995; Kozlov and Mogilner 2007). It claims that all the forces in biological tissues are balanced: Their vector sums are equal to zero (or, more precisely, are equalized within no more than milliseconds) so that the nonzero accelerations take extremely small time periods alternated by much longer periods when they are lacking. Suggest indeed that a mechanical force F which stretches or compresses a cable is generated in point N removed from the closest nodule M to the distance l. As a result, a wave of elastic deformation will move from N to M at the rate close to that of sound waves. When it reaches the

point *M,* the latter will start to move toward *N* with acceleration. This will last, however, only until similar waves of elastic deformations spread from *M* reach the next neighboring nodules *P, Q, R*. From this moment, the vector sum of all the forces applied to *M* turns into zero, and acceleration is abolished. Depending on the resistance of the surrounding medium, point *M* will either stop or continue to move with constant rate. As shown by the corresponding evaluations (Odell et al. 1981) due to high rate of elastic deformation wave and very small distances between the neighboring nodules, the periods of the force imbalance will last no more than 10^{-6}–10^{-7} s. This is negligibly small even compared to the duration of a single actomyosin sliding cycle (2×10^{-1} s). However, this imbalance period is already enough to reach the final velocity of about 3×10^{-5} m/s which largely exceeds typical rates of morphogenetic movements. All the above said permits to conclude that any forces generated within living tissues and providing morphogenetic movements are practically immediately balanced by the opposing ones. The morphogenetic consequences of the force balance are of primary importance. First, it means that the action of a single force is spread toward even far removed regions without considerable damping. Moreover, any changes in a single force value will affect the entire geometry of the network and vice versa: By deforming the web, both the values and the directions of the forces will be also changed: The forces and the geometry are mutually linked.

Under the condition of the force balance, it is enough to know the angular directions of all the forces applied to a given nodule for calculating their relative values and vice versa. This is especially easy if the number of forces is 3, which is the most probable case.[1] Consider indeed a nodule formed by the forces OA, OB, and OC and denote angle AOC as α, COB as β, and AOB as γ. As shown by simple trigonometric calculations, the relations between OA and OC will be

$$\left|\overrightarrow{\mathrm{OA}}\right| = \frac{\left|\overrightarrow{\mathrm{OC}}\right|(\cos\alpha \cdot \cos\gamma - \cos\beta)}{\sin^2\beta} \tag{2.1}$$

In Chap. 3, several examples using Eq. (2.1) will be discussed.

In math terms, the condition of the force balance within each force nodule enforces us to operate with tensors, rather than vectors. This provides some opportunities. First, the dynamic states described by tensors are self-supported, that is, do not require any external foothold demanded by vectors: It is especially important for mass cell movements described in Chaps. 3 and 4. Next, one-dimensional tensors are able to organize two-dimensional spaces, exemplified by cell layers. The best example is the Poisson's deformation (see Chap. 1, Box), widely spread and actively reinforced in morphogenesis. Another process described in terms of tensors is the transformation of oblique cells toward rectangular ones

[1] Because of its robustness: Any higher order nodule can be decomposed into third-order ones just by small shifts.

due to isotropic contraction of actin gel. This process plays the leading role in transformations of so-called cell fans (see Chap. 3).

Meanwhile, the vectors can emerge again on the upper structural level as the combinations of tensors. Suggest that we have an internally stressed conical or ovoid body with uniform density of the force nodules. If isolated from any external forces, such a shape (by the way, rather biomorphic) can be mechanically balanced only if the tensions are reversely proportional to the local diameters (i.e., they are greater in more narrow part). In other words, we get here the *gradient of the stress tensor*, (or briefly, the stress gradient) dictated by geometry (or the other way round). Within the entire body scale, this forms a vector, defining the body polarity. The important morphogenetic role of stress gradients will be discussed in the next chapter.

2.5 Final Remarks on Supramolecular Machines

Now, we return back to molecular level for mentioning some other mechanisms involved in generation and regulation of mechanical stresses.

The first of them is in transforming the fast actomyosin contractions typical for muscle activities to sustained contractions maintaining long-term stresses. The switch from fast to sustained regime is regulated by a family of universal regulators of cytoskeletal activity, RhoA proteins. It is realized by inhibition of phosphatase (associated with light chains of myosin molecule) by RhoA-dependent kinase (Weiser et al. 2007). Myosin II mediated support of sustained tensile stresses non-associated with fast contraction has been shown both for embryonic (Roh-Johnson et al. 2012) and pathologically modified tissues (Samuel et al. 2011).

While these and other data indicate the leading role of actomyosin machinery in supporting long-term stresses, their initiation in early embryos is owed to another mechanism: increase of osmotically driven turgor pressure in primary embryonic cavities (blastocoel and subgerminal cavity of Amniotes). The pressure reaching in amphibian blastula stage embryos ≈325 mosm = 70 N/cm^2 (Wilson et al. 1989) is created due to the inward (blastocoel directed) transport of sodium and chloride ions provided in its turn by predominant location of ionic channels at the apical (external) parts of the blastocoel roof cells and of ionic pumps at the basal (internal) parts (Stern 1984). Although this factor, unique in providing voluminous (three-dimensional) deformations and stretching stresses, is rare for animals (another example related to hydroid polyps will be discussed in Chap. 4), its role should not be underestimated: As shown later, its switching-off leads to grave developmental abnormalities. In plants, it is the dominating stress-producing agent (see Chap. 5).

By concluding this section, in addition to widely accepted and extensively explored supramolecular machines it would be proper to mention a non-conventional hypothesis suggested by Pollack (2001) and Del Guidice with coworkers (2005, 2011) proposing the existence of a quite different class of a similar scale mechanisms which may be called low entropy machines.

The authors assume that supramolecular structures (including cytoskeletal filaments) are surrounded by several layers of a structured water which consist of precisely oriented molecular dipoles, with their positively charged poles directed towards cytoskeletal filaments, and the negatively charged ones to the bulk of disoriented water. These hypothesized coherent water domains, or CWD (which should be the areas of very low entropy) may work as a kind of electrical batteries accumulating substantial energy and discharging it if being destructed. According to Del Guidice et al. calculations, CWD diameters extend up to 100 nm (corresponding to about 10^7 molecules) and by exceeding a certain density threshold, should oscillate in a coherent way with 10^{11} Hz frequency. Due to low entropy, CWD are able to absorb non-coherent (heat) energy from the surrounding bulk water and transform it into "high grade" coherent energy. Noteworthy, CWD absorb light in the infrared and ultraviolet spectral regions. The absorbed energy may reach 12.06 eV which is only ≈0.5 eV lower than the energy of water ionization. In other words, CWD possess a substantial amount of free or almost free electrons, which results in the electrical potential difference between CWD and bulk water. In such a way, CWD should work as real machines, by producing electrical energy and the movements of charged particles. These up to now purely theoretical considerations deserve to be experimentally tested.

2.6 From Self-assembly to Self-organization: Temporal and Spatial Symmetry Breaks

What was reviewed beforehand—with a great exception of chemo-mechanical transduction in McClare and Bluemenfeld sense—relates mostly to a classical self-assembly characterized by preservation and increase rather than decrease of the symmetry order. However, for creating a complicated space-temporal organization of embryos, the reverse dynamics associated with symmetry breaks will be necessary. Let us show that even a slight structural complication (as compared to isolate supramolecular machines) just leads to symmetry breaks and other signs of self-organization, while if mostly temporal.

We start from pointing to several experimental and theoretical papers (Plaçais et al. 2009; Howard 2009; Ishiwata et al. 2010; Vogel et al. 2009; Kruse and Riveline 2011) which describe and analyze phenomenologically simple models of actomyosin contraction under loading. Even a single actin filament brought in close proximity to a substrate densely coated with heavy meromyosin molecules, when loaded by several picoNewtons elastic force, exerts rhythmic oscillations with peak frequencies from 1.5 to 14 Hz (Plaçais et al. 2009). By changing mechanical parameters, different modes of oscillations can be traced (including bimodal ones). The authors found that the oscillatory characteristics were mostly determined by the stiffness of the system, while the number of involved meromyosin molecules affected the level of the random noise. With the increase of the number of myosin molecules involved, the oscillation pattern becomes more regular, approaching that of intact muscle (Ishiwata et al. 2010).

As will be shown below, most of intracellular oscillations have several minutes periodicity, being hence enormously delayed, as compared to characteristic rates of molecular events. This alone is sufficient for ascribing these events to the feedbacks

generated in large molecular collectives. There are meanwhile also more direct arguments favoring this point of view.

Some of the oscillations and feedback reactions are based upon activity of actomyosin structures while other upon kinesin–microtubule interactions (review: Kruse and Riveline 2011). The first ones are exemplified by so different systems as moving fibroblasts exerting several pN pulling forces upon their environment with 3–4 min periodicity and hair bundles of the inner ear cells, oscillating with about 100 ms periods. Several minutes periods oscillations in large cell collectives, some of them (but not all) based on actomyosin activity, will be reviewed in the next chapter. Periodic assembly–disassembly of cortical actin was observed in microtubule-depleted cells and in cell fragments. In these cases, the actin cortex locally tears and retracts due to the action of myosin. During retraction, actin is disassembled leaving a bulge of highly tensed cortex behind; in this very place, actin is assembled again.

Another example of microtubules–kinesin-based oscillations are the movements of the meiotic prophase spindle body in fission yeasts which have 5–10 min period and last for several hours. These movements are generated by periodic pulling of spindle poles by kinesins which are attached to the cortex and are moving toward "–" ends of astral microtubules (Vogel et al. 2009). Extensive oscillations of the length and positions of mitotic spindle during the first cleavage division of *C. elegans* eggs are based upon a similar mechanism (Kruse and Riveline 2011).

All these processes definitely belong to realm of self-organization because by establishing a regular periodicity, they break temporal symmetry at the macroscopic scale (far exceeding characteristic times of individual molecular events). Obviously, the oscillation patterns require negative feedbacks between the main dynamic components (which may be supplemented also by positive feedbacks acting at shorter time periods, as mentioned in Chap. 1). What might be their origin?

As shown in several cases (Vogel et al. 2009; Kruse and Riveline 2011), such oscillations well can proceed in vitro systems, free from any chemical regulators. Thus, without denying the latter's role in intact cells, we can conclude that in the "minimal" cases, the feedbacks can be purely mechanical, provided by any loads (either natural or artificial) resisting the pulling or pushing activity of the motor proteins. More concrete models which use this assumption split into two categories. The first one (Kruse and Riveline 2011) emphasizes the existence of the bimodal velocity distribution (the modalities having the opposite signs) in the simplest, however, necessarily loaded actomyosin systems. By this model, under certain values of mechanical parameters, the elastic load/velocity (of the motors movements along actin filament) dependence becomes nonlinear, permitting the motors to switch between two velocity values which correspond to the minimal energetic potential. The model takes into consideration the translational symmetry of actin filaments which affects the strength (energetic potential) of actin motor protein binding. The second model, shared by most of authors, connects the value of elastic load with the rate of detachment of the motor proteins from the loaded actin filament. A concrete scheme suggested for the case of microtubules may be the following (Vogel et al. 2009). Suppose that an intercellular structure is pulled by the

kinesins attached to the oppositely directed microtubules, A and B (which are oriented by their "+" ends to the opposite parts of the cell membrane). The balance between both opposite forces is unstable to small differences in microtubules lengths: A slightly longer microtubule (say A) possesses more motors and exerts hence a greater pulling force to B. This promotes increased detachment rate of the motors from B, which enhances even more the pulling force of microtubule A. In other words, a positive rate/rate feedback emerges. However, because of the finite cell diameter, microtubule A should shrink losing the motors, while the tensed microtubule B, becoming free from further stretching has more chances to attach the motors again. At this moment, the movement changes its direction.

By developing this idea, Howard (2009) puts a remarkable question: "Are the individual motors and filaments micromanaged like marionettes by a small group of cellular puppeteers (signaling pathways) …? Or is there a certain degree of autonomy of action of collectives of motors and filaments that allow them to self-organize and coordinate?" Being inclined to the second version, the author develops the idea of load-accelerated motors/filaments detachment. He adds to the previous models a new assumption of negative damping meaning that with increase of the body velocity, the resisting force should be decreased, rather than increased, so that acceleration occurs. This requires additional portion of energy which should be taken "from the surroundings" (op. cit.). Another addition to the initial model is in introducing a delay (a finite time period) between the application of the load and the detachment of motor proteins from the filaments. Together, these requirements are sufficient for providing non-damped oscillations.

We describe this concept with so many details because it contains a rudiment of the feedback scheme which by our view is the leading one for the supracellular level morphogenesis, although much greater space/temporal scales are involved in the latter case (see Chap. 4). Also, one can agree with Kruse and Riveline (2011) that the biological role of the above-described oscillations may be in probing the mechanics of cell environment (stiffness and/or elastic tensions); as it will be shown later, no organized morphogenesis can proceed without such a probing.

In addition to oscillations, well-expressed space-enfolded dissipative structures consisting either of actin or of tubulin have been recently reproduced. Actin filaments, propelled in a planar geometry by immobilized molecular motors (meromyosin) when exceeding a critical density, become self-organized to form coherently moving structures which include clusters, swirls, and interconnected bands (Schaller et al. 2010). With the density increase, the disordered phase is changed first by polar nematic clusters and then by wave-like structures with enhanced directional persistency. The ordered structures in the form of swirls span up to several millimeters diameter and have lifetime up to several minutes. These structures are considered as effects of essentially cooperative interactions of many filaments "based on the balanced uptake and loss dynamics of the individual constituents" (op. cit.) which require the input of mechanical energy. In other words, they are typical dissipative events.

The same is true for streams and vortex-like structures consisting of microtubules propelled by surface-bound dyneins (Sumino et al. 2012). The streams of the

microtubules moving in both directions appear in about 5 min after ATP addition and transform into vortices of about 400 μm in diameters after next 10–15 min. Individual microtubules non-trapped within the vortex could leave it for entering a neighbor one or to travel further.

These observations are instructive, since they demonstrate the capacity of energized collectives of filaments to be involved into dissipative structures. On the other hand, their variability and large linear dimensions sharply distinguish them from any structures of a living cell. Much better approximation to the dynamic structures of the real cells is provided by experimental and theoretical analysis of the structures created in active (removed from thermodynamic equilibrium) actin gels. This will be the topic of the next section.

2.7 Metastable ("Glassy") States of the Cytoskeleton and Energy Wells

We start with reviewing in vitro studies of several mechanical parameters (elastic or stiffness modulus and maximal strain values) belonging to cross-linked or bundled actin networks (Gardel et al. 2004; Storm et al. 2005). Let us remind that in the cross-linked actin networks, the filaments are intersected at different angles, while in the bundled networks they are oriented in antiparallel fashion. While measured as functions of either actin concentration, density of cross-linkages or the amplitudes of imposed strains, the above-mentioned mechanical parameters showed a biphasic behavior: When plotted in double logarithmic coordinates, these functions were first rested at the plateau stage and after exceeding a certain threshold demonstrated almost linear increase. The biphasic behavior is associated with two thermodynamically different steps of the samples reactions: enthalpic and entropic. The first phase is the rewinding (straightening) of large loops of actin filaments. This mode dominates under relatively low actin concentrations and large distances between cross-links. Due to random arrangement of the loops, the resulted strains are oriented isotropically. In contrast, the second (entropic) phase is associated with stretching of already rectified filaments. Such a behavior is favored by increased actin concentration and high density of cross-links. The main amount of energy imposed at this stage is spent for eliminating thermal fluctuations and further stretching of the filaments. The resulted strains become anisotropically oriented along the stretch direction.

In order to describe and distinguish both types of behavior, the notions of the so-called persisting and contour lengths of actin filaments are useful (L_p and L_c correspondingly) (Storm et al. 2005; Liverpool 2006). L_c is the "absolute" length of the filament between two neighboring cross-links, while L_p is the length at which the filament loses memory of its orientation (by another expression, L_p is the characteristic length of tangential memory damping). Obviously, the greater the filament is curved and/or the densely are the loops arranged, the smaller is L_p and vice versa.

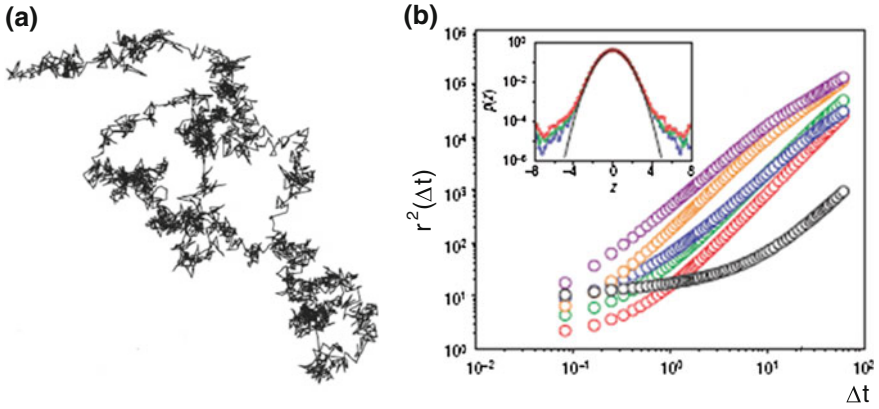

Fig. 2.2 Super-diffusive behavior and self-organized criticality in actomyosin gel. **a** A bead glued to a cell surface shows intermittent dynamics, being delayed in some locations/time moments and extensively translocated in between. **b** Statistics of spontaneous beads motions. *Horizontal axis* time lags Δt (s), *vertical axis* Areas covered during time lags ($r^2(\Delta t)(\mathrm{nm}^2)$). All the graphs except the lowest one which illustrates the effects of ATP depletion are almost linear in double log coordinates, indicating thus critical behavior. *Inset Colored lines* display deviations of beads displacements from a gaussian distribution. From Bursac et al. (2005)

Under $L_c > L_p$, the filament is considered to be flexible (such are the intermediate filaments composed of the protein vimentin), while in reverse case ($L_p > L_c$) the filament is stiff: Collagen and fibrillar actin belong to this category. Accordingly, actin filaments maintain tangential correlations over relatively large lengths, making them adequate conductors of tensile stresses.

Similar kinds of measurements have been performed on living cells with the use of ferromagnetic beads bound to the actin cytoskeleton via cell adhesion molecules (integrins) (Fabry et al. 2003; Bursac et al. 2005; Deng et al. 2006; Trepat et al. 2007). In Bursac et al. experiments, both spontaneous beads movements and those initiated by stepwise or oscillatory shear stresses were traced in several kinds of cells. In all of them, spontaneous movements of unloaded beads showed remarkable intermittent dynamics with periods of stalling (so-called "subdiffusive" behavior) and more extensive translational movements (the "super-diffusive" behavior, exceeding the limits of Gaussian distribution) (Fig. 2.2a, b). Within several seconds after application of a shear stress, the beads motility increased, but in several dozens minutes returned to a control level, indicating what is called solidification. Such a transformation requires ATP splitting energy.

This approach is remarkable, first of all, by treating the cytoskeleton as a unified dynamic system (rather than a mixture of specific components) including into it all the components which provide mechanical integrity of a cell (scaffold proteins, cell–cell and cell–matrix contacts, members of signaling cascades etc.). Such system is under permanent stress fluctuations of different degrees of randomness, which increases under diminishment of characteristic times: While under Tch < 10^{-2} s the movements are completely random, within 10^{-2}–10^{0} s range,

peculiar regime of the active non-equilibrial "stirring" is taking place, combining randomness and directionality. At the greater times, the latter dominates (Fakhri et al. 2014). The authors of the both above-mentioned papers emphasize the widespread of the power law relations between the duration of the action or the frequency of the applied forces on the one hand and the changes of mechanical parameters (stiffness, elasticity) of the sample on the other hand. As mentioned in Chap. 1, such systems should be close to the state of self-organized criticality. Applied to actin networks, it may indicate emergence of a large number of the different depth energy wells which the system can select while moving along the relaxation pathway: The deeper the well, the less it is available (because of being presented in smaller proportion or separated by higher energy barrier). In other words, power law dependence indicates metastability of actin networks and their readiness to select one of the available states. Moreover, a notion of metastability index can be introduced "as the level of mechanical agitation (noise) present in the microenvironment relative to the depth of the energy well" (Fabry et al. 2003). This index can be also defined as the "effective temperature of the matrix" (op. cit.) which is roughly proportional to the energy pumped into the cytoskeleton. When the index is high, the element can hop randomly between wells so that the system as a whole can flow and become disordered. Under smaller index values, the system's elements become trapped in deep wells from which they are unable to escape; under these conditions, the system behaves as an elastic solid body (op. cit.).

These suggestions, hardly familiar to developmental biologists, may be important for paving a way toward essentially new approaches to morphogenesis and cell differentiation. The matter is that metastability of the cytoskeleton (in its broadest meaning) may point to the cell's maintenance in a competent, but as yet non-differentiated state. Taking into consideration the increased evidences on the role of cytoskeletal transformations in cell differentiation (see Chap. 4 for more detailed discussion), one may assume that selection between cell differentiation pathways may be close if not identical to that between different energy wells of the cytoskeleton's energy relief.

2.8 Cell-Matrix and Cell–Cell Contacts: Mechanodependent Self-organization

One of the most important but as yet not properly evaluated recent achievements in cell biology was a discovery that the active responses to mechanical signals are necessary not only for differentiation but even for the very survival of metazoans cells: In the absence of such signals or under inability to respond by self-stretching, a cell is unable to proliferate (Chiquet et al. 2009) or even switches on the apoptotic program (Chen et al. 1997). In this section, we shall look what is known about molecular devices used by cells to percept and elaborate mechanical signals. As usual, we will make an accent to their dynamics, feedbacks, and self-organizing properties.

For percepting mechanical signals from the surroundings, a given cell should be bound with another one either by direct contacts, or via components of extracellular matrix (ECM). Most of the direct cell–cell contacts belong to the so-called adherent, or cadherin-mediated junctions while cell–ECM contacts are exhibited by the so-called focal junctions. Both of them are complicated multimolecular ensembles quite far from being static fastenings. Moreover, we shall see that they are active mechanosensitive formations far from thermodynamic equilibrium.

The first fundamental property of both types of contacts is that they become strengthened under loading (increased tensile forces) and weakened with the loading (tensions) decrease. This property belongs to the class of feedback reactions which are basic for all morphogenetic processes and will be discussed in more details in the next chapter. As applied to individual cells, it was studied in most details on cell–ECM (or cell-artificial substrate) contacts. Cells' bounding to ECM is provided by proteins of the integrin family which are at the same time connected with the so-called adaptor proteins linking them with actin filaments from the cytoplasmic side. The both side linkages are mechanodependent. Among them, fibronectin fibers (the main, if not the sole ECM component in embryonic tissues) are characterized by astonishing degree of extensibility (the greatest for biological fibers): They may be stretched in 8 times and when released return to the starting length within just a few minutes (Klotzsh et al. 2009). Although the Young modulus of fibronectin is extensively increased under stretching, the cells' produced forces (of several nN range) are estimated to be enough for at least moderate fibronectin stretching. Importantly, ECM stretching increases the exposure of cryptic sites which contain some imminent component of embryonic induction (Wipff et al. 2007).

At the cytoplasmic side, integrins are bound in a tension-dependent way to vinculin through the adaptor protein talin-1. Vinculin also links to actin and is recruited in response to applied forces. Another adaptor protein is $p130^{Cas}$ which, when being phosphorylated, activates small GTPases. As a result, hundreds of proteins functionally entangled to a net of positive and negative feedbacks are recruited to adhesion sites which are passing under loading from the state of small (<1 μm diameter) and unstable contacts to much larger (several μm diameter) contact zones, called focal adhesions (FAs). FA areas were shown to be in a strict linear proportionality to the amount of applied force, keeping thus a constant stress value estimated as 5.5 $nN/\mu m^2$ (Balaban et al. 2001). This again corresponds to several pN force applied to a single integrin molecule.

Recently, several physically based models have been proposed for FA growth (Shemesh et al. 2005; Ladoux and Nicolas 2012). In the context of this book, they are of a special interest because the suggested mechanisms of recruiting protein molecules to FA sites have some similarities with the models of stress-dependent behavior on the level of cell collectives (to be discussed in the next chapter). By Shemesh et al. model, the molecular dynamics of FA is driven by the tendency to minimize total free energy of the system which includes both the pool of free protein molecules and those aggregated onto a substrate stretched by actomyosin-generated pulling forces and anchored from the other side to ECM. The main system's parameters are the energy of pulling forces and the difference of chemical potentials

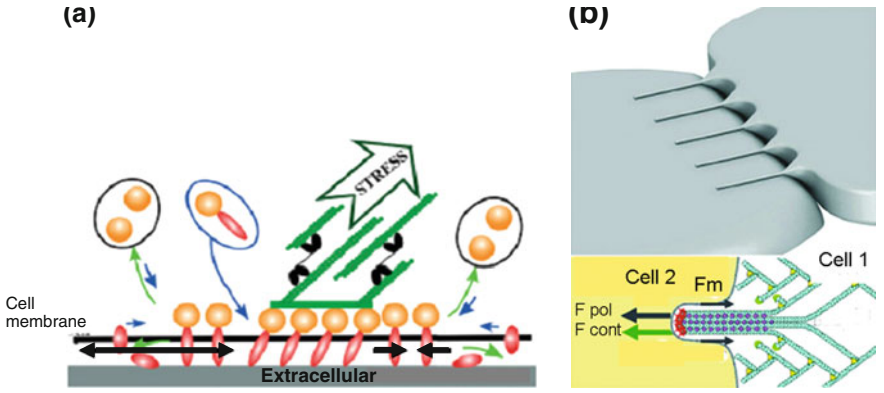

Fig. 2.3 Asymmetry in cell-substrate and cell–cell junctions. **a** Dynamics of protein turnover in focal junctions. By the action of asymetrically oriented actomyosin-generated pulling stress the leftward part of the cell membrane is stretched, while the opposite side is relaxed or slightly compressed. As a result, the protein insertion to the *left* part is expected to exceed that to the *right* one. **b** Apical junction between two epithelial cells as an asymmetric push–pull unit. Cell 1 exerts a pushing impulse generated by actin polymerization to cell 2. The latter responds by actomyosin contraction. F cont is the contractile (pulling) force and Fm is the membrane resistance of Cell 2. F pol is the pushing force of actin polymerization exerted by Cell 1. **a** From Ladoux and Nicolas (2012), modified. **b** From Brevier et al. (2008), © IOP Publishing, reproduced by permission of IOP Publishing. All rights reserved

of protein molecules in the aggregated and in the free state. Within a wide enough range of parameters, it becomes energetically favorable for free molecules to become inserted in the stretched substrate just in the direction of the pulling force because in this case, the elastic stress of the aggregated phase is relieved: This corresponds to the directed FA growth. Under relatively smaller pulling forces FA either becomes in a steady state or disassembles. If applying this model to the typical asymmetric arrangement of actomyosin bundles in relation to FA plane, one can see that the pulling bundles will be flanked by a stretched zone from the one side and by a compressed one from the other side. Under these conditions, it will be thermodynamically favorable to insert protein molecules into the stretched zone and remove them from compressed one. As a result, the molecular turnover will be accompanied by extension of the leading cell edge, that is, by crawling of the cell (Fig. 2.3a).

The local drive toward free energy decrease in no way mean that the entire mature FA is an equilibrial structure. Rather, it requires an imminent flow of directed energy being hence similar to a dissipative structure requiring tensile stress for its maintaining.

A requirement of non-dissipated portion of energy for stabilizing FA is confirmed by the properties of cell reaction known as rigidity sensing: FAs are reinforced and stabilized when establishing contacts with a rigid (non-deformable)

rather than a soft (deformable) matrix. Obviously, under the contacts with non-deformable substrate, the mechanical energy generated by actomyosin contraction is not dissipated, while in case of a relatively soft substrate, a substantial amount of energy is spent to deform the latter. This is quantified by a simple expression $W = F^2/2K$ where in our case, W is the amount of energy required by a cell to build the force F and K is the so-called spring constant, characterizing the substrate stiffness (Bischofs and Schwarz 2003). Accordingly, the greater the substrate stiffness, the smaller will be the amount of energy W spent by the cell to generate a given amount of force F and hence creating FA of a given square (which is, as mentioned above, proportional to F). In terms of time t and power N, the equivalent expression is $t = F^2/2KN$ which means that under the same power, the greater the substrate stiffness, the smaller the time period required for creating a given square FA. A widespread and morphogenetically important (but not universal) cells property to orient themselves onto anisotropic substrates along the maximal stiffness direction is explained by Bischofs and Schwarz as a tendency to minimize the energy and the time required for producing the given square FA.

On the other hand, the experiments by seeding cells onto dense arrays of pillars of a different rigidity (Saez et al. 2005) lead to somewhat other conclusions. It was found indeed that the force exerted by a cell upon each given pillar is linearly proportional to the pillar's spring constant, thus equally deforming the pillars of different stiffness. Meanwhile, according to the above-presented formula under constant energy production, the force should be proportional only to the square root of the spring constant ($F = \sqrt{2WK}$); in order to achieve a linear force/stiffness dependence, the energy produces by a cell should increase with the stiffness increase and be stored most probably in the actin cytoskeleton, making it pre-stressed. In other words, the stiffness increase should induce a positive feedback between aggregation of proteins promoting FA growth and the pulling force exerted by microfilaments. Such reactions were indeed observed. Moreover, by using ingenious single cell measuring device, the pulling force was shown to be initiated surprisingly soon ($t < 0.1$ s) after abrupt change in stiffness (Mitrossilis et al. 2010). So instead of always minimizing the energy spending, a cell behaves as an experienced stockbroker immediately increasing the investments in the projects promising greatest profits: In our case, these are the sites of greater stiffness. This emphasizes once again domination of non-equilibrium energy spending inside cells; more specifically, it points to a positive feedback between the proteins aggregation within FA and the amount of mechanical energy directed toward this FA.

Interestingly, both Bischofs and Schwarz model and Saez et al. results lead to the same qualitative conclusions about cell behavior on the substrates of regionally different stiffness: The cells will establish larger contacts with more stiff substrates and, in case of stiffness gradients move upwards the gradients, exemplifying the so-called durotaxis (Lo 2000). Coming now to unequally tensed elastic substrates and assuming that its stiffness is proportional to tension, we come to the idea of tensotaxis, that is, cell movement upwards the tension gradients. In addition, by spending its contacts-making energy in direct proportion to the local stiffness/tension of the

substrate, a cell should enhance, rather than smooth the regional differences in stiffness/tension. This will be shown to be of importance for the behavior of cell collectives.

The dynamics of cadherin-mediated cell–cell junctions share the main properties of focal adhesions in the sense that both maturation and maintenance of the first ones are also mechanodependent. These contacts transmit from one cell to another actomyosin-generated tension of pN range (Borghi et al. 2012) which promotes recruitment of vinculin to the sites of cell adhesions (Sumida et al. 2011). Another type of cell–cell contacts, hemidesmosomes of *C. elegans*, respond to muscle-generated tensions by switching on several signaling systems (Zhang et al. 2011). This may explain why muscle contraction is required for proper development of embryos of these species.

Similarly to focal junctions, cadherin-mediated ones are asymmetric, taking the shape of so-called push–pull units (Brevier et al. 2008). Their assembly starts from formation of a lamellopodia by one of the cells (called the donor cell) which exerts a pushing force (produced by actin polymerization) to the neighboring acceptor cell. As a result, the donor cell makes a protrusion, penetrating into acceptor cell. In response, this latter develops actomyosin contraction exerting thus a pulling force to the donor cell's protrusion; in the absence of this force, the protrusion fails to elongate (Fig. 2.3b).

A "push–pull" structure of the cadherin-mediated contacts indicates ones more regular lateral asymmetry of epithelial cells, which should be added to their universal apico-basal polarity. At the same time, by constantly requiring input of energy canalized by the action of actomyosin machine, these structures share non-equilibrial properties of focal junctions.

Recently, several refined fluorescent and computer imaging techniques were used for quantifying anisotropic stresses generated by mammary epithelial cells in 3D cultures (Campàs et al. 2014). Under all the reservation mentioned by the authors, this estimation seems to be the first reliable one related to the integrated tissue rather than single cells. The values obtained were 3 and 4 $nN/\mu m^2$ which is of the same order as those detected by Balaban et al. (2001) for focal junctions and twofold larger than the stresses generated by cells of embryonic tooth mesenchyme, either within cultured aggregates and in developing whole mouse mandibules.

Ingenious method for measuring forces acting across the vinculin molecules as depending upon the assembly or disassembly of the focal adhesions was elaborated by Grashoff et al. (2010). The tension sensors were constructed in which an elastic molecular domain derived from the spider silk protein was inserted between two fluorophores undergoing resonance energy transfer with wavelength changing as a function of applied force. By calibrating this device, the absolute values of forces could be measured which gave ≈2.5 pN values per vinculin molecule in stable focal contacts. Similar technique was used for evaluating the effects of the shear stress across cadherin-containing cell–cell contacts (Conway et al. 2013).

Further development of these methods opens quite new perspectives for estimating stress patterns at the level of macromorphology as well: It would be interesting to compare so-called maps of mechanical stresses based up to now upon

the local incisions techniques (see Chap. 3) with those obtained with the use of molecular sensors. This does not mean that the classical methods will lose their validity: For example, the molecular sensors hardly can detect the directions of tensions. In any case, however, the new techniques are the first ones permitting to measure the absolute values of preexisted, rather than artificially imposed stresses.

2.9 Transmission and Regulation of Mechanical Forces in Cell Cortex

As will be argued in more details in the next chapter, mechanical stresses, in most cases tensile, are transmitted in embryonic tissues over macroscopic distances, greatly exceeding individual cells diameters. Because of poor development of extracellular matrix and lack of connective tissue fibers in early embryos, the main root for transmitting tensions goes via complicated set of structures including cell (plasma) membrane proper (that is, lipid bilayer together with membrane proteins), submembrane actomyosin cytoskeleton, and membrane-to-cytoskeleton attachments linking these two components. For the sake of simplicity, we shall define this entire set of structures as cell cortex, although some authors relate this notion to submembrane components only.

The first paradox we are encountered with when speaking about tensions at the cell surface is that the lipid bilayer itself can be stretched no more than to about 2 % (Apodaca 2002), while many cells including embryonic ones, can be artificially elongated more than to 100 % of initial length, especially if the stretching is performed by several rounds separated from each other by 1–2 min intervals. It is the intervals requirement which gives the answer: During each round of stretching and immediately after it, the initial square of lipid bilayer is restored (and in some cases, restored with an overshoot) due to insertion of new portions of membrane taken from the so-called membrane reservoir. This is internalized and often folded membrane fraction, which, depending on mechanical state, may be either rapidly (within minutes or even seconds) inserted in the outer membrane plane or undergo the reverse transformation. The main part of reservoir is presented by caveolae, 60–80 nm in diameter membrane invaginations. Under physiological or experimentally induced (caused by hypotonicity) membrane stretch, the caveolae are flattened and disassembled within about 2 min in actin and ATP-independent way, considerably decreasing membrane tension. After returning to normal osmotic conditions and thus releasing membrane tension, caveolae are reassembled, now by slow actin and ATP-dependent process. As a result, cell volume becomes even smaller than it was before hypo-osmotic shock, demonstrating a certain overshoot (Sinha et al. 2011). Other sources of a membrane reservoir which are smaller than the caveolar compartment but important at some steps of cell reaction are membrane microfolds, the so-called vacuole-like dilations (spherical membrane invaginations), blebs (membrane evaginations nonsupported by actin cytoskeleton), and a pool of endomembranes undergoing exocytosis (Gauthier et al. 2012).

Tension regulation with the use of membrane reservoir is well studied and creates one of the most important morphogenetic feedbacks: Namely, cell surface stretching promotes externalization of some part of membrane pool while relaxation of cell surface tension and, the more, shrinkage of cell membrane switches on its internalization. During phagocytosis, a well-documented increase of exocytosis activation is taking place (Masters et al. 2013). Obviously, these reactions are directed toward restoring tensional homeostasis: Externalization of some part of membrane pool decreases overnormal tension, while its internalization works in the opposite way. The maintenance of membrane tension on a certain level is indispensable for polarization and directed migration of single cells (Houk et al. 2012). Let us discuss in more details the tensional dynamics during cell spreading, as studied by Gauthier et al. (2011, 2012).

After seeding a cell onto a substrate, the membrane tension is increased in 2–3 times. This is followed by unfolding of membrane reservoir, consisting at that time mostly of large membrane folds. At this stage, the tension is kept constant. Then, the next increase of membrane tension is taking place, in 2–3 min followed by a burst of exocytosis leading to extensive enlargement of membrane area and hence to its relaxation. At the same time, submembrane actomyosin network is contracted, producing membrane ruffles. As a result the tension is dropped again, now below the previous minimal level. The authors suggest that tensional dynamics is characterized by repetitive increase–decrease cycles of few dozens minutes each, each next of them occurring at lower membrane tension. As a result, cell surface becomes heterogeneous obtaining several protrusions which are anchored to the substrate and develop focal contacts associated with microfilament bundles. The latter's activity leads to another increase of tensions, now strictly localized along the protrusions. At last, due to protrusions competition, the cell acquires a single polar axis joining the leading and trailing edges. This process, also to great extent mechanically regulated, is described in more details in the next section.

At the moment, the following points from the above description are of the main interest. The first of them is that membrane tensions demonstrate a cyclic dynamics, indicating feedbacks between relaxation and strengthening; second, the feedbacks are associated with obvious overshoots; and the last point is that they drop down the cell symmetry order from almost spherical ($\infty/\infty \cdot m$) to polarized ($1 \cdot m$). This means that already at the single cell level we have a small and rudimentary, but nevertheless adequate model of morphogenesis expressed in its full scale at the level of cell collectives.

By summarizing, one may conclude that cell cortex shares both solid (elastic) and quasi-liquid properties. The elastic component is mostly coupled with submembrane actomyosin network, while the membrane proper (lipid bilayer) exhibits quasi-liquid behavior, maintaining its strain within quite narrow limits independently of preceded deformations. We use a prefix "quasi" because in the case considered the standard behavior of subsurface molecules in ordinary liquids is now reproduced on the scales of membrane vesicles and invaginations, exceeding those of single molecules in 3–4 orders at least. As we shall see later, such a dualistic

elasto-liquid behavior of cell surfaces provides a long-term maintenance of macroscopic tensile fields which are indispensable for multicellular morphogenesis.

Accordingly, as applied to cell cortex, the physical measures used traditionally both for solids and liquids can be employed. Although in most cases the estimations are of a relatively low precision, they permit to compare the values of the active forces generated within cells with those of passive resistance of cell cortex elements. As mentioned before, active forces produced by actin polymerization at the leading cell edge are enough for equilibrating resistance up to $\sim$300 nN without reducing the rate of cell advancement (Carey et al. 2011). Assuming that there are the forces generated at the leading cell edge due to actin polymerization which pull and stretch cell cortex, it will be relevant to compare them with the mechanical resistance of a lipid bilayer and submembrane actomyosin network. The elastic modulus of a lipid bilayer lays within 10^3 N/m^2 = 1 nN/μm^2 range (Diz-Muñoz et al. 2013), while that of actomyosin network extensively varies in a nonlinear fashion, depending upon its stretching (Rauzi and Lenne 2011). In the above described slightly stretched and loops-abundant enthalpic phase, actomyosin network is very soft (elastic modulus about 1 N/m^2), while under extensive stretching it passes to entropic phase with the filaments aligned in stretch direction: Now, elastic modulus can rise up to at least 10^3 N/m^2. The branched actin structures located at the leading cell edge are even stiffer: At least in vitro conditions, elastic modulus for these structures reaches 10^3 N/m^2. Suggest that the pulling force of actin polymerization is evenly spread throughout the cell membrane area having 10 nm = 10^{-2} μm thickness and several hundreds (say from 200 to 500) μm diameter (this is usual transversal cell diameter at the leading edge level). We can see that a force non-exceeding 10 nN range, that is a small part of what may be produced by actin polymerization at the leading edge, is enough for equilibrating the elastic resistance of lipid bilayer and for stretching at the same time the actomyosin network. This increases the probability of the cortical layer of an actively spreading cell to be stretched for most of the time.

Quasi-liquid properties of the cortical layer have been estimated by tension measurements giving for a nerve growth cone the value of 3×10^{-3} mN/m, for a neutrophil 3×10^{-2} nN/μm, and for erythrocyte an order greater (Hochmuth et al. 1996). Accordingly, for increasing the contour length of the cell membrane to 100 μm (and the average cell diameter to $100/\pi \approx 30$ μm), a similar force of few nanonewtons is enough. Under all reservations, these results indicate that just a small part of forces produced within a cell is enough to deform it.

2.10 Symmetry Breaks in Entire Cells

Is there anything in common in the symmetry breaks taking place at different stages of development—from its very start up to formation of specialized cells? A number of recent investigations (Munro 2006; Li and Gundersen 2008; Gucht Van der and Sykes 2009; Nance and Zallen 2011; Goehring and Grill 2013; Diz-Muñoz et al. 2013)

permitted to give a generally positive answer to this question. A brief generalized review of the main results is given below.

First, it was found that the key structural regulators of cell polarity belonged in all the cases to the family of so-called PAR (partitioning) proteins, which consist of two mutually extinct groups, displacing each other from cells cortex (mostly by phosphorylation). This already creates a negative feedback, promoting segregation of both groups. Next, extensive cross talks, mostly providing positive feedbacks, are also taking place between the members of PAR family and small GTPases Cdc-42 and Rac. It is also worth mentioning, that all the PAR proteins are highly dynamic, turning over on a timescale of tens of seconds, even if they support more stable structures.

Another member of the dynamic circuit leading to cell polarization is actomyosin network which undergoes unilateral contraction displacing the group of PAR proteins by means of so-called advection, that is, by involving them into a common flow. PAR proteins are far from being passive participants in their redistribution: For example, PAR-3 amplifies myosin activity. As a result, another positive feedback is added.

The third vectorizing member is exemplified by microtubules, the role of which largely varies in different cases of cell polarization.

One of the best studied examples of cell polarization is the establishment of antero-posterior (AP) axis in newly fertilized *C. elegans* eggs. Prior to fertilization, the entire egg cortex is occupied by one of PAR groups, the so-called Par-3/Par-6/Pkc-3 complex. The sperm marking by its entrance points the posterior pole of the future organism brings with itself the microtubule organizing center (MTOC) which relaxes the adjacent part of actomyosin network, stimulating its shift toward anterior and similarly directed advection of the above-mentioned PAR complex. Also, it stimulates the recruitment of the members of the alternative PAR proteins group to the posterior domain, possibly by its direct transportation along microtubules (Bastock and Johnston 2011). As a result, the posterior and anterior domains become sharply segregated from each other, providing strictly different fates of the blastomeres developed from each of these regions.

Either actomyosin flows or microtubules transport involving PAR proteins participate in almost all other cases of egg or cell polarization. Thus, in vertebrate oocytes (both in mouse and in *Xenopus*) the above-mentioned PAR proteins complex is involved by this flow toward the future animal pole and obviously determines the latter's position. In ascidian eggs, the oocyte polarity is first manifested by quite extensive Ca^{2+} triggered actomyosin flows displacing the so-called myoplasm, enriched by mitochondria, toward the vegetal pole. Later on a bundle of oppositely directed microtubules (their "+" ends located near the animal pole) comes into play, compressing the myoplasm at the site of the future posterior pole. The accumulation of PAR proteins complex starts from 2 blastomeres stage and goes up to 16-cell stages. Being at first microfilaments dependent, it becomes later on transported along microtubules (Sardet et al. 2007). Formation of the apico-basal polarity of mammalian epithelial cells is associated with the Par-3/Par-6/Pkc-3 loaded flows directed toward the sites of the cadherin-mediated cell–cell contacts,

while the abbreviated Par-6/Pkc complexes become bound with apical cell surfaces. In contrast, the apico-basal polarization of *Drosophila* cells is driven by the dynein-based transport of PAR-3 along microtubules toward apico-lateral cell boundaries. In reverse, PAR proteins are known to be implicated in localizing the force-generating interactions between microtubules and the cortex, being the main regulator of the polarized microtubules-based transport in *Drosophila* oocytes, *C. elegans* cleaving eggs and in both vertebrate and invertebrate epithelia (Munro 2006). By comparing the contributions of actin-based and microtubules-dependent mechanisms, it is suggested that the first ones play the key role in the initiation and rapid responses to external stimuli, while microtubules build on and stabilize the initial asymmetry (Li and Gundersen 2008).

So we see that cell polarization is always associated with a complicated network of positive and negative feedbacks between certain groups of proteins as well as between the force-generating devices—microfilaments and microtubules together with motor proteins. Taken themselves, these feedbacks are already directed toward segregating initially homogeneous cortical patterns into different domains, but are they enough to provide the global order on the single cell scale being an imminent component of cell polarization? Let us discuss this problem taking a typical polarization pattern of a crawling cell as example (Fig. 2.4). As one can learn from textbooks on the molecular biology of the cell, a polarized state is characterized by domination of the myosin II-free arborescent actin structures at the leading edge and of actomyosin contractile filaments at the rear end of a cell; this pattern is supported by negative feedbacks between the activity of two GTPases: Rac in the anterior region and Rho in the posterior one. However, this seems far from being the whole story. By casting again a glance to Fig. 2.4d–f, one can see that from the morphological point of view, the antero-posterior cell polarization means selection of a single axis out of the entire set of possible directions. This implies the competition

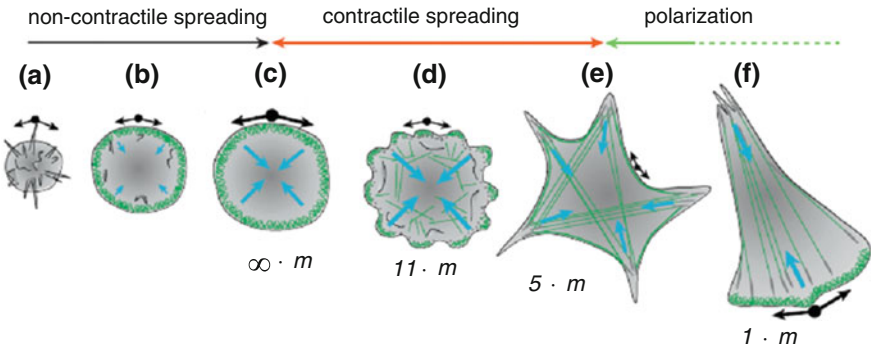

Fig. 2.4 Diagrams of mechanical stresses at successive stages of cell spreading and polarization. *Black diverged arrows* display tensions on cell surface; *blue converged arrows* display tensions generated by actomyosin contraction and maintained by newly established focal junctions. *Note* periodic ups and downs in surface tension and an overall decrease of the cell symmetry order (shown). From Gauthier et al. (2012), © Elsevier 2012, reproduced with permission from Elsevier

between several primary protrusions shown in Fig. 2.4d. Tensions look to be the best agents for making this job. Just this point of view is developed in several recent studies (Kozlov and Mogilner 2007; Houk et al. 2012; Diz-Muñoz et al. 2013). In particular, Houk et al. argued that diffusion-based mechanisms are non-sufficient for long-range interactions between frontal and rear parts of cells, while the membrane tension which by their estimations is increased twofold during leading edge protrusion may exert long-range inhibition of actin assembly going within a cell in antero-posterior direction. Within such a framework, the role of chemical attractants which are known to orient antero-posterior axes of several types of cells (from yeasts to lymphocytes) may be in triggering the internal mechanically based regulatory contour which in the second turn recruits the above-mentioned GTPases to their proper localizations.

A bold attempt to interpret the factors of cell polarity maintenance in mechanical terms without the use of chemical feedbacks has been performed by Kozlov and Mogilner (2007) employing keratinocyte fragments as a model. The fragments exhibit two most ubiquitous shapes, round and crescent-like. In the first case, just two mutually balanced forces are present: the passive tangential tension and the pushing force produced by the actin polymerization; in crescent-like forms, the contractile force of the active myosin-powered tension in the rear (concave) area is added. By the authors calculations, the free mechanical energy of the sample regarded as a function of the crescent's opening angle θ has two minima: the first one at $\theta = 0$ (corresponding to the round shape) and the other one laying between 100° and 150°. The height of the energy barrier between them depends on the ratio between the myosin-powered tension at the rear edge and the pushing force of actin polymerization at the frontal edge: The greater the latter force is, the easier is the transition from the non-polarized (round) to the polarized (crescent-like) form. The authors suggest that the main polarizing factor is just the drive toward energy minimum, while the role of more detailed chemical signaling is restricted in regulating the rate of this drive.

The next question related to symmetry breaks on the single cells level is to whether they require strong external cues or instead can go "spontaneously," that is, under infinitesimal perturbations. As far as many of symmetry breaks are started from disrupting the cortical actomyosin network, let us look for potential possibilities of such a net to be fractured "spontaneously."

In this respect, it is worth reminding a topological Brouwer theorem (for a biologically oriented review see Isaeva et al. 2012) which states that any vector field mapped onto three-dimensional surface (but not onto two- or one-dimensional ones) must have at least one singularity, that is, a point where a field continuity is broken. Just such a field is exemplified by actin network deposited on the cortex of three-dimensional egg cells: Thus, egg cortex should necessarily have at least one point of potential rupture. The demand of singularity may be the most fundamental cause for what we call cell polarization; certainly, this trend should be properly located and amplified by a set of regulatory factors.

As the first step toward more concrete models, we may consider the behavior of actin gel surrounding microscopic beads (Van der Gucht and Sykes 2009). Due to

actin polymerization, new monomers are incorporated at the bead surface underneath the preexisting gel, producing stresses and making the entire actin shell tensed. Thus, after some time the shell breaks, releasing the accumulated elastic energy. This may be an adequate model for a spectacular formation of the comet-like actin tails by a bacteria *Listeria* and to some extent for the break of actomyosin network in *C. elegans*, although in the latter case the site of the break is predetermined by the male centrosome. In any case, the value of tensile stress required for actin shell break was found to be of the same order (10^3–10^4 Pa) as for the cortical layer of most cells. By these estimations, cell cortex may be close to the break threshold, requiring no more than about 10 % of additional stress to be disrupted. The distance between the amount of the cortical tension and the instability threshold may regulate the number of ruptures to be performed: If the tension is far from threshold, the break requires a strong external influence: In this case, a single rupture is expected. Meanwhile, by approaching to the verge of stability, the appearance of numerous ruptures is more probable.

Coming back to cell polarization, we have to remind first of all that egg cells may acquire animal–vegetal polarity and, in particular, dorso-ventrality, either being induced from outside or spontaneously (see Chap. 4 for more detailed discussion). Same dualism holds true for other cases of cell polarization. For example, although by many evidences the establishment and maintenance of apico-basal polarity of an epithelial cell requires such external cues as contacts with neighboring cells and ECM, a spatially smoothed ectopic activation of the kinase LKB1 (related to the activation of myosin II based contractility) induces spontaneous symmetry breaking and establishes well-defined apical and baso-lateral domains in the absence of cell–cell contacts (Li and Gundersen 2008).

In the case of the planar cell polarity, the situation is similar. In a number of cases, it requires distinct dissymmetrizers. According to the above-presented calculations of Van der Gucht and Sykes, the moderate mechanical forces, able to overcome the energetic barrier of actomyosin net rupture, may play such a role. In this respect, some novel data on the effects of mechanical tensions on planar cell polarity may be of interest. Aigouy et al. (2010) proposed that the tensions exerted from the proximal part of *Drosophila* wing provide planar polarity of the distal part cells. In our laboratory, Evstifeeva (2013) demonstrated that in artificially stretched double explants of ventral ectoderm taken from early gastrula *Xenopus* embryos, the streams caused by ciliary beating are oriented in most cases perpendicular to stretch directions and in the intact embryos they orient at right angles to the tensions produced by gastrulation and neurulation movements. Meanwhile, in non-stretched explants, the streams are oriented much more randomly, although if tending to be grouped into several small uniformly oriented regions. So far as the direction of ciliary beating marks planar cell polarity, this indicates that the latter may be affected by mechanical force in vertebrate embryos as well.

By concluding, we may suggest that such an important and esthetically beautiful event as cell polarity is based upon a complicated and up to now not completely untangled web of negative and positive feedbacks related to different levels; among them, mechanical feedbacks seem to play the leading role in a large-scale

integration of lower levels ones. For these feedbacks to be effective, the entire cell should be either in an unstable state, or on the verge of stability: In both cases, it behaves as a nonlinear system. Being still rudimentary on the levels of cytoskeleton and single molecular machines, nonlinear properties are expressed in full scale at the upper structural levels of cell organization.

References

Aigouy B, Farhadifar R, Staple DB et al (2010) Cell flow reorients the axis of planar polarity in the wing epithelium of Drosophila. Cell 142(5):773–786

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (2003) Molecular biology of the cell. Garland Publishing Inc, New York

Apodaca G (2002) Modulation of membrane traffic by mechanical stimuli. Am J Physiol Renal Physiol 282:F179–F190

Balaban NQ et al (2001) Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nature Cell Biol 3:466–472

Bastock R, Johnston D (2011) Going with the flow: an elegant model for symmetry breaking. Devel Cell 21:981–982

Bishofs IB, Schwarz US (2003) Cell organization in soft media due to active mechanosensing. PNAS 100:9274–9279

Bluemenfeld LA (1983) Physics of bioenergetic processes. Springer, Berlin

Borghi N, Sorokina M, Sherbakova OG, Weis WL, Pruitt DL, Nelson WJ, Dunn AB (2012) E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. PNAS 109:12568–12573

Brevier J, Montero D, Svitkina T, Riveline D (2008) The asymmetric self-assembly mechanism of adherent junctions: a cellular push-pull unit. Phys Biol 5(1):016005

Bursac P, Lenormand G, Fabry B, Oliver M, Weitz DA, Viasnoff V, Butler JP, Fredberg JJ (2005) Cytoskeletal remodelling and slow dynamics in the living cell. Nat Mater 4:557–561

Campàs O, Mammoto T, Hasso S, Sperling RA, O'Connell D, Bischof AG, Maas R, Weitz DA, Mahadevan L, Ingber DE (2014) Quantifying cell-generated mechanical forces within living embryonic tissues. Nat Methods 11:183–189

Carey SP, Charest JM, Reinhart-King CA (2011) Forces during cell adhesion and spreading: implications for cellular homeostasis. Stud Mechanobiol Tissue Eng Biomatter 4:29–69

Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE (1997) Geometric control of cell life and death. Science 276:1425–1428

Chernavskii DS, Chernavskaia NM (1999) Protein-machine. Biological macromolecular constructions. Moscow University Printing House, Moskva

Chiquet M, Gelman L, Lutz R, Maier S (2009) From mechanotransduction to extracellular matrix gene expression in fibroblasts. Bioch Biophys Acta 1793:911–920

Conway DE, Breckenridge MT, Hinde E, Gratton E, Chen CS, Schwarts MA (2013) Fluid shear stress on endothelial cells modulate mechanical tension across VE-cadherin and PECAM-1. Curr Biol 23:1024–1030

Danilchik MV, Brown EE, Riegert K (2006) Intrinsic chiral properties of the Xenopus egg cortex: an early indication of left-right asymmetry? Development 133:4517–4526

Del Giudice E et al (2005) Coherent quantum electrodynamics in living matter. Electromagnetic Biol Med 24:199–210

Del Giudice E, Stefanini P, Tedeschi A, Vitiello G (2011) The interplay of biomolecules and water at the origin of active behavior of living organisms. J Phys Conf Ser 329:012001

Deng L, Trepat X, Butler JP, Millet E, Morgan KG, Weitz DA, Fredberg JJ (2006) Fast and slow dynamics of the cytoskeleton. Nat Mater 5:636–640

Diz-Muñoz A, Fletcher DA, Weiner OD (2013) Use the force: membrane tension as an organizer of cell shape and motility. Trends Cell Biol 23:47–53

Evstifeeva AJu (2013) Topology and planar polarity of Xenopus embryonic ciliary epithelium. Izvestia Russ Acad Sci Ser Bio 40(6):661–667 (in Russian)

Fabry B, Maksym GN, Butler JP, Glogauer M, Navajas D, Taback NA, Millet EJ, Fredberg JJ (2003) Time-scale and other invariants of integrative mechanical behavior of the living cell. Phys Rev E 68:041914

Fakhri N, Wessel AD, Willms C, Pasquali M, Klopfenstein DR, MacKintosh FC, Schmidt CF (2014) High-resolution mapping of intracellular fluctuations using carbon nanotubes. Science 344:1031–1034

Forgacs G (1995) On the possible role of cytoskeletal filamentous networks in intracellular signaling: an approach based on percolation. J Cell Sci 108:2131–2143

Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira P, Weitz DA (2004) Elastic behavior of cross-linked and bundled actin networks. Science 304:1301–1305

Gauthier NC, Fardin MA, Roca-Cusachs P, Sheetz MP (2011) Temporary increase in plasma membrane tension coordinates the activation of exocytosis and contraction during cell spreading. PNAS 108:11467–11472

Gauthier NC, Masters TA, Sheetz MP (2012) Mechanical feedback between membrane tension and dynamics. Trends Cell Biol 22:527–536

Goehring NW, Grill SW (2013) Cell polarity: mechanochemical patterning. Trends Cell Biol 23:72–80

Goodwin BC, Trainor LEH (1985) Tip and whorl morphogenesis in Acetabularia by calcium-regulated strain fields. J Theor Biol 117:79–106

Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, McLean MA, Sligar SG, Chen CS, Ha T, Schwartz MA (2010) Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nature 466:263–266

Gurwitsch AG (1944) A theory of biological field (in Russian). Nauka, Moskva

Hochmuth F-M, Shao J-Y, Dai J, Sheetz MP (1996) Deformation and flow of membrane into tethers extracted from neuronal growth cones. Biophys J 70:358–369

Houk AR, Jilkine A, Mejean CO, Boltyansky R, Dufresne ER, Angenent SB, Altshuler SJ, Wu LF, Weiner OD (2012) Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. Cell 148:175–188

Howard J (2009) Mechanical signaling in networks of motor and cytoskeletal proteins. Ann Rev Biophys 28:217–234

Igamberdiev AU (2012) Physics and logic of life. Nova Science Publishers, New York

Isaeva VV, Kasyanov NV, Presnov EV (2012) Topological singularities and symmetry breaking in development. BioSystems 109:280–298

Ishiwata S, Shimamoto Yuta, Suzuki Madoka (2010) Molecular motors as an auto-oscillator. HFSP J 4:100–104

Klotzsh E, Smith ML, Kubow KE, Muntwyler S, Little WC, Beyeler F, Gourdon D, Nelson BJ, Vogel V (2009) Fibronectin forms the most extensible biological fibers displaying switchable force-exposed cryptic binding sites. PNAS 106:18267–18272

Kozlov MM, Mogilner A (2007) Model of polarization and bistability of cell fragments. Biophys J 93:3811–3819

Kruse K, Riveline D (2011) Spontaneous mechanical oscillations: implications for developing organisms. Curr Top Devel Biol 95:67–91

Ladoux B, Nicolas A (2012) Physically based principles of cell adhesion mechanosensitivity in tissues. Rep Prog Phys 75:116601 (25 p)

Li R, Gundersen GG (2008) Beyond polymer polarity: how the cytoskeleton builds a polarized cell. Nat Rev Mol Cell Biol 9:860–873

Lindemann ChB, Lesich KA (2010) Flagellar and ciliary beating: the proven and the possible. J Cell Sci 123:519–528

Liverpool T (2006) Active gels: where polymer physics meets cytoskeletal dynamics. Phil Trans R Soc A 364:3335–3355

Lo C (2000) Cell movement is guided by the rigidity of the substrate. Biophys J 79(1):144–152
Masters TA, Viasnoff V, Gauthier N (2013) Plasma membrane tension orchestrates membrane trafficking, cytoskeletal remodeling, and biochemical signaling during phagocytosis. Proc Natl Acad Sci USA 110:11875–11880
Matsuno K (2006) Forming and maintaining a heat engine for quantum biology. BioSystems 85:23–29
McClare CWF (1971) Chemical machines, Maxwell's demon and living organisms. J Theor Biol 30:1–34
Mitrossilis D, Fouchard J, Pereira D, Postic F, Richert A, Saint-Jean M, Asnacios A (2010) Real-time single cell response to stiffness. PNAS 107:16518–16523
Munro EM (2006) PAR proteins and the cytoskeleton: a marriage of equals. Curr Opin Cell Biol 18:86–94
Nance J, Zallen JA (2011) Elaborating polarity: PAR proteins and the cytoskelenon. Development 138:799–809
Odell GM, Oster G, Alberch P, Burnside B (1981) The mechanical basis of morphogenesis. I Epithelial folding and invagination. Dev Biol 85:446–462
Plaçais P-Y, Balland M, Guerin T, Joanny J-F, Martin P (2009) Spontaneous oscillations of a minimal actomyosin system under elastic loading. Phys Rev Lett 103:158102
Pohl C, Bao Z (2010) Chiral forces organize left-right patterning in *C. elegans* by uncoupling midline and antero-posterior axis. Dev Cell 19(3):402–412
Pollack GH (2001) Cells, gels and the engines of life: a new, unifying approach to cell function. Ebner, Seattle
Rauzi M, Lenne P-F (2011) Cortical forces in cell shape changes and tissue morphogenesis. Curr Topics Dev Biol 95:93–121
Roh-Johnson M, Shemer G, Higgins CD, McClellan JH, Werts AD, Tulu US, Gao L, Betzig E, Kiehart DP, Goldstein B (2012) Triggering a cell shape change by exploiting pre-existing actomyosin contractions. Science 335(6073):1232–1235
Saez A, Buguin A, Silberzan P, Ladoux B (2005) Is the mechanical activity of epithelial cells controlled by deformations or forces? Biophys J: Biophys Lett 89:L52–L54
Samuel MS, Lopez JI, McGhee EJ, Croft DR, Strachan D, Timpson P, Munro J, Schröder E, Zhou J, Brunton VG, Barker N, Clevers H, Sansom OJ, Anderson KI, Weaver VM, Olson MF (2011) Actomyosin-mediated cellular tension drives increased tissue stiffness and β-catenin activation to induce epidermal hyperplasia and tumor growth. Cancer Cell 19:776–791
Sardet C, Paix A, Prodon F, Dru P, Chenevert J (2007) From oocyte to 16-cell stage: cytoplasmic and cortical reorganizations that pattern the ascidian embryo. Dev Dyn 236:1716–1731
Schaller V, Weber Chr, Semmrich Chr, Frey E, Bausch AR (2010) Polar patterns of driven filaments. Nature 467:73–77
Shemesh T, Geiger B, Bershadsky AD, Kozlov MM (2005) Focal adhesions as mechansors: a physical mechanism. PNAS 102:12383–12388
Schroedinger E (1944) What is life? The physical aspect of the living cell. Cambridge University Press, Cambridge
Sinha B et al. (2011) Cells respond to mechanical stress by rapid disassembly of caveolae. Cell 144:402–413
Speder P, Noselli S (2007) Left-right asymmetry: class I myosins show the direction. Curr Opin Cell Biol 19:82–87
Stern CD (1984) A simple model for early morphogenesis. J Theor Biol 107:229–242
Storm C, Pastore JJ, MacKintosh FC, Lubensky TC, Janmey PA (2005) Nonlinear elasticity in biological gels. Nature 435:191–194
Sumida GM, Tomita TM, Shih W, Yamada S (2011) Myosin II activity dependent and independent vinculin recruitment to the sites of E-cadherin-mediated cell-cell adhesion. BMC Cell Biol 12:48 (9 p)
Sumino Y, Nagai KH, Shitaka Y, Tanaka D, Yoshikawa K, Chate H, Oiwa K (2012) Large-scale vortex lattice emerging from collectively moving microtubules. Nature 483:448–452

Tsikolia N, Schroeder S, Schwartz P, Viebahn C (2012) Paraxial left-sided nodal expression and the start of left-right patterning in the early chick embryo. Differentiation 84:380–391

Trepat X, Deng L, An SS, Navajas D, Tschumperlin DJ, Gerthoffer WT, Butler JP, Fredberg JJ (2007) Universal physical responses to stretch in the living cell. Nature 447:592–595

Vandenberg LN, Levine M (2013) A unified model for left-right asymmetry? Comparison and synthesis of molecular models of embryonic laterality. Dev Biol 379:1–15

Van der Gucht J, Sykes C (2009) Physical model of cellular symmetry breaking. Cold Spring Harb Perspect Biol 1:a001909

Vogel SK, Pavin N, Maghelli N, Julicher F, Tolic-Nerrelykke IM (2009) Microtubules and motor proteins: mechanically regulated self-organization in vitro. Eur Phys J Spec Topics 178:57–69

Weiser DC, Row RH, Kimelman D (2007) Rho-regulated Myosin phosphatase establishes the level of protrusive activity required for cell movements during zebrafish gastrulation. Development 136:2375–2384

Wilson PA, Oster G, Keller R (1989) Cell rearrangements and segmentation in Xenopus: direct observations of cultured explants. Development 105:155–166

Wipff P-I, Rifkin DB, Meister J-J, B.Hinz (2007) Myofibroblast contraction activates latent TGF-beta from the extracellular matrix. J Cell Biol 179:1311–1323

Zhang H, Landmann F, Zahreddine H, Rodriguez D, Koch M, Labouesse M (2011) Tension-induced mechanotransduction pathway promotes epithelial morphogenesis. Nature 471:99–103

http://www.springer.com/978-3-319-13989-0

Morphomechanics of Development
Beloussov, L.V.
2015, XV, 195 p. 55 illus., 45 illus. in color., Hardcover
ISBN: 978-3-319-13989-0