4.5 Toward a quantitative understanding of life

Raymond Goldstein[1] and Patricia Bassereau[2]
[1]University of Cambridge, Cambridge, UK
[2]Institut Curie, Paris, France

4.5.1 General overview

More than half a century ago, physics and biology came together to understand the DNA double-helix structure, one of the most important discoveries of the 20th century. At present, the complexity of biological structure and function and the observed emergent phenomena in biology delineates a well-defined research domain where physics meets biology. Emergent phenomena include processes where larger entities exhibit properties that their simpler constituent entities (and the naive superposition of their properties) do not exhibit and, as a consequence, something new emerges from collective behaviour that could not be predicted from its constituent parts. Complexity applies to key open questions in the physics of the many-body interacting systems, but it finds its most natural setting in biology, from the synchronized dynamical behaviour of the human brain or the beating of heart cells to the concerted action across multiple scale of biomolecules and cells in tissues and organs. The cascade of energy and information across the many biological levels defines a new field of research, the thermodynamics of information, which starts at the Maxwell demon paradox and the interpretation of the second law and may lead us far beyond.

4.5.2 Physics meets biology over the last 60 years

Physics and biology have been intertwined since the dawn of modern science (figure 4.15), from Antony van Leeuwenhoek's use in 1700 of advanced optics to reveal the hidden world of microscopic life [322], to Bonaventura Corti's (1774) discovery of the persistent fluid motion inside large eukaryotic cells [323], Robert Brown's 1828 study of random motion at the microscale [324], and Theodor Engelmann's determination in 1882 of the wavelength dependence of photosynthetic activity [325]. Although at the time it might have been difficult to define the precise disciplines of each of these scientists—perhaps they were all 'natural philosophers'—in hindsight we can see clearly the way in which their discoveries impacted both biology and physics. Despite this long history of discoveries at the boundary between the two fields, and the innumerable fundamental contributions to both disciplines over the long arc of time, the field of *biological physics* as a discipline within the research enterprise of physics has only risen to great prominence since the postwar era, particularly since the mid-1980s. We are now at the point that most academic physics departments have an identifiable group in biological physics alongside those in high energy, condensed matter, atomic and astrophysics. In this introductory section we will review some of key developments over the past 60 years in order to identify what we see as key intellectual threads that run through that history, to set the stage for the forward-looking sections that follow.

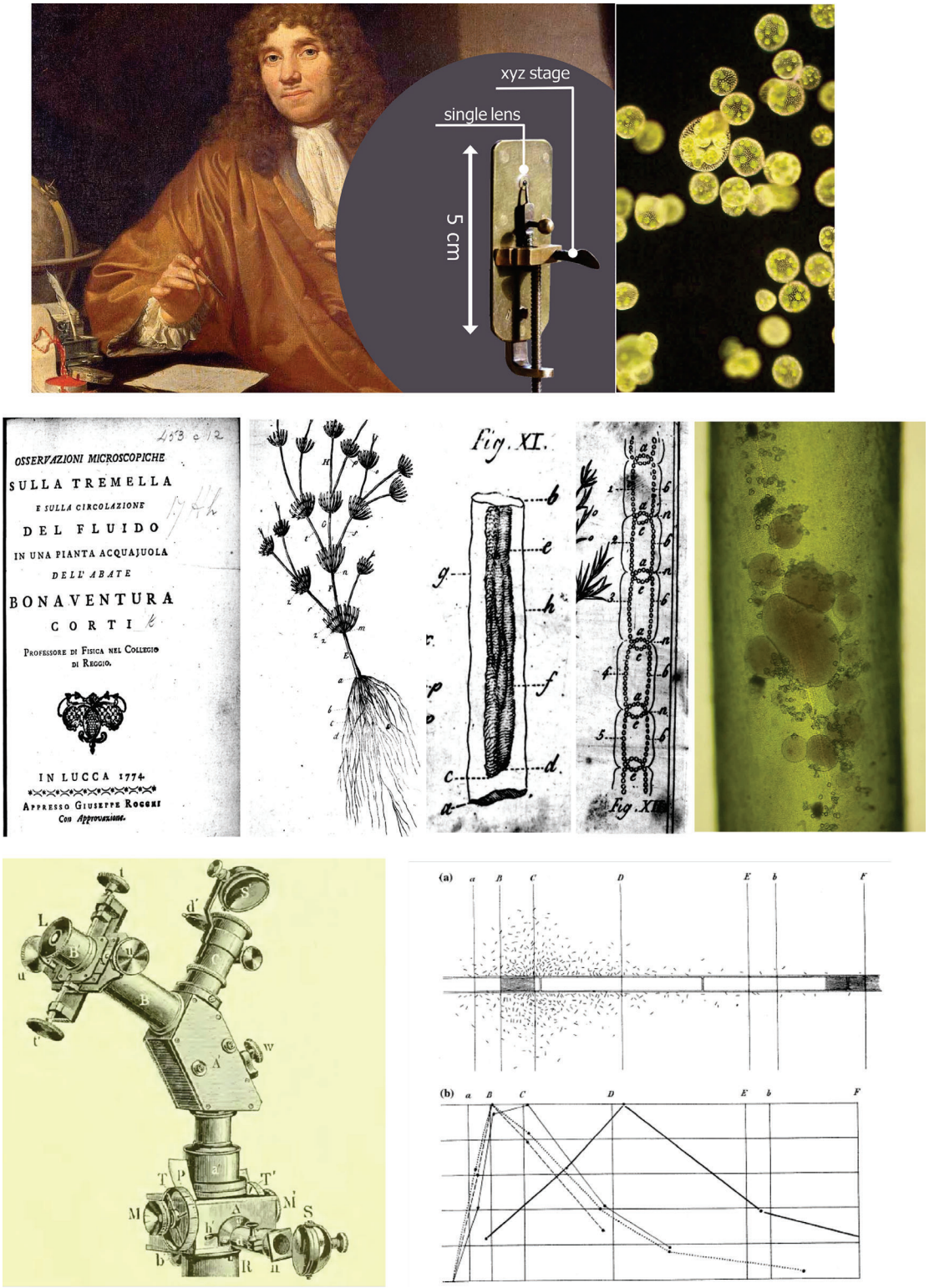

Figure 4.15. Historical connections between physics and biology. Top row: Antony vanLeeuwenhoek, his microscope, and the alga Volvox that he discovered. Middle: BonaventuraCorti's celebrated treatise on cytoplasmic streaming, with drawings of aquatic plants he studied,and a modern image of the plant Chara corallina. Bottom: The micrscope with which Theodor Engelmann's determined the action spectrum of photosynthesis by visualizing the accumulation of aerotactic bacteria along an alga illuminated by the solar spectrum. This image has been obtained by the authors from the Wikipedia website, where it is stated to have been released into the public domain. It is included within this article on that basis.

We begin by noting that each of the discoveries highlighted above was made possible by state-of-the-art *microscopy* that was able to reveal phenomena that had escaped previous notice. That technological advances often translate into scientific discoveries is a familiar pathway in science, but it takes more than just a new piece of kit to lead to true progress. Indeed, there is often a separate but equally important collective aspect of the scientific community at work, in defining the questions, bridging disciplines, and training students.

Our choice of 60 years for this overview was made to capture several of the most important immediately postwar advances at the interface of biology and physical sciences which, in an interesting historical twist, clustered around the same time (figure 4.16). These were the 1952 elucidation of the dynamics of action potentials in neurons by Hodgkin and Huxley [332] and the theoretical work by Turing in that same year showing that chemical reaction-diffusion systems can exhibit spatio-temporal patterns, followed in 1953 by the discovery of the structure of DNA by Franklin, Watson, and Crick [333, 334] using x-ray scattering methods. As in the discussion above, the work of Hodgkin and Huxley built very much on developments in experimental methods; in this case it was the invention of the 'voltage clamp' by Cole [335], a device that utilizes feedback to maintain the voltage across a membrane at a set value, that allowed the properties of ion channels in membranes to be studied as a function of (controlled) voltage. The idea of a voltage-dependent channel conductance was the key to understanding not only neuronal dynamics but 'excitable media' in general [336]. Experimental methods from physics were of course also key to the DNA structural work, which was an offshoot of the original development of x-ray scattering to determine crystal structures based on Bragg's law [337].

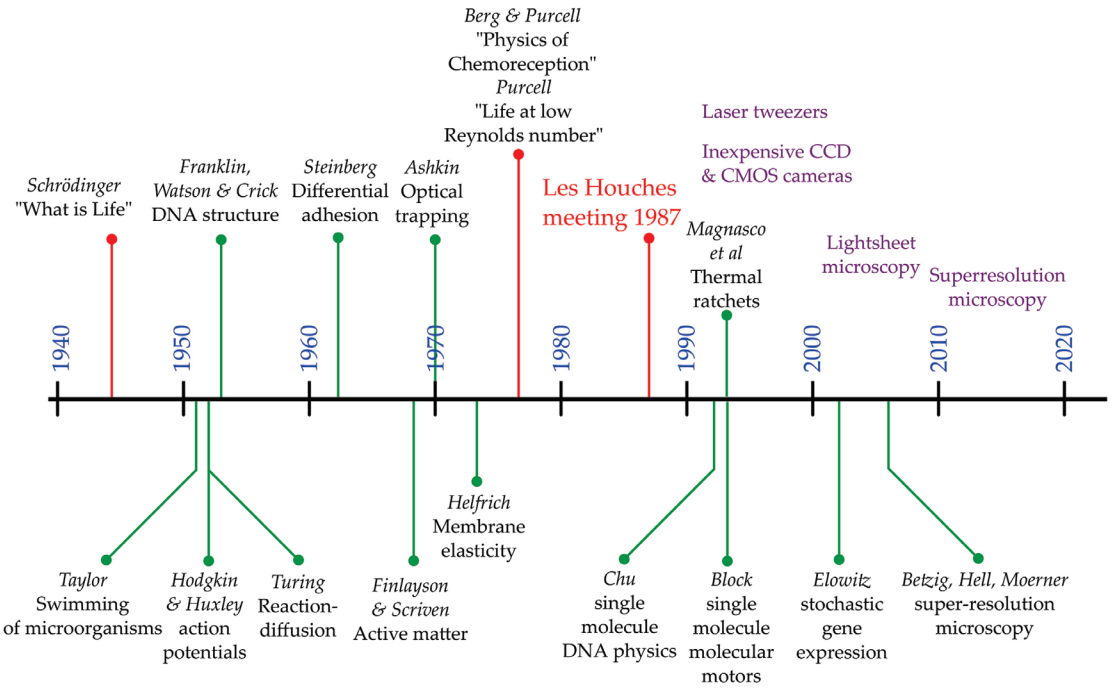

Figure 4.16. Timeline of significant developments in biological physics from the mid 20th century onwards.

Turing's work had little to do with technological advancements and his research would not generally be considered originally to be part of academic physics; it might be categorized instead as one of the earliest works in *mathematical biology* [338]. Using very simple mathematical models of two interacting chemical species, he showed that a system of chemical reactions that would, in the absence of diffusion, be linearly stable, could be rendered unstable by diffusion, leading to spatially periodic patterns. In this context, this mechanism was a revolutionary idea, although spatio-temporal symmetry breaking had already been understood in fluid mechanical contexts at least since the time of Rayleigh's work on thermal convection [339]. Yet, the relevance of the Turing mechanism to actual biological systems has been debated ever since [340], in part because in its standard form the mechanism requires the diffusivities of the two chemical species to differ by what seems to be an unphysical amount. It was physicists who provided the first experimental realizations of the Turing instability by reducing the diffusivity of one of the chemical species through binding to a gel substrate [341–343]. While it has also been unclear how to square highly regulated biological patterning with the concept of spontaneous symmetry breaking, it is important to emphasize that Turing's work introduced most of the concepts now used in the study of how biological form develops, the most important of which is that a system of reacting and diffusing chemicals can form spatio-temporal patterns [344]. It was within applied mathematics that reaction-diffusion dynamics were first applied to pattern formation in biological populations such as the spiral waves and chemotaxis exhibited by the slime mold *Dictyostelium discoideum* [345], but these ideas eventually resonated with physicists studying such pattern formation [346]. There is now ample evidence that these basic ideas are correct.

Like Turing's paper, Taylor's (1951) work [347] that offered the first explanation for the swimming of microscopic organisms in a viscous fluid sat not within physics proper, but rather in fluid dynamics (in the British school of applied mathematics). This and subsequent works by other applied mathematicians such as Lighthill [348] clarified the physics behind self-propulsion in the absence of inertia by exploiting the theoretical simplifications inherent in the dynamics of asymptotically slender filaments such as eukaryotic and prokaryotic flagella. These developments were later taken up by physicists and caught the attention of the community thanks to Purcell in his celebrated 1977 essay on 'Life at low Reynolds number' [349], and in his work with Berg [350] that highlighted physical considerations in the phenomenon of chemoreception. This is one of the many important examples in which scientists who came from more established areas within physics moved into biological physics, helping to legitimize it within the broader community.

These intellectual strands that were developing in the 1970s were very much apart from the molecular-biology-centered world of mainstream biology at the time, and indeed they were distinct from much of the field known as 'biophysics' that was focused on proteins, ion channels, and electrophysiology, but they would prove to be harbingers of the development of biological physics as a distinct discipline. Another excellent example of the biological research beyond the molecular level is that of Steinberg on the cellular organization in tissues. In his 1962 paper on this subject

[351], he formulated a differential adhesion hypothesis for the self-organization of cell types within tissues. Although the dominance of the molecular view of biology at the time meant this work gained little traction in the community, its enthusiastic uptake by physicists in the 1990s [352] led to an explosion of work on the subject of tissue biomechanics that continues to this day.

The gradual transformation of the field of solid-state physics into what we now term condensed matter physics began in the 1960s and early 1970s with the intense interest both in phase transitions and critical phenomena and also in the physics of liquid crystals. In moving away from the static description of precisely ordered solids, the field naturally began to focus on phenomena on length scales larger than molecular, and to utilize continuum descriptions such as those familiar from the Landau–Ginzburg theory for superconductivity, with particular emphasis on the formulation of scaling laws [353] in polymer physics, building on the foundational work of the theoretical chemist Flory [354]. An important strand of this research concerned the description of interfaces, as involved in phenomena such as wetting, and also in pattern formation during solidification [355]. The emphasis on continuum approaches was particularly significant in the field of liquid crystals, where the intellectual leadership of the French school led by De Gennes proved so important in creating a worldwide community of experimentalists and theorists. It was out of this field that came the seminal work of Helfrich in 1973 [356] on the elasticity of fluid membranes. The particularly simple form of this energy functional, dependent only on the shape of the membrane, allowed for an explosion of analytical and numerical work on a range of problems in membrane physics. With these advances, it was but a small step to move from the description of idealized membranes as found in lipid vesicles to real biological membranes with all their complexity.

On the experimental side, the arrival of affordable and relatively easy-to-build optical trapping setups in the early 1990s enabled quantitative experiments at the single-molecule level that were simply not possible previously. The first single-molecule experiments on the stretching of DNA in 1992 [357], combined with later theory [358], enabled the precise quantitative understanding of semiflexible polymers, opening up the understanding of aspects of chromosome structure and function in the cell. Likewise, pioneering work in 1993 on the stepping of motor proteins along biofilaments [359] revealed the stochastic nature of the motion of molecular motors and almost overnight placed their dynamics squarely within the field of non-equilibrium statistical physics. At roughly the same time, the paradigm of stochastic rectified motion on periodic potential energy landscapes (aka 'Brownian ratchets') received intense focus as the model for molecular motors [360–363] and also for the unidirectional motion exhibited by plants [364]. Together these helped to launch a subfield of stochastic thermodynamics, leading to principles for complex systems such as synthetic machines [365], including collective effects [366], with applications to such systems as hair cells in the ear [367]. From the mid-1990s onward, the advent of relatively inexpensive CCD and CMOS cameras and associated image processing techniques, including particle tracking methods [368], meant that the barrier to experimental work in this area was dramatically lowered.

Later developments in the new millennium saw a whole new generation of affordable high-speed cameras, further enabling the worldwide study of fast phenomena such as flagellar synchronization [369] and cell motility [370]. Finally, the development of soft lithography for microfluidics [371] further broadened the experimental base for studies at the microscale.

From all of the above, we see that fields that in the postwar period were considered apart from the core of physics, including fluid mechanics, transport theory, and much of continuum physics), were embraced by the biological physics community as its focus turned toward phenomena on scales from nanometers to millimeters, where so many cellular phenomena occur. It was perhaps only natural that physicists in this new era would reject [372] the view [373] that their role in biological research was simply to provide instrumentation or better intermolecular potentials in the service of the questions biologists had framed. Rather, the field has prospered precisely because physicists and biologists have joined together to pose the questions that guide the field. Finally, as the ranks of academia working on biological physics grew throughout the 1990s to the point that a new generation of PhD students was trained and themselves moved into academia, the whole field gained critical mass to the point of being among the fastest growing divisions within national physics societies.

4.5.3 Challenges and opportunities for the future

In this section we speculate on the future directions of a few of the many areas within biological physics, aiming for an overview rather than an encyclopedic account.

4.5.3.1 Soft matter and self-organization: from active matter to cell and tissues

In a remarkably insightful article in 1969 [374], Finlayson and Scriven considered the possibility of various types of hydrodynamic instabilities arising from what they termed ‘active stresses’. These are contributions to the stress tensor arising from gradients of scalar fields (e.g., the concentration of some solute) that represent the conversion of chemical energy to kinetic energy, typically manifest by a pattern-forming instability. This is the essence of what we now term ‘active matter’; systems in which there is injection of energy at small scales that display coherent structures on scales large compared to the microscopic constituents [375–378]. Early examples of this notion are found in new physical models for membranes that include the non-equilibrium activity of proteins that transfer ions across membranes via external sources of energy (light, ATP hydrolysis, electric fields) [379, 380]. Other examples include collections of molecular motors and microtubules [381], where the consumption of ATP by the motors powers translocation that leads to filament motion and self-organization, and in collections of self-propelled particles, in theory [382–384] and experiment [385], where coherent structures arise from hydrodynamic interactions between the organisms (figure 4.17).

These general concepts and tools were used in parallel to study the organization of living systems and of engineered ones. On the biological side, ‘active membranes’

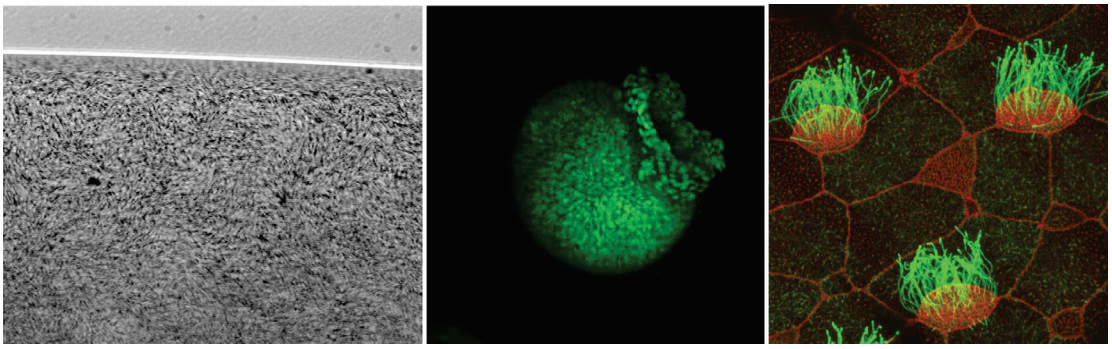

Figure 4.17. Active matter on multiple scales, from individual cells to confluent tissues. From left to right: bacterial 'turbulence' in a suspension of B. subtilis (reproduced from [326], CC BY 4.0), embryonic inversionin Volvox (reproduced from [327] CC BY 4.0), and a tissue with multiciliated cells (Mitchell Lab, Northwestern University).

have been generalized to membranes with a cortex and in contact with polymerizing actin filaments [386, 387]; they contributed to elucidate the non-equilibrium origin of the flickering of the red blood cells [388], although a consensus is still not reached. They have been applied to active gels [389, 390] made of polar dynamical cytoskeleton filaments and active crosslinkers like molecular motors that generate flows and stresses, features that do not exist in passive gels. Active gels have been instrumental for understanding actin flows in different cellular features: cell motility, blebbing, division, etc [390]. Liquid crystals principles have been extended to active nematics, smectics, or cholesterics. The power of this continuous description is that the model of active nematics can describe as well the self-organization of patterns and the flows for actively moving entities such as bacteria in films, anisotropic motile synthetic particles, animals flocks, or that of cells in living tissues during development, embryos [391]. Of course, non-equilibrium activity also affects phase separation [392]. A rapidly growing body of work has shown that liquid–liquid phase separation of multivalent assemblies occurs in the cytoplasm of cells, forming membraneless compartments [393], or in the nucleus [394]. However, Oswald ripening and thus droplet size is probably limited by active enzymatic reactions [395].

While many of the ideas and experiments on active matter systems implicitly use as a reference dilute suspensions of the motile entities, in recent years there has been a gradual shift of focus toward "confluent' tissues, typically quasi-two-dimensional sheets whose constituent cells are in space-filling contact with their neighbors. This leads to a wholly different class of phenomena and theoretical models that touch on some of the most significant issues in developmental biology. During development, tissues like epithelia are very dynamic since cells continuously die or divide and in addition, active forces are exerted at the cell–cell junctions. Thus, in spite of their morphological similarity with foams, epithelia differ strongly from them [396, 397], and non-equilibrium principles are necessary to describe their homeostasis [398, 399]. In addition, many tissues bend, fold, and even invert their topology during

embryogenesis [327]. Through a combination of advances in experimental methods such as light-sheet imaging [400] and use of 'organoids' [401] to study the early stages of multicellularity, and theoretical models addressing geometric rearrangements of tissues [402], we anticipate that the near future will see significant progress in understanding many key issues in the biomechanics of development and differentiation.

So far, very little work has been done to integrate at the cell scale all active exchanges that occur between compartments, with the plasma membrane and with other cells. At a single compartment level such as the Golgi apparatus, they directly affect its shape [403]. Apart from during division, cells have to maintain their shape, area, and volume in spite of these multiple fluxes, and how they manage is a recurring issue in biology. Modeling these exchanges and understanding how homeostasis at the cellular scale is achieved remains one of the future challenges for physicists and cell biologists. The following section addresses how homeostasis is maintained in tissues.

4.5.3.2 Deciphering the physical principles of mechano-chemical networks that control homeostasis, shape, and size of living entities

In his book *On Growth and Form* [404], D'Arcy Wentworth Thompson raised the question of how physical forces contribute to determine the size and shape of living organisms and thus initiated the field of 'mechanobiology'. In addition to the non-equilibrium principles that govern cellular assemblies, mechanics also plays a key role. Indeed, cells exert, sense, and respond to external forces. For instance, the spreading velocity of cellular migration depends on the stiffness of the underlying substrate [405]. Cells exert forces on their environment generally using dynamical actin networks and the contractile actomyosin machinery. To a large extent, cell mechanosensitivity depends on proteins embedded in the plasma membranes that are linked on the extracellular side to specific ligands of the external matrix or to similar membrane proteins of a neighbouring cell in tissues. On the intracellular side, they have cryptic binding sites that unfold and allow connection to the actin cytoskeleton in a load-dependent manner. Actin structures are themselves mechanosensitive [406]. Moreover, mechanical forces trigger biochemical response and signalling pathways (i.e., cascades of biochemical reactions with positive and negative feedback loops). A revealing illustration is provided by stem cells (i.e., non-differentiated, pluripotent cells) that differentiate into very different cell types—neurons, muscle, or bones—depending on the stiffness of their micro-environment [407]. Mechanical cues are thus transduced into biochemical signals, and integrated with genetic and chemical signals to modulate diverse physiological processes. In addition, there is constant cross-talk between biochemistry and mechanics during mechanotransduction, which is itself a part of the early development since developmental genes can be switched on by internal stresses accumulated during the growth of the embryo [408]; it is also involved in cancer development [409]. Physics and bioengineering have strongly contributed to this field, in particular by developing many tools for measuring forces at all scales, from the single mechanosensitive molecules to

stresses in tissues [410]. On the biochemical side, complex signalling networks have been identified in cell-extracellular matrix adhesion mediated by integrins [411, 412], or adherens-junction in cell–cell contacts [413]. Mechanosensitive channels, in particular 'piezo' channels, the main type of molecular force sensor in eukaryotes [414], are present in cell membranes; they let ions flow when they mechanically activated, which also triggers a cascade of biochemical signals, but have been less studied.

Cross-talk exists between these different signalling pathways that are all integrated at the cell level. Systems biology approaches are certainly essential in understanding these complex regulatory networks and how cells manage their mechanical interactions with their environment. But models based only on gene ON/OFF circuits are insufficient to understand how tissue integrity is preserved against mechanical stresses, extensile, or compressive, and during rearrangements of cells, and how tension homeostasis is set. The existence of cellular rearrangements in tissues implies the remodeling, destruction, or creation of the connecting structures between cells in a coordinated fashion (figure 4.18). In some rare cases, the feedback loops between mechanics and signaling are known [415]. Since ever-more force sensors are available to quantify stresses across scales, these measurements have to be integrated with the networks in cells to develop models that explain the emergence of larger-scale behavior from the interactions of their molecular components inside cells. Thus, it appears that it will still be some time before we have a final answer to D'Arcy Thompson's questions: what limits the growth and division process, and what determines the size and shape of organs or animals?

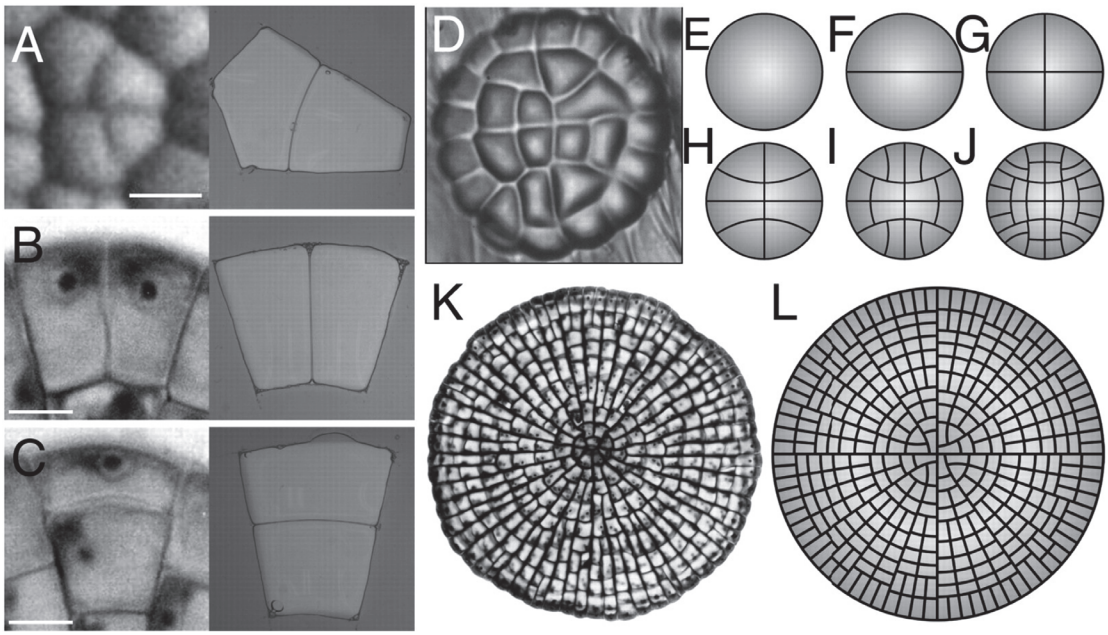

Figure 4.18. Cell division in the plant cells and similarity to shapes of soap bubble. Reproduced from [328] CC BY 4.0. (A–C) Patterns of cell division in various plants (left) compared to shapes of soap bubbles (right). (D) A large-scale geometry compared to mathematical results (E–J) in which a rule derived from soap bubble physics is iterated for uniform growth in the region. (K, L) as in (D–J), but for marginal growth.

4.5.3.3 Some perspectives on brain functions, for soft matter and computational neuroscience

Neuroscience has a singular place in biology. It is at the cross-road between disciplines, since the brain is not only an organ but controls locomotion, sensing, memory, decision making, and, at least in humans, feelings, consciousness, etc. The brain is a complex, temporally, and spatially multiscale structure. From the perspective of physics, it can be of course investigated at the cellular level. But since neurons communicate and form circuits and interconnect functional areas in the brain, it is better described as a hierarchical network (termed the 'human connectome' [416]). In addition, neurons are embedded in glial cells that protect them, but also contribute to some signaling functions. Two of the most challenging goals in science continue to be on the one hand the generation of the complete map of the neural connections in a brain and, on the other, to understand from a molecular point of view how signals are produced and transmitted and how the network builds up, in order to decipher how this incredibly complex structure can produce complex cognitive functions. The breadth of challenges for physicists of the future is too wide to list in totality; here we mention two of them, on axonal signaling and for computational neuroscience.

Neuronal cells have unique properties—mechanical, geometrical, and internal organization—and are actively studied *per se*. Strikingly, not much has been done since the work of Hodgkin and Huxley [332] to revisit their model of axonal transmission based on electrical signals. In the standard action potential model, signal propagation is achieved by the voltage-dependent opening and closing of ion channels, largely ignoring the specific physical properties of cell membranes. Only T Heimburg has challenged it, by suggesting that a lipid phase transition occurs in the membrane in the course of the action potential, increasing membrane conductance [417]. However, no study has included yet the non-equilibrium effect due to the activity of the channels we discussed above. Thus, a comprehensive model of the propagation of the electric axonal signal is still missing.

Various methods are routinely used to image whole-brain activity and detect dysfunction and disease, including x-rays (CT scan), radioactivity (PET scan), and NMR (MRI). However, to image the neuronal networks with better resolution, neurosciences have greatly benefited from the most advanced developments in microscopy and imaging: methods for imaging in diffusing media allow deep imaging in the brain, light-sheet microscopy for volumetric imaging, and optogenetics to control neuronal circuits [418]. Model animals with small brain volume or that are moderately transparent (*Drosophila*, zebrafish) have also facilitated these studies. It is now possible to follow neural algorithms in living and freely moving animals as they vary their behavior [419]. Using whole-brain functional imaging, the brain of a zebrafish can be imaged during the different stages of the decision-making process about its swimming direction [420]. Betzig and collaborators achieved the *tour de force* of imaging the whole brain of a fruit fly with molecular contrast and nanoscale resolution using combined light-sheet and expansion microscopy [421]. It is thus now possible to locate individual neurons, trace connections between them, and visualize organelles inside neurons, over large volumes of brain tissue in 3D,

albeit on fixed brains. These are only a few examples showing how the field is developing with the blooming of new optical techniques, pushing the frontiers of the observations. One obvious consequence is that with these advances will come enormous quantities of data, as in many other areas of cell biology in which volumetric imaging is used. One strategy to manage and analyse such 'big data' is obviously to use artificial intelligence and deep learning methods [422] to extract meaningful information. In addition, there is now a timely opportunity for computational sciences to develop approaches based on network science to provide integrated models of interactions in neurobiological systems. In fact, a new field termed 'network neuroscience' is growing, bridging network theory and experiments [423]. One might hope that creative developments in computational sciences in theory and in functional imaging will eventually allow us to unlock the neuronal code.

4.5.3.4 Emergence of life and physics of biological evolution: from the second law of thermodynamics to the selection of structures

In his 1944 book *What Is Life? The Physical Aspect of the Living Cell* [424], Erwin Schrödinger stressed the apparent paradox behind life: how can living organisms maintain an organized state and grow complex structures without violating the second law of thermodynamics that predicts an evolution towards maximized entropy? He resolved it by pointing out that Earth is not an isolated system, but receives energy from the Sun, and that living systems absorb energy. Moreover, he also used thermodynamic arguments to explain why an internal organizing factor that carries information (that eventually turned out to be DNA) is necessary for living systems to develop in an organized manner and replicate faithfully.

Likewise, the emergence of life on Earth cannot be explained by the second law, but rather (in part) by far-from-equilibrium thermodynamics and the concept of dissipative structures highlighted by Prigogine and others. While this issue is covered elsewhere in this volume, we note that there is a strong school of research, by no means universally accepted, supporting the idea that the evolution of life began from 'soup' of RNA molecules before DNA appeared. Yet, the mechanisms by which the nucleotide bases and sugars could be formed beforehand by prebiotic reactions are still not elucidated. There are promising attempts to produce artificial cells [425] and a significant body of work on life-inspired and out-of-equilibrium systems at the nanoscale [426]. Compartmentalization and the appearance of membranes are key steps during evolution. Recent work on active membraneless droplets suggested that they could have formed the protocells from which cell membranes could have appeared [427]. Bottom-up reconstitution of a synthetic cell with well-characterized functional molecular entities in vesicles can also help to understand the origin of life [428]. Conversely, with a top-down approach, the Craig Venter Institute has shown it is possible to recreate artificially genomes and minimal cells [429]. A synthetic minimal organism has been reproduced *in silico* by reconstruction of a complete set of chemical reactions [430]. Hydrodynamic models have also been used to understand how DNA may have replicated in early times: laminar thermal convection, present in submarine hydrothermal vents, can very efficiently accelerate the DNA replicating polymerase chain reaction (PCR) [431], which is enhanced when the

molecules are trapped in porous rocks [432]. We expect these *in vitro* approaches will become ever-more important in the future. On the modeling side, considering the complexity of this interdisciplinary problem, there is a clear need for further development of related aspects of non-equilibrium physics.

4.5.3.5 Evolution of biological complexity

High on the list of fundamental problems in biology, just behind the origin of life and the nature of consciousness, is the origin of multicellularity. While the simplest organisms to appear on earth were no doubt unicellular, eventually life evolved to become larger, in the sense of having more cells, and also more complex, dividing up life's processes into ever-more specialized cell types [433]. It has been recognized since the time of Weismann [434] in the late 19th century that a great challenge is to understand the driving forces behind the transition to multicellularity, and, as pointed out by Huxley some years later [435], to identify the biological entities on which evolution acts [435]. While there can be obvious advantages to larger and more complex organisms, such as greater motility, avoidance of predators, and larger uptake rates of nutrients, there are also metabolic costs associated with the regulatory networks that control the organism and the cellular scaffolding that holds it together [436]. Recent work has begun to address these issues using green algae [437] and choanoflagellates (figure 4.19), the closest uni- and multicellular relatives of animals [438].

The ability to track *single* living objects (bacteria, yeast, or cells) using microfluidic devices and to analyze their lineage during multiple rounds of cell division will open the way for new discoveries when these experiments will be coupled to external perturbations. Among the most promising approaches to understanding the origins of biological complexity involve the use of artificial methods to put evolutionary pressure on extant organisms. A prime example of this is recent work on yeast, in which repeated rounds of centrifugation of growing cultures, selection of

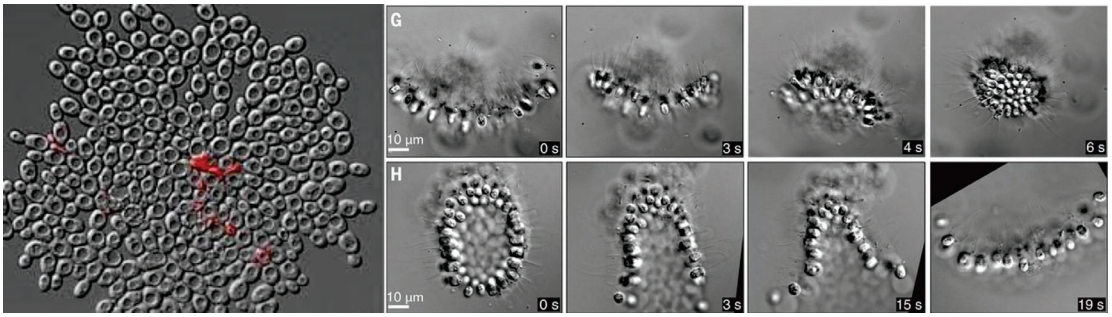

Figure 4.19. Novel multicellular organisms. Left: 'snowflake yeast'. Reproduced from [329] CC BY 4.0 formed via repeated rounds of selection for faster settling speed under centrifugation. Right: snapshots during the curvature inversion of a sheet of choanoflagellate cells comprising the organism C. flexa, as triggered by light. Reproduced from [330] CC BY 4.0.

the fastest sedimenting fraction (containing the largest organisms), and subculturing of that fraction produces 'snowflake yeast' (figure 4.19), a genuine multicellular variant [329]. This 'experimental evolution of multicellularity' enables a whole range of questions in the origins of multicellularity to be addressed, and we anticipate significant developments in this area in the coming years. It is clear that ideas from statistical physics [439, 440] will be important for the analysis of the data arising from these studies. In sum, we can see the emergence of a field centered around the physics of biological evolution, using concepts from condensed matter such as frustrated states and glasses to describe transitions during evolution [441]. With the appearance of ever-more data from advances in experimental methods due to technical developments, we can imagine that more theoretical models will be designed to understand how living systems cope with the second law to adapt to their environment.

4.5.4 The future

While predictions are always difficult, especially about the future, we offer a few final words on the greatest challenges in the field of biological physics may address in coming years. Clearly, the most fundamental, unsolved problem is the origin of life. As we have touched on here and as discussed in greater detail elsewhere in this volume, a range of highly interdisciplinary efforts appears poised to make significant progress on this problem. The issues concern the bootstrapping problem of how a truly self-replicating system can arise biochemically, and also the geophysical conditions that are amenable to such a development. Likewise, the nature of consciousness remains mysterious, but we can anticipate that the continued development of probes of neuronal structure and organization will point the way toward a deep understanding of this emergent phenomenon. In the realm of developmental biology, it is clear that the rapid explosion of experimental methods to probe cellular fate determination and global regulation combined with physical concepts regarding spatio-temporal patterning will continue apace, and we can look forward to a deeper understanding of the *regulation* of development. Even such issues as the regulation of limb size are, at this point in time, not resolved; their study in model organisms will continue to be an important research endeavor. Ultimately, we may hope that these physical methods will contribute to understanding the origins and control of the unregulated cell division that is at the heart of cancer. At the subcellular scale we still lack a comprehensive understanding of such complex machines such as the ribosome, which work in confined environments or with a limited energy supply. At the more macroscopic scale, the use of *in vitro* evolution methods will surely continue, providing a platform for the true quantitative understanding of evolution in the natural world. Coupled closely to this will be an increasing focus on what might be termed 'physical ecology', the study of communities of organisms coexisting with their natural habitat (figure 4.20). As of this writing, the world is wrestling with a global pandemic and it seems natural to expect much future research on the interplay between viruses and their hosts.

Figure 4.20. Two aspects of physical ecology. Left: marine algal blooms (green) in the Baltic Sea as seen from a European Space Agency satellite (reprinted from [331], courtesy of ESA). Right: the blue glow of bioluminescence triggered in breaking waves at a beach (photo courtesy of Gergo Rugli).

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