Resource Letter: Bio-molecular Nano-machines: where Physics, Chemistry, Biology and Technology meet

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Abstract

Cell is the structural and functional unit of life. This Resource Letter serves as a guide to the literature on nano-machines which drive not only intracellular movements, but also motility of the cell. These machines are usually proteins or macromolecular assemblies which require appropriate fuel for their operations. Although, traditionally, these machines were subjects of investigation in biology and biochemistry, increasing use of the concepts and techniques of physics in recent years have contributed to the quantitative understanding of the fundamental principles underlying their operational mechanisms. The possibility of exploiting these principles for the design and control of artificial nano-machines has opened up a new frontier in the bottom-up approach to nanotechnology.

Some are to be read, some to be studied, and some may be neglected entirely, not only without detriment, but with advantage. - Anonymous

1 Introduction

Motility is the hallmark of life. From the sprinting leopard to flying birds and swimming fish, movement is one of life’s central attributes. The mechanisms of motility at the level of macroscopically large organisms are the main topics of investigation in biomechanics and insights gained from these investigations find applications, for example, in robotics. Not only animals, but even plants also move in response to external stimuli. Results of pioneering systematic study of this phenomenon were reported already in the nineteenth century by Charles Darwin in a classic book, titled The power of movement in plants, which was co-authored by his son.

In living systems, movements take place at all levels of biological organization- from molecular movements at the subcellular levels and cellular movements to movements of organs and organ systems. However, in this article, we focus exclusively on the molecular mechanisms of motility at the level of single cells (both unicellular organisms and individual cells of multicellular organisms) and those at the subcellular level.

1. L. Chong, E. Culotta and A. Sugden, On the move, Science 288, 79 (2000).

2. A.C. Leopold and M.J. Jaffe, Many modes of movement, Science 288, 2131-2132 (2000).

3. D.W. Maughan and J.O. Vigoreaux, An integrated view of insect flight muscle: genes, motor molecules, and motion, News Physiol. Sci. 14, 87-92 (1999).
1.1 Cell movements: molecular mechanisms of motility

Antonie van Leeuwenhoek made the first systematic study of the motility of unicellular microorganisms using his primitive microscope. Since then, over the last three centuries, swimming, crawling, gliding and twitching of single cells have fascinated generations of biologists. However, investigation of the molecular mechanisms of cellular motility began only a few decades ago. The motility of a cell is the outcome of the coordination of many intracellular dynamical processes. Interestingly, intracellular movements also drive motility and division of the cell itself. We’ll present a systematic list of these developments from the perspective of physicists.

4. H.C. Berg, *E. coli in Motion*, (Springer, 2003).

5. D. Bray, *Cell Movements: from molecules to motility* (Garland Publishig, Taylor and Francis, 2001).

6. D.A. Fletcher and J.A. Theriot, *An introduction to cell motility for the physical scientist*, Phys. Biol. 1, T1-T10 (2004).

1.2 Intracellular movements: machines and mechanisms

“Nature, in order to carry out the marvelous operations in animals and plants, has been pleased to construct their organized bodies with a very large number of machines, which are of necessity made up of extremely minute parts so shaped and situated, such as to form a marvelous organ, the composition of which are usually invisible to the naked eye, without the aid of the microscope”.

Marcelo Malpighi, 17th century (as quoted by Marco Piccolino, Nat. Rev. Mol. Cell Biol. 1, 149-153 (2000)).

Imagine an under water “metro city” which is, however, only about 10µm long in each direction! In this city, there are “highways” and “railroad” tracks on which motorized “vehicles” transport cargo to various destinations. It has an elaborate mechanism of preserving the integrity of the chemically encoded blueprint of the construction and maintenance of the city. The “factories” not only supply their products for the construction and repair works, but also manufacture the components of the machines. This eco-friendly city re-charges spent “chemical fuel” in uniquely designed “power plants”. This city also uses a few “alternative energy” sources in some operations. Finally, it has special “waste-disposal plants” which degrade waste into products that are recycled as raw materials for fresh synthesis. This is not the plot of a science fiction, but a dramatized picture of the dynamic interior of a cell.

In an influential paper, published in 1998, Bruce Alberts emphasized that “the entire cell can be viewed as a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines”. Just like their macroscopic counterparts, molecular machines have an “engine”, an input and an output. Some of these machines are analogous to motors whereas some others are like pumps; both linear and rotary motors have been identified. Some motors move on protein filaments whereas others move on nucleic acid strands (i.e., DNA or RNA).

7. M. Piccolino, *Biological machines: from mills to molecules*, Nature Rev. Mol. Cell Biol. 1, 149-153 (2000).

8. C. Mavroidis, A. Dubey and M.L. Yarmush, *Molecular Machines*, in: Annual Rev. Biomed. Engg., 6, 363-395 (2004).

9. T.D. Pollard, *Proteins as machines*, Nature 355, 17-18 (1992).

10. B. Alberts and R. Miake-Lye, *Unscrambling the puzzle of biological machines: the importance of the details*, Cell, 68, 415-420 (1992).

11. B. Alberts, *The cell as a collection of protein machines: preparing the next generation of molecular biologists*, Cell 92(3), 291-294 (1998).

12. A. Baumgartner, *Biomolecular machines*, in: *Handbook of Theoretical and Computational
In spite of the striking similarities, it is the differences between molecular machines and their macroscopic counterparts that makes the studies of these systems so interesting from the perspective of physicists. Biomolecular machines are usually protein or macromolecular complex. These operate in a domain far from thermodynamic equilibrium where the appropriate units of length, time, force and energy are, nano-meter, milli-second, pico-Newton and $k_B T$, respectively ($k_B$ being the Boltzmann constant and $T$ is the absolute temperature). The viscous forces and random thermal forces on a nano-machine dominate over the inertial forces. These are made of soft matter and are driven by “isothermal” engines. Molecular motors can convert chemical energy directly into mechanical energy.

13. D’Arcy Thompson, On Growth and Form, vol.I reprinted 2nd edition (Cambridge University Press, 1963).

### 1.3 Outline of organization

We divide the intracellular molecular cargoes into three different types: (i) membrane-bound cargoes, e.g., vesicles and organelles; (ii) macromolecules, e.g., DNA, RNA and proteins; (iii) medium-size organic molecules and small inorganic ions. In part I we study motor proteins which transport the membrane-bound cargoes. In part II we consider all those machines which are involved in the synthesis, export/import, packaging, other kinds of manipulations and degradation of the macromolecules. In part III we focus on machines which transport medium-size organic molecules and small inorganic ions across plasma membrane or internal membranes of eukaryotic cells; transporters of ions are usually referred to as pumps because ions are transported against their natural electro-chemical gradients. Finally, in part IV we present machines and mechanisms which drive cell motility and cell division.

Based on the nature of input and output energies, machines can be classified. For example, the motor of hair dryer is an electro-mechanical machine. But, in this article we’ll not consider purely chemo-chemical machines although some of these perform important biological functions.

14. J. Howard, Mechanics of motor proteins and the cytoskeleton, (Sinauer Associates, Sunderland, 2001).

15. M. Schliwa, (ed.) Molecular Motors, (Wiley-VCH, 2003).

16. D. D. Hackney and F. Tanamoi, The Enzymes, vol.XXIII Energy Coupling and Molecular Motors (Elsevier, 2004).

17. J.M. Squire and D.A.D. Parry, Fibrous proteins: muscle and molecular motors, (Elsevier 2005).

18. A.B. Kolomeisky and M.E. Fisher, Molecular motors: a theorist’s perspective, Annu. Rev. Phys. Chem. 58, 675-695 (2007).

19. J. Howard, Molecular mechanics of cells and tissues, Cellular and Molecular Bioengineering 1, 24-32 (2008).

### 1.4 Criteria for selection

We have used the following guidelines for selection of papers for this resource letter:
(i) To our knowledge, at present, there is no single book where a reader can find a comprehensive coverage of all the molecular machines. Therefore, in this resource letter, we list monographs and edited collections of reviews on specific machines and mechanisms.

(ii) Review articles usually provide a critical overview of progress in an area of research and, normally, remain useful to both beginners as well as experts for a relatively longer period of time as compared to original papers. Therefore, in this resource letter, review articles guide the reader through the enormous literature on experimental works on molecular machines. Occasionally, we also list original experimental papers; most of these are either classic or too recent to be discussed in any review article, or introduce new models.

(iii) Since the emphasis of this resource letter is on quantitative models of mechanisms of molecular machines, many original papers on theoretical works have been listed together with the review articles.

(iv) Results of fundamental research on the structure and function of molecular machines not only have important biomedical implications but may also find practical applications in bottom-up approach to designing and manufacturing artificial nano-machines. Therefore, papers on bio-nanotechnology which satisfy the criteria (ii) or (iii) above have also been listed.

(v) Unpublished manuscripts (including those posted in public domain archives) have not been listed. But, the final version of some Ph.D. theses have been included because these provide technical details which are not available in the papers published elsewhere by the author.

1.5 List of review series and journals

In this multidisciplinary area of research, articles appear in journals that cover physics, chemistry, biology and (nano-)technology. We list here only a few major sources for review articles as well as original papers. But, this list is neither exhaustive nor in the order of any ranking.

(1) “Annual Review” Series (e.g., Annual Reviews of Biophysics and Biomolecular Structure).
(2) “Trends” series (e.g., Trends in Cell Biology).
(3) “Current Opinion” series (e.g., Current Opinion in Structural Biology).
(4) Bioessays,
(5) “Nature Reviews” series (e.g., Nature Reviews in Microbiology).
(6) Nature,
(7) Nature Cell Biology,
(8) Nature Structural and Molecular Biology,
(9) Science,
(10) Proceedings of the National Academy of Sciences, USA, (PNAS),
(11) Cell,
(12) Molecular Cell,
(13) Current Biology.
(14) Journal of Molecular Biology,
(15) Journal of Cell Biology,
(16) Journal of Biological Chemistry,
(17) Biophysical Journal,
(18) Physical Review Letters,
(19) Physical Review E,
(20) Physical Biology,
(21) Europhysics Letters,
(22) European Physical Journal E.
(23) EMBO Reports,
(24) EMBO Journal,
(25) European Biophysical Journal.

2 Experimental techniques for studying operational mechanisms of molecular machines

The most profound scientific revolutions are those that provide an entirely new way of viewing and studying a field. These are the ones that provoke new questions and question old answers and in the end give us a new understanding of what we thought we understood. Often they are occasioned by the invention of novel instruments of techniques; the telescope, the microscope, and X-ray diffraction come to mind. It may be that such a revolution is occurring in biochemistry today through the development of methods that allow us to investigate the dynamics of single
Seeing is believing. Telescopes opened up the celestial world in front of our eyes. The invention of the optical microscopes in the seventeenth century made it possible to have a glimpse of the world of micro-organisms (bacteria, etc.). But these microbes are typically micron-size objects; it would be ideal if we could have “nanoscopes” for seeing nano-machines. In addition to the requirement of high spatial resolution, such nanoscopes should also have sufficiently high temporal speed so that the dynamics of the nano-machines can be monitored under the nanoscope.

But, it is impossible to see a molecule directly under an optical microscope of conventional design because nature has imposed a limit on the resolution that can be achieved with these optical instruments. This fundamental limit on the resolution is a consequence of the wave nature of light and it depends on the wavelength of the radiation used for observation.

But, optical microscopes merely enhance the power of our visionary perception. Therefore, in principle, it should be possible to achieve higher resolution if X-rays or γ-rays are used for imaging although we can no longer use our eyes as detector for these probes. Moreover, vision is only one of the several sensory perceptions humans possess. A blind person can construct a mental image of an object by running his fingers along the contours of the object. Furthermore, in principle, it is possible to reconstruct the shape of an object, without seeing or touching it, by throwing balls at it from all sides and, then, analyzing the way the balls are scattered by the object.

2.1 Ensemble-averaged techniques

X-ray crystallography and electron microscopy

The basic principle of X-ray scattering for the determination of the structure of macromolecules is as follows: an atomic constituent of the macromolecule absorbs some energy of the X-ray incident on it and then re-radiates the same in all directions. A protein crystal has a periodic array of identical atoms. The X-rays re-radiated by these atoms interfere constructively in some directions whereas they interfere destructively in all the other directions. Therefore, the detectors record a “pattern” in the intensity of X-ray scattered by the protein crystal sample. But, such a “diffraction pattern” provides an indirect, and static, image of a molecular machine. However, microscopes (optical as well as electron) have some advantages over the X-ray scattering technique: microscopes produce the images directly in real space whereas X-ray diffraction requires Fourier transform from momentum space to real space.

The deBroglie wavelength associated with a material particle is given by $\lambda = h/p$ where $p$ is the momentum of the particle. A sufficiently high resolution microscope can be constructed if a charged particle is selected and it is accelerated to the required momentum by applying an external electric field. Electrons are most convenient for this purpose; an electron beam can be easily bent and focussed using a suitable magnetic field configuration. Electron microscopy is one of the most powerful experimental techniques for determination of the structures of molecular machines. In spite of all the technological advances in electron microscopy, it is still lot more cumbersome to use than an optical microscope. In an optical microscope, the sample does not require as elaborate preparation as in an electron microscope. Besides, the intense beam of electrons often damage or destroy the sample itself. Moreover, image obtained from an electron microscope requires special expertise to interpret. Furthermore, the generation and control of the electro-magnetic fields makes the electrom microscope costly as well as much less user friendly than optical microscopes.

20. J. Frank, Three-dimensional electron microscopy of macromolecular assemblies, (Academic Press, 1996).

21. E. Nogales and N. Grigorieff, Molecular machines: putting the pieces together, J. Cell Biol. 152, F1-F10 (2001).

22. A.J. Koster and J. Klumperman, Electron microscopy in cell biology: integrating structure and function, Nat. Rev. Mol. Cell Biol. 4, SS6-SS10 (2003).
23. V. Lucic, F. Forster and W. Baumeister, Structural studies by electron tomography: from cells to molecules, Annu. Rev. Biochem. 74, 833-865 (2005).

24. W. Chiu, M.L. Baker, W. Jiang, M. Dougherty and M.F. Schmidt, Electron cryomicroscopy of biological machines at subnanometer resolution, Structure 13, 363-372 (2005).

25. W. Chiu, M.L. Baker and S.C. Almo, Structural biology of cellular machines, Trends Cell Biol. 16, 144-150 (2006).

26. M. Rossmann, M.C. Morais, P.G. Leiman and W. Zhang, Combining X-ray crystallography and electron microscopy, Structure 13, 355-362 (2005).

27. G.J. Jensen and A. Briegel, How electron cryotomography is opening a new window onto prokaryotic ultrastructure, Curr. Opin. Struct. Biol. 17, 260-267 (2007).

28. A. Hoenger and D. Nicastro, Electron microscopy of microtubule-based cytoskeletal machinery, Methods in Cell Biol. 79, 437-462 (2007).

29. L. Wang and F.J. Sigworth, Cryo-EM and single particles, Physiology 21, 13-18 (2006).

2.2 Single-molecule techniques

We got our first glimpse of the macromolecules via X-ray diffraction and, then, electron microscopy. But, what one got from those probes were static pictures. Moreover, most of the traditional old experimental techniques of biophysics relied on collection of data for a large collection of molecules and thereby getting their ensemble-averaged properties. The amplification of the signals caused by the presence of large number of such molecules makes it easier to detect and collect the data. The average value of a variable is valuable information. There are practical limitations of the bulk measurements in the specific context of understanding the operational mechanisms of cyclic molecular machines because it is practically impossible to synchronize their cycles. The recently developed single-molecule techniques can be broadly classified into two groups: (i) methods of imaging, and (ii) methods of manipulation.

30. J. Zlatanova and K. van Holde, Single-molecule biology: what is it and how does it work?, Mol. Cell 24, 317-329 (2006).

31. P.V. Cornish and T. Ha, A survey of single-molecule techniques in chemical biology, ACS chemical biology, 2, 53-61 (2007).

• Techniques of single-molecule imaging

For visualization of the conformational changes or movements of the molecule under investigation in a single molecule experiment, a prior attachment of a label to the molecule is essential. Based on these labels, the single molecule imaging of molecular motors can be divided into two groups: (i) techniques where the label is a relatively large light-scattering object (for example, a dielectric bead of 1 micron diameter); and (ii) techniques where the label itself emits light (e.g., a fluorophore). Fluorescence microscopy provided a glimpse (howsoever hazy) of single molecules. Imaging a fluorescently labelled molecular motor in real time enables us to study its dynamics just as ecologists use “radio collars” to track individual animals.

32. R.Y. Tsien, Imagining imaging’s future, Nat. Rev. Mol. Cell Biol. 4, SS16-SS21 (2003).

33. J.W. Lichtman and J.A. Conchello, Fluorescence microscopy, Nat. Methods 2, 910-919 (2005).

34. Y. Garini, B.J. Vermolen and I.T. Young, From micro to nano: recent advances in high-resolution microscopy, Curr. Opin. Biotechnol. 16, 3-12 (2005).

35. W.E. Moerner, A dozen years of single-molecule spectroscopy in physics, chemistry and biophysics, J. Phys.Chem. B 106, 910-927 (2002).

36. W.E. Moerner and D.P. Fromm, Methods of single-molecule fluorescence spectroscopy and
microscopy, Rev. Sci. Instr. 74, 3597-3619 (2003).

37. E.J.G. Peterman, H. Sosa and W.E. Moerner, Single-molecule fluorescence spectroscopy and microscopy of biomolecular motors, Annu. Rev. Phys. Chem. 55, 79-96 (2004).

38. W.E. Moerner, New directions in single-molecule imaging and analysis, PNAS 104, 12596-12602 (2007).

39. X. Michalet and S. Weiss, Single-molecule spectroscopy and microscopy, C.R. Physique 3, 619-644 (2002).

40. X. Michalet, A.N. Kapanidis, T. Laurence, F. Pinaud, S. Doose, M. Pflughoeft and S. Weiss, The power and prospects of fluorescence microscopies and spectroscopies, Annu. Rev. Biophys. Biomol. Struct. 32, 161-182 (2003).

41. S.W. Hell, Towards fluorescence nanoscopy, Nat. Biotechnol. 21, 1347-1355 (2004).

42. D.J. Stephens and V.J. Allan, Light microscopy techniques for live cell imaging, Science 300, 82-86 (2005).

43. Y. Ishii and T. Yanagida, How single molecule detection measures the dynamic actions of life, HFSP journal, 1, 15-29 (2007).

44. E. Toprak and P.R. Selvin, New fluorescent tools for watching nanometer-scale conformational changes of single molecules, Annu. Rev. Biophys. Biomol. Struct. 36, 349-369 (2007).

45. H. Park, E. Toprak and P.R. Selvin, Single molecule fluorescence to study molecular motors, Q. Rev. Biophys. 40, 87-111 (2007).

46. C. Joo, H. Balci, Y. Ishitsuka, C. Buranachai and T. Ha, Advances in single-molecule fluorescence methods for molecular biology, Annu. Rev. Biochem. 77, 51-76 (2008).

Let us also not forget that experiments are also carried out with molecular machines to understand their mechanisms; such experiments should answer questions like “what if...”. Such controlled experiments would need some means of manipulating the nanomachines of life. The mechanical transducers like, for example, cantilevers of scanning force microscopes (SFM) and microneedles, require physical contact with the biomolecule. In contrast, manipulators that utilize electromagnetic fields do not require any contact forces.

The operation of optical tweezers is based on a very simple physical principle. Photons are massless (more precisely, rest mass is zero), but have momentum. Photons are capable of exerting very weak force called radiation pressure in the terminology of classical physics. Utilizing this property of photons (or, equivalently, electromagnetic radiation) in a laser beam with high, but inhomogeneous, intensity, it has been possible to trap dielectric particles (e.g., a latex bead) at the focal point of the beam. When a motor attached to such a bead walks on its filamentary track, the laser trap pulls it back thereby applying a load force on the motor.

In magnetic tweezers, the macromolecule is attached between a surface and a superparamagnetic bead. Stretching force can be applied on the macromolecule by controlled alterations of the external magnetic field. A major advantage of the magnetic tweezers is that the same setup can be used also to apply torque on the molecule by merely rotating the magnetic field.

47. J.M. Imhof and D.A. vanden Bout, Resource Letter: LBMOM-I: Laser-based modern optical microscopy, Amer. J. Phys. 71, 429-436 (2003).

48. M.J. Lang and S.M. Block, Resource Letter: LBOT-I: Laser-based optical tweezers, Amer. J. Phys. 71, 201-215 (2003).

49. D.C. Appleyard, K.Y. Vandermeulen, H. Lee and M.J. Lang, Optical trapping for undergraduates, Amer. J. Phys. 75, 5-14 (2007).

50. T. Strick, J.F. Allemand, V. Croquette and D. Bensimon, The manipulation of single
From the evolutionary point of view, cells can be broadly divided into two categories, viz., prokaryotes and eukaryotes. Most of the common bacteria (like, for example, Escherichia Coli and Salmonella) are prokaryotes. Animals, plants and fungi are collectively called eukaryotes. The main difference between prokaryotic and eukaryotic cells lies in their internal architectures; the main distinct feature of eukaryotic cells is the cell nucleus where the genetic materials are stored. The prokaryotes are mainly unicellular organisms. The eukaryotes which emerged first through Darwinian evolution of prokaryotes were also unicellular; multi-cellular eukaryotes appeared much later.

In biology, often the simplest among a family of objects is called a model system for the purpose of experimental investigations.

- **Model eukaryotes**:

The most popular model *animals* for biological studies are as follows: (i) the fruit fly *Drosophila melanogaster*, a model insect, (ii) *Caenorhabditis elegans* (C-elegans), a transparent worm, (iii) the zebra fish *danio rerio*, a model vertebrate; (iv) the mouse, however, is more important for practical use of cell biology in medical sciences. *Arabidopsis thaliana* is the most popular model *plant* while *Chlamydomonas reinhardtii* is a model of green algae. *Saccharomyces cerevisiae* (Baker’s yeast) and *Schizosaccharomyces pombe* (Fission yeast) are most widely used models for fungi. However, for studying filamentous fungi,
Neospora crassa is used most often as a model system.

- **Model prokaryotes:**
  Bacteria are divided into two separate groups on the basis of their response to a staining test invented by Hans Christian Gram. Those which respond positively are called Gram-positive bacteria whereas those whose response is negative are called Gram-negative. One of the main differences between these two groups of bacteria is the nature of the cell wall.

  The commonly used models for Gram-positive bacteria are Bacillus subtilis, Listeria monocytogenes, etc. The bacterium Escherichia coli (E-coli), which is normally found in the colon of humans and other mammals, and the bacterium Salmonella are the most extensively used model for Gram-negative bacteria. Another prominent member of the group of Gram-negative bacteria is Proteus mirabilis.

- **Model viruses and bacteriophages:**
  Human immunodeficiency virus (HIV) is the most dreaded among the viruses that can infect homo sapiens (humans). Among the other viruses which can infect eukaryotes are Tobacco mosaic virus, etc. Bacteriophages are also viruses, but these infect prokaryotes. T-odd (e.g., T7) and T-even (e.g., T4) bacteriophages, phage λ, φ29, etc. are some of the extensively used model bacteriophages.

  65. E.M. Meyerowitz, *Prehistory and history of Arabidopsis research*, Plant Physiol. **125**, 15-19 (2001).

  66. E.H. Harris, *Chlamydomonas as a model organism*, Annu. Rev. Plant Physiol. and Plant Mol. Biol. **52**, 363-406 (2001).

  67. R.H. Davis and D.D. Perkins, *Neurospora: a model of model microbes*, Nat. Rev. Genetics, **3**, 7-13 (2002).

  68. I.J. van der Klei and M. Veenhuis, *Yeast and filamentous fungi as model organisms in microbiology research*, Biochim. Biophys. Acta **1763**, 1364-1373 (2006).

  69. M.A. Sleigh, *The biology of protozoa* (Edward Arnol, London, 1973).

  70. K.B.G. Scholtzof, *Tobacco mosaic virus: a model system for plant biology*, Annu. Rev. Phytopathology, **42**, 13-34 (2004).

  71. B.D. Harrison and T.M.A. Wilson, *Milestones in the research on tobacco mosaic virus*, Phil. Trans. Roy. Soc. Lond. B **354**, 521-529 (1999).

  72. A.L. Wang and C.C. Wang, *Viruses of the protozoa*, Annu. Rev. Microbiol. **45**, 251-263 (1991).

  73. H.W. Ackermann and H.M. Krisch, *A catalogue of T4-type bacteriophages*, Arch. Virol. **142**, 2329-2345 (1997).

  74. P.G. Leiman, S. Kanamaru, V.V. Mesyanzhinov, F. Arisaka and M.G. Rossmann, *Structure and morphogenesis of bacteriophage T4*, Cell. Mol. Life Sci. **60**, 2356-2370 (2003).

  75. V.V. Mesyanzhinov, P.G. Leiman, V.A. Kostyuchenko, L.P. Kurochkina, K.A. Miroshnikov, N.N. Sykilinda and M.M. Shneider, *Molecular architecture of bacteriophage T4*, Biochemistry (Moscow), (translated from Biokhimiya), **69**, 1190-1202 (2004).

  76. E.S. Miller, E. Kutter, G. Mosig, F. Arisaka, T. Kunisawa and W. Rüger, *Bacteriophage T4 genome*, Microbiol. Mol. Biol. Rev. **67**, 86-156 (2003).

  77. M.E. Gottesman and R.A. Weisberg, *Little lambda, who made thee?*, Microbio. Mol. Biol. Rev. **68**, 796-813 (2004).

  78. W.J.J. Meijer, J.A. Horcajadas and M. Salas, *φ29 family of phages*, Microbiol. Mol. Biol. Rev. **65**, 261-287 (2001).
3 Techniques for theoretical modeling of molecular machines

Theory provides understanding and insight. These allow us not only to interpret the empirical observations and recognize the importance of the various ingredients but also to generalize, to create a framework for addressing the next level of question and to make predictions which can be tested in in-vivo/in-vitro or in-silico experiments.

Theorization requires a model of the system. A theoretical model is an abstract representation of the real system which helps in understanding the real system. This representation can be pictorial (for example, in terms of cartoons or graphs) or symbolical (e.g., a mathematical model). Qualitative predictions may be adequate for understanding some complex phenomena or for ruling out some plausible scenarios. But, a desirable feature of any theoretical model is that it should make quantitative predictions. The predictions of a theory, at least in principle, can be tested by in-vitro and/or in-vivo experiments in the laboratory.

The predictions of a mathematical model can be derived analytically in terms of abstract symbols; for specific sets of values of the model parameters, the predictions can be shown numerically or graphically. The predictions of a theoretical model can be obtained numerically by carrying out computer simulations (i.e., in-silico experiments) of the model. Thus, simulation is not synonymous with modeling. When a model is too complicated to be formulated in abstract notations and to be treated analytically, it is called a computer model of the system. Since fully analytical treatment of a model can be accomplished exactly only in rare cases, one has to make sensible approximations so as to get results as accurate as possible. Simulation of a model also tests the validity of the approximations made in the analytical treatments of the model. We should also make a distinction between the two different “computational methods”, namely, (i) computer simulations which, as we have mentioned above, test hypotheses; and (ii) Knowledge discovery (or, data mining) which extracts hidden patterns or laws from huge quantities of experimental data, forming hypotheses.

A model can be formulated at different physical or logical levels of resolution. The physical resolution can be spatial resolution or temporal resolution. Every theoretical model is intended to address a set of questions. The modeler must choose a level of description appropriate for this purpose keeping in mind the phenomena that are subject of the investigation. Otherwise, the model may have either too much redundant details or it may be too coarse to provide any useful insight. Since physicists most often focus only on generic features of the various classes of machines, rather than specific features of individual members of these classes, they normally develop minimal models which may be regarded as mesoscopic, rather than molecular, i.e., their status in somewhere in between those of the macroscopic and molecular models.

79. A. Mogilner, R. Wollman and W.F. Marshall, Quantitative modeling in cell biology: what is it good for?, Developmental Cell 11, 279-287 (2006).

3.1 Mechanics of molecular machines: noisy power stroke versus Brownian ratchet

If the input energy directly causes a conformational change of the protein machinery which manifests itself a mechanical stroke of the machine, the operation of the machine is said to be driven by a “power stroke” mechanism. This is also the mechanism used by all man made macroscopic machines. Let us contrast this with the following alternative scenario: suppose, the machine exhibits “forward” and “backward” movements because of spontaneous thermal fluctuations. If now energy input is utilized to prevent “backward” movements, but allow the “forward” movements, the system will exhibit directed, albeit noisy, movement in the “forward” direction. Note that the forward movements in this case are caused directly by the spontaneous thermal fluctuations, the input energy rectifies the “backward”
movements. This alternative scenario is called the Brownian ratchet mechanism.

80. R.D. Vale and F. Oosawa, Protein motors and Maxwell’s demons: does mechanochemical transduction involve a thermal ratchet?, Adv. Biophys. 26, 97-134 (1990).

81. R.D. Astumian, Making molecules into motors, Sci. Am. 285, 56-64 (2001).

82. R.D. Astumian and P. Hänggi, Phys. Today 55, 33-39 (2002).

83. R.D. Astumian, Design principles of Brownian molecular machines: how to swim in molasses and walk in a hurricane, Phys.Chem.Phys. 7, 5067-5083 (2007).

84. R.D. Astumian, Thermodynamics and kinetics of a Brownian motor, Science 276, 917-922 (1997).

85. F. Jülicher, A. Ajdari and J. Prost, Modeling molecular motors, Rev. Mod. Phys. 69, 1269-1281 (1997).

86. P. Reimann, Brownian motors: noisy transport far from equilibrium, Phys. Rep. 361, 57-265 (2002).

87. J. Howard, Protein power strokes, Curr. Biol. 16, R517-R519 (2006).

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3.2 Chemical reactions relevant for molecular machines: ATP hydrolysis

To understand molecular machines, we also have to consider chemical reactions, which most often supply the (free-) energy required to drive these machines.

In other words, in order to understand the mechanisms of biomolecular machines, it is necessary to understand not only how these move in response to the mechanical forces but also how these are affected by chemical reactions. In fact, the machines are usually enzymes (i.e., catalysts).

Adenosine triphosphate (ATP) contains three phosphate groups as compared to two phosphate groups in the adenosine diphosphate (ADP). Hydrolysis of ATP to ADP releases free energy and, therefore, plays a crucial role in running a wide range of chemical processes in a living organism. Therefore, ATP is sometimes also referred to as the “energy currency” of the cell.

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3.3 General mechano-chemistry of molecular machines

From the perspective of (bio-)physics, the mechanical force required for the directed movement of the motors are generated, most often, from the energy liberated in chemical reactions, e.g., in ATP hydrolysis. On the other hand, from the perspective of (bio-)chemistry, most of the machines are enzymes (i.e., proteins which act as catalysts for many chemical reactions); the rate of enzymatic reactions, including that of ATP hydrolysis, is strongly influenced by external forces. Thus, the mechanisms of molecular machines are governed by a nontrivial combination of the principles of nano-mechanics and those of chemical reactions. Therefore, quantitative modeling of molecular machines require theoretical formalisms of mechano-chemistry or chemo-mechanics.

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• Efficiency of molecular machines: general discussions

The efficiency of molecular motors can be defined in several different ways: while one of the definitions is very similar to that of its macroscopic counterpart, the other definitions are unique to motors operating under different conditions and characterize different aspects of its movement.

Not all molecular motors are designed to pull loads. Moreover, in contrast to the macroscopic motors, viscous drag forces strongly influence the function of molecular motors. Therefore, there is a need for a generalized definition of efficiency that does not necessarily require the application of any external load force. Such a measure of efficiency, which is different from the thermodynamic efficiency defined above, has also been suggested; it is called “Stokes efficiency” because the viscous drag is calculated from Stokes law.

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Allosterism usually refers to the change of conformation around one location of a protein in response to binding of a ligand to another location of the same protein. A motor protein has separate sites for binding ATP and the track. Therefore, the mechanochemical cycle of a motor can be analyzed from the perspective of allosterism.
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4 Eukaryotic cytoskeleton: structure and dynamics

4.1 Protein constituents of eukaryotic cytoskeleton

The protein constituents of the cytoskeleton of eukaryotic cells can be broadly divided into the following three categories: (i) Filamentous proteins, (ii) accessory proteins, and (iii) motor proteins. The three classes of filamentous proteins, which form the main scaffolding of the cytoskeleton, are: (a) actin, (b) microtubule, and (c) intermediate filaments. On the basis of functions, accessory proteins can be categorized as follows: (i) regulators of filament polymerization, (ii) filament-filament linkers, (iii) filament-plasma membrane linkers. Since accessory proteins do not play any crucially important role in the operation of the cytoskeleton-based molecular machines, we shall not consider accessory proteins in this article.

The three superfamilies of motor proteins are: (i) myosin superfamily, (ii) kinesin superfamily, and (iii) dynein superfamily. Both kinesins and dyneins move on microtubules; in contrast, myosins either move on actin tracks or pull the actin filaments.

- **Structures of microtubules and actin filaments**

  Microtubules are cylindrical hollow tubes whose diameter is approximately 20 nm. The basic constituent of microtubules are globular proteins called tubulin. Hetero-dimers, formed by α and β tubulins, assemble sequentially to form a protofilament. 13 such protofilaments form a microtubule. The length of each α − β dimer is about 8 nm. Since there is only one binding site for a motor on each dimeric subunit of MT, the minimum step size for kinesins and dyneins is 8 nm.

  Although the protofilaments are parallel to each other, there is a small offset of about 0.92 nm between the dimers of the neighbouring protofilaments. Thus, total offset accumulated over a single looping of the 13 protofilaments is $13 \times 0.92 \approx 12$nm which is equal to the length of three $\alpha-\beta$ dimers joined sequentially.

Part I: Cytoskeletal motors: porters and rowers, shuttles and muscles

The cytoskeleton of an eukaryotic cell maintains its architecture. Counterparts of some molecular components of the eukaryotic cytoskeleton have been discovered recently also in prokaryotic cells. It is a complex dynamic network that can change in response to external or internal signals. The cytoskeleton is also responsible for intra-cellular transport of packaged molecular cargoes as well as for the motility of the cell as a whole. The cytoskeleton plays crucially important role also in cell division and development of organisms. In this part we focus almost exclusively on the motility and contractility driven by molecular motors at the sub-cellular level; motor-driven motility and contractility of the cell as a whole will be taken up in the last part of this article.
Therefore, the cylindrical shell of a microtubule can be viewed as three helices of monomers. Moreover, the asymmetry of the hetero-dimeric building block and their parallel head-to-tail organization in all the protofilaments gives rise to the polar nature of the microtubules. The polarity of a microtubule is such an α tubulin is located at its - end and a β tubulin is located at its + end.

Filamentous actin are polymers of globular actin monomers. Each actin filament can be viewed as a double-stranded, right handed helix where each strand is a single protofilament consisting of globular actin. The two constituent strands are half staggered with respect to each other such that the repeat period is 72 nm.

The spatial organization and function of the cytoskeletons of plants and algae differ significantly from those of eukaryotic cells.

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MAPs and ARPs

Microtubule-associated proteins (MAPs) and Actin-related proteins (ARPs) play important roles in controlling the structure and dynamics of microtubules and filamentous actin, respectively. Microtubule plus-end tracking proteins (+TIPs) are special MAPs that accumulate at the plus end of microtubules; members of a few families of motor proteins are also +TIPs. Biological functions of some of these proteins will be considered later in this part of this resource letter.

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4.2 Prokaryotic cytoskeleton

Unlike eukaryotes, bacteria do not have any obvious need for a cytoskeleton. First, their cell walls are rigid enough to provide mechanical strength to the cell. Second, the size of bacterial cells is so small that transportation of cargo by pure diffusion would be sufficiently rapid for the survival of the cell. These general considerations and the lack of direct evidence for cytoskeletal structures in the early experiments on prokaryotes led to the common belief that the prokaryotic cells lack a cytoskeleton. However, more recent experimental evidences strongly indicate the existence of bacterial homologs of the filamentous proteins in eukaryotic cells. For example, FtsZ is a bacterial homolog of tubulin whereas MreB and ParM are those of actin. Moreover, CreS (crescentin)
is considered to be a strong candidate for the bacterial counterpart of intermediate filaments of eukaryotic animal cells. However, so far it has not been possible to identify any bacterial homolog of the eukaryotic motor proteins. Nevertheless, the existence of such homologs with very low sequence identity with their eukaryotic counterparts cannot be ruled out.

FtsZ polymerize to form protofilaments. But, unlike eukaryotic tubulins, these protofilaments do not cooperate to form higher order tube-like structures which would be analogous to microtubules. On the other hand, ParM polymerizes to form a double-stranded helical filament which is also very similar to filamentous F-actin.

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4.3 Nucleation of MT and actin filaments

The role of γ-tubulin in the nucleation of MT filaments has been known for quite some time. Two classes of actin nucleating proteins are: (i) formin protein family; and (ii) Arp2/3 complex.

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4.4 *Dynamics of polymerization/depolymerization of MT
and actin: treadmilling and dy-
namic instability*

The dynamics of polymerization and depolymerization of microtubules is quite different from those of most of the common proteins. *Dynamic instability* is now accepted as the dominant mechanism governing the dynamics of microtubule polymerization. Each polymerizing microtubule persistently grows for a prolonged duration and, then makes a sudden transition to a depolymerizing phase; this phenomenon is known as “catastrophe”. However, the rapid shrinking of a depolymerizing microtubule can get arrested when it makes a sudden reverse transition, called “rescue”, to a polymerizing phase. It is now generally believed that the dynamic instability of a microtubule is triggered by the loss of its guanosine triphosphate (GTP) cap because of the hydrolysis of GTP into guanosine diphosphate (GDP). But, the detailed mechanism, i.e., how the chemical process of cap loss induces mechanical instability, remains far from clear.

Some of the fundamental quantitative questions on this phenomenon are as follows: Does the system reach a steady state under the given conditions, and if so, what is the distribution of the lengths of the microtubules in that state? It has been discovered that some small molecules can suppress the dynamic instability and influence the rates of growth and/or shrinkage of the microtubules when bound to the tubulins. These molecules are potential anti-cancer drugs because of the corresponding implications of the dynamic instability in cell division. What are the quantitative effects of these drug molecules on the nature of the steady state (if it still exists) and on the corresponding distribution of the microtubule lengths?

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5 Push and pull of cytoskeletal filaments: nano-pistons

In this section, we focus only on the mechaism of force generation by polymerizing cytoskeletal filaments, namely, microtubules and actin. However, these phenomena will be reconsidered again in part IV in the broader context of cell motility and cell division.

A microtubule can keep growing even when it encounters a microscopically light obstacle; its action in such situations is reminiscent of a piston. Unlike microtubules, the protofilaments of FtsZ do not exhibit dynamic instability. On the other hand, unlike actin, filamentous ParM exhibits a dynamic instability which is very similar to that of microtubules except that the instability of ParM filaments is caused by the hydrolysis of ATP rather than that of GTP. Moreover, unlike actin, whose polar polymer grows asymmetrically through treadmilling, ParM exhibits a symmetrical bidirectional growth where rate of elongation at both ends are identical.

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5.1 Spring-like force generated by cytoskeletal filaments

The piston-like action of polymerizing filaments is not the only mode of motor-independent force generation. Spring-like actions of filamentous structures are known to drive fast motility of some biological systems. One well known example of such biological spring is the vorticellid spasmoneme whose major protein component is spasmin. The sperm cell of the horse-shoe crab Limulus polyphemus also utilizes the spring-like action of a coiled bundle, which consists mainly of actin filaments, to penetrate into an egg for its fertilization.

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6 Processive cytoskeletal motors: porters

Many cytoskeletal motors carry molecular cargo over distances which are quite long on the intracellular scale. Because of their superficial similarities with porters who carry load on their heads, these motors are often colloquially referred to as “porters”.

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6.1 Architectural designs of the porters: common features

All the cytoskeletal motor proteins have a head domain; this domain contains a site for ATP hydrolysis and another site for binding to a cytoskeletal filament which serves as a track for the motor. Binding of ATP to the head alters the affinity of the motor for its track. The head domain of the kinesins is the smallest (about 350 amino acids), that of myosins is of intermediate size (about 800 amino acids) whereas the head of dyneins is very large (more than 4000 amino acids).

The “identity card” for members of a superfamily is the sequence of amino acids in the motor domain. The members of a given superfamily exhibit a very high level of “sequence homology” in their motor domain. But, the amino acid sequence as well as the size, etc. of the other domains differ widely from one member to another of the same superfamily. All kinesin and dyneins have a tail domain which binds with the cargo. The tail domain exhibits much more diversity than the head domain because of the necessity that the same motor should be able to recognize (and pick up) wide varieties of cargoes.

Myosins are actin-based motor proteins. According to the widely accepted nomenclature, myosins are classified into families bearing numerical (roman) suffixes (I, II, ..., etc.). According to the latest standardized nomenclature of kinesins, the name of each family begins with the word “kinesin” followed by an arabic number (1, 2, etc.). Moreover, large subfamilies are assigned an additional letter (A, B, etc.) appended to the family name. For example, kinesin-14A and kinesin-14B refer to two distinct subfamilies both of which belong to the family kinesin-14.

Dyneins are microtubule-based motor proteins. Dyneins can be broadly divided into two major classes: (i) cytoplasmic dynein, and (ii) axonemal dynein. Structural features of these motors is quite different from those of kinesins and myosins.
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6.2 Mechano-chemistry of cytoskeletal motors: general aspects

Even for a given single motor domain, a large number of molecular motors are involved in each enzymatic cycle. In principle, there are, many pathways for the hydrolysis of ATP, i.e., there are several different sequences of states that defines a complete hydrolysis cycle. Although, all these pathways are allowed, some paths are more likely than others. The most likely path is identified as the hydrolysis cycle.

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6.3 Fundamental questions

We phrase the questions in such a way that these may appear to be directly relevant only for the cytoskeletal motors. But, these can be easily rephrased for the other types of motors including, for example, those which move on nucleic acid strands. These questions are as follows:

(i) Fuel: What is the fuel that supplies the (free-)energy input for the motor? The free energy released by the hydrolysis of ATP is usually the input for cytoskeletal motors.

(ii) Engine, cycle and transmission: The site on the motor, where ATP is hydrolyzed, can be identified as its engine. What are the distinct states of the cyclic engine in various stages of each cycle? Which step of the cycle is responsible for the generation of force (or, torque)? How is the structural (conformational) change, caused by this force (or torque), amplified by the architecture of the motor? In other words, how does the transmission system of the motor work, i.e., what are the analogues of the clutch and gear of automobiles?

(iii) Track and traction: Are they filamentous tracks static or dynamic, i.e., do the lengths and/or orientations of the tracks change with time? What is the traction mechanism used by a motor head for staying on track?

(iv) Number of engines and coordination of their cycles: The state of oligomerization of the motor subunits has important functional implications. Majority of the members of myosin and kinesin superfamilies are homodimers although monomeric, heterodimeric and tetrameric kinesins have also been discovered. Some members of myosin and kinesin superfamilies are known to self-assemble into higher-order structures; the most well known among these higher-order structures is the myosin thick filaments in muscles which will be described later in the context of muscle contraction. What functional advantages arise from oligomerization? Are the cycles of the different engines of a motor coordinated in any manner and, if so, how is this coordination maintained?

(v) Stroke and step sizes: The separation between the two successive binding sites on the track is the small-
est possible step size of the motor. On the other hand, a stroke is a conformational change of the motor bound to the track. In general, the stroke size need not be equal to the step size. What is the stroke size of a given motor? If the motor covers only a fraction of the distance to the next binding site by the stroke, how does it manage to cover the remaining distance? Can the same motor adopt different step sizes under different circumstances?

(vi) Directionality and processivity: Majority of myosins are + end directed i.e., move towards the barbed end of actin filaments. Similarly, majority of the kinesins are also + end directed motors whereas most of the dyneins are - end directed motor proteins. What determines the direction of movement, i.e., why are some motors +end directed whereas the others are -end directed? Can a motor reverse its direction of motion (a) spontaneously, or (b) under an opposing (load) force? Do the motors possess reverse gears and is it possible to reverse the direction of their movement by utilizing the reverse gear mechanism? What is the minimal change (e.g., mutation) required to reverse the direction of motion of a motor?

One of the key features of the dynamics of cytoskeletal motors is their ability to attach to and detach from the corresponding track. A motor is said to be attached to a track if at least one of its heads remains bound to one of the equispaced motor-binding sites on the corresponding track. Moreover, a motor can detach completely from its track.

One can define processivity in three different ways:
(i) Average number of chemical cycles in between attachment and the next detachment from the filament;
(ii) attachment lifetime, i.e., the average time in between an attachment and the next detachment of the motor from the filament;
(iii) mean distance spanned by the motor on the filament in a single run.

The first definition is intrinsic to the process arising from the mechano-chemical coupling. But, it is extremely difficult to measure experimentally. The other two quantities, on the other hand, are accessible to experimental measurements.

To translocate processively, a motor may utilize one of the two following strategies:

strategy I: the motor may have more than one track-binding domain (oligomeric structure can give rise to such a possibility quite naturally). Most of the cytoskeletal motors like conventional two-headed kinesin use such a strategy. One of the track-binding sites remains bound to the track while the other searches for its next binding site.

strategy II: it can use a “clamp-like” device to remain attached to the track; opening of the clamp will be required before the motor detaches from the track. Many motors utilize this strategy for moving along the corresponding nucleic acid tracks. The duty ratio is defined as the average fraction of the time that each head spends remaining attached to its track during one cycle. The typical duty ratios of kinesins and cytoplasmic dynein are at least 1/2 whereas that of conventional myosin can be as small as 0.01. What is the mechanism that decides the processivity (or the lack of processivity) and the duty ratio of a motor?

(vii) Stepping pattern of a double-headed motor: Does the motor move like an “inchworm” or does the stepping appear more like a “hand-over-hand” mechanism? Moreover, two types of hand-over-hand mechanism are possible: symmetric and asymmetric. In the symmetric pattern, the two heads exchange positions, but the three-dimensional structure of the molecule is preserved at all equivalent positions in the cycle. In contrast, in the asymmetric pattern, the two heads exchange position, but alternate steps differ in some way, e.g., what happens in “limping” which involves alternate faster and slower stepping phases. Can a motor switch from one track to a neighbouring track and, if so, how does it achieve that? What prevents a motor from changing lane on a multi-lane track?

(viii) Speed and efficiency: Is the average speed of a processive motor determined by the track or the motor or fuel or some external control mechanism? Recall that the average speed of a car on a highway in sparse traffic can be decided either by the smoothness of the highway, or by the model of the car (whether it is a Ferrari or a heavy truck), or by the quality of the fuel. Similarly, how does the molecular constitution of the track and the nature
of the motor-track interaction affect the speed of the motor? Is the mechano-chemical coupling tight or loose? If hydrolysis of ATP provides the input free energy, then, how many steps does the motor take for every molecule of ATP hydrolyzed, or, equivalently, how many ATP molecules are consumed per step of the motor? What is the maximum speed it can attain? Can an external force applied to a motor in the forward direction speed it up? How does the speed of the motor depend on the opposing “load” force? As the load force increases, the velocity of the motor decreases. The magnitude of the load force at which the average velocity of the motor vanishes, is called the stall force. What happens when the load force is increased beyond the stall force? Three possible scenarios are as follows: (i) the motor may detach from the track, or (ii) the motor may reverse its direction of motion (and move in the direction of the load force) (a) without hydrolyzing ATP, or (b) hydrolyzing ATP. The force-velocity relation is one of the most fundamental characteristic property of a motor. What is the most appropriate definition of efficiency of the motor and how to estimate that efficiency?

(ix) Regulation and control: How is the operation of the motor regulated? For example, how is the motor switched on and off? Recall that the speed of a car can also be regulated by imposing the same speed limit or by traffic signals. Are there molecular signals that control the motor’s movement on its track and how? How does the motor pick up its cargo and how does it drop it at the target location? How do motors get back to their starting points of the processive run after delivering their cargo?

(x) Motor-motor interactions: How do different types of motors interact while moving on the same track carrying their cargo? How do different classes of motors, which move on different types of tracks, coordinate their functions and even transfer or exchange their cargoes?

6.4 Motility assays

There are two geometries used for in-vitro motility assays: (i) the gliding assay and (ii) the bead assay. In the gliding assay, the motors themselves are fixed to a substrate and the filaments are observed (under an optical microscope) as they glide along the motor-coated surface. In the bead assay, the filaments are fixed to a substrate. Small plastic or glass beads, whose diameters are typically of the order of 1µm, are coated with the motors. These motors move along the fixed filaments carrying the bead as their cargo. The movements of the beads are recorded optically.

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6.6 Kinesin porters

- Homodimeric conventional kinesin: members of Kinesin-1 family
  
  Kinesin-1, the prototypical kinesin motor consists of three major domains:
  (i) The *motor domain*: this domain can be further subdivided into the globular catalytic core, the adjacent neck linker and the neck region. The core motor domain consists of about 325 amino acids.
  (ii) The *stalk* which is a α-helical coiled-coil domain.
  (iii) The globular *tail* domain at the end of the stalk which can bind with cargo.

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• Heterodimeric kinesins: members of Kinesin-2 family

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• Kinesin-13 and kinesin-8 families: MT polymerases and depolymerases

Some kinesin motors move diffusively to target one of the two ends of the MT track and, then, start depolymerizing the track itself; MCAK, a member of the kinesin-13 family, is an example of such kinesins. The diffusive motion of the MCAK does not require ATP, but it hydrolyzes ATP to power its depolymerase activity. These depolymerases play important roles in some crucial stages of cell division which we’ll take up in the last part of this resource letter.

Kip3p, a member of the kinesin-8 family, is also a MT depolymerase. But, unlike, MCAK, it “walks”, rather than diffusing, in a specific direction on the MT track by hydrolyzing ATP and, after reaching the target end, starts depolymerizing the MT.

A single depolymerase can peel off more than one MT subunit from the tip of the MT; the larger the number of MT subunits it peels off, the higher is its processivity.

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6.7 Dynein porters

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In the preceding section we have considered operational mechanisms of processive single cytoskeletal motors. Even in multimeric porters, different motor domains coordinate in a certain way that leads to the processive directed motion of the motor as a whole. In this section, we focus on their collective transport properties which arise from their coordination, cooperation, competition, etc.; collective properties of rowers will be taken up later.

The situation envisaged here corresponds to a typical bead-assay where the filamentary tracks are fixed to a substrate and motors are attached to a micron-sized bead (usually made of glass or plastic). The movement of the bead in the presence of ATP is monitored using appropriate optical microscopic methods. In such situations, each bead is likely to be covered by \( N \) motors where, in general, \( N > 1 \). More than one motor is also used for transportation of vesicles and large organelles in-vivo.

7.1 Load sharing on fixed MT track: single cargo hauled by many kinesins

Normally, a vesicle or an organelles can be hauled by more than one motor simultaneously. If all the motors carrying the cargo are plus-end directed (or, if all are minus-end directed) they share the load. In order to understand the cooperative effects of such multiple motors, in-vitro experiments are easier to perform than in-vivo experiments. The force-velocity relations for such cargoes can be measured using, for simplicity, micron-size dielectric beads, instead of vesicles or organelles.

7.2 Load sharing on fixed MT track: single cargo hauled by many dyneins

Cooperative effects of multiple dynein motors exhibits richer variety of phenomena.

7.3 Tug-of-war on fixed MT track: bidirectional transport of a single cargo hauled by kinesins and dyneins

It is well known that some motors reverse the direction of motion by switching over from one track to another which are oriented in anti-parallel fashion. In contrast to these types of reversal of direction of motion, we consider in this section those reversals where
the cargo uses a “tug-of-war” between kinesins and dyneins to execute bidirectional motion on the same MT track. Several possible functional advantages of bidirectional transport have been conjectured.

Wide varieties of bidirectional cargoes have already been identified so far; these include organelles (for example, mitochondria) as well as secretory vesicles and even viruses. If motion in one direction dominates overwhelmingly over the other, it becomes extremely difficult to identify the movement unambiguously as “bidirectional” because of the limitations of the spatial and temporal resolutions of the existing techniques of imaging.

The main challenge in this context is to understand the mechanisms of this bidirectional transport and those which control the duration of unidirectional movement in between two successive reversals. This insight will also be utilized for therapeutic strategies. For example, the motor or the motor-cargo link may be targeted blocking the virus that hijacks the motor transport system to travel towards the nucleus. On the other hand, a virus executing bidirectional movements can be turned away from the outskirts of the nucleus by tilting the balance in favour of the kinesins.

At least three possible mechanisms of bidirectional transport have been postulated. (i) One possibility is that either only + end directed motors or only - end directed motors are attached to the cargo at any given instant of time. Reversal of the direction of movement of the cargo is observed when the attached motors are replaced by motors of opposite polarity. (ii) The second possible mechanism is the closest to the real life “tug-of-war”; the competition between the motors of opposite polarity, which are simultaneously attached to the same cargo and tend to walk on the same filament generates a net displacement in a direction that is decided by the stronger side. (iii) The third mechanism is based on the concept of regulation; although motors of opposite polarity are simultaneously attached to the cargo, only one type of motors are activated at a time for walking on the track. In this mechanism, the reversal of the cargo movement is caused by the regulator when it disengages one type of motor and engages motors of the opposite polarity. For experimentalists, it is a challenge not only to identify the regulator, if such a regulator exists, but also to identify the mechanism used by the regulator to act as a switch for causing the reversal of cargo movement. Dynactin has been identified as a possible candidate for the role of such a regulator.

7.4 Unidirectional traffic of many cargoes on a single track: Molecular motor traffic jam

Most of the multi-motor phenomena we have considered in the preceding section are restricted to sufficiently low densities where direct interaction of the cargoes did not occur. As the cargoes are always much bigger than the motors (in-vitro as well as in-vivo), direct steric interactions of the cargoes become significant when several cargoes are carried by sufficiently dense population of motors along the same track. Such situations are reminiscent of vehicular traffic where mutual hindrance of the vehicles cause traffic jam at sufficiently high densities. In analogy with vehicular traffic, we shall refer to the collective

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movement of molecular motors along a filamentary track as “molecular motor traffic”; we shall explore the possibility of molecular motor traffic jam and its possible functional implications.

Most of the minimal theoretical models of interacting molecular motors utilize the similarities between molecular motor traffic on MT and vehicular traffic on highways both of which can be modeled by appropriate extensions of the totally asymmetric simple exclusion process. In such models the motor is represented by a “self-propelled” particle and its dynamics is formulated as an appropriate extension of the dynamics of the totally asymmetric simple exclusion process. In such models, in addition to forward “hopping” from one binding site to the next, the motor particle is also allowed to detach from the track. Moreover, attachment of a motor particle to an empty site is also allowed.

In reality, a molecular motor is an enzyme that hydrolyses ATP and its mechanical movement is coupled to its enzymatic cycle. In some recent works on cytoskeletal motor traffic, the essential features of the enzymatic cycle of the individual motors have been captured.

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7.5 Bidirectional traffic of many cargoes on a single track: Molecular motor traffic jam

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7.6 Cargoes at crossings and on park-and-ride transport system

Filamentous actin forms branched networks. Therefore, naturally, a fundamental question on transport of cargoes by unconventional myosins on actin networks is what happens to the cargo when it reaches a point where a single track branched out in two different directions. Moreover, similar situation also arises when a cargo hauled by kinesins reaches a crossing of MT tracks.
Furthermore, the networks of microtubules and actin filaments are not disconnected. The cytoskeleton is microtubule-rich near the cell center whereas dense actin filaments dominate the cytoskeleton near the cell periphery. On their way to destinations near the cell periphery, cargoes cover some distance by taking ride on microtubule-based kinesin motors and then switch to actin-based myosin motors; this is sometimes referred to as the park-and-ride transport system in analogy with that in metro cities of developed nations. Similar transfer of cargoes from actin network to microtubule network during the transport in the reverse direction is also well documented.

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7.8 Examples of intracellular transport and traffic: axonal transport

In a human body, the axon can be as long as a meter whereas the corresponding cell body is only about 10 microns in length. Almost all the proteins needed to maintain the synapses are synthesized in the cell body. How are these proteins transported to the synapse along the long axon? The problem is even more challenging in animals like elephant and giraffe which have even longer axons. A bundle of parallel MTs usually run along the axons and dendrites; these form the track for the motorized transport of vesicles and organelles. In axons the plus end of the MTs point towards the axonal presynaptic terminus (the growth cone in developing neurons and the end-plate in motor neurons), i.e., from the center to the periphery of the cell. Movement of the cargo in a direction away from the cell body is called anterograde whereas that in the reverse direction is called retrograde; the former is driven by kinesins while the latter is dominated by dyneins.

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7.10 Examples of intracellular transport and traffic: tip growth

Cytoskeleton plays crucial role also in creating and growing tip-like cell surface protrusions. Examples of such tip growth phenomena include axonal elongation in mammals, growth of root hairs and pollen tubes in plants, hyphal growth in filamentous fungi, etc.

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### 7.11 Diseases caused by malfunctioning of cytoskeletal motor transport system

Just as occasional disruption of work in any department of a factory can bring entire operation factory to a standstill, defective molecular machines can cause diseases. For example, malfunctioning of the track and/or motor can cause breakdown of the intracellular molecular motor transport system leading to a traffic-jam-like situation

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### 7.12 Hijacking of cytoskeletal transport system by viruses

Viruses are known to hijack the motors to travel from the cell periphery to the cell nucleus.

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8 Processive cytoskeletal motors: cross-linkers and sliders

8.1 Cross-linking and relative sliding of two MT filaments by kinesin

Eg5, a tetrameric kinesin, one of the most prominent members of the kinesin-5 family, has been studied experimentally because of its ability to slide two microtubule filaments with respect to each other in the mitotic spindle. Although Eg5 is processive, its processivity is quite low. Sliding of MT filaments, driven collectively by Eg5, has some similarities with myosin-driven muscle contraction, which we will consider in a later section.

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7.13 Drug delivery using cytoskeletal motors

The molecular motor transport system can be utilized even for targeted drug delivery where molecular motors can be used as vehicles for the drug.

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8.2 Cross-linking and relative sliding of two actin filaments by processive myosin

Cross-linking and relative sliding of actin filaments by myosin motors can, in principle, create fingerlike cell protrusions called filopodia. The sliding of actin filaments by nonprocessive muscle myosins will be considered later in the context of muscle contraction.

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8.3 Axonemal dynein and beating of flagella

The molecular composition, structure and dynamics of eukaryotic flagella are totally different from those of bacterial flagella. Moreover, structurally, eukaryotic flagella and cilia are qualitatively similar cell appendages; their quantitative differences lie in their size and distribution on the cell.

In this subsection we consider only the physical processes driven by the cytoskeletal filaments and the motors which lead to the beating of the flagella. How the various patterns of these beatings in a fluid medium propels the eukaryotic cell is a problem of fluid dynamics and will be taken up later in the section on swimming of eukaryotic cells.

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9 Nonprocessive cytoskeletal motors: collective dynamics of rowers

The oars of rowers come in contact with water for a very brief period, giving a stroke and then comes out of water, completing one cycle. All the rowers of the same group try to synchronize their stroke cycle in such a way as to provide the maximum thrust to the boat. Similarly, “rower” molecular motors also remain attached to their track for a small fraction of their ATPase cycle, i.e., the duty ratio of these nonprocessive motors is usually small. However, the collective stroke of a very large number of such tiny motor molecules can generate forces large enough to contract a muscle.

9.1 Nonprocessive myosin and muscle contraction

There are some chemical differences between the muscles of vertebrates and invertebrates (e.g., flight muscles of insects). Muscle cells of vertebrates can be broadly classified into “striated” and smooth (“non-striated”) types. Vertebrate striated muscle cells can be further divided into two categories—skeletal and cardiac. Although skeletal muscles of vertebrates (e.g., those of frog and rabbit) were used in most of the early investigations on the mechanism of muscle contraction, the cardiac muscle has been getting attention in recent years because of its implications in cardiac disease control.

Each muscle fiber is actually an enormous multinucleated cell produced by the fusion of many mononucleated precursor cells during development whose nuclei are retained in the adult muscle cell. The diameter of muscle cells is typically 10 – 100 µm and the length can range from less than a millimeter to a centimeter. Each of these cells is enclosed by a plasma membrane. The nuclei are squeezed to the peripheral region just beneath the plasma membrane.

About 80 percent of the cytoplasm of a skeletal muscle fiber (i.e., muscle cell) is occupied by cylindrical rods of protein and are known as myofibrils. Many myofibrils, each about 1µm in diameter, are contained within the cross section of a single muscle cell. The muscle cells also contain mitochondria sandwiched between the myofibrils.

Myofibrils are the structures that are responsible for muscle contraction. The most distinctive feature of myofibrils is their banded appearance; the dark bands correspond to higher density of protein. The spatial periodicity of the alternating light and dark bands is 2.3-2.6 µm in the resting state of a muscle; the entire repeating structure, from one Z-disc to the next, is known as sarcomere.

The banded appearance of the sarcomere is produced by hundreds of protein filaments bundled together in a highly ordered fashion. The two main types of filament are:

(i) thick filaments, about 15 nm in diameter, are made mostly of myosin;
(ii) thin filaments, about 7 nm in diameter, consist mostly of actin.

Both these types of filaments contain also other types of proteins which help to hold them in correct steric arrangement and regulate the process of contraction. Arrays of thin and thick filaments overlap in the
sarcomere in a manner similar to that of two stiff bristle brushes. Myosin molecules are arranged in such a way on the thick filament that their heads point away from the mid-zone towards either end of the filament. The thick filaments come within about 13 nm of the adjacent thin filament which is close enough for the formation of cross-bridges between the myosin heads belonging to the thick filament and actin molecules constituting the thin filaments.

In two landmark papers published in 1954, A.F. Huxley and Niedergerke and, independently, H. E. Huxley and Hanson proposed the sliding filament hypothesis of muscle contraction. According to this hypothesis, it is the sliding of the thick and thin filaments past each other, rather than folding of individual proteins, that leads to the contraction of the muscle. This theory was formulated clearly and quantitatively in another classic paper of A.F. Huxley in 1957.

In the original version of the sliding filament model, developed in the nineteen fifties, it was generally assumed that the cross bridges moved back and forth along the backbone of the thick filaments remaining firmly attached to it laterally. However, later X-ray studies demonstrated that the filament separation could vary without apparently interfering with the actin-myosin interactions. On the basis of this observation, in 1969, H.E. Huxley proposed the myosin “lever arm” hypothesis. This model was developed further and formulated quantitatively by A.F. Huxley and Simmons in 1971.

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9.2 Bidirectional motion of MT driven collectively by nonprocessive kinesins

Consider a group of identical motors bound to an elastic backbone. Even if each individual motor is non-processive, such a system of elastically coupled motors can move collectively on a filamentary track in a processive manner in one direction for a period of time and, then, spontaneously reverse its direction of motion. Such spontaneous oscillations can account for the dynamics of axonemes, which are core constituents of eucaryotic cilia, as well as oscillatory motions of flight muscles of many insects.

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10 Cooperative extraction of membrane tubes by cytoskeletal motors

Cytoskeletal motors carry membrane-bounded vesicles and organelles as cargoes while walking along their respective tracks as porters. Interestingly, motors can also extract membrane tubes from vesicles. The nature of the dynamics of the tube, however, depends on the extent of processivity of the motors.

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11 Effects of defect and disorder on shuttles and muscles

So far we have implicitly regarded the microtubule track for the cytoskeletal motors to be a perfectly periodic array of motor-binding sites. However, in reality, the MAPs can introduce “defect” and “disorder” into this perfectly periodic lattice; the lattice constant being 8 nm. In particular the Tau proteins are known to block the kinesin-binding sites on the microtubules. Binding of Tau affects at least two different rate constants, namely, those corresponding to: (a) the attachment of a new motor to the track, and (b) the forward stepping of the motor.

12 Self-organization of microtubule-motor complex in-vitro

In the earlier sections in this part we have focussed attention on situations where motors move on filamentous tracks that neither change length nor orientation during the entire period of movement of the motors. We have also separately considered the dynamic instability of microtubules because of which microrubules can grow or shrink. In this section we study the interplay of the dynamics of both microtubules and motors, addressing the question of the structures that emerge from the self-organization of microtubule-motor complex in-vitro. These are also relevant for the phenomenon of cell division which will be taken up in the last part of this resource letter.

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**Part II: Molecular machines for synthesizing, manipulating and degrading macromolecules of life**

The individual *monomeric residues* that form proteins and nucleic acids are *amino acids* and *nucleotides*, respectively. Both these types of macromolecules are *unbranched* polymers. The complete *covalent* structure is called the *primary* structure of the macromolecule. It would be extremely time- (and space-) consuming to write a chemical formula for the entire primary structure. Therefore, it is customary to express primary structures in terms of abbreviation using an alphabetic code. The most common convention uses one-letter code for each nucleotide and three-letter code for each amino acid. For proper biological function, these macromolecules form appropriate *secondary* and *tertiary* structures. The term *conformation* is synonymous with tertiary structure.

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In this part we consider molecular machines which either synthesize, or manipulate, or degrade macromolecular constituents of a cell, namely, DNA, RNA or protein. Among these machines, some translocate along a macromolecular track whereas others, whose spatial positions are more or less fixed, translocate macromolecules. This difference, of course, corresponds to a mere change of reference frame. Many of these machines are involved in all the major biological functions of genetic materials, e.g., transcription, replication, repair and recombination as well as in defence system of the cell against invading foreign genetic materials.
The constituent monomers, the primary structures as well as the spatial organization of the higher-order structures of nucleic acids and proteins are quite different. However, in spite of these differences, there are some common features in the birth, maturation and death:

(i) The sequence of the monomeric subunits to be used for synthesis are dictated by the corresponding template.
(ii) both nucleic acids and proteins are made from a limited number of different species of monomeric building blocks.
(iii) these polymers are elongated, step-by-step, during their birth by successive addition of monomers, one at a time.
(iv) Synthesis of each chain (polynucleotide and polypeptide) begins and ends when the machine encounters well-defined start and stop signals on the template strand.
(v) The primary product of the synthesis, namely, polynucleotide or polypeptide, often requires “processing” whereby the modified product matures into functional nucleic acid or protein, respectively.
(vi) DNA, the genetic blueprint of life, needs repair every now and then to maintain its integrity. During cell division, it is faithfully replicated and passed onto the daughter cells. But, the other macromolecules of life are not so lucky. Because of “wear and tear”, these aged macromolecule becomes less useful with the passage of time. Finally, these are degraded, i.e., shredded into its constituent subunit pieces which are, then, recycled for synthesis of fresh macromolecules.

Interestingly, all these processes are driven by molecular machines which nature has designed for the specific purpose.

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13 Packaged organization of nucleic acids

In every cell the genetic information is encoded in the sequence of the nucleotides. Thus, at some stage of biological evolution, Nature chose an effectively linear device (namely, a NA strand) and a quaternary code (i.e., four symbols, namely, A, T, C, G) for storing genetic information. This was not the most efficient choice! The fewer is the number of letters of the alphabet the longer is the string of letters required to express a given message. One serious consequence of nature’s choice of the memory device and coding system is that even for the most primitive organisms like an E.coli bacterium, the total length of the DNA molecule is orders of magnitude longer than the organism itself! The problem is more acute in case of eukaryotic cells where an even longer DNA has to be
accomodated within a tiny nucleus! Moreover, random packaging of the DNA into the nucleus would not be desirable because, for wide variety of biological processes involving DNA, specific segments of the DNA molecules must be “unpacked” and made accessible to the corresponding cellular machineries. Furthermore, at the end of the operation, the DNA must be re-packed. Nature has solved the problem of packaging genetic materials in the nucleus of eukaryotic cells by organising the DNA strands in a hierarchical manner and the final packaged product is usually referred to as the chromatin.

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Even in bacteria and viral capsids, the genome has to be packaged in a manner which allows efficient access during various processes of DNA and RNA metabolism. Most often, packaging or unpackaging and repackaging of the genome requires specific molecular machines. We’ll consider some of these machines later in this part of this article.

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14 Elasticity of macromolecules of life

Nature must have extracted some advantage from the synthesis of macromolecules during the course of biological evolution. If it could manage all biological functions with small molecules, living systems would not consist with such a large component of macromolecules. What new features did macromolecules introduce? It not only introduced a new length scale (characterized by its size) and a time scale (associated with its dynamics) but also brought in its “flexibility” which is not possible with only small molecules. This flexible nature of macromolecules also gives rise to the importance of conformational entropy. In fact, many biological processes are driven by entropic elasticity. Apriori, it is not at all obvious that the phenomenological concepts of classical theory of elasticity, which were developed for macroscopic objects, should be applicable even for single molecules of DNA, RNA, etc. Technological advances over the last two decades made it possible to stretch, bend and twist a single macromolecule and the corresponding moduli of elasticity have been measured.

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Thus, elasticity of macromolecules of life is an interesting topic of research in its own right. Moreover, most often genome (DNA or RNA) are stored in bent conformation. For example, in eukaryotic cells, DNA is bent and wrapped around histones. Similarly, in viral capsids, nucleic acids are strongly bent for efficient packaging. Furthermore, temporary bending of macromolecules take place in many biological processes driven by molecular motors. Therefore, the elasticity of the macromolecules of life is also interesting in the study of molecular machines which polymerize, manipulate and degrade these molecules.

15 Rings and bracelets

A large number of molecular machines, which perform diverse functions in DNA metabolism, have toroidal architecture that is a characteristic feature of their multi-domain or multi-meric structure. The possible functional advantages of the toroidal architecture might have been exploited by nature in its evolutionary design of its intracellular toolbox.

15.1 Clamps and clamp loaders

Not all processive motors have a naturally strong grip on the nucleic acid track. Such motors hold onto the track during their translocation because they are attached to ring-like special clamp; such clamps are loaded onto the track by some other special purpose ATP-consuming machines called clamp loaders.

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15.2 SMC proteins

Members of the SMC (Structural Maintenance of Chromosomes) family of proteins are bracelet-like devices which are found in both prokaryotes and eukaryotes. These form core components of the cohesin and condensation complexes in eukaryotes. A common feature of their architectural design is the two “arms” which are connected at a hinge. ATP-driven conformational transformations of these machines manifest as transitions from “opening” of the two arms of a bracelet about the hinge which joins them.

Now we focus exclusively on the ATP-dependent operational mechanism of the SMC proteins. Later, we’ll consider their role in important processes of DNA metabolism, e.g., in chromosome segregation.

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16 Helicase and unzipping of nucleic acids

Helicases are molecular motors that unzip double-stranded nucleic acids and translocate along one of the two strands. Some Helicases also function as “sweepers” in the sense that non-helicase proteins
bound to the nucleic acid strand are dislodged by a helicase.

Nucleic acid translocases either move along nucleic acid tracks or, if anchored, move a nucleic acid strand. Helicases are special types of nucleic acid translocase as these translocate along single strands of nucleic acids by unzipping double-stranded nucleic acids. There are many nucleic acid translocases which, in spite of structural similarity with helicases, do not unzip nucleic acids.

Now we focus only on the mechanisms of operation of helicases. Later we’ll examine their operational mechanisms in broader contexts like, for example, replication, repair and recombination.

Helicases have been classified in various ways using different criteria. (i) Several conserved amino-acid sequences have been discovered in helicases. On the basis of these “helicase signature motifs”, DNA helicases have been classified into superfamilies SF1, SF2, SF3, etc. (ii) On the basis of the nature of the nucleic acid (DNA or RNA) track, i.e., the nucleic acid which they unwind, helicases have been classified into (a) DNA-helicases, (b) RNA-helicases and (c) hybrid helicases. Some helicases are, however, hybrid in the sense that these can unwind both DNA and RNA. (iii) Some helicases move from 3’ to 5’ end of a ssDNA whereas others move in the opposite direction. On the basis of directionality, helicases have been classified into two groups: 3’ to 5’ helicases and 5’ to 3’ helicases. (iv) Helicases have also been grouped according to the the source of these proteins, i.e., humans, plants, bacteria, viruses, etc.

In this chapter, we study the mechanisms of helicases separately for monomeric, dimeric and hexameric helicases. (v) On the basis of the number of ATPase domains, helicases have been classified into monomeric and multimeric types; dimeric and hexameric being the most common multimeric helicases. Here we utilize this last scheme of classification for highlighting the unity of mechanisms of helicases in spite of their diverse functions.

One of the fundamental questions on the mechanochemistry of helicases is the mechanism of energy transduction—does it unzip the nucleic acid actively or does it utilize the transient opening of the double strands by thermal fluctuations in a manner which can be identified as a thermal ratchet mechanism?

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A large number of helicases are hexameric and have an approximate ring-like architecture. For hexameric helicases, at least three alternative mechanisms of enzymatic activities have been suggested; these include, activities of all the ATP-binding domains in (i) parallel, (ii) random, (iii) sequential manner.

(i) Parallel: In this mechanism all the subunits hydrolyze dTTP and exert power stroke simultaneously.

(ii) Random: There are at least two possible different scenarios:

(a) Random in time, where power stroke of each subunits starts and finishes at random times independent of other units; (b) Random in space, where power strokes are sequential in time (i.e., each subunit can begin only after another finishes), but the order of power strokes around the ring is random.

(iii) Sequential: There are at least two different sequences in which the subunits can exert power stroke:

(a) paired sequential, i.e., sequentially around the ring, but with diametrically opposite subunits in the same state; (b) ordered sequential, i.e., sequential in the strict order 1,2,...6 around the ring.

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17 Topoisomerases and untangling of nucleic acids

During various processes in DNA metabolism, often DNA strands get entangled. Topoisomerases untangle nucleic acids thereby changing their topology. The extent of supercoiling is expressed quantitatively by the linking number which is the sum of the twist and writhe of the DNA molecule. The linking number is an integer and is a topological characteristic property of the molecule. DNA molecules with different linking numbers are called topoisomers. The topoisomerase interconverts topoisomers and hence the name.

Topoisomerases are divided into two classes which are named type I and type II. Type I topoisomerases can change the linking number of a closed circular DNA in steps of ±1 whereas type II topoisomerases change the linking number in steps of ±2. This
is achieved by type I topoisomerases by first cleaving one strand of the DNA and, then, after passing the other strand through this break, resealing the break. In contrast, a type II topoisomerase cleaves both strands of a dsDNA and passes another intact segment of dsDNA through this break. Type I and II topoisomerases are further classified into subfamilies designated as IA, IB, IIA, IIB, etc. on the basis of primary sequence and operational mechanism. DNA gyrase of *E. coli* are among the most extensively studied topoisomerases. Reverse gyrase, as the name suggests, introduces supercoiling opposite to that introduced by the gyrase. Besides positive and negative supercoiling, the two other types of reactions catalyzed by topoisomerases are (i) knotting or unknotting, and (ii) catenation or decatenation.

The mechanisms of type I topoisomerases are simpler than those of type II topoisomerases. Each of the type II topoisomerase machines consist of two identical halves and two “gates”. The untangling of DNA occurs through sequential opening and closing of these gates appropriately coordinated with a transient nicking and subsequent ligation of one of the two strands which are thus made to pass through each other.

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18 Membrane-associated machines for macromolecule translocation: exporters and importers

We now consider the translocation of three types of macromolecules, namely, DNA, RNA and proteins across cell membranes as well as internal membranes of eukaryotic cells. In the next part, we’ll consider active transport of small molecules and ions across membranes by molecular machines.

18.1 Export and import of macromolecule across membranes: general principles

Macromolecules to be translocated across the pore may be hydrophobic or may be electrically charged. Therefore, it is not surprising if it encounters an energy barrier while trying to translocate across the pore. However, what makes macromolecule translocation even more interesting from statistical physics
perspective is that the macromolecule also encounters an entropic barrier. The number of allowed conformations of the macromolecular chain, and hence its entropy, is drastically reduced when it translocates across a narrow pore. Therefore, in general, the barrier encountered by the translocating macromolecular chain is a free energy barrier.

So far as the process of macromolecule translocation is concerned, it can be divided into two subprocesses: in the first, the tip of the macromolecule just enters the pore and, then, in the second subprocess the entire length of the chain crosses the pore. The first process is analogous to putting the tip of a thread through the hole of a needle whereas the second is the analogue of pulling a length L of that thread through the same hole after successful insertion of the tip. Both power stroke and Brownian ratchet mechanisms have been proposed. Power stroke can manifest itself either as a “push” or a “pull” in the appropriate direction.

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18.2 Export and import of DNA

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- **DNA transfer across cell membranes: viral and bacterial DNA**

Three basic mechanisms of intercellular DNA transfer in bacteria are:

(i) **Transformation**, i.e., uptake of naked DNA (DNA which is not associated with proteins or other cells) from extracellular environment;

(ii) **Transduction**, i.e., indirect transfer of bacterial DNA into a new cell by a bacteriophage;

(iii) **Conjugation**, i.e., direct transfer of DNA between two bacteria which are in physical contact with each other.

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• DNA transport through eukaryotic nuclear pore complex

The nuclear pore complex (NPC) is itself a large assembly of proteins; the individual protein components of this assembly are called nucleoporins. This assembly has an eight-fold symmetry about an axis normal to the plane of the membrane. On the cytoplasmic (i.e., exterior) side of the membrane, eight fibrils extend from the eight lobes which are arranged in the form of a ring. On the nucleoplasmic (i.e., interior) side of the membrane these eight fibers join to form a basket-like structure at a distance of approximately 100 nm from the inner membrane. DNA uptake into the nucleus through the nuclear pore complex are received little attention so far.

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18.3 mRNA export from eukaryotic nucleus

The m-RNA must be exported from the nucleus before it can be translated into proteins.

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18.4 Export and import of proteins

• Protein translocation across membranes: general principles

Protein translocation can take place (a) during synthesis (co-translation, e.g., in ER), or (b) after completion of synthesis (post-translation, e.g., in mitochondria).

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Machines for protein translocation across membranes of organelles

There are two distinct major pathways of protein transport in eukaryotic cells: (i) the vesicular pathway, and (ii) non-vesicular pathway. In the vesicular pathway, proteins are transported from one membrane-bound organelle to another after packing the protein in a vesicle. The vesicle buds out from the donor organelle and, after reaching the destination, fuses with the acceptor organelle. In this pathway, the vesicle is transported in the cytoplasmic environment by cytoskeletal motor transport system which we have discussed earlier in part I of this article.

Machines for protein translocation across membranes of endoplasmic reticulum

Next we focus on the non-vesicular pathway where proteins are translocated across membranes of organelles by protein-translocating machines.

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- **Machines for protein translocation across membranes of mitochondria and chloroplasts**

Most of the proteins are translocated into mitochondria post-translationally. Mitochondria have a translocase of the outer membrane (called TOM) and a translocase of the inner membrane (called TIM). Similarly, the corresponding translocases of chloroplasts are names as TOC and TIC, respectively. The twin-arginine translocation (Tat) pathway of the thylakoid membrane of chloroplasts and their prokaryotic counterparts share some common features.

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19 Genome packaging machines of viral capsids

As stated earlier, the viral genomes may consist of DNA or RNA. There are two alternative mechanisms
for packaging of the genome. In case of some viruses, the genome is encapsulated by molecules that self-assemble around it. In contrast, the genome of other viruses are packaged into a pre-fabricated empty container, called viral capsid, by a powerful motor. As the capsid gets filled, the pressure inside the capsid increases which opposes further filling. The effective force, which opposes packaging, gets contributions from three sources: (a) bending of stiff DNA molecule inside the capsid; (b) strong electrostatic repulsion between the negatively charged strands of the DNA; (c) loss of entropy caused by the packaging.

One of the model systems, which has been very popular among the researchers, is the bacteriophage φ29; its genome consists of a double-stranded DNA. For understanding the mechanism of packaging double-stranded RNA into the viral capsids, the bacteriophage φ6 has been used as model system.

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The highest pressure generated inside the capsid of the φ29 is about 60 times the normal atmospheric pressure (i.e., about 10 times the pressure in a typical champagne bottle!) and the corresponding force applied by the packaging motor is about 60 pN. Thus, genome packaging motors of viral capsids are among the strongest discovered so far. What is the mechanism used by these motors to generate such a relatively large force (large compared to the forces generated by most of the other motors)?

At first sight, the phenomenon seems to have (at least superficially) several similarities with translocation of macromolecules into eukaryotic organelles. Therefore, questions on the mechanisms of translocation motors can also be reformulated for understanding the mechanisms of packaging motors of viral capsids. Are the nucleic acids “pulled” or “pushed” into the capsid head by the motor? Or, is the mechanism better described by a “nut-like” rotation of the packaging motor on the “bolt-like” nucleic acid strand?

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20 Polynucleotide polymerases

Among the macromolecules of life, nucleic acids and proteins are polymerized by machines which use the respective tracks also as templates for the synthesis. In this section, we consider polymerase machines which synthesize nucleic acids while translocating on another nucleic acid. The main quantities of interest in this context is the rate of synthesis of the macromolecules. Although most of the works initially focussed on the average rates, the fluctuations in the rate of synthesis is receiving more attention in recent years because of two recent developments: (a) the availability of experimental techniques for detection of individual macromolecular products as they are synthesized and released, and (b) the relevance of transcriptional and translational noise in the study of overall noise in gene expression.

The free energy released by the polymerization of the polynucleotide products serve as the input energy for the driving the mechanical movements of the corresponding polymerase. Therefore, these are also regarded as molecular motors. Polymerase motors generate forces which are about 3 to 6 times stronger than that generated by cytoskeletal motors. But, the step size of a polymerase is about 0.34 nm whereas that of a kinesin is about 8 nm. Moreover, the polymerase motors are slower than the cytoskeletal motors by two orders of magnitude. Furthermore, natural nucleic acid tracks are intrinsically inhomogeneous because of the inhomogeneity of nucleotide sequences whereas, in the absence of MAPs and ARPs, the cytoskeletal tracks are homogeneous and exhibit perfect periodic order.

The polymerase is expected to have binding sites for (a) the template strand, (b) the nascent polynucleotide strand, and (c) the NTP subunits. It must have a mechanism to select the appropriate NTP dictated by the template and a mechanism to catalyze the addition of the NTP thus selected to the growing polynucleotide. It must be able to step forward by one nucleotide on its template without completely destabilizing the ternary complex consisting of the polymerase, the template and the product. Finally, it must have mechanisms for initiation and termination of the polymerization process for which, most often, it requires assistance of other proteins.

Most of the fundamental questions we raised in the context of the cytoskeletal motors remain valid also for polynucleotide polymerases. Some further questions, that are unique for polymerases, are posed below:

(i) Are the two translocations, namely nucleotide addition and forward movement of the polymerase, tightly coupled? Or, is it possible to add nucleotide to the growing product without forward movement of the polymerase? In principle, the latter seems to be possible provided the conformation of the TEC changes accordingly.

(ii) What are the paths of the template and product polynucleotide chains within the polymerase? If the template one of the two strands of a double-stranded nucleic acid, what path does the non-template strand follow?

(iii) Does the template and the nascent product polynucleotide form any hybrid structure and, if so, what are the (free-)energetics of the that determine the maximum size of the hybrid? What causes the product polynucleotide to separate from the corresponding template?

(iv) Do the secondary structures of the template and the product play any role in the process of polymerization?

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On the basis of the nature of the template and product polynucleotides, polymerases can be broadly divided into four classes: DNA-dependent RNA polymerase (DdRP), DNA-dependent DNA polymerase (DdDP), RNA-dependent DNA polymerase (RdDP) and RNA-dependent RNA polymerase (RdRP).

There are several common architectural features of all polynucleotide polymerases. The shape of the polymerase has some resemblance with the “cupped right hand” of a normal human being; the three major domains of it are identified with “fingers”, “palm” and “thumb”. There are, of course, some crucial differences in the details of the architectural designs of these machines which are essential for their specific functions. The most obvious functional commonality between these machines is that these add nucleotides, the monomeric subunits of the nucleic acids, one by one following the template encoded in the sequence of the nucleotides of the template. However, in spite of the gross architectural similarities between the polymerases in prokaryotic and eukaryotic cells, there are significant differences in the primary sequences of these machines.

The main stages in the synthesis of polynucleotides by the polymerase machines are common:

(a) initiation: Once the polymerase encounters a specific sequence on the template that acts as a chemically coded start signal, it initiates the synthesis of the product. This stage is completed when the nascent product becomes long enough to stabilize the macromolecular machine complex against dissociation from the template.

(b) elongation: During this stage, the nascent product gets elongated by the addition of nucleotides.

(c) termination: Normally, the process of synthesis is terminated, and the newly polymerized full length product molecule is released, when the polymerase encounters the terminator (or, stop) sequence on the template. However, we shall consider, almost exclusively, the process of elongation.

20.1 DdRP and transcription

In all kingdoms of life, the DdRP are multi-subunit enzymes. The eukaryotic DdRP machines are not only larger in size than their bacterial counterparts, but also consist of larger number of subunits. There are three different types of DdRP in eukaryotic cells, namely, RNAP-I, RNAP-II and RNAP-III. The mRNA, which serves as the template for protein synthesis, is polymerized by RNAP-II whereas rRNA and tRNA are synthesized by RNAP-I and RNAP-III, respectively.

A common architectural feature of all DdRPs is the “main internal channel” which can accommodate of DNA/RNA hybrid that is typically 8 to 9 bp long. The NTP monomers enter through another pore-like “entry channel” while the nascent transcript emerges through the “exit channel”. The formation of the bond between the newly arrived NTP and the RNA chain takes place at a catalytically active site located at the junction of the entry pore and the main channel. In principle, during actual transcription, it may be necessary first to unwind the DNA, at least locally, to get access to the nucleotide sequence on a single-stranded DNA. Interestingly, the RNAP itself exhibits helicase activity for this purpose.

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The translocation of a polymerase along its template resembles that of a device that moves along a digital tape and reads information from it. The synthesis of the product polymerase is, then, analogous to writing of new information. However, unlike digital logic of a computer, decisions made by a polymerase are governed by competing rates and equilibria among alternative conformations and complexes. The decisions which regulate its operation are dictated by two types of input: intrinsic and extrinsic. Discrete segments of the template and product polynucleotides, with which the polymerase interacts, provide intrinsic inputs. Extrinsic inputs come from small ligands and other regulatory proteins.

Single molecule studies of DdRP have provided quantitative data on the force-velocity relation for these motors.

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Quantitative modeling of the DdRP in transcription began almost two decades ago. The collective movement of DdRP on a given track is interesting from several different perspectives. For example, a stalled DdRP can be restarted by another approaching it from behind and such a scenario can lead to polymerization of the transcripts in a “burst”.

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### 20.2 Primase: a unique DdRP

DdDP cannot begin polymerization of a polynucleotide from scratch. First, a DNA primase polymerizes a short RNA primer using the DNA template. Then, a DdDP adds nucleotide subunits to the primer thereby continuing DNA replication. One of the fundamental questions is how does the primase coordinate its operation with those of the DdDP machines?

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20.3 DdDP and DNA replication

Two DdDP machines have to replicate the two complementary strands of DNA both of which serve as templates. However, each DdDP translocates unidirectionally ($5' \rightarrow 3'$) elongating the product strand. As a result, the “leading strand” is synthesized progressively, whereas the “lagging strand” is replicated discontinuously; the “Okazaki fragments” synthesized by this discontinuous process are then joined together (ligated). The coordination of the operation of the two polymerases is one of the interesting aspects of the operational mechanism of the DdDP machines.

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Single molecule manipulation of DdDP have elucidated the operational mechanisms of these machines and resulted in the recent progress in their quantitative modeling.

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The DdDP alone cannot replicate the genome; together with DNA clamp and clamp loader, DNA helicase and primase, it forms a large multi-component complex machinery which replicates the DNA and is often referred to as the replisome.

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Transcription of a gene is carried out a large of times during the life time of a single cell. In contrast, a distinct feature of DNA replication is that, during its lifetime, a cell must not replicate its genome more than once. Only recent investigations have explored how cell achieves this requirement.

A reverse transcriptase is a RdDP which uses a RNA template to polymerize a DNA. The most common example of RdDP is the HIV-1 reverse transcriptase which synthesizes DNA from the RNA genome of the human immunodeficiency virus (HIV). HIV-1 reverse transcriptase is one of key targets for some of the drugs which are being tried against AIDS.

Telomeres, i.e., telomeric DNA, are the terminal DNA at chromosome ends. Telomerase is a unique reverse transcriptase that uses an RNA template to polymerize telomeric DNA. In the absence of telomerase operation, telomerers would gradually shorten in each round of DNA replication because the DdDP cannot replicate these end portions of the DNA. Shortening of telomere is believed to be a cause of premature ageing and other age-related diseases. Therefore, understanding the operational mechanism of telomerase will help in the control of premature ageing as well as in developing cancer therapeutics.
20.6 RdRP and RNA replication

In spite of strong resemblance of the overall shape of all the RDRPs with a “cupped right hand”, viral RDRPs have some special architectural features. The most notable distinct feature of these polymerases is that, in contrast to the “open hand” shape of the other polynucleotide polymerases, the RDRP resembles a “closed hand”. The closing of the “hand” is achieved by loops, called “fingertips”, which protrude from the fingers and connect with the thumb domain at their other end. The fingertip region forms the entrance of the channel where the RDRP binds with the RNA template. In addition, there is a small positively charged tunnel through which the nucleotide monomers required for elongation of the RNA enter. The genome of some of the viruses consist of double stranded RNA; the corresponding RDRPs have some additional unique structural elements which unzip the two strands and feed the appropriate strand to the catalytic site.

20.7 Nucleic-acid analogs as templates for polynucleotide polymerase

Nucleic acid analogs with altered backbones or bases have been synthesized artificially. Threose nucleic acid (TNA) is a nucleic acid analog whose backbone consists of repeating threose units linked by phosphodiester bonds. Glycerol nucleic acid (GNA) is another analog of natural nucleic acids. GNA is based on glycerol-phosphate backbone repeat unit. The operational mechanism of polynucleotide polymerases using these nucleic acid analogs as templates has become a subject of experimental investigation in recent years. Understanding these processes may shed new light on the origin of life as some of the nucleic acid...
analogs might have been used the genetic material by
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20.8 Coordination between transcription and replication

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20.9 Transcription and replication
of DNA in mitochondria and chloroplast

Mitochondrial DNA (mtDNA) is replicated by a mt-
DdDP called DNAPol γ. However, the mechanism of
replicating the lagging strand of mtDNA is different
from that of replicating the nuclear DNA.

Surprisingly, in majority of the eukaryotes, the
mitochondrial DdRP is structurally closer to single-
subunit polymerases of bacteriophages, rather than
the multi-subunit polymerases of bacteria, although
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21 Ribosomes and polymerization of polypeptides

Synthesis of each protein from the corresponding messenger RNA (mRNA) template is carried out by a ribosome and the process is referred to as translation (of genetic code). Ribosome is one of the largest and most sophisticated macromolecular machines within the cell. Even in the simplest organisms like single-cell bacteria, a ribosome is composed of few rRNA molecules as well as several varieties of protein molecules.

Each ribosome consists of two parts which are usually referred to as the large and the small subunits. The small subunits binds with the mRNA track and assists in decoding the genetic message encoded by the codons (triplets of nucleotides) on the mRNA. But, the actual polymerization of the protein (a polypeptide) takes place in the large subunit. The operations of these two subunits are coordinated by a class of adapter molecules called tRNA.

The “head” and the “body” are the two major parts of the small subunit. Two major lobes, which sprout upward from the “body”, are called the “platform” and the “shoulder”, respectively. The decoding center of the ribosome lies in the cleft between the “platform” and the “head” of the small subunit. The incoming template mRNA utilizes a “channel” formed between the “head” and the “shoulder” as a conduit for its entry into the ribosome. Through the cleft between the “head” and the “platform” the mRNA exits the ribosome.

The characteristic “crown-like” architecture of the large subunit arises from three protuberances. On the flat side of the large subunit exists a “canyon” that runs across the width of the subunit and is bordered by a “ridge”. Halfway across this ridge, a hole leads into a “tunnel” from the bottom of the “canyon”. This “tunnel” penetrates the large subunit and opens into the solvent on the other side of the large subunit. This “tunnel” serves as the conduit for the exit of the nascent polypeptide chain. This “tunnel” is approximately 10 nm long and its average width is about 1.5 nm.

Several intersubunit “bridges” connect the two subunits of each ribosome. This bridges are sufficiently flexible so that relative movements of the two subunits can take place in each cycle of the ribosome. The intersubunit space is large enough to accommodate just three tRNA molecules which can bind, at a time, with the three binding sites E, P and A. Moreover, the shape of intersubunit space is such that it allows easy passage of the L-shaped tRNA molecules.

Just like the synthesis of polynucleotides (e.g., transcription and replication), synthesis of polypeptides (i.e., translation) also goes through three stages, namely, initiation, elongation, and termination. During the elongation stage, the three major steps in the
chemo-mechanical cycle of a ribosome are as follows: In the first, the ribosome selects a aa-tRNA whose anticodon is exactly complementary to the codon on the mRNA. Next, it catalyzes the reaction responsible for the formation of the peptide bond between the existing polypeptide and the newly recruited amino acid resulting in the elongation of the polypeptide. Finally, it completes the mechano-chemical cycle by translocating itself completely to the next codon and is ready to begin the next cycle.

Elongation factors (EF), which are themselves proteins, play important roles in the control of these major steps which require proper communication and coordination between the two subunits. The need for coordination between the two subunits can be appreciated from the following considerations. The formation of the peptide bond between the growing polypeptide and the newly arriving amino acid (which can take place only in the larger subunit) can be allowed only after it is recognized as the correct species implied by the genetic code. During the process of checking its identity through the codon-anticodon matching (which takes place in the smaller subunit), the formation of the peptide bond is prevented by an elongation factor Tu (EF-Tu). However, once a cognate tRNA is identified, the smaller subunit sends a “green signal” (by a molecular mechanism that remains unclear), the EF-Tu separates out by a process driven by GTP hydrolysis thereby clearing the way for the peptide bond formation. Similarly, elongation factor G (EF-G) coordinates the translocation of the mRNA by one codon and the simultaneous movement of the tRNA molecules from one binding site to the next one.

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21.1 Single-molecule experiments to probe single ribosome mechano-chemistry

Thus, each ribosome has three different functions which it performs on each run along the mRNA track: (i) it is a decoding device in the sense that it “reads” the sequence of codons on the mRNA and selects a
aa-tRNA whose anticodon is exactly complementary to the codon on the mRNA.

(ii) it is a peptidyltransferase that catalyzes the reaction responsible for the formation of the peptide bond between the existing polypeptide and the newly recruited amino acid resulting in the elongation of the polypeptide.

(iii) it is a conveying machine that, while moving along a mRNA chain, passes tRNA molecules through itself during elongation. Interestingly, function (i) is performed exclusively by the smaller subunit while the function (ii) is carried out in the larger subunit. This division of labour between the larger and the smaller subunit may be related to the fact that there is a relatively large (8 nm) separation between the anti-codon and the amino-acid-carrying end of the tRNA molecules. But, the function (iii) requires coordinated movement of the two subunits.

Some specific steps in the mechano-chemical cycle of a ribosome are driven by the hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP). Therefore, ribosome is often regarded as a motor. However, a ribosome is not merely a “protein-making motor protein” but it serves as a “mobile workshop” which provides a platform where a coordinated action of many tools take place for the selection of the appropriate subunits and for linking them to synthesize each of the proteins. As this mobile workshop moves along the “assembly line” (mRNA), new subunits (amino-acids) are brought to it by the “workers” (tRNA molecules).

Some of the fundamental questions on the mechanism of translation are the following:
(i) How does the tRNA move on the ribosome (a) before, and (b) after the peptide bond formation? (ii) How does the ribosome modulate the stability of its binding with the mRNA so that it can step forward on its track once in each cycle during the elongation stage without destabilizing the ribosome-mRNA-tRNA complex itself? (iii) How is the movement of the ribosome on mRNA coordinated with the movements of the tRNA molecules on the ribosome? (iv) What are the sources of energy required for these movements and how are these energies transduced? Most of these questions can be addressed using single-ribosome techniques; however, very few such experiments have been reported in the literature.

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Theoretical modeling of single-ribosome operation has made very little progress so far.

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Most often many ribosomes move simultaneously on a single mRNA strand while each synthesizes a separate copy of the same protein. Such a collective movement of the ribosomes on a single mRNA strand has superficial similarities with vehicular traffic and is, therefore, referred to as ribosome traffic. Most of the theoretical models of ribosome traffic represent the mRNA as a one-dimensional lattice, where each site corresponds to a single codon. Since an individual ribosome is much larger than a single codon, the ribosomes are represented by hard rods in these models. So, ribosome traffic is treated as a problem of non-equilibrium statistical mechanics of a system of interacting “self-driven” hard rods on a one-dimensional lattice. Moreover, in these models the inter-ribosome interactions are captured through hard-core mutual exclusion principle: none of codons can be covered simultaneously by more than one ribosome. Thus, these models of ribosome traffic are essentially totally asymmetric simple exclusion process for hard rods: a ribosome hops forward, by one codon, with probability $q$ per unit time, if an only if the hop does not lead to any violation of the mutual exclusion principle.
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But, strictly speaking, a ribosome is neither a particle nor a hard rod. Moreover, in all the works mentioned above, the entire complexity of the mechano-chemistry of each ribosome is captured by a single parameter $q$. Only a few attempts have been made in recent years to capture the mechano-chemistry of individual ribosomes in the quantitative models of interacting ribosomes in traffic-like situations.

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22 Spliceosome; closely related to ribosome?

In eukaryotic cells, pre-mRNA require several types of processing before it matures to a functional mRNA. One of the key processes is splicing whereby non-coding segments (introns) are removed and the resulting strands are joined. Just like the ribosome, spliceosome is also composed of proteins as well as RNA.

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23 Fidelity of template-dictated polymerization

Nature’s evolutionary design has successfully optimized two competing demands: accuracy and speed of template-dictated synthesis of nucleic acids and proteins. The typical probability of the errors is about 1 (i) in $10^3$ polymerized amino acids, in case of protein synthesis, (ii) in $10^4$ polymerized nucleotides in case of mRNA synthesis and (iii) in $10^9$ polymerized nucleotides in case of replication of DNA. The mechanisms of proof reading and quality control also has to optimize two other mutually conflicting demands: maintaining the integrity of the genome and tolerance for some errors (genetic mutations) which is necessary for diversification of species. What are the mechanisms used by the intracellular machinery for manufacturing macromolecules of life to simultaneously achieve these conflicting goals?

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24 Machines for non-template-dictated biopolymerization

24.1 Machines for polymerization of polysachharides

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• Starch synthesizer machines

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• Starch synthesizer machines

Biosynthesis of cellulose have also received lot of attention.

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• Chitin synthesizer machines

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There are some common features, in spite of wide range of differences, in the organization of eukaryotic and prokaryotic chromosomes.

25.1 Histone modifying enzymes

In order to get access to the relevant segments of DNA for various processes in DNA metabolism, eukaryotic cells use a class of machines which alter the DNA-histone interactions. These machines fall in two different classes: (i) enzymes that covalently modify histone proteins (histone modifying enzymes), and (ii) ATP-dependent chromatin-remodeling enzymes (CRE) which alter the structure and/or position of the nucleosomes.

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Although, at first sight, chromatin-assembly and remodeling may appear to be opposite processes, there is a step common to both—sliding of the nucleosome on a DNA. Therefore, it should not be surprising that several CRE participate in both these processes.

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Some of the CRE repress chromatin, instead of activating it. Therefore, a general definition of chromatin remodeling should be as follows: chromatin remodeling is a change in the state of chromatin that facilitates its activation or repression.
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26 Machines for DNA Repair and recombination

For hereditary transmission of the genome, repair of damaged DNA is as essential as the high fidelity of the replication of the genome itself. Recombination can be viewed as a process whose sole purpose is to rearrange genetic material thereby generating genetic diversity. However, recombination can also be used to repair damaged DNA. Recombinational repair is not the only method of DNA repair; there are other methods of DNA repair which do not exploit recombination. Just as DNA replication requires coordinated operation of several machines, DNA repair and recombination also needs similar coorporation of another set of machines.

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27 Machines for degrading macromolecules of life

27.1 Machines for degrading DNA

Nucleases are enzymes which function as “scissors” by cleaving the phosphodiester bonds on nucleic acid molecules. Endonucleases cleave the phosphodiester bond within the nucleic acid thereby cutting it into two strands whereas exonucleases remove the terminal nucleotide either at the 3’ end or at the 5’ end.

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Erythrocytes (red blood cells) and lens fiber cells in the eyes possess no nucleus! In reality, DNA is removed from the precursors of these cells during their maturation. Moreover, during the development of an animal, some cells are deliberately killed; this phenomenon, known as apoptosis (programmed cell death), also involves degradation of the DNA of the target cells. Furthermore, those cells which become toxic or senescent are also killed actively and their DNA are degraded. Finally, bacteria have evolved a mechanism of degrading DNA of invading bacteriophages. In this subsection we list references of some relevant papers on the molecular architecture and mechanisms of operation of the machines which degrade DNA.

27.2 DNA degradation by restriction-modification enzymes

R-M systems consist of two components which perform two competing functions. Restriction involves an endonuclease that breaks a DNA by hydrolyzing the phosphodiester bond in backbone of both the strands. On the other hand, modification involves a methyltransferase which adds a chemical group to a DNA base at a position that blocks the restriction activity. Both these activities are specific for the same DNA sequence. The main biological function of the R-M system is to defend the bacterial host against bacteriophage infection by cleaving the phage genome while the DNA of the host are not cleaved. The restriction endonucleases have been classified into three groups: type I, type II and type III. Both type I and type III are molecular machines in the true sense because these require ATP for their operation.

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27.3 Machines for degrading RNA

Ribonucleases (whose commonly used abbreviation is RNase) are also nucleases and function as “scissors” that cleave the phosphodiester bonds on RNA molecules. Like all other nucleases, RNases are also broadly classified into endoribonucleases and exoribonucleases.

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In eukaryotes, a barrel-shaped multi-protein complex, called exosome, degrades RNA molecules. The bacterial counterpart of exosome is usually referred to as the RNA degradosome. The fundamental questions on the operational mechanism of these machines are of two types. The first types of questions are essentially identical to those raised earlier in the context of import/export of macromolecules by translocation motors. The second type of questions are similar to those raised in the context of (ribo-)nucleases, namely, the mechanism of shredding or mincing and the resulting size distribution of the products.

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### 27.4 Machines for degrading proteins

Proteases are enzymes which perform functions that are analogous to nucleases. Just as nucleases cleave the phosphodiester bonds on nucleic acids (i.e., polynucleotides), proteases cleave peptide bonds on polypeptides and, hence, sometimes also called peptidase.

Simple proteases in the extracellular space, e.g., the pancreatic proteases, digest proteins derived from diets. However, such non-specific proteases are not expected to operate in the intracellular space because they would indiscriminately cleave all the essential and non-defective proteins to their amino acid subunits thereby destroying the cell itself. Evolution has designed intracellular machines for protein degradation which mince only the unwanted proteins in very specialized chambers whose gates open to allow only for only such unwanted proteins. Moreover, since mitochondria and plastids had bacterial ancestors, it is not surprising to find very similar proteases in these compartments.

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- **Proteasome: a protein degrading machine**

  Proteasome is a large and complex machine for protein degradation. It has structural and functional similarities with exosome; what exosome does for RNA, proteasome does for proteins. Obvously, the fundamental questions to be addressed are very similar to those in the case of exosomes.

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28 Mechanisms for searching target sequence on NA

In most of the processes we have discussed so far a protein or a macromolecular complex has to bind a specific site on a DNA. For example, in order to initiate transcription, the transcription factor must bind with a specific site on the DNA. Similarly, sequence-specific binding is required for the operation of restriction enzymes. How does the machine target the specific site? Is search through an effectively one-dimensional diffusion sufficiently rapid? Or, does the search become more efficient by a combination of the one-dimensional diffusion with other processes?
29 Effects of inhomogeneities, defects and disorder

The sequence of nucleotides on naturally occurring nucleic acid strands are intrinsically inhomogeneous. Numerical calculations for the given inhomogeneous sequence of any specific nucleic acid strand is not very difficult. But, for the simplicity of analytical calculations, two extreme idealizations are sometimes considered: in one of these the actual sequence is replaced by a hypothetical homogeneous sequence whereas in the other the sequence is assumed to be completely random.

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Part III: Membrane associated ion transporters and related machines
A wide variety of machines are associated with either the plasma membrane of the cell or with the internal membranes that enclose various organelles like, for example, mitochondria. In part II we have considered machines which translocate macromolecules across cell membranes. Now, in this part, we focus on machines which transport small and medium size molecules and ions across membranes.

These “transporters” can be broadly divided into two categories- active and passive. Channels are passive transporters because these allow the passage of molecules or ions down their electro-chemical gradients and do not require input energy for performing this task. In contrast, active transporters drive molecules or ions against their electro-chemical gradients by utilizing some input energy directly or indirectly.

The active transporters can be further divided into two classes- primary and secondary active transporters. Primary active transporters include (a) ATP-binding cassette (ABC) transporters, (b) ion pumping P-type ATPases. Primary active transporters use light or chemical energy as input to transport molecules and/or ions across a membrane. In fact, one of the major roles of pumps is to create and maintain electrochemical gradients by actively transporting ions. Secondary active transporters use the spontaneous flow of the ions along such electro-chemical gradients to drive other species of molecules “uphill” (i.e., against their natural own electro-chemical gradients). Interestingly, in spite of their mode of operation (i.e., active versus passive), ion channels and pumps share one common feature, namely, the ability to transport ions in a selective manner; ion-selectivity is crucial for the survival of the cell.

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30 ATP-binding cassette (ABC) transporters: two-cylinder engines of cellular cleaning pumps

An ATP-binding cassette (ABC) transporter is a membrane-bound machine. These machines are found in all cells from bacteria to humans. In prokaryotic cells, ABC transporters are located in the plasma membrane. In eukaryotes, ABC transporters have been found in the internal membranes of organelles like mitochondria, peroxisomes, golgi and endoplasmic reticulum. These translocate ions, nutrients like sugars and amino acids, drug molecules, bile acids, steroids, phospholipids, small peptides as well as full length proteins.

In spite of wide variations in their functions and substrates translocated by them, they share some common features of structure and dynamics. Each ABC transporter consists of four core domains. Out of this four, two transmembrane domains (TMDs) are needed for binding the ligands which are to be transported while the two nucleotide-binding domains (NBDs) bind, and hydrolyze, ATP. Many ABC transporters are single four-domain proteins. In contrast, “half-size” ABC transporters consist of one
TMD and one NBD; many ABC transporters are actually homo-dimers or hetero-dimers of “half-size” transporters.

Some of the fundamental questions specifically related to the mechanisms of ABC transporters are as follows:

(i) why do these machines need two ATP-binding domains although it consumes only one molecule of ATP for transporting one ligand?

(ii) Do the two NBDs act in alternating fashion, like a two-cylinder engine where the cycles of the two cylinders are coupled to each other? Or, do the two NBDs together form a single ATP-switch?

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### 31 Membrane associated ion-pumps: P-type ATPases

P-type ATPases form a superfamily of machines which transport *cations* across membranes.

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### 31.1 Na/K pumps

This pump plays crucial roles and is mainly responsible for maintaining electrolyte balance in almost all cells in humans. It takes in K\(^+\) ions and ejects out Na\(^+\) ions.

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Cytosolic Ca$^{2+}$ concentration is maintained below about 10 $\mu$M for normal metabolism of the cell. On the other hand, Ca$^{2+}$ is one of the most important carriers of signals. During signaling, brief opening of Ca$^{2+}$ channels in the plasma membrane (or organelar membrane) allow Ca$^{2+}$ to enter spontaneously because of the existing electro-chemical gradient. However, this increase of Ca$^{2+}$ inside is only transient as Ca$^{2+}$ pump ejects the Ca$^{2+}$ ions out. It is this Ca$^{2+}$ pump that maintains the high electro-chemical gradient (low Ca$^{2+}$ concentration inside and high Ca$^{2+}$ concentration outside).

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### 31.3 Proton pumps

In the fungal plasma membrane (e.g., of yeast) a proton pump hydrolyzes ATP to pump out the protons thereby creating an electro-chemical gradient. This electro-chemical gradient is utilized to provide the energy to the proton-coupled co-transporters for sugars, amino acids and other nutrients. The gastric H+/K+-ATPase is most closely related to Na+/K+-pump; it pumps out H+ ions and takes in K+ ions.

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### 31.4 Copper pumps

Malfunctioning of the copper-transporting ATPases can lead to Menkes and Wilson diseases in humans.

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The input energy for both bacteriorhodopsin (BR) and halorhodopsin (HR) is light. But, BR pumps protons whereas HR pumps chloride ions. The analog of mechano-chemical cycle of motors is the photocycle of BR and HR.

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- Xanthorhodopsin

Like bacteriorhodopsin, xanthorhodopsin is also a light-driven proton pump. But, has a special feature in its structure which, like antenna, enables it to collect light more efficiently than what is possible by a bacteriorhodopsin.

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32 ATP synthase and related machines

ATP synthase is the smallest rotary motor and is embedded in the membrane of the organelles mitochondria (in animal cells) and chloroplasts (in plant cells). It consists of two coupled parts which are called \( F_0 \) and \( F_1 \) and, therefore, ATP synthase is also referred to as \( F_0F_1 \)-ATPase. This motor in reversible. In the normal mode, \( F_0 \) is rotated by a protonmotive torque which, in turn rotated \( F_1 \) during which the latter synthesizes ATP from ADP and phosphate. In the reverse mode, \( F_1 \) consumes ATP for its own rotation in the reverse direction thereby rotating also \( F_0 \) in reverse while the latter operates effectively as a proton pump.

There are also some interesting architectural similarities between the ATP synthase and the TrwB DNA translocase.

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32.2 From nutrient to ATP: energy production by mitochondria

For the synthesis of ATP by mitochondria, protons must translocate across the $F_0$ subunit of the ATP synthases, which are bound to the mitochondrial membranes, down their electro-chemical gradient. But, how is the concentration gradient of protons created? This is achieved primarily by a process called oxidative phosphorylation during which electrons, derived from nutrients, are passed through a sequence of enzyme complexes located in the mitochondrial membranes.

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The concentration gradient of protons required for ATP synthesis by chloroplasts in plants, by harvesting sunlight, is the result of a sequence of processes, the primary one being photophosphorylation.

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32.5 Na$^+$-ATP synthase

We have already seen that Na$^+$ can substitute for H$^+$ as the coupling ion in secondary transporters. Now we point out that even for the operation of ATP synthases, H$^+$ is not essential and in some ATP synthases Na$^+$ used instead of H$^+$.

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### 32.6 Vacuolar ATPase

Vacuolar ATPases were initially identified in plant and fungal vacuoles and hence the name. Later these were found also in plasma membrane and organelle membranes of mammalian cells and plants. Therefore, it is more appropriate to link the letter “V” in V-ATPase with “various” (various membranes) rather than “vacuoles”. V-ATPases are ATP-dependent proton pumps that regulate pH (acidify) intracellular compartments in eukaryotic cells.

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32.7 Pyrophosphatase

Membrane-bound pyrophosphatase (PPase) usually couples pyrophosphate (PP$_i$) hydrolysis to ion translocation across the membrane.

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- \( Na^+ \)-pyrophosphatase
  
  However, very recently, it has been argued that some bacteria, which live under extreme conditions, translocate sodium ion, instead of proton, by coupling it to the hydrolysis of pyrophosphate.

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32.8 Historical notes on ATP synthase and related machines

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33 Bacterial flagellar motor

In this section, we consider only the rotary motor that drives the bacterial flagella. The architecture of the full flagellum and how its movements propels the bacterium in the fluid medium will be taken up in the section on swimming of bacteria.

33.1 Bacterial flagellar motor driven by proton-motive force

Normally, the bacterial flagellar motor is driven by a protonmotive force, i.e., protons driven by a transmembrane electro-chemical gradient.

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### 33.3 Comparison between ATP synthase and bacterial flagellar motor

Both the bacterial flagellar motor and ATP synthase are driven by a torque arising from a force which is of electro-chemical origin. However, there are also crucial differences between these two rotary motors.

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### Part IV: Machine-driven cellular processes

Cells exist in wide variations in their sizes, shapes and internal structures. For example, among the bacteria, cocci (spherical), bacilli (rod-shaped) and spirochetes (spiral) reflect the shapes of these unicellular organisms. Among the unicellular eukaryotes, protozoa exhibit some of the most complex and exquisite forms. The animal cells also exhibit widely
different characteristics. The linear dimension of a typical eukaryotic cell is about 10 µm. But, a neuron can be as long as a meter. Each skeletal muscle cell has more than one nucleus whereas a red blood cell has none at all. Germ cells have only one set of chromosomes whereas all other cells have two sets. Hair cells of the inner ear act as mechano-sensors while rod cells of the retina of the eye are photo-sensors. However, in spite of such diversities, there is unity in some of the common cellular processes. In this part we consider mainly cell motility and cell division both of which are also closely related to changes in cell shape.

34  Machine-driven cell motility

"From whale sperm to sperm whales, locomotion is almost always produced by appendages that oscillate or by bodies that undulate, pulse, or undergo peristaltic waves". M.H. Dickinson et al., Science 288, 100 (2000).

34.1  Cell motility: some general principles

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34.2  Motility of prokaryotic cells

Unicellular microorganisms have developed diverse molecular mechanisms of locomotion. The actual mechanism used by a specific type of organism depends on the nature of the environment in the natural habitat of the organisms. In this section, we consider exclusively the prokaryotes.

If a bacterium lives in a bulk fluid, it’s natural mode of motility is swimming. In contrast, is a bacterium lives in a thin fluid film close to a solid surface (i.e., in a wet surface), gliding should be its mechanism of movement. Of course, some bacteria may be capable of utilizing both these modes of motility. However, there are subtleties of swimming and gliding that an uninitiated reader may not be able to anticipate. Moreover, there are mechanisms of motility other than swimming and gliding.

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34.3 Bacterial shape and shape changes during motility

The shapes of bacterial cells depend, at least partly, on the cytoskeleton.

**Bacterial shape**

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**Transient and permanent appendages for motility of prokaryotic cells**

The shape changes during motility and division of cells. Therefore, it is not surprising that components of the cytoskeleton play crucial roles in both these processes.

Microorganisms exploit the movements of permanent appendages like flagella, cilia, pili and fimbriae for their movements. We have already considered the rotary motor which drives bacterial flagella. Although the motor is driven by either proton motive force or sodium-motive force, the number of flagella and their spatial arrangements on the cell vary widely from one bacterial species to another. Many bacteria have only one flagellum whereas some species of bacteria possess more than one. Perhaps the most unusual is the periplasmic flagella of spirochetes. Moreover, some bacterial species possess dual flagellar systems which are suitable for movement under different conditions; the polar flagellum is used for swimming in bulk fluids whereas the lateral flagella for swarming close to solid surfaces.

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**Swimming of bacteria: flagella driven by rotary motor**

Depending on the species, a bacterium may have a single flagellum, or one flagellum at each end, or a tuft of flagella at one or both ends. Each flagellum consists of a filament, a hook and a basal body. The filament is helical and is composed of eleven protein fibrils arranged like the strands of a rope; a fine channel (≈ 70 Å in diameter) runs through the axis of the filament. The hook is a hollow, flexible, proteinaceous structure. The basal body consists mainly of the flagellar motor. The flagellar motor of a bacterium (say, E-coli) is about 50 nm in diameter and consists of about 20 different components. The speed of this rotary motor could be of the order of 100 Hz.
A large class of single-cell bacteria “swim” in their aqueous environment using their flagella which, in turn, are rotated by the flagellar motors driven by proton-motive (or sodium-motive) force, as we have already explained earlier.

The Reynolds number, that characterizes the swimming of bacteria in aqueous media, is normally very small. Perhaps, one can appreciate the situation better by comparing with swimming of a human being; a comparable Reynolds number would be realized if a human being tried to swim in honey!

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- **Swimming of spirochetes: hidden flagella driven by rotary motor**

  Spirochetes have periplasmic flagella (i.e., flagella which are located in the periplasmic space between the outer cell membrane and the cell wall). Thus, unlike E-coli, spirochetes do not stick their flagella out into the fluid outside the cell. But, the flagella of spirochetes are also driven by proton-motive force. Rotation of these flagella deform the cell body which, consequently, rolls. It is this corkscrew-like motion of the spirochete that propels it through the external fluid medium. However, one counterintuitive consequence of this mechanism of the motility of spirochetes is that spirochetes move faster in gel-like media than in water.

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- **Swimming without flagella: linear motor of spiroplasma**

  The mollicutes (*Spiroplasma, Mycoplasma* and *Acholoplasma*) are the smallest free-living organisms. Their structure is unusual in the sense that they do not have cell wall and the standard form of prokaryotic flagella. The *Spiroplasma* are unique among the mollicutes because, as the name suggests, their spiral shape can be viewed as a dynamic helical membrane tube (of typical radius of about 0.1µm). They maintain their helical structure by the internal cytoskeletal filaments.

  The motility of the spiroplasma is driven by its contractile cytoskeleton. Thus, in contrast to the rotary motors of flagellated bacteria, the machinery driving the spiroplasma are linear motors. Just like spirochetes, spiroplasma move faster in media with higher viscosity. But, in contrast to spirochetes, spiroplasma move with higher viscosity irrespective of whether or not it is gel-like.

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- Gliding of over surfaces: push of linear motors
  For some bacteria, like Myxococcus xanthus, hydration of a slime secreted by the bacterium through a nozzle, generates the force required for their own movement.

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Gliding of Mycoplasma mobile resembles motion of centipedes where tiny “leg-like” appendages are powered by ATP hydrolysis.

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- Twitching: pull of linear motor type IV pili
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34.4 Taxis
So far we have discussed the machineries used by prokaryotic cells for motility. But, how does the cell sense its environment and decide the direction of its motion?

Chemotaxis refers to the directional movement in response to the gradient of concentration of a chemical. Substances which attract a cell are called chemoattractant while those repelling a cell are called...
chemorepellant. Strictly speaking, aerotaxis is a special case of chemotaxis where the motile cells respond to a gradient of concentration of the dissolved oxygen. Mechanotaxis is cell migration controlled by the rigidity of an underlying substrate. Phototaxis is the corresponding response to light gradient whereas galvanotaxis is the ability to move in response to electric potential gradient. Finally, haptotaxis is the motility in response to gradient in adhesion of ligands.

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34.5 Motility of eukaryotic cells

Unicellular eukaryotes, like free-living protozoa, move primarily for food. In multicellular prokaryotes, cell locomotion is essential in development. Moreover, leukocytes move to offer immune response. Furthermore, fibroblasts, which are normally stationary, move during wound healing.

One of the fundamental questions on cell motility is the molecular mechanisms involved in the generation of required forces. Broadly speaking, three different mechanisms have been postulated and their possibility in specific contexts have been explored: (i) Force generated by polymerization of cytoskeletal protein filaments (actin and microtubules), (ii) Force generated by cytoskeletal motors by their interactions with filamentous tracks, and (iii) forces of osmotic of hydrostatic origin.

- Taxis of eukaryotic cells

Chemotaxis is not restricted only to prokaryotes. Eukaryotic cells are also guided by an appropriate guidance system. Chemotaxis is involved in wide varieties of biological processes starting from embryogenesis to wound healing and immune response. Several different models of eukaryotic chemotaxis have also been proposed.

Chlamydomonas reinhardtii is a unicellular biflagellate green alga. It has been used extensively as a model experimental system for investigating swimming of eukaryotic cells. These cells swim towards light by beating their flagella. In contrast to phototaxis of C. reinhardtii, the sperm cells of eukaryotes are guided by chemotactic signals. Interestingly, the guidance of the axonal growth cones is also guided by chemotaxis.

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•Crawling of eukaryotic cells: dynamic protrusions

The crawling of eukaryotic cells involves the formation and movement of transient cell protrusions like lamellipodia, filopodia, etc. A lamellipodium is a thin sheet-like protrusion whose typical thickness varied between 0.1 and 0.2 µm. In contrast, a filopodium is a finger-like structure whose typical diameter varies between 0.1 and 0.3 µm. Structurally, there are crucial differences between these two types of cell protrusions. Lamellipodia are filled with a branched network of actin filaments whereas parallel bundles of filamentous actin run along the length of filopodia. Quite often filopodia protrude from a lamellipodium. Therefore, two different models for the formation of the actin-bundles of filopodia have been proposed. In the “convergent elongation model”, the filopodial actin filaments are assumed to originate from the lamellipodial actin network. But, in the “de novo filament nucleation model”, the filopodial actin filaments are assumed to nucleate separately in the filopodia. A common feature of the actin networks in lamellipodia and filopodia is that the fast growing (barbed) ends of the actin filaments are oriented towards the membrane which gets pushed by the piston-like action of the polymerizing actin filaments. This piston-like pushing by polymerizing actin is very similar to piston-like action of polymerizing microtubules, which we discussed earlier, except that actins can form branched structures whereas microtubules do not. Other protrusions of the eukaryotic cell include pseudopodia, ruffles, microvilli, invadopodia, etc.

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Two different types of models have been developed in the context of cell crawling. Some models focus exclusively on the dynamics of the cell protrusions.

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**Crawling of eukaryotic cells: full cyclic dynamics**

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**Motility of bacterial pathogens driven by actin comets**

Bacterial pathogen *Listeria Monocyte* uses a simplified mechanism of motility based on force generation by actin polymerization. In this case a comet-like tail of polymerizing actin filaments push the pathogen in the host cell. Unlike, cell crawling, which is also driven by actin-polymerization, neither adhesion to a slid substrate nor retraction of the rear of the cell is required.

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• Motility of nematode sperm by actin-like MSP

In contrast to other types of sperm cells which swim using flagella (and which we’ll consider soon), sperm of several nematode species crawl. However, unlike most of the crawling cells, these nematode sperms do not contain actin. Instead, another protein, called major sperm protein (MSP) acts like actin forming dynamic filaments which drive the crawling of the nematode sperm.

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Swimming of eukaryotic cells: beating of eukaryotic flagella

Earlier we have already pointed out that the beating of the eukaryotic flagella are driven by axonemal dynein motors which move by hydrolyzing ATP. Eukaryotic cells beat their cilia not only for motility, but, in some circumstances, also to move the surrounding medium with respect to the cell surface. For example, the cilia on the epithelium of the upper respiratory tracts beat to remove the dust and other foreign particles.

Now, the main question is: how does a flagellated eukaryotic cell exploit the patterns of beating of its flagellum for its swimming? This question is addressed by analyzing the hydrodynamic effects of the different patterns of beating of the flagellum.

Motility of flagellate protozoan from termites
35 Machine-driven cell division

35.1 Cell cycle

During its lifetime, before complete division of a parent cell into its two daughters, a cell goes through a sequence of states which are identified primarily by its shape and internal architecture.

35.2 Brief introduction to cell division: eukaryotes versus prokaryotes

Although each stage of the cell cycle is of interest to cell biologists, we are mainly interested in the machines and mechanisms involved in the different stages of cell division. In particular we focus attention on mitosis and cytokinesis.

35.3 Mitosis and chromosome segregation in eukaryotes: machines and mechanisms

Mitosis is a complex process whereby identical copies of the replicated genome are segregated so as to form the separate genomes of the two daughter cells which would result from the cell division. The bipolar machinery which carries out this process is called the mitotic spindle. A similar machinery, called the meiotic spindle, runs the related process of meiosis, which reduces the size of the genome by half to produce a haploid gamete from a diploid one. We shall consider separately a few important sub-steps of mitosis.

A large number of coordinated processes are involved in mitosis. These include, for example, spindle morphogenesis, chromosome condensation, sister chromatid separation, dynamic instability of the microtubules, depolymerase-driven length control of microtubules, walking of MT-associated motors on their tracks, etc. We have studied several of these active processes separately in the preceding sections. It is the integration of so many processes within a single theory of mitosis that poses the main conceptual challenge to theoretical modelers.

There are three different sources of forces which govern the dynamics of the mitotic spindle: (i) Forces generated by cytoskeletal motors which can capture microtubules and can also slide microtubules with respect to each other; (ii) pushing and pulling forces exerted by polymerizing and depolymerizing microtubules; (iii) spring-like forces which arise from the elastic stretching of the chromosomes. Moreover, the bending of the microtubules may have important consequences.

- Spindle morphogenesis

Both the mitotic spindle and the meiotic spindle are formed by microtubules (MT), MAPS and cytoskeletal motors. In principle, a spindle can form by one of the two different pathways. In the centrosome-directed pathway, the spindle starts from centrosomes (which are eventually located at the poles of the spindle) and grow towards the center by adding tubulin subunits at their plus ends. In contrast, in the chromosome-directed pathway, chromosomes induce MT assembly; but, the randomly oriented MTs require assistance of motor proteins to reorient properly so as to form the bipolar spindle.
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- **Chromosome motility**

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35.4 Chromosome segregation in prokaryotes: machines and mechanisms

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Normally *Bacillus subtilis*, a rod shaped bacterium, divides to two similar daughter cells. However, under some special circumstances, which leads to spore formation, a *Bacillus subtilis* divides asymmetrically into a small prespore and a larger mother cell. The translocation of the chromosome into the small prespore compartment is carried out by the motor protein
SpoIIIE. Most of the fundamental questions on its operational mechanism are similar to those generic ones for helicases and translocases (including packaging motors for viral capsids). In particular, how does SpoIIIE, which anchors itself at the septum between the two compartments, translocate the DNA in the desired direction, namely, from the larger to the smaller compartment? in the

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35.5 Eukaryotic cell cytokinesis: machines and mechanisms

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- Cytokinesis in animal cells

In dividing cells of animals and fungi, an actomyosin ring forms in the middle of the cell and its contraction generates the force required for cytokinesis. In other words, cytokinesis in animals and fungi is driven by a coordinated operation of the cell membrane and a cytoskeletal motor-filament system. How is the equatorial plane recognized by actin? Do the actin filaments nucleate in the equatorial plane of the cell itself or are the actin filaments transported there from elsewhere? How are the actin filaments and the myosin motors organized and how do they interact so as to generate the force responsible for furrow ingress? Do the actin filaments work like a tightening “purse string” or do the actin filaments work like a radially shrinking spokes of a bicycle wheel?

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For bacterial cells, the mechanisms of locating the mid-cell and those of cytokinesis are now quite well understood. An interesting finding of the experiments is that eukaryotic cells use microtubule-based machiriy for chromosome movements and actin-based machinery for cytokinesis. In sharp contrast, prokaryotic cells do just the reverse.

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35.8 Division of peroxisomes

Just as mitochondria are the powerhouses of eukaryotic cells, peroxisomes are often regarded as the garbage pail of the cell. The division of peroxisomes can be divided roughly into three stages: (a) elongation of the peroxisome, (b) constriction of peroxisomal membrane, and (c) fission of the peroxisome. In spite of fundamental differences in their structure and function, mitochondria and peroxisomes share quite a few components of the machineries which drive their fission. For example, dynamin-like proteins, which are involved in the fission of mitochondria, also form the ring that is required for the fission of peroxisomes.

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36 Miscellaneous natural and artificial molecular machines

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38 Molecular biomimetics—bottom-up approach to nano-technology

Initially, technology was synonymous with macro-technology. The first tools applied by primitive humans were, perhaps, wooden sticks and stone blades. Later, as early civilizations started using levers, pulleys and wheels for erecting enormous structures like pyramids. Until nineteenth century, watch makers were, perhaps, the only people working with small machines. Using magnifying glasses, they worked with machines as small as 0.1mm. Micro-technology, dealing with machines at the length scale of micrometers, was driven, in the second half of the twentieth century, largely by the computer miniaturization.

In 1959, Richard Feynman delivered a talk at a meeting of the American Physical Society. In this talk, entitled “There’s Plenty of Room at the Bottom”, Feynman drew attention of the scientific community to the unlimited possibilities of manipulating and controlling things on the scale of nano-meters. This famous talk is now accepted by the majority of physicists as the defining moment of nano-technology. In the same talk, in his characteristic style, Feynman noted that “many of the cells are very tiny, but they are very active, they manufacture various substances, they walk around, they wiggle, and they do all kinds of wonderful things—all on a very small scale”.

From the perspective of applied research, the natural molecular machines opened up a new frontier of nano-technology. The miniaturization of components for the fabrication of useful devices, which are essential for modern technology, is currently being pursued by engineers following mostly a top-down (from larger to smaller) approach. On the other hand, an alternative approach, pursued mostly by chemists, is a bottom-up (from smaller to larger) approach. We can benefit from Nature’s billion year experience in nano-technology. We have given a long list of studies completed so far on the architectural design of a natural nanomachine, identification of its components and monitoring the spatio-temporal coordination of these components in the overall operation of the machine. The lessons learnt from such investigations can then be utilized to design and synthesize artificial nanomachines. In fact, the term biomimetics has already become a popular buzzword; this field deals with the design of artificial machines utilizing the principles of natural bio-machines. Even nanobotics may no longer be a distant dream.

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