TIMELINE

Biological machines: from mills to molecules

Marco Piccolino

Although scientific progress is usually represented as being linear, it may, in fact, have a cyclical character — some discoveries may be forgotten or lost (at least temporarily), and themes may reappear through the centuries. Consider, for example, the concept of 'molecular machines', from the exciting phase of research that flourished in the seventeenth century, to the idea of machines that is at centre stage in modern cell biology.

More than three centuries ago, the birth of modern life sciences was marked by the idea that body function is based on organic machines whose performance can be explained by similar laws to those operating in man-made machines. In the seventeenth century, this concept was used not only to explain functions that obviously reflected those of mechanical devices (such as skeletal and articular motion or the action of muscles), but also for other operations — digestion, sensation, fermentation and production of blood, for example[1–3]. To account for these more delicate operations of animal economy, body machines were thought to involve tiny components that could escape detection by the naked eye. This view derived, in part, from a recurrence of the physicists' view that the Universe is composed of atoms. In Greek classical science this view was advocated by Democritus, and in the seventeenth century by the French philosopher and scientist Pierre Gassendi. As Marcello Malpighi (FIG. 1), one of the greatest seventeenth-century life scientists[1,2], put it[4]:

Figure 1 | **Marcello Malpighi** (from the *Opera Postuma*, 1798 Venetian in Folio edition). Malpighi, a prominent scientist in the seventeenth century, was one of the first to attribute body function to an organized series of minute 'organic machines'. The concept underlying his metaphor of the 'angel and the mill' prompted anatomical investigations, which laid the foundation for modern microscopic anatomy. (Image courtesy of the library *G. Romiti* of the Anatomical Institute of the University of Pisa.)

> "Nature, in order to carry out the marvellous 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 marvellous organ, the composition of which are usually invisible to the naked eye, without the aid of the microscope."

The rise of machines

Until the sixteenth and seventeenth centuries, most progress in the life sciences and medicine elaborated on classical doctrines, dating from Hippocrates, Aristotle and Galen (BOX 1, overleaf). But in the seventeenth century, interest in experimental studies exploded because, as had happened in astronomy and physics, new investigations cast doubt on the infallibility of the Ancients. In particular, the discovery of blood circulation, published in 1628 by William Harvey[5] — and the subject of some debate at the moment[6–8] — questioned the very foundation of classical physiology on which the whole body structure was interpreted.

In the wave of the scientific revolution promoted by Galileo[9], a conceptual breakthrough was the idea that body function can be explained by similar physical laws to those that account for the action of artificial machines. This idea was elaborated on philosophical grounds by René Descartes[10], and developed into a scientific manifesto by investigators such as Giovanni Alfonso Borelli and Malpighi[11,12]. As a result, physiology no longer needed to depend on metaphysical theories for the interpretation of body functions. Instead, like astronomy and new physics, it could become a 'true' science — an investigation that combined experimental study with the application of the 'laws of mathematics and geometry' to body machines.

One result of this new scientific attitude was that scientists were discouraged from searching for the ultimate causes of 'vital processes'. This was vividly expressed by Malpighi, in a beautiful passage from his *Opera postuma*[4]:

> "The way our soul uses the body in operating is ineffable, yet it is certain that in the operations of growth, sensation and motion the soul is forced in conformity with the machine on which it is acting, just as a clock or a mill is moved in the same way by a pendulum or lead or stone, or by an animal or by a man; indeed if an angel moved it, he would produce the same motion with changes of positions as the animals or agents do. Hence, even though I did not know how the angel operates, if on the other hand I did know the precise structure of the mill, I would understand this motion and action, and if the mill were out of order, I would try to repair the wheels or the damage to their structure without bothering to investigate how the angel moving them operated."

To know how a machine operates, you need to know its structure. So the idea of 'organic machines' prompted anatomical investigations — both classical, descriptive macroscopic anatomy, and a new, 'subtle anatomy', based on the use of newly invented techniques (some of which were the precursors of modern histological methods). It is no surprise, then, that the basis of the modern microscopic anatomy of animals and plants emerged in the seventeenth century, owing to the work of Malpighi and many others[4,12–17]. As had happened with Galileo's astronomical observations, this new investigative attitude was not due simply to the availability of new technology, but also to the new cultural climate.

Decline and fall

The climate changed in the eighteenth century, as interest in microscopic studies dwin-

Box 1 | The four humours

Although anatomy was a part of classical medicine, it was not used to investigate body function. According to the doctrine of four humours — blood, yellow bile or choler, phlegm, and black bile or melancholy — the body and its organs were conceived as the stage where the humours interacted (depending on astronomical, atmospheric, climatic or other influences). Health and good temper resulted when humours were in correct proportions and mixing was appropriate ('to mix' in Latin is *temperare*). Conversely, diseases, or bad tempers, were produced when one humour was in excess or the mixture was inappropriate. This idea hardly favoured anatomical investigation and certainly did not promote the study of the structure of organs and tissues. Indeed, many organs, including the liver and lung, were considered to consist of effused blood (parenchyma), and so were thought to be devoid of a real internal structure. Similarly, small animals, such as insects, were thought to lack an internal structural organization.

dled. This was partly due to the apparent failure of the investigative programme, based on mechanistic explanations of body function, that had dominated the seventeenth century. Although many discoveries were made during that time, such as the structures of blood capillaries and alveoli in the lungs, the possibilities of explaining life processes on a simple, mechanical basis were limited.

So the idea of mechanical body machines was largely abandoned. Instead, interest moved towards new forces — particularly electricity which, together with the study of gas ('airs') and chemistry, took centre stage. Electricity was particularly attractive as a principle for explaining vital processes because its application could produce movements in paralytic limbs or in animal preparations. As well as muscle contraction it was easy to evoke an electrical mechanism for nerve conduction, owing to the easy and rapid propagation of electricity, which seemed to match the rapid flow of sensation along nerve fibres[18].

Sensibility and irritability

It was a conceptual advance in the second half of the eighteenth century that sowed the seeds for the modern idea of machines. This came from the idea of 'irritability', conceived by the Swiss physiologist Albrecht von Haller (FIG. 2) in 1752. On the basis of animal experiments, Haller concluded that 'sensibility' (the ability to perceive a stimulus) and 'irritability' (the ability to react to that stimulus with a contraction) were different properties of living tissue, pertaining typically to nerves (sensibility) and muscles (irritability)[19].

Haller confessed that he could not ascertain the mechanism of irritability, but suggested that it depended on an essential constituent of living tissue (the gluten). He distinguished irritability — a vital property — from elasticity, which has purely physical properties and is unrelated to vital processes. Different kinds of stimuli (chemical, mechanical or electrical) could excite muscle irritability, which was normally brought about by the action of a nerve. But Haller believed that the nerve's influence was not the real cause of the muscle contraction — instead, it acted only as a stimulating (or exciting) factor that activated the intrinsic irritability of muscles.

Haller's ideas spread over Europe, causing lively discussions and dividing physiologists into 'Hallerians' or 'anti-Hallerians'. Hallerians claimed that irritability had the same function in living matter as the Newtonian idea of gravity had in the inorganic world. In fact, Haller's reluctance to propose a mechanistic explanation for irritability paralleled Newton's aversion to proposing hypotheses about the mechanism of gravity. The point to emerge from these discussions was that an organism's response to a stimulus is not a purely physical consequence of that stimulus, but that it reflects the organism's internal organization. In other words, the response (in the case of irritability, contraction of a muscle) is what the organism is prepared — we would now say 'programmed' — to produce. The energy of the response is 'enclosed' in the organism, and does not come from the energy of the stimulus. So what really matters is the information encoded by the stimulus. In the first half of the nineteenth century, a similar idea was behind the development, by Johannes Müller, of the doctrine of 'specific nervous energy', according to which the sensation aroused by the stimulation of a sensory structure does not depend on the characteristics of the stimulus, but on the type of structure stimulated[20].

Figure 2 | **Albrecht von Haller** (from the first edition of his *Elementa physiologiae*[42]). Haller, of Swiss origin, was a leading figure in eighteenth-century physiology. He conceived the idea of 'sensibility' and 'irritability' to explain the body's reaction to stimulus. In his formulation of the concept of irritability to account for muscle contraction, he first acknowledged, although in an implicit way, the importance of information flow in biological systems. (Image courtesy of the library *G. Romiti* of the Anatomical Institute of the University of Pisa.)

Machines revisited

Haller's ideas laid the foundation for the development of another fundamental idea in the nineteenth century: that of the 'internal milieu', developed by Claude Bernard[21] in 1865. Bernard attempted to found medicine as a true science, based on the laws of physics and chemistry. He studied the characteristics of living organisms that seemed to elude physico-chemical principles, such as their relative independence of the conditions of the external environment (*milieu cosmique*). He attributed these characteristics to the organizational complexity of organisms, and often referred to the body or its working components as 'machines' (although his machines were more operational than structural devices). For example, he discovered the liver's ability to synthesize sugar not because he studied the morphological structure of this organ, but because he used chemical analysis to follow the fate of blood sugar passing through the liver.

This typifies the study of body machines in the eighteenth and nineteenth centuries — the emphasis was not on knowing the minute structures responsible for physiological responses such as contractions or sensations, but rather on studying their operation. In part, this was due to the lack of knowledge about the organization of living tissues. For instance, cellular theory was

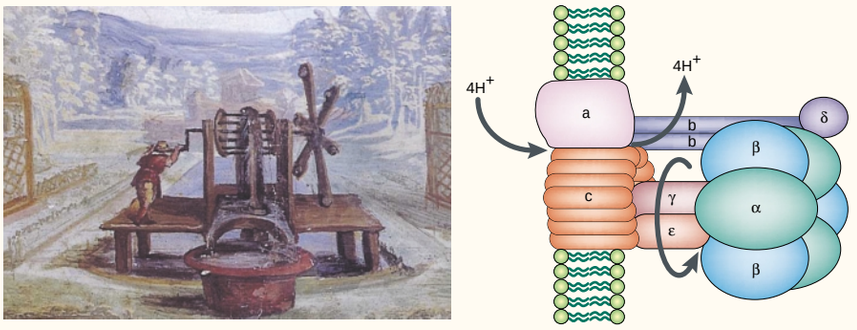

Figure 3 | **The metaphor of a 'machine', applied to living organisms.** Compare an old, manually operated hydraulic machine (left) to the rotary ATP synthase of modern molecular biology (right). Both machines are reversible with minor readjustment. In the molecular machine, electrochemical energy in a proton gradient is normally used to produce rotary movement and ATP, but the machine can also work in reverse to produce an electrochemical gradient at the expense of ATP (figure adapted from REF. 43). In the man-made machine, the hydraulic potential energy could be converted into mechanical work that the man could use (from the '*Stanzino delle Matematiche*' Museo degli Uffizi, Florence; © by 'Ministero Affari Culturali' of Italy).

developed only around 1839 by Matthias Schleiden[22] and Theodor Schwann[23], and much time elapsed before there was any real knowledge about genetic laws, the existence and structure of membranes, the functions of proteins and enzymes, and the existence of hormones and other chemical messengers. In the absence of adequate knowledge, attempts to devise mechanistic hypotheses of biological phenomena were likely to fail.

First-generation biochemists

Another reason for the lack of interest in the minute organization of body structure was the growing importance of chemistry in biological studies in the eighteenth century. For example, the discovery by Antoine Laurent Lavoisier, Pierre Simon de Laplace[24] and Lazzaro Spallanzani[25], that a process akin to combustion occurs in living tissues, had great biological relevance[26]. In the following century it became increasingly evident that many functions of living organisms depend on chemical reactions. A chemical reaction typically occurs in a solution, and involves particles that move by diffusion and collide randomly with one another. Similarly, within an organism, chemical reactions seemed to require a liquid medium, and did not depend on the existence of particular structures. So it is not by chance that Claude Bernard developed his idea of a liquid internal milieu just when biologists were becoming interested in chemistry.

Interest focused on those reactions that could, potentially, produce the energy necessary for life. In his book *Reflections on Muscle*[27], Andrew Huxley remarked that the relative lack of interest in the structural details of biological processes partly explains why the observations made around 1880 by Theodor Engelmann[28] — of characteristic changes in the dimensions of band patterns in muscle fibres during contraction — did not immediately result in an anticipation of the 'sliding theory' that was eventually formulated in the 1950s. Many scientists were neither interested nor confident in microscopy, preferring to visualize muscle contraction as the result of shortening of a muscular protein, fuelled by a chemical process akin to those being discovered in fermentation reactions[27,29]. The first generation of biological chemists were more interested in breaking down the cellular and subcellular components of living tissues to make them amenable to chemical analysis than in adjusting their chemical techniques to the complex components of biological materials.

On the other hand, a biochemical approach, combined with physiological and clinical investigation, was fundamental in developing the concept of a 'hormone' at the turn of the nineteenth century (the word was introduced by Bayliss and Starling in 1905). It soon became clear that hormones, together with nervous reactions, were essential for regulating body function and maintaining stability of the internal milieu. This led Walter Cannon, in 1925, to propose the idea of 'homeostasis'[30]. Through the study of hormones and other chemical messengers it became clear that, besides being involved in metabolic processes and in other chemical actions, molecules can carry important information in biological systems. Moreover, these molecules might relay information through specific receptor and effector systems.

The idea of catalysis also emerged through chemistry. Biological materials were found to have specific and highly efficient catalysts, termed 'enzymes' by Willy Kühne[31] in 1877. The study of enzymes (and of other proteins, as well as large molecules such as nucleic acids) was, in fact, behind the resurgence of interest in the idea of 'minute machines' during the twentieth century[32].

Twentieth-century machines

It became increasingly clear that the function of enzymes depends not only on their elementary chemical composition, but also on the configuration of their components. For example, effective interactions between enzymes, substrates and cofactors depend on the spatial arrangement of the interacting elements. This insight led to interest in the structure of complex molecules. It was also evident that the function of enzymes and other biological molecules could be regulated through specific control mechanisms. For instance, in 1963, Jacques

Box 2 | Modern molecular machines

Today, biology is revealing the importance of 'molecular machines' and of other highly organized molecular structures that carry out the complex physico-chemical processes on which life is based. There are many diverse molecular machines:

- The photosynthetic system and complex devices that produce ATP.
- DNA replication and protein translation apparatus.
- Enzymatic cascade of phototransduction.
- The integrated membrane system, involving ionic pumps and channels, that produces ionic gradients and generates electric differences across membranes; this underlies the production of electric signals in nerve fibres.
- Machines that convert chemical energy into mechanical energy during muscle contraction or flagellar motion.
- Finely integrated metabolic cycles and networks, including the system involved in antigen recognition and antibody production, the integrated system of hormones, extracellular molecules and intracellular messengers that are connected by many control pathways.

Monod, Jean Pierre Changeux and François Jacob[33] introduced the concept of allosteric regulation — that enzymatic action can be regulated by chemical signals acting on sites other than the enzyme's catalytic site. This has since provided a reference for interpreting mechanisms involving molecules and systems that differ from those based on typical enzymatic actions; for instance, ligand–receptor interactions and various modulatory actions. An important advance has been the recognition that complex receptor assemblies are linked to second-messenger systems through specialized proteins[34,35] and that there is a flux of biological information. This information is carried by specific messengers, which act on systems that recognize them and develop specific responses. Through this complex flux of information, different mechanisms can be organized in more complex systems, resulting in highly integrated and efficient processes.

The concept of information flux is also central to one of the biggest advances of twentieth-century biology — recognition of the molecular mechanisms responsible for transmitting genetic information and protein synthesis. These mechanisms involve the coding of genetic information by nucleic acids; transmission of this information through complex molecular devices that work at high rates with few errors; transcription of this information; translation into an amino-acid sequence; and finally, post-translational editing of this sequence[32,36]. Although these devices carry out basically chemical reactions, these reactions can no longer be considered as purely chemical processes due to unrestricted encounter-limited diffusion in a liquid medium. In fact, cellular compartments can hardly be considered typical liquid media.

The idea now is that 'structure' is fundamental to the operation of modern molecular devices: for example, take the three-dimensional arrangement of individual molecules; the spatial arrangement of proteins in sequential operations; and the arrangement of different proteins in a given process with respect to the membranes surrounding subcellular organelles or the cell as a whole. Given the importance of structure, modern biological pathways fully deserve the names "molecular and supramolecular machines"[36,37].

Ancient versus modern machines

To an extent, these extraordinary biological machines (BOX 2) realize the dream of the seventeenth-century scientists — a dream that led Malpighi to suppose, more than three centuries ago, that "machines will be eventually found not only unknown to us but also unimaginable by our mind"[38]. If we consider that basically the same molecular device underlies ATP synthesis and bacterial flagellar motion, we see that modern biological machines correspond to the uniformity of nature pictured by Malpighi when he said[4]:

> "In its things Nature operates by necessity always in a uniform way. . . . Even though they appear disparate, the things of Nature are not so disconnected that one cannot observe a concatenation and uniformity in operating."

However, in the importance of information flow, modern biological (and non-biological) machines differ from old machines, and surpass the expectations of the early life scientists. The old biological machines were supposed, at a minute level, to be ". . . made up of cords, filaments, beams, levers, tissues, fluids coursing here and there, cisterns, canals, filters, sieves and similar mechanisms"[4]. Besides the "fluids coursing here and there", energy — rather than information — was thought to circulate through such components. No feedback mechanisms or control processes were predicted. The lack of an adequate concept of 'information' explains other difficulties encountered by early life scientists. For instance, it was impossible to come up with a reasonable theory of body development and the transmission of hereditary characteristics[14,39,40].

Some modern molecular devices, such as the rotary mechanism involved in ATP synthesis, may visually resemble the artificial machines that inspired the scientific revolution more than three centuries ago (FIG. 3). However, as well as the intrinsic regulatory mechanisms in what Paul Boyer called these new "splendid molecular machines"[41], there are the regulatory actions based on information flux which, in this case, control the various phases of energetic metabolism culminating in ATP synthesis. With reference to Malpighi's metaphor of the angel and the mill, we could perhaps say that, besides trying to understand the mechanisms of these biological wheels, modern scientists have started to picture how, by controlling a flux of signals through information networks, the angel regulates the complex machine of the living mill.

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OPINION

Slow axonal transport: stop and go traffic in the axon

Anthony Brown

Efforts to observe the slow axonal transport of cytoskeletal polymers during the past decade have yielded conflicting results, and this has generated considerable controversy. The movement of neurofilaments has now been seen, and it is rapid, infrequent and highly asynchronous. This motile behaviour could explain why slow axonal transport has eluded observation for so long.

Neurons communicate with other cells by extending cytoplasmic processes called axons and dendrites. Remarkably, axons can attain lengths of one metre or more, although they lack ribosomes and Golgi complexes. Axonal proteins and Golgi-derived vesicles are formed in the neuronal cell body and are shipped along the axon by a process called axonal transport. This movement is essential for the growth and survival of axons, and continues throughout the life of the nerve cell.

Studies on axonal transport in laboratory animals with radioisotopic pulse labelling have shown that there are hundreds of axonally transported proteins, but that these proteins move at a small number of discrete rates, which can be categorized as either fast or slow. Each discrete rate component represents the movement of a largely distinct subset of proteins that are transported together throughout their journey along the axon. To explain these observations, Lasek and colleagues proposed the structural hypothesis of axonal transport, which postulates that all axonal proteins move by association with, or as integral parts of, subcellular carrier structures[1]. According to this hypothesis, each rate component represents the movement of a unique type of macromolecular structure (TABLE 1).

The fast components of axonal transport are now known to represent the anterograde and retrograde movement of distinct types of membranous organelles along microtubules at average rates of roughly 50–400 mm day^{-1} (~0.5–5 μm s^{-1}), propelled by the action of molecular motor proteins[2]. Membranous organelles can therefore be considered to be the carrier structures for fast axonal transport. In contrast, the slow components of axonal transport represent the movement of cytoskeletal and cytosolic proteins at much slower rates, and the nature of the carrier structures for these proteins is not known. Proteins that associate with neurofilaments and microtubules move in slow component 'a' at average rates of roughly 0.3–3 mm day^{-1} (~0.004-0.04 μm s^{-1}), and proteins that associate with microfilaments, as well as many other cytosolic proteins, are transported in slow component 'b' at average rates of roughly 2–8 mm day^{-1} (~0.02–0.09 μm s^{-1}) (TABLE 1).

No movement *en masse*

In radioisotopic pulse-labelling experiments, slow components 'a' and 'b' form unimodal asymmetrical waves, often loosely described as 'bell-shaped', which spread as they move along the axon towards the axon tip (FIG. 1). Each wave represents the concerted movement of many distinct proteins whose individual waveforms coincide. Early studies on slow axonal transport stressed the coherence of these transport waves but not the spreading, and this gave rise to the idea that cytoskeletal and cytosolic proteins move along the axon *en masse*, that is, in a slow and synchronous manner[1].

The expectation of a slow and synchronous movement has had a profound influence on the design of experiments aimed at detecting slow axonal transport. For example, many studies have used fluorescence photobleaching or photoactivation strategies in which fluorescent or caged fluorescent cytoskeletal proteins are injected into nerve cells and then a popula-

Table 1 | The moving structures of axonal transport*

Rate class	Average rate	Moving structures	Composition (selected examples)
Fast components			
Fast anterograde	200–400 mm day^{-1} (≈2–5 μm s^{-1})	Golgi-derived vesicles and tubules (secretory pathway)	Synaptic vesicle proteins, kinesin, enzymes of neurotransmitter metabolism
Bi-directional	50–100 mm day^{-1} (≈0.5–1 μms^{-1})	Mitochondria	Cytochromes, enzymes of oxidative phosphorylation
Fast retrograde	200–400 mm day^{-1} (≈2–5 μm s^{-1})	Endosomes, lysosomes (endocytic pathway)	Internalized membrane receptors, neurotrophins, active lysosomal hydrolases
Slow components			
Slow component 'a'	0.3–3 mm day^{-1}	Neurofilaments, microtubules‡	Neurofilament proteins, tubulin, spectrin, tau proteins
Slow component 'b'	2–8 mm day^{-1} (≈0.02–0.09 μm s^{-1})	Microfilaments, supramolecular complexes of the cytosolic matrix	Actin, clathrin, dynein, dynactin, glycolytic enzymes

*Data compiled from REFS 1,41,44. ‡ In some neurons, microtubule proteins are transported in slow component 'b' as well as slow component 'a'.