Cell and molecular mechanics of biological materials

Living cells can sense mechanical forces and convert them into biological responses. Similarly, biological and biochemical signals are known to influence the abilities of cells to sense, generate and bear mechanical forces. Studies into the mechanics of single cells, subcellular components and biological molecules have rapidly evolved during the past decade with significant implications for biotechnology and human health. This progress has been facilitated by new capabilities for measuring forces and displacements with piconewton and nanometre resolutions, respectively, and by improvements in bio-imaging. Details of mechanical, chemical and biological interactions in cells remain elusive. However, the mechanical deformation of proteins and nucleic acids may provide key insights for understanding the changes in cellular structure, response and function under force, and offer new opportunities for the diagnosis and treatment of disease. This review discusses some basic features of the deformation of single cells and biomolecules, and examines opportunities for further research.

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As material systems, living organisms are far more complex than engineered materials such as metals, ceramics, polymers and semiconductors. They are dynamic and provide integrated functions that include metabolism, control, sensing, communication, growth, remodelling, reproduction and apoptosis (programmed cell death)[1]. During the past few decades, studies have established the connections between structure, mechanical responses and biological functions of different organs and tissues including, for example, the heart, lung, bone, cartilage, blood vessel, and skeletal and cardiac muscles[2–4]. These studies have led to better diagnosis and treatment of orthopaedic, cardiovascular and respiratory diseases by providing a greater understanding of how the biological functions of the body are related to biosolid and biofluid mechanics. To decipher the fundamental mechanisms of biological materials, however, more systematic studies of deformation, structural dynamics and mechanochemical transduction in living cells and biomolecules are needed.

A CHANGING PARADIGM: SMALL IS IMPORTANT

The past decade has seen the development of instruments capable of mechanically probing and manipulating single cells and biomolecules at forces and displacements smaller than a piconewton ($1\ \text{pN} = 10^{-12}\ \text{N}$) and a nanometre ($1\ \text{nm} = 10^{-9}\ \text{m}$), respectively. These advances have provided opportunities for examining the processes responsible for operation of cellular machinery, the forces arising from molecular motors and the interactions between cells, proteins and nucleic acids[5,6]. Such progress has been accompanied by a rapid accumulation of structural information, and better modelling and simulation[7,8], arising from developments in computational biology and bioinformatics.

The biological cell constitutes the basic unit of life and performs a variety of functions: the synthesis, sorting, storage and transport of molecules; the expression of genetic information; the recognition, transmission and transduction of signals; and the powering of molecular motors and machines[1]. The cell also converts energy from one form to another and responds to external environments by continually altering its structure[9]. For example, endothelial cells lining the interior walls of blood vessels alter the expression of 'stress-sensitive' genes in response to shear flow in the blood[10]. For the cell body to move forward during cell migration, contractile forces must be generated within the cell[11]. Adhesion of cells to extracellular matrix (ECM) through focal adhesion complexes provides both signalling and structural functions[12–14]. Cells also undergo mechanical deformation when subjected to external forces and geometric constraints, in a similar manner to engineering materials. Consider, for example, the human red blood cell (erythrocyte) with a diameter of 7.0–8.5 μm, which is subjected to about 100% elastic deformation as blood flows through narrow capillaries

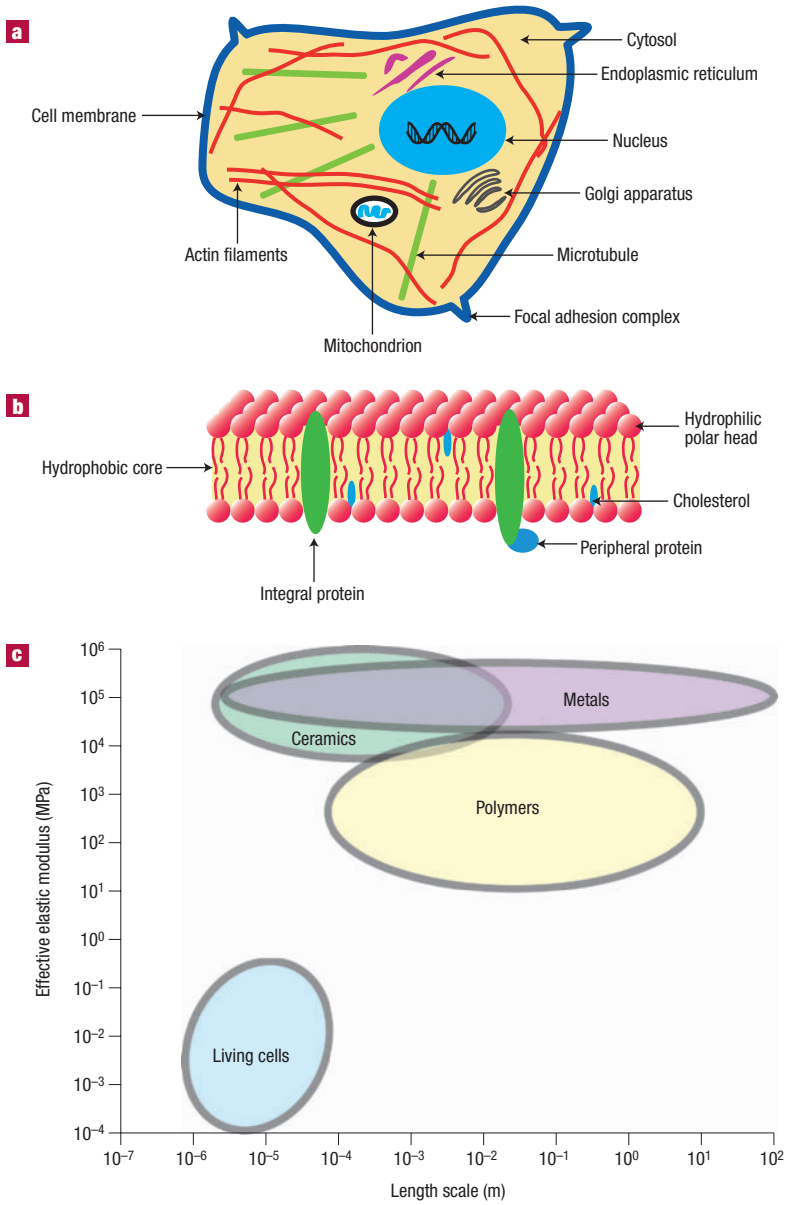

Figure 1 Cell structure and elastic properties. a, Schematic diagram of a typical eukaryotic cell, which contains many subcellular elements. **b**, The membrane comprises a phospholipid bilayer, with hydrophilic heads and hydrophobic tails, whose mechanical rigidity is altered by the presence of integral protein molecules and cholesterol. The interior of the cell consists of the nucleus, containing the DNA, and the cytoplasm, which contains the cytosol, endoplasmic reticulum, microtubules, actin filaments, intermediate filaments, mitochondria and the Golgi apparatus. The cytosol is the fluid inside the cell, in which the various organelles reside. The actin filament and intermediate filament are about 8 and 13 nm in diameter, respectively. The microtubule, in the shape of a hollow tube, has a diameter of 25 nm. The Golgi apparatus comprises a series of sacs with folded membranes whose function involves protein sorting. Mitochondria are organelles, ~0.5 μm in diameter and 1 μm in length, where the cell's energy metabolism takes place. Transmembrane protein receptors, such as integrins, provide a link between the extracellular matrix (ECM) and the cell interior. **c**, Approximate range of values for the elastic modulus of biological cells and comparisons with those of engineering metals, ceramics and polymers.

with inner diameter smaller than 3 μm. Many normal and diseased conditions of cells are dependent on or regulated by their mechanical environment, and the deformation characteristics of cells can provide important information about their biological and structural functions.

Most of the biological cells are 1–100 μm in size, and they comprise many constituents (Fig. 1). The cell is covered by a phospholipid bilayer membrane reinforced with protein molecules, and the interior of the cell includes a liquid phase (cytosol), a nucleus, the cytoskeleton consisting of networks of microtubules, actin and intermediate filaments, organelles of different sizes and shapes, and other proteins. The resistance of single cells to elastic deformation, as quantified by an effective elastic modulus[15,16], ranges from 10^2 to 10^5 Pa (Fig. 1c), orders of magnitude smaller than that of metals, ceramics and polymers. The deformability of cells is determined largely by the cytoskeleton, whose rigidity is influenced by the mechanical and chemical environments including cell–cell and cell–ECM interactions.

FORCE AND DEFORMATION OF LIVING CELLS

Why are mechanical forces essential to living cells? One model is that cells such as those in bone and endothelium are subjected to specific forces as part of their 'native' physiological environment. Any alteration of such forces is likely to cause a disruption in their normal functioning, thereby producing a diseased state. Another model is that specialized cells, such as the cochlear outer hair cell, realize their functions by converting an electrical or chemical stimulus into a mechanical force that has a more direct role in cell function[17]. Yet another model is that for certain cells, such as muscle cells, a mechanical signal in the form of force or deformation transmitted during an activity, such as exercise, facilitates a function that is not necessarily a mechanical one.

Mechanical loading of cells induces deformation and remodelling, which influence many aspects of human health and disease. Heart failure is often due to the loss of contractility of heart muscle cells[2]. Severe stretching of the axon of neural cells during traumatic brain injury causes cell death[18], whereas slow stretching of the axon helps neural cell growth[19]. The compliance of the blood vessel wall, which is crucial to hypertension and other cardiovascular diseases, is controlled to a large extent by the deformability of smooth muscle cells and by the content of elastin and collagen[2]. During cell locomotion, the crawling of cells can be altered markedly by the stiffness of the substrate; cells have the ability to recognize the mechanical environment (for example stiffness) and adjust their behaviour (for example direction of motion) accordingly[20]. Cell deformation, viscoelastic or otherwise, can be studied under uniaxial and biaxial tension or compression, pure shear, hydrostatic pressure, bending, twisting, and a combination of these. Clearly, the determination of constitutive behaviour of single cells will contribute significantly to the growing field of single-cell mechanics. Here the constitutive behaviour includes multiaxial stress–strain relations and changes of mechanical properties with time and/or in response to biochemical or electrical stimulus.

EXPERIMENTAL METHODS FOR MECHANICALLY PROBING CELLS

Experimental techniques developed over the years to study the mechanical behaviour of cells can be broadly classified into three types (Fig. 2): first, local probes in which a portion of the cell is deformed (type A); second, mechanical loading of an entire cell (type B); and third, simultaneous mechanical stressing of a population of cells (type C).

Atomic force microscopy (AFM) and magnetic twisting cytometry (MTC) are common methods of type A. In AFM, a sharp tip at the free end of a flexible cantilever generates a local deformation on the cell surface (Fig. 2a)[16]. The resulting deflection of the cantilever tip can be calibrated to estimate the applied force. In MTC, magnetic beads with functionalized surfaces are attached to a cell and a magnetic field imposes a twisting moment on the beads, thereby deforming a portion of the cell (Fig. 2b)[21]. An appropriate analysis of deformation provides the elastic or viscoelastic properties of the cellular component.

In the micropipette aspiration experiment of type B, a cell is deformed by applying suction through a micropipette placed on the surface of the cell (Fig. 2c). By recording geometry changes of the cell, the elastic response of the cell is inferred, usually by ignoring friction between the cell membrane and micropipette walls[22]. Another type B method involves optical tweezers or a laser trap, in which an attraction force is created between a dielectric bead of high refractive index and a laser beam, pulling the bead towards the focal point of the trap[23]. In one adaptation of the optical tweezers to deform a single cell, a trap is used with two microbeads (typically 1 μm to several micrometres in diameter) attached to the opposite ends of a cell[24] (Fig. 2d). As shown later, the techniques illustrated in Fig. 2a–d can also be suitably modified to probe the mechanical response of single biomolecules.

Shear-flow methods (Fig. 2e) and stretching devices (Fig. 2f) are type C methods used to study the mechanical response of an entire population of 10^2–10^4 cells. Shear-flow experiments are usually conducted with either a cone-and-plate viscometer, consisting of a stationary flat plate and a rotating inverted cone with which laminar and turbulent flows can be applied, or a parallel-plate flow chamber in which cells are subjected to laminar flow[25]. In both cases the shear stress applied to cells can be readily quantified. Different uniaxial, biaxial and pressure-controlled elastic-membrane stretching devices have also been used to deform cells[26–28]. In one adaptation, cells are cultured on a thin-sheet polymer substrate, such as silicone, which is coated with ECM molecules for cell adhesion. The substrate is then mechanically deformed while maintaining the cell's viability *in vitro*. In this manner, the effects of mechanical loading on cell morphology, phenotype and injury can be examined. Furthermore, by systematically altering the mechanical properties of the substrate material through, for example, changing the degree of crosslink in the polymeric gel, the individual and collective interactions of the cells with the substrate can be studied[20]. Such studies have been performed to investigate the propensity for migration of a group of cells towards or away from the region of localized tension or compression in the substrate. By using elastic micropatterned substrates, the relationship between force applied by the cell to the substrate and the assembly of focal adhesions can be investigated. The contractile forces generated by cells during locomotion and mitosis have also been measured with a deformable-substrate method[30,31].

As an alternative approach, attempts have been made to quantify cellular forces using microfabricated or MEMS (microelectromechanical systems) devices, such as that based on a cluster of microneedles[32] (Fig. 3a, b) or a cantilever beam[33] (Fig. 3c). It might also be possible to apply a pointwise mechanical force or deformation in a controlled fashion by using a MEMS device (Fig. 3d). However, it is still difficult to quantify accurately the forces generated by cells or the distribution of forces between various subcellular structures inside a cell. The reason is that a significant portion of forces is supported as well as generated by the cell cytoskeleton; however, cells are active and the cytoskeletal structures are dynamic — they could undergo remodelling or re-organization in response to mechanical perturbations. This raises a fundamental paradox: how can we measure the mechanical behaviour of living cells if they react to our measurement tools? We need to study systematically how such reactions depend on the type, history and intensity of the associated mechanical forces or deformations, and how quickly (or slowly) the specific cellular property of interest changes after mechanical perturbation. Further, owing to the dynamic nature and complex geometry of living cells, even for the same cell type studied under similar conditions, the measurement of the mechanical behaviour of individual cells can give rise to different results depending on cell morphology, the stage in the cell cycle and the response of different subcellular structures to mechanical perturbation. These issues are fundamental to the study of the mechanics of cells.

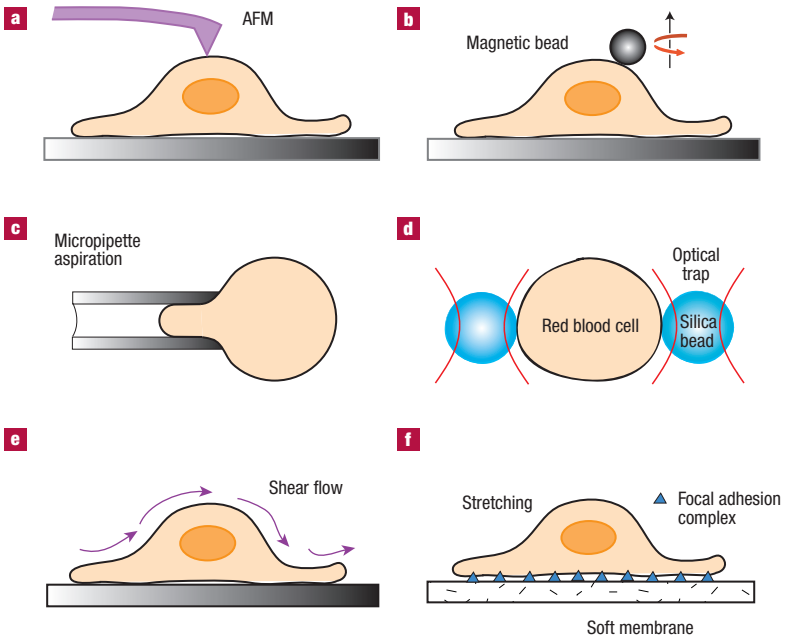

Figure 2 Schematic representation of the three types of experimental technique used to probe living cells. a, **b**, Atomic force microscopy (AFM) (**a**) and magnetic twisting cytometry (MTC) (**b**) are type A methods that can probe cell components at a force resolution of 10^{-10} and 10^{-12} N, respectively, and a displacement resolution of at least 1 nm. **c**, **d**, Micropipette aspiration (MA) (**c**) and optical trapping (**d**) are type B techniques that can deform an entire cell at a force resolution of 10^{-10} and 10^{-11} N, respectively. **e**, **f**, Shear flow (**e**) and substrate stretching (**f**) methods are capable of evaluating the mechanical response of a population of cells.

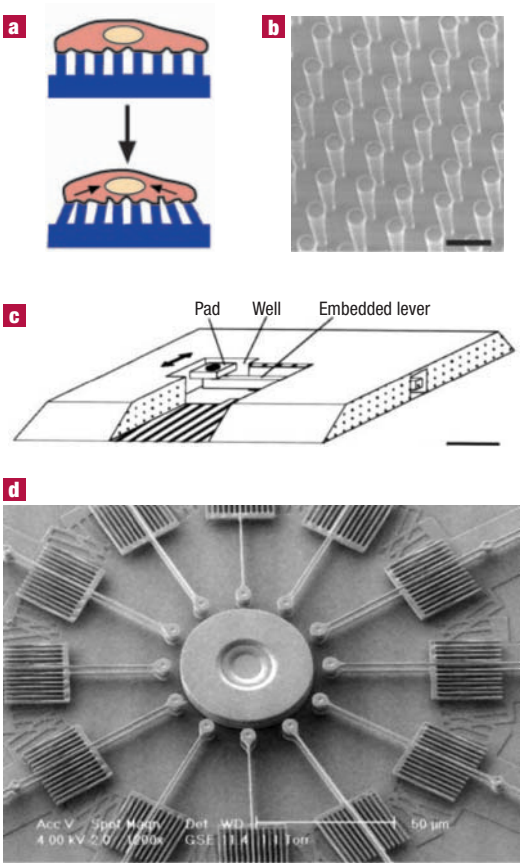

Figure 3 Microfabricated and MEMS devices for cell mechanics measurement. **a**, **b**, With proper attachment (**a**), the mechanical interactions between cells and their underlying substrates can be measured by using microfabricated arrays of elastomeric, microneedle-like posts (**b**) (from ref. 33). Scale bar, 10 μm. **c**, A cut-away view of a micromachined device consisting of a pad, a cantilever and a well. It has been used to measure the traction forces generated by fibroblasts (from ref. 32). Scale bar, 10 μm. **d**, A microfabricated MEMS device consisting of multiple passive and active cantilever beams for measuring the forces generated by a cell at different locations, and for applying a localized shear force to a single cell that would 'sit' on the cluster of circle pads at the ends of the cantilever beams. The shear displacement of the active cantilever beam is generated by an electrostatic actuator.

CELLULAR STRUCTURES THAT SENSE, GENERATE AND SUPPORT FORCES

Most living cells sense, support or generate forces. For example, skeletal, heart and smooth muscle cells generate contractile forces on excitation, performing many essential functions of the body[1]. Endothelial cells can recognize the magnitude, mode (steady or pulsatile), type (laminar or turbulent) and duration of applied shear flow[34,35], and respond accordingly, maintaining healthy endothelium or leading to vascular diseases including thrombosis and atherosclerosis[36]. Fibroblast cells 'crawl' like an inchworm by pulling the cell body forward using contractile forces[11]. These and many other examples indicate that the mechanical functions of living cells are closely related to human health and diseases. It is not well understood how cells sense mechanical forces and deformation and convert such signals to biological responses[14]. Further, living cells are different from typical engineering materials in that cells are dynamic and their structures can change in response to mechanical load. This raises many fundamental questions essential to cell mechanics: How do forces applied to a cell, either directly or through the focal adhesion complex, induce reorganization of the cytoskeleton, thus changing its mechanical properties? How does the dynamics of the cytoskeleton affect cell spreading, rounding, crawling and adhesion? How does the interaction between ECM and focal adhesion complexes transduce a mechanical signal (force or deformation) into cells? Answering these and other questions will be crucial in understanding the structural basis of cellular function.

Modelling the constitutive behaviour of cells poses a particular challenge. The deformation of some relatively simple types of cell, such as the human red blood cell without a nucleus, has traditionally been studied by using continuum models[37]. However, for most living cells, deformation models require proper accounting for internal structure, spatial granularity, heterogeneity and the active features unique to cells. For example, the shape, motility and mechanical properties of tissue cells depend largely on cell cytoskeleton[38], which is a dynamic system undergoing structural changes during cell spreading and rounding or due to mechanical or chemical signals[39–41]. The changes in the microtubule and actin filament systems of a fibroblast cell during cell spreading are illustrated in Fig. 4a. Different actin assemblies, including stress fibres, cell cortex and filopodium, form an active structure of the cell, generating forces through the actin–myosin complex and facilitating the contractility of the cell[1]. Essential to many cellular functions, this active apparatus of the cell generates, supports and responds to mechanical forces. Continuum-based models[42] might therefore be incapable of capturing the dynamic structural changes that occur inside the cell. The cytoskeleton might also have a signalling function by providing mechanical linkages from the ECM to the cell membrane to the nucleus[43,44], as well as a structural basis for mechanical force balance[45,46], thus having a critical role in force sensing and mechanochemical transduction[40]. In addition to cell cytoskeleton, the spatial granularity and heterogeneity of cells, including cell nucleus, mitochondria, endoplasmic reticulum and Golgi in the cytoplasm, complicates the mechanical behaviour of the cell.

Studies of whole-cell mechanical properties, influenced by cytoskeleton and viscous cytoplasmic fluid, should include the mechanics of subcellular structures: stress fibres, filopodium, microtubule and actin filament networks[47], and focal adhesion complexes (FACs) formed mainly by integrin, talin, vinculin, α-actinin, paxillin and tensin[40]. FACs provide a mechanical linkage and relay signals between the cytoskeleton and ECM[48]. Usually attached to the FACs, stress fibres are bundles of actin filaments with myosin; they are components of the cytoskeleton and generate contractile forces during cell crawling, thus serving as a mechanical actuator. Stress fibres are very dynamic: they form in response to tension generated in cells and are disassembled at mitosis when the cell rounds up and loses its attachment to the substratum. Figure 4b,c illustrates the marked changes in spatial arrangement of stress fibres in endothelial cells after 3 hours of uniaxial stretching.

Taken together, the examples shown in Fig. 4 indicate the two important features of the structural dynamics of cells. The first is that cells change their structures actively as part of normal biological function, including cell division, crawling, spreading, rounding and actin-based motility[49]. Although an applied force might not be involved in these processes, the force generation and internal force balance between cell cytoskeleton and ECM, between different cytoskeletal

filament networks, or even within a single cytoskeletal system such as microtubules have a crucial role and merit more systematic studies. The second is the structural alterations and remodelling of cell cytoskeleton in response to mechanical forces or chemical stimuli, which might be essential in mechanosensing, signal transduction and the adaptation to a mechanically or chemically stressful environment. Although both features described above involve signalling, force generation and balance, the polymerization and depolymerization of filament systems, and reorganization, the second feature is particularly important for the measurement of mechanical behaviour of cells, as mentioned earlier. However, this might give engineers and biologists a mechanical means with which to probe as well as control the cellular responses and functions.

THE NEED FOR MOLECULAR BIOMECHANICS

The mechanics of biological molecules, including proteins and nucleic acids, is crucial to understanding the issues in cellular and subcellular mechanics in the preceding sections. Such issues include the manner in which cells sense mechanical forces or deformation and transduce mechanical signals into alterations in biological processes such as cell growth, differentiation and movement, and protein secretion. Still in its infancy, this emerging field of mechanics strives to investigate issues such as, first, how the structural and mechanical properties of DNA (deoxyribonucleic acid), RNA (ribonucleic acid) and proteins under stretching, twisting, bending and shearing conditions affect DNA–protein, RNA–protein and protein–protein interactions, and, second, what function the deformation of proteins and nucleic acids has in DNA condensation, gene replication, transcription and regulation. Further, mechanochemical coupling in enzymes as nanomachines, the use of DNA and proteins as a component of nanosystems and the attendant interface considerations inevitably require an understanding of the deformation and mechanics of biomolecules[50].

DNA MECHANICS

A typical DNA molecule (Fig. 5a) is a right-handed double helix with about 10 base pairs per helical turn, which has a pitch of 3.4 nm (ref. 1). Arranged in hereditary units of genes, the DNA contains all the information necessary to construct cells and tissues. In a cell nucleus, enzymes constantly pull, twist and bend DNA molecules in order to transmit and express genetic information. The bending and twisting rigidities of DNA affect how it wraps around histones to form chromosomes, supercoils during replication, bends upon interactions with proteins, and packs into the confined space within a virus[51–53]. During the past decade, single-molecule biomechanics experiments of DNA under bending, stretching, twisting and shearing conditions have revealed many intriguing features of the deformation and dynamics of DNA. The development of such models as the worm-like-chain (WLC) model[54] have helped to establish the theory of entropic elasticity of DNA.

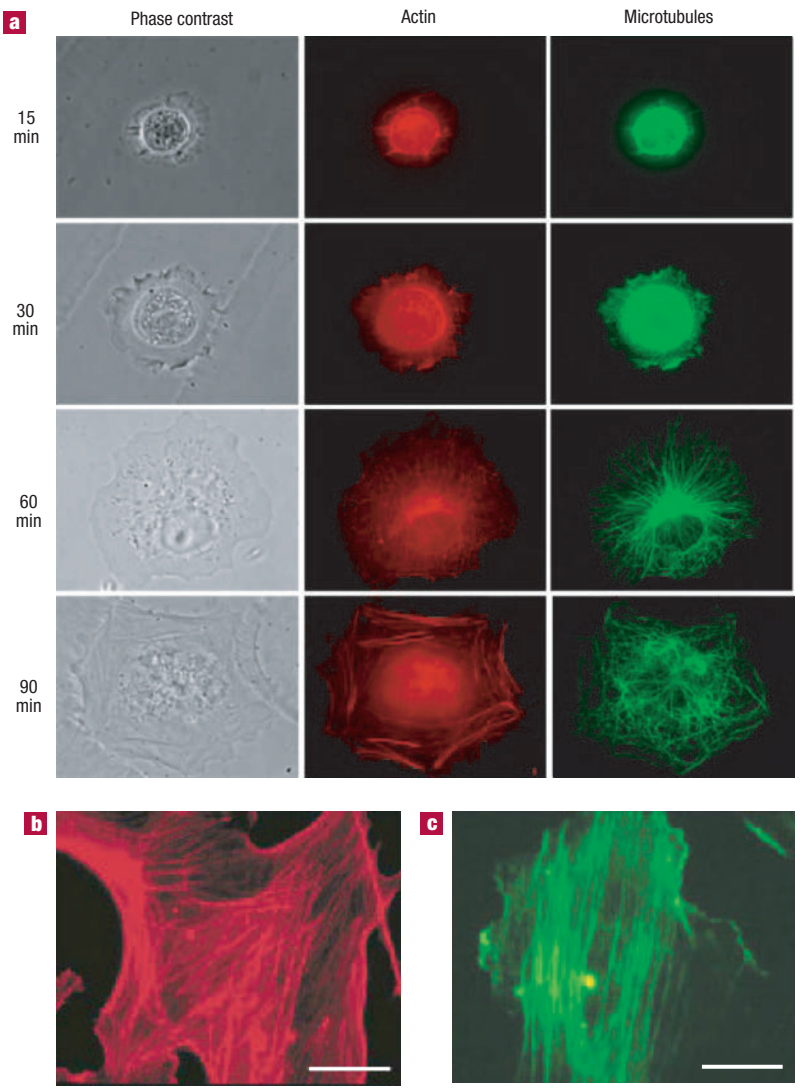

Figure 4 Cytoskeleton dynamics in living cells, as illustrated by changes in the microtubule and actin filament network during cell spreading and the rearrangement of stress fibres after cyclic stretching. **a**, When a rounded mouse primary fibroblast cell was placed on a glass surface, its spreading was accompanied by marked changes in both microtubules (green, right panels) and actin filaments (red, middle panels) as recorded after 15, 30, 60 and 90 min. The phase-contrast image of the same cell is shown as a reference (left panels). Reproduced from http://cellmotility.genebee.msu.ru/html/video.htm. **b**, Unstretched human aortic endothelial cells have dense, randomly oriented stress fibres. **c**, After 3 hours of pure uniaxial stretching, the stress fibres in endothelial cells are rearranged and oriented nearly perpendicular to the stretching direction (reproduced from ref. 27). Scale bar, 15 μm.

The material parameter controlling DNA elasticity is its flexural persistence length ξ, which is defined as the distance over which two segments of a DNA chain remain directionally correlated. In a physiological buffer at 25 °C, $\xi \approx 50$ nm for double-stranded DNA (dsDNA) and $\xi \approx 1$ nm for single-stranded DNA (ssDNA)[5]. The bending rigidity κ of a DNA molecule is given by $\kappa = \xi k_B T$ (ref. 55), where k_B is Boltzmann's constant and T is temperature. Linear elasticity theory implies that the

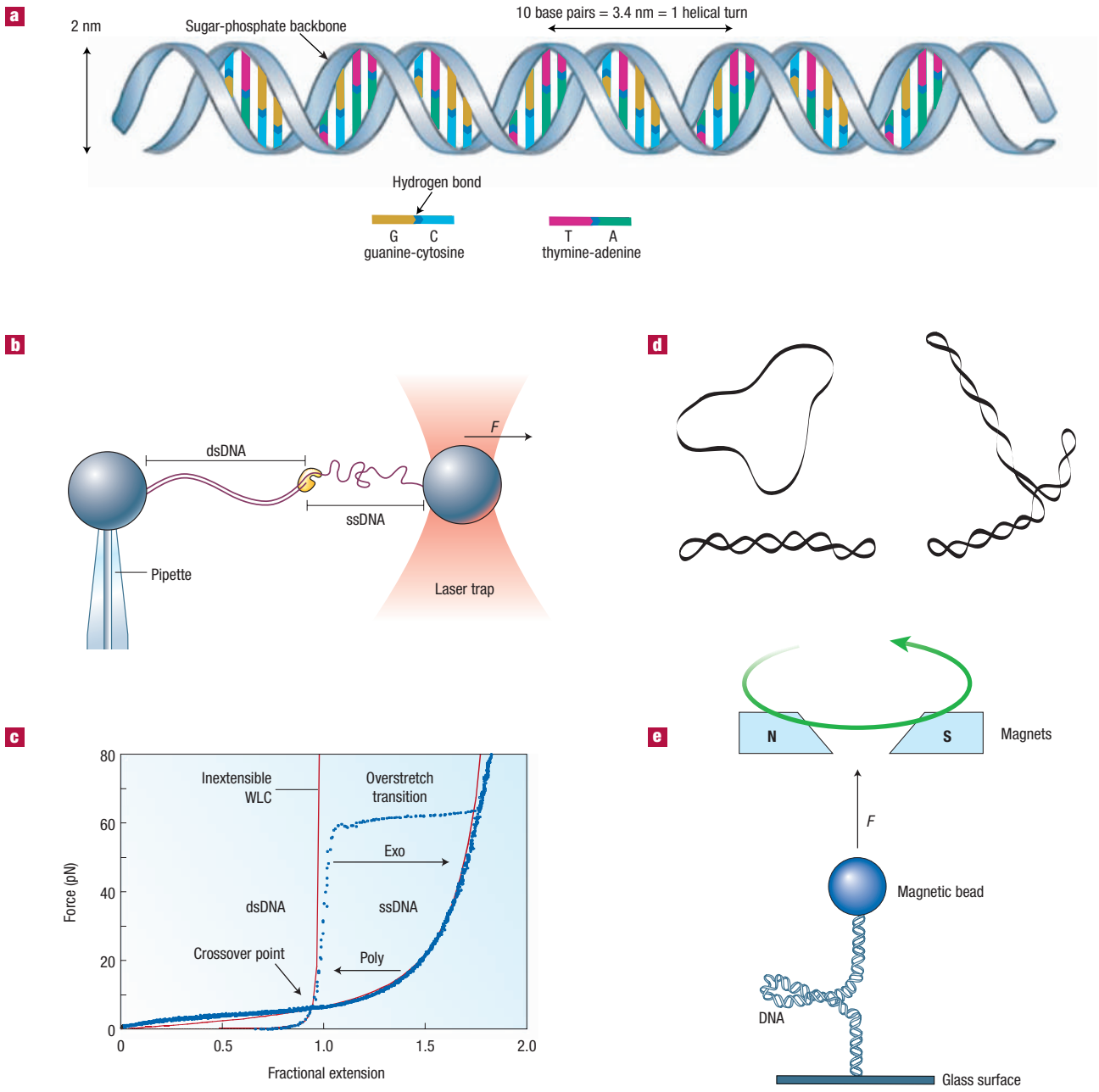

Figure 5 DNA and its elastic behaviour under stretching and twisting. a, A double-helical DNA is a linear molecule formed by Watson–Crick base-pairing (G-C and A-T), 2 nm in thickness and ~0.34 nm per base pair. **b**, A DNA molecule is being stretched between beads held in a micropipette and a force-measuring optical trap. The measured extension is the sum of contributions from the single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) segments. **c**, Force–extension curves for dsDNA and ssDNA molecules. Arrows show changes in extension observed at constant tension during polymerization (poly) or force-induced exonuclease activity (exo). A comparison between the measurements and prediction using the WLC model (red curves) is also shown. **d**, A relaxed circular DNA is supercoiled. **e**, The effect of tension on the supercoiling of DNA is studied with controlled stretch and twist by using a magnetic device (**b**, **c** and **e** are reproduced from ref. 5).

energy ΔG required to bend a short segment of DNA through an angle $\Delta\theta$ is $\Delta G = k_B T(\Delta\theta)^2(\xi/2L)$ (ref. 5), where L is the contour length ($L < \xi$) of DNA. This suggests, first, that it is energetically unfavourable to bend DNA into small loops or sharp turns, and, second, that in wrapping DNA around a histone ~11 nm in diameter at 37 °C, proteins must convert ~18 kcal mol^{-1} of their binding energy into mechanical work.

The bending stiffness of DNA has a crucial role in protein–DNA interactions during DNA packaging or

recombination and gene transcription[56]. However, mechanisms responsible for the stiffness of DNA remain elusive. Mutual repulsions between phosphate groups might give rise to DNA rigidity by resisting deformed conformations, and the tendency to maximize base stacking might lead to resistance to DNA bending. Consequently, the local bending stiffness of a short segment of dsDNA is dependent on the base sequence[57]. Clearly, quantifying the relative contributions of base stacking and electrostatic repulsion to DNA stiffness is an important research area in DNA mechanics[58].

Entropic elasticity is the tendency of a chain to bend and shorten owing to thermal forces. The lowest enthalpy of a DNA chain is at an entirely extended state, that is, no curves, where $\Delta H = k_B T(\Delta\theta)^2(\xi/2L)$ and $\Delta\theta = 0$. However, the lowest free-energy ΔG of a DNA chain in a thermal bath must include entropy from various bent configurations of that chain. For long molecules in which $L >> \xi$, the chain has many bent states and is virtually always crumpled in solution. Such behaviour can be approximated by the 'freely jointed chain' (FJC) model. Here the continuum rod-like nature of DNA is made discrete (coarse-grained) into unbendable straight links, which are connected by perfectly flexible joints. If each link is given a length 2ξ, then the average dimensions of a DNA molecule (such as its end-to-end distance) are recovered as those of a three-dimensional random walk of connected links. When a coiled DNA molecule is stretched (Fig. 5b) by an external force f acting to align the FJC in solution, the chain's mean extension x/L becomes that of a dipole aligned in an external field, i.e. the Langevin function[59]

$$\frac{x}{L} = \left[\coth\frac{2f\xi}{k_B T} - \frac{k_B T}{2f\xi}\right] \quad (1)$$

However, single-molecule experiments[54] have revealed that equation (1) fails to describe DNA behaviour, especially at forces $f > k_B T/\xi$ with moderately high extensions ($x/L > 1/2$). This result is perhaps not surprising, because dsDNA contains no free joints. A more subtle analysis must be made to count all possible curved states of a smooth-bending rod in solution, that is, of a WLC (Fig. 5c). The partition function of such a chain then includes bending energy for different states, as well as end-to-end contraction against an external force. Such a model has been solved numerically[60]. A handy approximation to the exact solution is given by

$$\frac{f\xi}{k_B T} = \frac{x}{L} + \frac{1}{4(1 - x/L)^2} - \frac{1}{4} \quad (2)$$

Note that equations (1) and (2) converge to the same result for low extensions ($x/L << 1$), that is, $f = (k_B T/\xi)(3x/2L)$, but that they behave differently when the force becomes high and the residual contraction $(1 - x/L)$ becomes small. Then the force goes as $(1 - x/L)^{-1}$ for the FJC but as $(1 - x/L)^{-2}$ for the WLC (and for real dsDNA). The infinitely large force f as $x/L = 1$ in equations (1) and (2) is a result of the inextensible assumption in both models.

For a DNA molecule in a canonical B-form under applied forces of 12 pN < f < 65 pN, the contour length L increases and the resistance to stretching can be characterized by a stretch modulus S. For λ-phage DNA in a solution containing 150 nM Na^+, $S \approx 1{,}000$ pN (ref. 61). Finally, when $f \rightarrow$ ~65 pN, a dsDNA can undergo a phase transition from B-form DNA (0.34 nm per base pair) to S-form DNA (0.58 nm per base pair), with a marked increase in length similar to metal yielding[62,63].

Supercoiling of linear or circular DNA (Fig. 5d) is another important deformation mode that is usually described by the linking number, namely the sum of the number of helical turns along the molecule, and the coiling of the DNA axis about itself[64]. DNA supercoiling, together with long-range transmission of torsional strain, might have a crucial function in gene replication and transcription and in recombination[65]. When transcription begins, the formation of the RNA polymerase–promoter complex unwinds the DNA, resulting in negative supercoils[66]. Although different promoters have different sensitivities to supercoiling, in general the rate of gene expression can depend on the degree of supercoiling[67]: increased (decreased) negative supercoiling leads to higher (lower) gene expression rates. To understand the supercoiling of DNA better, studies of single linear dsDNA molecules under twist were performed with an applied tension[68,69]. When DNA is stretched and twisted by magnetic tweezers (Fig. 5e), it was found that for $f > 0.3$ pN with negative supercoiling there was a coexistence of B-DNA and

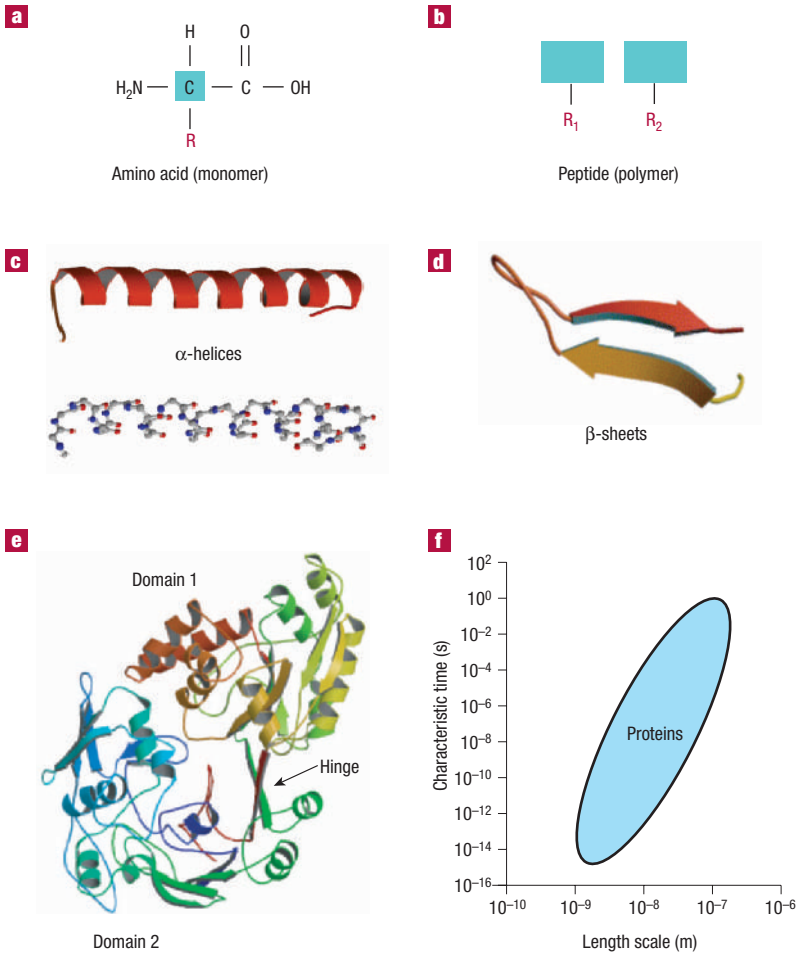

Figure 6 Basic structural features, characteristic length and time scales and typical force ranges of proteins. a, Almost all protein structures are constructed from 20 different amino acids. A monomer unit of an amino acid is shown with the side-chain unit labelled as R. **b**, A short peptide comprising two different amino acids for which there are 20^2, or 400, possible sequences. **c**, **d**, α-Helices (**c**) and β-sheets (**d**) form a protein's secondary structure. **e**, The secondary structures are folded into globular domains that form the three-dimensional structure of a protein. **f**, The characteristic time scales associated with protein motion and deformation span some 15 orders of magnitude, from 1 femtosecond to a few seconds.

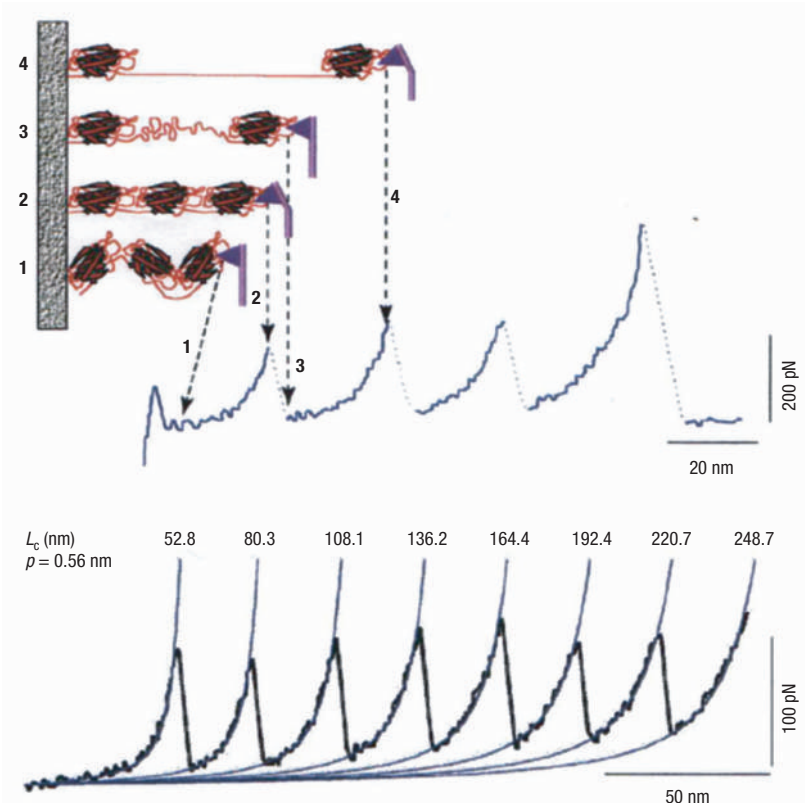

Figure 7 Domain deformation and unfolding of a multidomain protein under stretching with AFM. The saw-tooth pattern of peaks that is observed when force is applied to extend the protein corresponds to a sequential deformation and unfolding of individual domains. When the distance between substrate and cantilever increases (from state 1 to state 2), the protein elongates, generating a restoring force that bends the cantilever. When a domain unfolds (state 3), the free length of the protein increases, reducing the force on the cantilever to almost zero. Further extension again results in force on the cantilever (state 4). The consecutive domain deformation of the molecule (recombinant human tenascin-C) obeys the WLC model, with the persistence length p fixed at 0.56 nm and the contour length L_c for each peak adjusted as shown. (From ref. 100.)

denatured DNA. For positively supercoiled DNA with $f \sim 3$ pN, the coexistence of B-DNA and a Pauling-like, highly twisted structure was discovered[69]. Evidently, twisting DNA leads to shortening due to torsional buckling instability, analogous to the buckling of a coiled rubber tube, whereas unwinding causes local denaturation when the applied stretching force is small.

To conduct gene transcription, replication and DNA condensation, cells rely on many motor proteins, including RNA polymerase, DNA polymerase and topoisomerases. *Escherichia coli* RNA polymerase, for example, performs the synthesis of an RNA copy of the template DNA by progressing along it at speeds more than 10 nucleotides per second. T7 DNA polymerase, in contrast, catalyses DNA replication at rates greater than 100 bases per second. It has been discovered over the past few years that forces applied to enzymes can affect DNA replication and transcription in cells: at saturating nucleotide triphosphate concentrations, *E. coli* RNA polymerases stall at applied forces of 14–25 pN (ref. 70), and the transcription velocity decreases with applied force[71]. Further, torsion in DNA molecules seems to influence the relaxation of DNA supercoils by topoisomerase molecules[72], indicating that force in DNA can alter DNA–protein interactions during gene expression and transmission.

PROTEIN MECHANICS

Proteins perform a diverse array of tasks in living cells, including signal transduction, metabolic and catalytic functions, and mechanical support. Proteins are formed by an assortment of 20 different amino acids (Fig. 6a) arranged in a specific polypeptide sequence (primary structure, Fig. 6b). Hydrogen bonding leads to the formation of secondary structures of a protein, including α-helices and β-sheets (Fig. 6c,d) which, together with polypeptide loops, fold into globular domains owing to short-range forces such as hydrophobic and electrostatic interactions, van der Waals force and hydrogen bonding. A native, folded protein typically consists of multiple domains (Fig. 6e) and has a size range of 1–100 nm. Proteins in a cell undergo constant motion and structural changes, including large-scale (~5–50 Å) movements of domains as well as small-scale (~0.5 Å) random movements of secondary structures or domains, or 'breathing'. The timescales of protein conformational dynamics can span many orders of magnitude, ranging from one femtosecond to a few seconds (Fig. 6f)[73].

The unique three-dimensional structure (that is, the conformation) of a protein determines its function. However, proteins in a cell are deformable and can assume different (altered) conformations under physical forces[74]. Just as proteins can transform from a native or biologically active state to a denatured or inactive state in response to small changes in temperature or pH of the surroundings[64], the application of mechanical forces can lead to protein domain deformation and unfolding[75–78] (Fig. 7). Further, driven by brownian force, mechanical load or chemical reactions, protein molecules can also undergo domain hinge motion in cells[79], which can have significant biological implications. The motor molecule myosin 'moves' an actin filament by generating hinge motion of its head, whereas the rotation of the γ-subunit of the F_1-ATPase might be driven by the hinge motion of the β-subunit[80]. Some proteins can use hinge motion to transduce signals, regulate interactions and facilitate enzymatic activities.

Protein deformation, or conformational change due to mechanical force, can affect protein–protein and protein–DNA recognition, binding and unbinding, causing changes in downstream biochemical processes that control cellular behaviour. The underlying reason is that the forces that control molecular interactions including electrostatic double-layer force, van der Waals force, 'steric' repulsion forces, hydrogen bonding and hydrophobic interactions operate mainly within short ranges[81]. The three-dimensional geometry local to the binding pocket of a receptor–ligand pair or the protein–DNA binding site therefore contributes significantly to the characteristics of their binding. Good conformational matches usually lead to strong and long-lasting bonds. However, the conformational match at the binding site can change when the protein domains are deformed or unfolded under mechanical forces[82]. In certain cases, an applied force can alter the affinity and lifetime of a receptor–ligand pair[83,84]. In some other cases, only protein deformation can expose (or bury) the binding site, thus switching between the 'on' and 'off' states of protein, as illustrated by the extension and unfolding of fibronectin[85]. Protein deformation is therefore an important concept in molecular biomechanics[14,50].

ENGINEERING NANOSCALE BIOLOGICAL SYSTEMS WITH MECHANICS

Nanotechnology has facilitated the development of nanodevices using, or powered by, biomolecular machines. The use of current technologies of batteries, electromagnetic motors or hydraulic energy sources does not provide appealing possibilities for powering functionalized nanoscale inorganic machines with moving parts, especially when the nanodevices are used in the human body. In contrast, progress in molecular biology has revealed intriguing features of the structures, mechanisms and functions of many biomolecular motors, including kinesin, myosin, dynein, ATP synthase, DNA polymerase and RNA polymerase. Specifically, kinesin is a linear motor with two heads that transports vesicles along microtubules over a distance of several micrometres. A force of the order of 5–7 pN is needed to block the motion of kinesin[86]. Myosin is also a double-headed linear motor that produces sliding between actin and myosin thick filament in muscle. ATP synthase is a special enzyme that can either pump protons across a membrane by using ATP hydrolysis, or manufacture ATP from ADP and phosphate by using the energy derived from a transmembrane protonmotive gradient. These biomotors directly convert chemical energy derived from ATP hydrolysis into mechanical force or motion, with extremely high efficiency. With their nanometre size they have the potential for use in multifunctional, self-powered nanosystems.

Figure 8 shows an ATP synthase consisting of a transmembrane component, F_0, and a soluble component, F_1-ATPase, bearing catalytic sites for ATP hydrolysis and synthesis that are coupled with the proton flow in the F_0 domain through a shaft consisting of γ, δ and ε subunits[87]. The γ subunit is surrounded by three α and three β subunits, arranged alternately (Fig. 8a). Sequential ATP hydrolysis on the three β subunits induces rotation of the γ subunit through hinge motion. When ATP is supplied, the F_1-ATPase rotates 120° stepwise, with one ATP molecule hydrolysed per step[88]; it can rotate at ~130 r.p.s. at saturating ATP concentration without load. The torque generated by the F_1-ATPase motor is ~20–40 pN nm (refs 89, 90). As perhaps the world's smallest rotary engine, ATP synthase is fully reversible, with an energy efficiency of almost 100%.

Recently, efforts have been made to explore engineering applications of F_1-ATPase by positioning the rotary motor on a nickel post through histidine tags and attaching a nickel rod 750–1,400 nm long to the γ-subunit of F_1-ATPase[90] (Fig. 8b). To observe the rotational motion of the motor, actin filaments 500–3,400 nm long were similarly attached to F_1-ATPases[91] (Fig. 8b). Although such approaches are potentially useful, it may be difficult to use nickel rods or actin filaments to interface with other nanosystems. There is evidently a critical need to develop strategies to interface biomolecular motors with other nanodevices and to realize linear and rotational motion in a controlled, reversible fashion.

DNA-based nanostructures are another class of engineered biosystems that involve mechanics. For example, DNA stem–loop hairpin probes such as molecular beacons transduce the recognition of DNA or RNA targets into an optical, electrical or magnetic signal on the basis of the ~50-fold increase in bending rigidity between ssDNA (probe alone) and a double-stranded probe–target duplex[92,93]. These probes have been used in a range of applications from quantitative polymerase chain reaction[94] to the visualization of RNA expression in living cells[95]. The high specificity of Watson–Crick base pairing also permits the generation of nanostructures such as DNA arrays and nanomachines that, when combined with other biomolecules or microelectronic components, can be used to perform chemical synthesis, signal transduction, and computation[96–98].

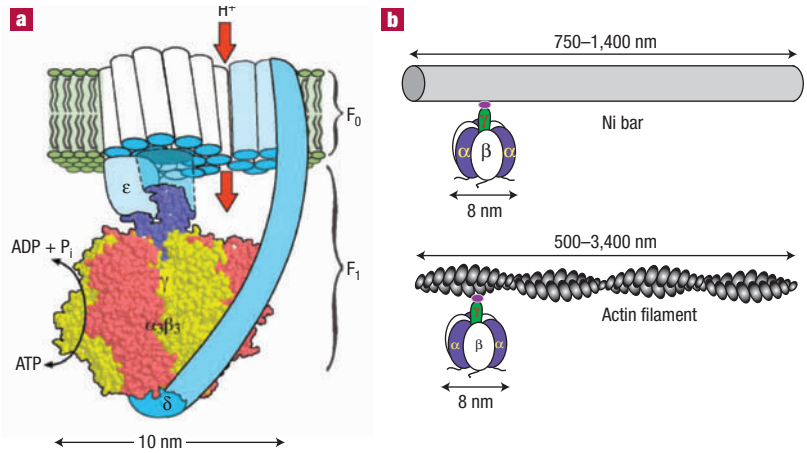

Figure 8 The molecular motor ATP synthase. a, The structure of ATP synthase (based on ref. 80). It has a transmembrane portion, F_0, and a soluble component, F_1, which contains catalytic sites located at the αβ interfaces. **b**, Current approaches of converting the F_1-ATPase into a rotational device by using actin filaments and nickel rods. In both cases the attached component (the actin filament of the nickel rod) is too large in the lateral direction, causing instabilities.

CLOSING REMARKS

In a manner analogous to studies of mechanics of engineering materials, cell and molecular mechanics of biological materials seeks to establish essential linkages between structure, mechanical properties and functions. Although methods have been developed to measure cell responses during deformation, cell adhesion, locomotion and mitosis, reliable experimental tools are currently unavailable for quantifying the distribution of mechanical forces between various subcellular structures as well as on individual proteins and nucleic acids inside a cell.

Advances in single-cell and molecule biomechanics have resulted in instrumentation (Figs 2 and 5) capable of measuring small forces. Despite this progress, experimental probing of single proteins is severely limited by at least three factors. First, it is difficult to image the deformation of protein molecules during active deformation because the geometric changes range only from a few ångströms to tens of nanometres. Advances in protein mechanics therefore require further progress in bioimaging technology. Second, it is difficult to hold, position and manipulate single protein molecules by conventional means. As a result, attaching them to devices that impose force or deformation in a controlled manner is a major experimental challenge. Rapid advances in micro-electromechanical and nano-electromechanical systems (MEMS and NEMS, respectively) offer opportunities for further progress[99]. Last, the characteristic time for motion and relaxation

of proteins and nucleic acids, which spans the wide range of picoseconds to seconds, may render experimental studies of the underlying deformation mechanisms a rather difficult task.

Despite the foregoing challenges, advances in experimental techniques to probe cellular and molecular mechanics have outpaced mechanistic interpretations and theoretical modelling efforts. Several possible reasons can be attributed to this trend. First, unlike engineering materials with characteristic microstructural length scales typically above 1 μm, subcellular and molecular structures of biomaterials are not amenable to characterization through continuum formulations because of small dimensions, mechanochemical coupling, and difficulties in ascribing local stresses and strains to different structural units of cells and biomolecules. Second, many mechanical functions of the cell are derived from and controlled by the cytoskeleton, which serves as an intracellular scaffold that supports movement and force and provides a physical basis for direct mechanosensing. Thus, simply lumping the specific cytoskeletal structures that bear, generate and sense forces into a structureless continuum or treating them merely as load-carrying structural members is usually inadequate for the analysis of living cells. One difficulty is that a significant portion of forces is supported as well as generated by the cell cytoskeleton, and major cytoskeleton components undergo simultaneous polymerization and depolymerization. This poses difficulties in developing a mechanistic model of the whole cell. Third, protein interactions and diffusion in the cytoplasm, protein–DNA interactions and energy conversion processes in cells are all known to be influenced by the brownian motion of biomolecules. The forces associated with brownian motion, as well as stochastic processes and non-equilibrium thermodynamics, should be accounted for in the overall interpretation of the mechanical response. Last, molecular dynamics simulations of protein binding and unbinding, deformation and unfolding, and protein–DNA interactions offer appealing prospects for studying the connection between structure and mechanical response. Despite advances in computational biology, such simulations are currently restricted to maximum time scales of only a few nanoseconds, which is much faster than the characteristic relaxation times of many proteins and nucleic acids. Additionally, the potential functions or force fields that describe interatomic interactions and binding energies might not be applicable to many biomolecular interactions.

In summary, progress in experimental and computational biology and biomechanics during the past decade has provided unprecedented opportunities to probe the mechanical responses of cells, proteins and DNA molecules. How the forces and deformations associated with these basic structural units of life can be influenced and engineered by chemistry is a topic of substantial scientific excitement and opportunity.

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Competing financial interests

The authors declare that they have no competing financial interests.