

## Introduction: Biology and physics

Physics deals with natural phenomena and their explanations. Biological systems are part of nature and as such should obey the laws of physics. However correct this statement may be, it is of limited value when the question is how physics can help unravel the complexity of life.

Physicists are intellectual idealists, drawing on a tradition that extends back more than 2000 years to Plato. They try to model the systems they study in terms of a minimal number of “relevant” features. What is relevant depends on the question of interest and is typically arrived at by intuition. This approach is justified (or abandoned) after the fact, by comparing the results obtained using the model system with experiments performed on the “real” system. As an example, consider the trajectory of the Earth around the Sun. Its precise details can be derived from Newton’s law of gravity, in which the two extensive bodies are each reduced to a point particle characterized by a single quantity, its mass. If one is interested in the pattern of earthquakes, however, the point-particle description is totally inadequate and knowledge of the Earth’s inner structure is needed.

An idealized approach to living systems has several pitfalls – something recognized by Plato’s student Aristotle, perhaps the first to attempt a scientific analysis of living systems. In the first place, intuition helps little in determining what is relevant. The functions of an organism’s many components, and the interactions among them in its overall economy, are complex and highly integrated. Organisms and their cells may act in a goal-directed fashion, but how the various parts and pathways serve these goals is often obscure. And because of the enormous degree of evolutionary refinement behind every modern-day organism, eliminating some features to produce a simplified model risks throwing the baby out with the bath water.

Analyzing the role of any component of a living system is made all the more difficult by the fact that whereas many cellular and organismal features are functional adaptations resulting from natural selection, some of these may no longer serve the same function in the modern-day organism. Still others are “side effects” or are characteristic (“generic”) properties of all such material systems. To a major extent, therefore, living systems have to be treated “as is”, with complexity as a fundamental and irreducible property.

One property of a living organism that sets it apart from other physical systems is its ability and drive to reproduce. When physics is used to understand biological systems it must be kept in mind that many of the physical processes taking place in the body will be organized to serve this goal, and all others must at least be consistent with it. The notion of goal-directed behavior is totally irrelevant for the inanimate world.

Physics and biology differ not only in their objects of study but also in their methods. Physics seeks to discover universal laws, valid everywhere in time and space (e.g., Newton's laws of motion, the laws of thermodynamics). Theory expresses these general laws in mathematical form and provides "models" for complex processes in terms of simpler ones (e.g., the Ising model for phase transitions, diffusion-limited aggregation models of crystal growth). Biology also seeks general principles. However, these are recognized either to be mechanisms or modes of organization limited to broadly defined classes of organisms (eukaryotes vs. prokaryotes; animals vs. plants) or to be molecular commonalities reflecting a shared evolutionary history (the use of DNA as hereditary material; the use of phospholipids to define cell boundaries). Biological "laws," where they exist (e.g., the promotion of evolution by natural selection, the promotion of development by differential gene expression) are rarely formulated in mathematical terms. (See Nanjundiah, 2005, for a discussion of the role of mathematics in biology.) There are very few general laws in biology and the ones that exist are much less exact than in physics.

This is not to say that there are no good models for biological processes, but only that they have a different function from models in physics. Biologists can study subcellular systems, such as protein synthesis or microtubule assembly, in a test tube, and cellular interactions, such as those producing heartbeats and skeletons, in culture dishes. Both types of experimental set-up – for cell-free systems and for living tissues outside the body – have been referred to as *in vitro*. It is always acknowledged, however, that, unlike in physics, the fundamental process is the *in vivo* version in its full complexity, not the abstracted version. There is always the hope that the experimentally accumulated knowledge of biological systems will lead eventually to the establishment of fundamental organizing principles such as those expressed in physical laws. It is however possible that the multileveled and evolutionarily established nature of cells and organisms will continue to defeat this hope.

Physicists and biologists also look at the same things in different ways. For a physicist, DNA may simply be a long polymer with interesting elastic properties. For the biologist, DNA is the carrier of genetic information. The sequence of bases, irrelevant to the physicist's concerns, becomes of central importance to the biologist studying how this information is stored in the molecule and how it is processed to produce specific RNAs and proteins. Because biologists must pay attention to the goal-directed aspects of living systems, the properties studied are always considered in relation to possible contributions toward the major goal of reproduction and subsidiary goals such as locomotion toward nutrients and increase in size and complexity.

Because biological systems are also physical systems, phenomena first identified in the nonliving world can provide models for biological processes too. In many instances, in fact, we may assume that complexity and integration in living organisms have evolved in the context of forms and functions that originally emerged by

straightforward physical means. In the following chapters we will introduce physical mechanisms that may underlie and guide a variety of the processes of early animal development. In certain cases simple physical properties and driving forces are the determining factors in a developmental episode. In other cases developmental causality may be multifactorial, which is to say that evolution has recruited physico-chemical properties of cells and tissues on many levels. An appreciation of the connection between physics and biology and the utility of biological physics for the life scientist will ultimately depend on the recognition of both the “simple” and the multifactorial physical determination of biological phenomena. When we come to consider the evolution of developmental mechanisms we will discuss scenarios in which simple physical determination of a biological feature appears to have been transformed into multifactorial determination over time.

The role and importance of physics in the study of biological systems at various levels of complexity (the operation of molecular motors, the architectural organization of the cell, the biomechanical properties of tissues, and so forth) is being recognized to an increasing extent by biologists. The objective of the book is to present a framework within which physics can be used to analyze biological phenomena on multiple scales. In order to bring coherence to this attempt we concentrate on one corner of the living world – early embryonic development. Our choice of this domain is not entirely arbitrary. During development, cells and tissues undergo changes in pattern and form in a highly dynamic fashion, using a wider range of physical processes than at any other time during the organism’s life cycle.

Physics has often been used to understand properties of fully formed organisms. The mechanics of locomotion in a vertebrate animal, for example, involves the suspension and change in orientation of rigid bodies (bones) connected by elastic elements (ligaments, tendons, muscles). The ability of some of the elastic elements (the muscles) to generate their own contractile forces distinguishes musculoskeletal systems from most nonliving mechanical systems – hence “biomechanics.” A developing embryo, in contrast, is much less rigid: rather than simply changing the orientation of its parts, it continuously undergoes remodeling in shape and form. Embryonic cells can slip past one another or be embedded in pliable, semi-solid matrices. Thus, the physical processes acting in an early organism are predominantly those characterizing the behavior of viscoelastic “soft matter” (a term coined by the physicist Pierre-Gilles de Gennes; de Gennes, 1992), rather than the more rigid body systems typical of adult organisms.

Another reason to concentrate on early development is that it is here that the role of physics in constraining and influencing the outcomes of biological processes is particularly obvious. Early developmental phenomena such as blastula formation and gastrulation are examples of *morphogenesis*, the set of mechanisms that create complex

biological forms out of simpler structures. While each episode of developmental change is typically accompanied by changes in the expression of certain genes, it is clear that gene products – RNA and protein molecules – must act in a specific physical context in order to produce three-dimensional forms and patterns. The laws of physics establish that not every structure is possible and that programs of gene expression can only produce shapes and forms of organisms and organs within defined limits.

The development of the embryo is followed from the fertilized egg to the establishment of body plan and organ forms. We close the developmental circle by discussing fertilization, the coming together of two specialized products of development – the egg and sperm – whose interaction employs certain physical processes (such as electrical phenomena) in a fashion distinct from other developmental events.

We conclude with a discussion of how developmental systems were likely to have originated from the physical properties of the first multicellular forms. The topology and complexity of gene regulatory networks may have had independent evolutionary histories from their associated biological forms. We therefore also review in this section computational models that test such possibilities.

Major stages of the developmental process and the major participating cellular and molecular components are introduced in terms familiar to students of biology, and sufficient background is provided to make these descriptions accessible to non-biologists. These developmental episodes are then analyzed from the viewpoint of physics (to the extent allowed by our present knowledge). No preparation beyond that of introductory calculus and physics courses will be needed for an understanding of the physics presented. Physical quantities and concepts will be introduced mostly as needed for the analysis of each biological process or phenomenon. Complex notions that are important but not essential for comprehending the main ideas are collected in boxes in the text and a few worked examples are included in the early chapters. We avoid presenting the basics of cell and molecular biology (for which many excellent sources already exist), beyond what is absolutely needed for understanding the developmental phenomena discussed. Each chapter concludes with a “Perspective,” which briefly summarizes its major points and, where relevant, their relation to the material of the preceding chapters.

We have drawn on an abundant and growing literature of physical models of biological processes, including phenomena of early development. The choice of models reflects our attempt to introduce the biology student to the spirit of the physical approach in the most straightforward fashion and to help the physics student appreciate the range of biological phenomena susceptible to this approach. We continually emphasize the constraints associated with any realistic application of physical models to biological systems. In line with this, we focus on the biology-motivated formulation of quantitative

models, rather than the solution of the resulting mathematical equations.

We have attempted, as far as possible, to make each chapter self-contained (with ample cross-referencing). Moreover, because biological development employs a wide range of physical processes at multiple spatial and temporal scales, we have made a point, wherever relevant, of introducing novel physical concepts and models for each new biological topic addressed.

Finally, biological physics is a relatively young discipline. With the constant improvement of experimental and computational techniques, the possibility of studying complex biological processes in a rigorous and detailed fashion has emerged. To be capable in this endeavor one has to be versatile. Biologists and medical researchers today and in the future will increasingly use sophisticated investigative techniques invented by physicists and engineers (e.g., atomic force microscopy, magnetic resonance, neutron scattering, confocal microscopy). Physicists will be called on to characterize systems of increasing complexity, of which living systems are the ultimate category. There is no way in which anyone can be an expert in all aspects of this enterprise. What will be required of the scientist of tomorrow is the ability to speak the language of other disciplines. The present book attempts to help the reader to become at least bilingual.

## Chapter I

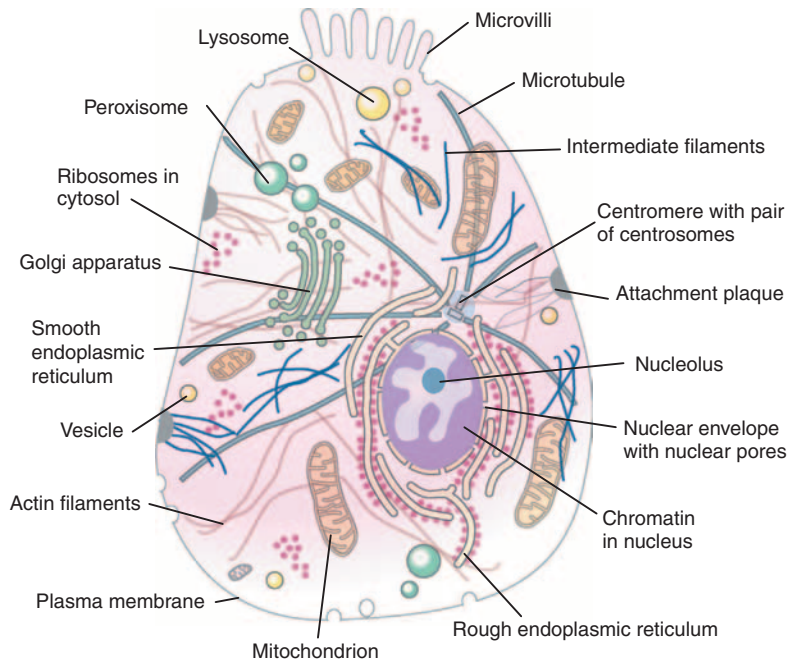
# The cell: fundamental unit of developmental systems

For the biologist the cell is the basic unit of life. Its functions may depend on physics and chemistry but it is the functions themselves – DNA replication, the transcription and processing of RNA molecules, the synthesis of proteins, lipids, and polysaccharides and their building blocks, protein modification and secretion, the selective transport of molecules across bounding membranes, the extraction of energy from nutrients, cell locomotion and division – that occupy the attention of the life scientist (Fig. 1.1). These functions have no direct counterparts in the nonliving world.

For the physicist the cell represents a complex material system made up of numerous subsystems (e.g., organelles, such as mitochondria, vesicles, nucleus, endoplasmic reticulum, etc.), interacting through discrete but interconnected biochemical modules (e.g., glycolysis, the Krebs cycle, signaling pathways, etc.) embedded in a partly organized, partly liquid medium (cytoplasm) surrounded by a lipid-based membrane. Tissues are even more complex physically – they are made up of cells bound to one another by direct adhesive interactions or via still another medium (which may be fluid or solid) known as the “extracellular matrix.” These components all have their own physical characteristics (elasticity, viscosity, etc.), which eventually contribute to those of the cell itself and to the tissues they comprise. To decipher the working of even an isolated cell by physical methods is clearly a daunting task.

## The eukaryotic cell

The types of cells discussed in this book, those with true nuclei (“eukaryotic”), came into being at least a billion years ago through an evolutionary process that brought together previously evolved “prokaryotic” living units. Among these were “eubacteria” and “archaeobacteria,” organisms of simpler structure in which information specifying the sequence of proteins was inherited on DNA present as naked strands in the cytosol, rather than in the highly organized DNA-protein complexes known as “chromatin,” found in the nuclei of eukaryotes.



**Fig. 1.1** Schematic representation of an animal cell. Most of the features shown are characteristic of all eukaryotic cells, including those of protists (e.g., protozoa and cellular slime molds), fungi, and plants. Animal cells lack the rigid cell walls found in fungi and plants, but contain specializations in their plasma membranes that permit them to bind to other cells or to extracellular matrices, hydrated materials containing proteins and polysaccharides that surround or adjoin one or more surfaces of certain cell types. These specializations are depicted in Fig. 4.1.

Modern eukaryotic cells, whether free-living or part of multicellular organisms (“metazoa”), have evolved far beyond the ancient communities of prokaryotes in which they originated. Cellular systems with well-integrated subsystems are at a premium in evolution, as are organisms with flexible responses to environmental changes. The result of millions of years of natural selection for such properties is that a change in any one of a cell’s subsystems (by genetic mutation or environmental perturbation) will have repercussions in other subsystems that tend either to restore the original functional state or to bring the subsystem in question to another appropriate state. The physicist’s aim of isolating relevant variables to account for the system’s behavior would seem to be all but impossible under these circumstances.

Despite these complications, no one questions that all cellular processes are subject to physical laws. For a cell to function properly, the values of its physical parameters must be such that the governing physical laws serve the survival and reproduction of the cell. If the osmotic pressure is too high inside the cell, it may lyse. If the voltage across the membrane is not appropriate, a voltage-gated ion channel will not function. If the viscosity of the cytoplasm is too

large, diffusion across it slows down and processes that are normally coordinated by diffusing signals become incoherent.

One way in which a cell sets the values of its physical parameters is by regulated expression of the cell's genes. Genes specify proteins, which themselves control or influence every aspect of the cell's life, including the values of its physical parameters. The embryo begins as a fertilized egg, or zygote, a single cell that contains the full complement of genes needed to construct the organism. The construction of the organism will necessitate changes in various physical parameters at particular stages of development and typically this will be accomplished by new gene expression. These changes will be set off by signals that may originate from outside the embryo or by interactions between the different parts of the embryo itself. As cells reach their definitive differentiated states later in development their appropriate functioning will require stability of their physical properties. If these change in an unfavorable direction then other signals are generated and transmitted to the nucleus to produce further proteins that reverse or otherwise correct the altered physical state.

In the remainder of this chapter we will consider certain transport and mechanical properties that are essential to an individual cell's existence. Because these properties have physical analogues at the multicellular level they will also figure in later discussions. We will begin with the "default" physical description, the simplest characterization of what might be taking place in the interior of the cell. We will then see how this compares with measurements in real biological systems. Where there is a disparity between the theoretical and actual behaviors we will examine ways in which the physical description can be modified to accommodate biological reality.

## Diffusion

### Free diffusion

The interior of the zygote, like most other cells, represents a crowded aqueous environment (Ellis and Minton, 2003) with many thousands of molecules in constant motion in the presence of complex structures such as organelles. Metabolism, the transformation of small molecules within the zygote, requires nutrients to be brought in and waste molecules to be carried out. In the course of intracellular signaling, information is passed on from one molecule to another and finally delivered to various destinations. How do biological molecules move inside the cell? Unless they are bound to a surface these molecules typically bump into each other and collide with water molecules and thus are constantly changing their direction of motion. Under such conditions how can they reach their destinations and carry out their tasks with high fidelity?

Motion requires energy. In general it can be active or passive. Of the two, only active motion has a preferred direction. Molecular motors moving along cytoskeletal filaments use the chemical energy of adenosine triphosphate (ATP) and transform it into kinetic

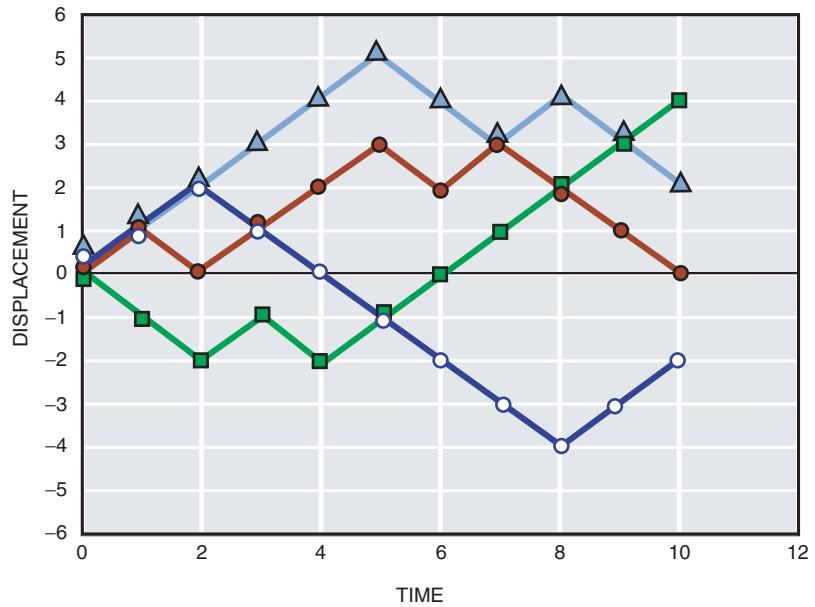
energy. They shuttle from the plus to the minus end of microtubules (“plus-directed motor”) or in the opposite direction (“minus-directed motor”).

As an example of passive motion we may consider a molecule inside the cell, kicked around by other molecules, just as a stationary billiard ball would be by a moving ball, and triggering a cascade of collisions. In the case of a billiard ball, motion is generated in the first place by a cue; cytoplasmic molecules are not set in motion by any such external device. Rather, they have a constant supply of energy that causes them to be in constant motion. The energy needed for this motion is provided by the environment and is referred to as “thermal energy.” In fact, any object will exchange kinetic energy with its environment, and the energy content of the environment is directly proportional to its absolute temperature  $T$  (measured in kelvins, K). For everyday objects (e.g., a billiard ball weighing 500 g) at typical temperatures (e.g., room temperature, 298 K = 25 °C) the effect of thermal energy transfer on the object’s motion is negligible. A cytoplasmic protein weighing on the order of  $10^{-19}$  g, however, is subject to extensive buffeting by the thermal energy of its environment.

For a particle allowed to move only along the  $x$  axis the kinetic energy  $E_{\text{kin}}$  imparted by thermal motion is given by  $E_{\text{kin}} = m\langle v_x^2 \rangle / 2 = k_B T / 2$ , where  $m$  is the mass of the particle,  $v_x$  is its velocity, and  $k_B$  is Boltzmann’s constant. The symbol  $\langle \rangle$  denotes an average over an ensemble of identical particles (Fig. 1.2). Averaging is necessary since one is dealing with a distribution of velocities rather than a single well-defined velocity. To understand better the meaning of  $\langle \rangle$ , imagine following the motion of a particle fueled exclusively by thermal energy, for a specified time  $t$  (at which it is found at some point  $x_t$ ) and measuring its velocity at this moment. If the particle is part of a liquid or gas it moves in the presence of obstacles (i.e., other particles) and thus its motion is irregular. Therefore, repeating this experiment  $N$  times will typically yield  $N$  different values for  $v_x^2$ . The average over these  $N$  values (i.e., the ensemble average) gives us the interpretation of  $\langle v_x^2 \rangle$  (Fig. 1.2). For a G-actin molecule at 37 °C, thermal energy would provide a velocity  $\langle v_x^2 \rangle^{1/2} = 7.8$  m/s. (For a billiard ball at room temperature the velocity would be a billion times smaller.) In the absence of obstacles, this velocity would allow an actin monomer to traverse a typical cell of 10  $\mu\text{m}$  diameter in about 1 microsecond.

The interior of a cell represents a crowded environment. A molecule starting its journey at the cell membrane would not get very far before bumping into other molecules. Collisions render the motion random or diffusive. When discussing diffusion (referred to as Brownian motion in the case of a single particle), a reasonable question to ask is, what distance would a molecule cover on the average in a given time? For one-dimensional motion the answer is (see, for example, Berg, 1993 or Rudnick and Gaspari, 2004)

$$\langle x_t^2 \rangle = 2Dt, \quad (1.1)$$



**Fig. 1.2** The meaning of the ensemble average  $\langle \cdot \rangle$ . The figure can be interpreted as showing either the diffusive trajectories of four random walkers in one dimension, allowed to make discrete steps of unit length in either direction with equal probability along the vertical axis, or four different trajectories of the same walker. The trajectories are shown up to 10 time steps. The walkers make one step in one (discrete) unit of time. (Displacement and time are measured in arbitrary units.) The average distance after 10 time steps is  $\langle x(t=10) \rangle = \frac{1}{4}(4 + 2 + 0 - 2) = 1$ , the average squared displacement is  $\langle x^2(t=10) \rangle = \frac{1}{4}(16 + 4 + 0 + 4) = 6$ . The mean first passage time of arrival at  $x = 2$  is  $\langle T_1(x=2) \rangle = \frac{1}{4}(2 + 2 + 4 + 8) = 4$ . These values are independent of how the trajectories are interpreted. Note the difference between  $\langle T_1 \rangle$  and  $t$ . The latter is a fixed quantity (10 in the figure), whereas the former is a statistical quantity.

assuming that at  $t = 0$  the particle was at  $x = 0$ . (Diffusion in three dimensions can be decomposed into one-dimensional diffusions along the main coordinate axis, each giving the same contribution to the three-dimensional analogue of Eq. 1.1. Thus, when  $x^2$  above is replaced by  $r^2 = x^2 + y^2 + z^2$ ,  $r$  being the length of the radius vector, the factor 2 on the right-hand side of Eq. 1.1 changes to 6.) Here  $D$  is the diffusion coefficient, whose value depends on the molecule's mass, shape and on properties of the medium in which it diffuses (temperature and viscosity). The average is calculated as above for the case of  $v$ . The diffusion coefficient of a G-actin molecule in water at 37°C is approximately  $10^2 \mu\text{m}^2/\text{s}$ , which allows its average displacement to span the 10  $\mu\text{m}$  distance across a typical cell in about 1 second. Comparing with the earlier result, obtained assuming unimpeded translocation with the thermal velocity, we see that collisions slow down the motion a million-fold.

There are several remarks to be made about Eq. 1.1. The most striking observation is that distance is not proportional to time: there