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Is the cell *really* a machine?

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ABSTRACT

It has become customary to conceptualize the living cell as an intricate piece of machinery, different to a man-made machine only in terms of its superior complexity. This familiar understanding grounds the conviction that a cell's organization can be explained reductionistically, as well as the idea that its molecular pathways can be construed as deterministic circuits. The machine conception of the cell owes a great deal of its success to the methods traditionally used in molecular biology. However, the recent introduction of novel experimental techniques capable of tracking individual molecules within cells in real time is leading to the rapid accumulation of data that are inconsistent with an engineering view of the cell. This paper examines four major domains of current research in which the challenges to the machine conception of the cell are particularly pronounced: cellular architecture, protein complexes, intracellular transport, and cellular behaviour. It argues that a new theoretical understanding of the cell is emerging from the study of these phenomena which emphasizes the dynamic, self-organizing nature of its constitution, the fluidity and plasticity of its components, and the stochasticity and non-linearity of its underlying processes.

> *Through its properties, by the microscopic clockwork function that establishes between DNA and protein, as between organism and medium, an entirely one-way relationship, this system obviously defies any 'dialectical' description. It is not Hegelian at all, but thoroughly Cartesian: the cell is indeed a machine.*
>
> (Monod, 1972, pp. 110–111)

1. Introduction: The Machine Conception of the Cell

Over the past half a century, molecular biology has generated vast amounts of knowledge at a rate that is surely unprecedented in the history of science. However, our progress in translating this ever-growing repository of information into a deeper theoretical understanding of what living systems are and how they function as coordinated wholes has been far less impressive. Now it may be that this is simply a reflection of the extraordinary complexity of the cell, and that it is only a matter of time before all cellular components are characterized and all of their interconnections are fully mapped out, at which point we will finally have a total grasp of the internal workings of the cell. Alternatively, it is possible that the problem lies not so much in the complexity of the cell as in the interpretive framework—the theoretical presuppositions, conceptual categories, and explanatory models—routinely used to make sense of this complexity. This paper explores this second possibility.

The main interpretive framework in molecular biology is *mechanicism*, a highly influential research program with many forms and incarnations that can be traced all the way back to the natural philosophy that gave rise to the Scientific Revolution (Hall, 1969; Nicholson, 2012; Loison, 2015)[1]. Modern proponents of mechanicism conceive of the cell as an intricate piece of machinery whose organization reflects a pre-existing design, whose structure is wholly intelligible in reductionistic terms, and whose operation is governed by deterministic laws, rendering its behaviour predictable and controllable—at least in principle. I shall hereafter refer to this pivotal mechanicist notion as the *machine conception of the cell* (MCC).

The MCC long predates the rise of molecular biology—its history runs parallel to that of mechanicism, which is why one can find rudimentary expressions of the MCC dating back to the seventeenth century, when analogies between machines and organ-

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[1] Since Descartes, biological theory has oscillated between the mechanicist view of the organism as a complex machine and a vitalist—and more recently *organicist*—view of the organism (inspired by Kant among others) as an agential, non-mechanical, intrinsically purposive system (Allen, 2005; Normandin and Wolfe, 2013; Esposito, 2013; Nicholson and Gawne, 2015).

isms first began to acquire currency. Malpighi, one of the founders of microscopical anatomy, attributed the bodily functions of animals and plants to "a very large number of machines, which are of necessity made up of extremely minute parts […] invisible to the naked eye" (Malpighi, quoted in Piccolino, 2000, p. 149). In a similar vein, Leibniz, the early modern natural philosopher, characterized organisms as machines of divine origin, which are hierarchically composed of ever-smaller machines *ad infinitum*. He contrasted organisms to machines of human origin, whose component parts are not themselves also machines in their own right (Smith, 2011).

By the turn of the twentieth century, the cell was being variously characterized as "a little engine with admirably adapted parts" (Conn, 1899, p. 126), a "chemical machine" capable of "automatically developing, preserving, and reproducing [itself]" (Loeb, 1906, p. 1), and "a battery, with a series of resistances and condensers, made up of conductors and dielectrics" (Matthews, 1924, p. 15). But most influential of all was the understanding of the cell as a "small chemical laboratory" (Hertwig, 1895, p. 126) or a miniature factory, with proteins and other macromolecules arranged like machine tools on an assembly line (Reynolds, 2007, 2018).

Following the Second World War, the pioneering ideas of cybernetics, information theory, and computer science captured the imagination of biologists, providing a new vision of the MCC that was translated into a highly successful experimental research program, which came to be known as 'molecular biology' (Keller, 1995; Morange, 1998; Kay, 2000). At its core was the idea of the computer, which, by introducing the conceptual distinction between 'software' and 'hardware', directed the attention of researchers to the nature and coding of the genetic instructions (the software) and to the mechanisms by which these are implemented by the cell's macromolecular components (the hardware). Early molecular biologists openly conjectured about the structure and function of the cell along these lines, deliberately transgressing the boundaries between the technological and the biological, as the following excerpt from a paper published in 1962 illustrates:

> Taking then, as an engineering definition of a living cell, 'A completely automatic factory for fabricating automatic factories like itself', we may profitably consider what components might be found in such a system. Passing over such trivia as a power station for utilizing whatever energy source might be available, it is clear that a large computer would be the control mechanism at the centre of our design. In its store would be an encyclopaedia of programmes which would give the proper response to all possible sets of external circumstances, and these would be activated by input devices which would record the external conditions and the supply position. Other input channels would monitor the progress of the various factory processes, forming the feedback loops which are essential to control mechanisms. Output from the computer would go […] to a set of automatic machine tools which would perform the various operations required for construction of a duplicate factory. Here the complex task of converting the information stored in the computer into solid matter would be performed. (Blow, 1962, p. 177)

It is quite remarkable to observe that, despite the enormous empirical advances that have been made since 1962, our basic theoretical picture of the cell has remained essentially unchanged (see, e.g., Bray, 2009; Danchin, 2009). The standard view nowadays is that the cell coordinates its functions by virtue of a 'genetic program' encoded in the DNA that directs and controls the expression of a specific set of RNAs and proteins, which assemble deterministically into stable 'molecular machines' that reliably and efficiently execute predetermined operations according to the mechanisms of cell division, endocytosis, signal transduction, etc. Machine analogies and metaphorical references to 'locks', 'keys', 'gates', 'pumps', 'motors', and 'engines' continue to pervade the technical literature (e.g. Piccolino, 2000; Frank, 2011), as does talk of the 'machinery' (e.g. Goodsell, 2009) and 'circuitry' (e.g. Alon, 2007) that underlies the cellular organization. The MCC itself is seldom explicitly defended; it has become so engrained in our minds that we simply take it for granted.

But why have we relied so heavily on machine metaphors to ground our theoretical understanding of living systems? What is so special about machines that make them such apposite analogues for thinking about cells? Although there are many different kinds of machines, a machine can be characterized in very general terms as a device with fixed interacting parts that operate in a coordinated fashion to produce a predetermined outcome. More specifically, one can identify four distinctive properties of machines that are particularly relevant in contemporary formulations of the MCC. First, machines can be described in terms of a list of parts and a blueprint indicating how those parts fit together, meaning that someone who has never seen a particular kind of machine should in principle be able to assemble any number of copies—each virtually identical in appearance and performance—provided they can consult the machine's design specifications. Second, as machines are designed to perform highly specific functions, their operation is tightly constrained, which is why it is possible to predict and control their behaviour. Third, machines are highly efficient in what they do because they always follow the exact same sequence of steps in every cycle of their operation. And fourth, the operation of machines is not continuous; their functioning can be interrupted and their parts examined without thereby jeopardizing their structural integrity. The first and fourth of these characteristics account for why the MCC justifies the belief in the sufficiency of reductionistic explanations of cellular phenomena, whereas the second and third show why the MCC provides support for a deterministic view of cellular processes.

In recent years, however, the MCC has come under attack from various fronts. Ironically, the very successes of molecular biology that were instigated by mechanicism have resulted in the accumulation of experimental data that are difficult to assimilate within its interpretive framework. As a result, critical reviews have begun to appear that explicitly challenge the reductionistic and deterministic presuppositions of mechanicism and question the coherence of the familiar clockwork image of the cell. Notable examples include Kirschner et al. (2000), Astumian (2001), Woese (2004), Cornish-Bowden (2006), Longo and Tendero (2007), Karsenti (2008), Huang (2009), Mayer et al. (2009), Kupiec (2010), Moore (2012), Bizzarri et al. (2013), Talbott (2013), Heams (2014), Longo and Montévil (2014), Soto and Sonnenschein (2018), and a series of articles by Kurakin (2005, 2006, 2009, 2010). Drawing and building on this burgeoning body of literature, the aim of this paper is to establish the inadequacy of the MCC. From a theoretical perspective, the MCC offers a poor and rather misleading representation of biological reality—or so I will argue[2].

The MCC fails to make appropriate sense of cellular phenomena for two basic reasons. The first has to do with the fact that cells, unlike machines, are self-organizing, fluid systems that maintain themselves in a steady state far from thermodynamic equilibrium by continuously exchanging energy and matter with their surroundings. And the second has to do with the fact that by virtue of their microscopic size, cells (and their molecular constituents, even more so) are subject to very different physical conditions

[2] For complementary critiques of the machine conception of living systems in other areas of biology—such as physiology, development, and evolution—see Nicholson (2013, 2014, 2018).

compared to macroscopic objects, like machines. Although both of these facts are incontrovertible—indeed, they may strike some readers as painfully obvious—the theoretical implications they have for our understanding of life are far from familiar, and it is these implications that shall be concerning me here. What I will contend is that they lead to a conception of the cell that is completely at odds with the mechanicist, reductionistic, and deterministic view that was championed by the founding fathers of molecular biology, such as Monod in his hugely influential *Chance and Necessity* (Monod, 1972), quoted in the epigraph of this paper.

If the facts that underlie the inadequacy of the MCC really are indisputable, why has it taken us so long to start taking serious notice of them? I suspect that part of the answer has to do with the resistance that many biologists intuitively feel towards denunciations of mechanicism. Perceived inconsistencies and contradictions in the established paradigm are often downplayed—or dismissed altogether—in order to safeguard the familiar assumptions that the research community works under. But an even more important factor, I believe, is that we have been blinded by traditional biochemical and biophysical methods. Until relatively recently, it was only possible to examine the cell's interior with crude in vitro techniques, looking at average behaviours of large populations of macromolecules under conditions usually remote from those existing in the cell. However, the introduction of novel methods capable of tracking and manipulating individual molecules within cells has allowed us to observe for the first time the real-time dynamics of biological macromolecules and the surprisingly wide range of behavioural repertoires they exhibit in in vivo conditions (Zlatanova and van Holde, 2006; Xie et al., 2008; Tinoco and Gonzalez, 2011). As I will discuss in more detail later, single-molecule studies are yielding results not anticipated by the use of population-averaged methods. These results are bringing about a radical shift in how we think about the cell, replacing a mechanical, neatly ordered, rigid picture with one that is inherently stochastic, more plastic, and less predictable. What we are witnessing, in effect, is a conceptual revolution being triggered by a methodological revolution.

Despite the historical predominance of mechanicism, a new interpretive framework is now required to understand what our recent findings are telling us about the nature of the cell. This framework is already arising, as more molecular biologists are becoming aware of the numerous problems plaguing the MCC. This paper will examine in detail four specific domains of research where the incompatibilities with the MCC are becoming particularly pronounced. The first is the study of the cellular architecture, which in line with the MCC has long been construed as a static, highly ordered structure. The second is the study of protein complexes, which have generally been characterized as remarkably specialized, exquisitely designed molecular machines. The third is the study of intracellular transport, which has tended to be explained in terms of miniature engines propelled by mechanical forces. And the fourth is the study of cellular behaviour, which has long been assumed to be governed by a deterministic program encoded in the genome.

Increasingly, all of these mechanicist interpretations are being called into question, and a fundamentally different conception of the cell is emerging. As I will show, according to this alternative view, the cellular architecture is regarded as a fluid, self-organizing process; protein complexes are considered to be transient, pleomorphic ensembles; intracellular transport is deemed to result from the harnessing of Brownian motion; and cellular behaviour is viewed as a probabilistic affair, subject to constant stochastic fluctuations. Taken together, these four case studies will illustrate how a rejection of the MCC—along with the mechanicist assumptions that underlie it—is contributing to the development of a more theoretically compelling picture of the cell.

2. Cellular Architecture: Static Structure or Stabilized Process?

Much of what we know about the cell's organization derives from snapshots of fixed, stained, or desiccated biological samples obtained by conventional microscopy techniques. A representative example is shown in Fig. 1. Historically, the interpretation of images of this kind naturally led to an understanding of the internal architecture of the cell in terms of clearly delineated, neatly compartmentalized structures that closely resemble machineries. These permanent structures were eventually assigned functions to make sense of their role in the overall economy of the cell, which in accordance with the MCC was viewed as a factory with highly specialized compartments.

But how are these structures that constitute the cellular architecture formed and maintained? What is it that determines their different shapes and sizes, as well as their respective locations and functions in the cell? For decades, the basis for our understanding of macromolecular order was the principle of *self-assembly* (Kushner, 1969; Inouie, 1982; Whitesides and Grzybowski, 2002). Self-assembly involves the physical association of molecules into a static equilibrium structure in the absence of an external energy source. It is driven by local stereospecific interactions between the aggregating 'building blocks', which remain unchanged throughout the process. As the properties of the resulting structure are determined by the properties of its parts, self-assembly can be regarded as "an extension of the central dogma of molecular biology, bringing us from the realm of linear information to the realm of protein assemblies" (Kirschner et al., 2000, p. 80). Classical, well-studied examples of self-assembly include viral capsid formation (Caspar and Klug, 1962) and ribosome biogenesis (Nomura, 1973).

However, self-assembly is not the only theoretical principle that can be invoked to explain the spontaneous generation of macromolecular order. There is also the principle of *self-organization* (Nicolis and Prigogine, 1977; Kauffman, 1993; Karsenti, 2008). Self-organization refers to the collective behaviour of molecules when these interact nonlinearly to generate a dynamic far-from-equilibrium structure (sometimes called a 'dissipative structure'), which maintains itself in a low-entropic 'steady state' by constantly expending energy and exchanging matter with its surroundings. So, while self-assembled systems are closed, as their material constitution is conserved, self-organizing ones are open, as they rely for their preservation on the continuous replenishment of the material that composes them.

Of course, we have known for a long time that self-organization is essential for living systems, given that the cell as a whole—how ever else one may wish to describe it—is, thermodynamically speaking, a far-from-equilibrium dissipative structure: in the absence of a steady supply of energy, it reaches equilibrium and dies. Nevertheless, it has proven surprisingly difficult to identify particular instances of self-organization inside the cell. This is due to the fact that self-assembly and self-organization tend to lead to similar observable patterns, albeit through totally different means. Specifically, both generate stable structures; the difference being that those generated by the former exhibit static stability whereas those generated by the latter exhibit dynamic stability (sometimes referred to as 'meta-stability'). The problem remained that conventional microscopy methods prevented us from distinguishing them.

Recent technological innovations have changed all of this. The development of in vivo microscopy techniques using genetically-encoded fluorescent tags of individual molecules has provided new insights into the spatiotemporal configuration of the cell. Perhaps the most surprising discovery that has emerged from these studies is the unexpectedly high degree of dynamism observed for a wide range of macromolecular structures. It appears that many—perhaps most—subcellular compartments are more appropriately described as dynamic self-organizing steady states than

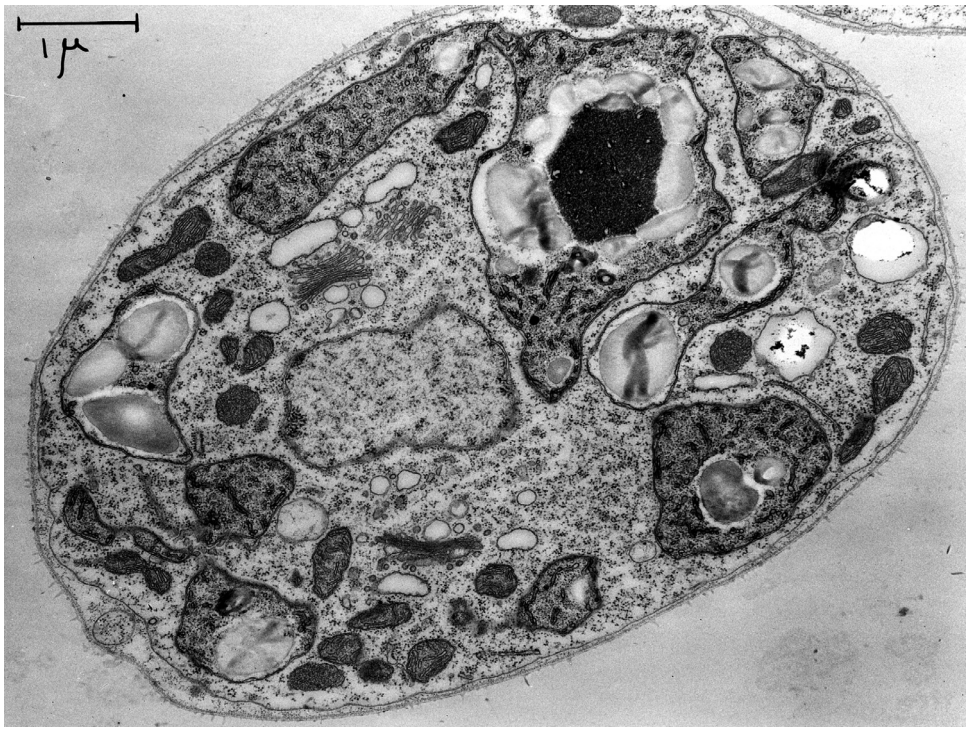

Fig. 1. Electron micrograph of a longitudinal section of the unicellular green alga *Chlamydomonas*, which conveys a static, clearly compartmentalized impression of the cell's interior. (Image courtesy of J. D. Jamieson and the Department of Cell Biology, Yale University School of Medicine; reproduced under a Creative Commons License.)

as static self-assembling machineries. The molecular constituents of the cell, it turns out, have a tendency to spontaneously self-organize into morphologically and functionally distinct organizations through inherently stochastic interactions. These transient meta-stable systems are sustained by the incessant flow of energy and matter passing through them, with their respective components displaying different recruitment probabilities, residence times, and turnover rates (Misteli, 2001a; Kurakin, 2009). Let me now discuss some specific examples of intracellular entities that are currently being completely reconceptualized as a result of recent empirical findings (prompted by the use of new methods).

The mitotic spindle of eukaryotic cells is one such example. The spindle is an ordered array of microtubules, associated proteins, and chromosomes that forms during cell division, and which distributes the duplicated genetic material to the daughter cells with stunning precision. Owing to its remarkably stable—almost crystalline—appearance in cross-sections of cells undergoing mitosis, the mitotic spindle is often characterized as "a fascinating protein machine" (Mogilner et al., 2006, p. 88) capable of assembling and disassembling according to genetically encoded instructions. However, recent research has shown that the mitotic spindle is actually a self-organizing system, displaying high degrees of flexibility and robustness (Nédélec et al., 2003; Pavin and Tolić, 2016). Architecturally speaking, the microtubules that compose the mitotic spindle are constantly polymerizing and depolymerizing, repeatedly undergoing cycles of GTP hydrolysis to maintain it in a steady state far from equilibrium. As a consequence of these findings,

> The traditional view of the mitotic spindle apparatus as a molecular machine which is built through a defined irreversible set of instructions is gradually being replaced. It can instead be envisaged as a self-regulating dynamic structure where multiple pathways of MT [microtubule] generation are spatially and temporally controlled and integrated, constantly 'talking' to one another and modifying the behaviour of their MTs in order to maintain a flexible yet robust steady-state spindle. (Duncan and Wakefield, 2011, p. 330)

It has further been suggested that not only the mitotic spindle, but the entire cytoskeleton is better characterized as a meta-stable flux dynamically responding to changes in its environment than as a static macromolecular construction. "Despite the connotations of the word 'skeleton'", Fletcher and Mullins (2010, p. 485) write, "the cytoskeleton is not a fixed structure whose function can be understood in isolation. Rather, it is a dynamic and adaptive structure whose component polymers and regulatory proteins are in constant flux".

Self-organization appears to be similarly crucial for intracellular membrane compartments, such as those involved in the secretory transport pathway, in which proteins targeted to the cell's exterior are transported from the endoplasmic reticulum through the Golgi complex to the plasma membrane. Although the compartments of this pathway have traditionally been regarded as static structures, the recent tracking of resident and cargo molecules through the pathway using in vivo microscopy has revealed that they are in fact constantly exchanging material (Lippincott-Schwartz et al., 2000). The Golgi complex, for instance, resembles the mitotic spindle in that its stability is a consequence of the balanced turnover of the molecules that flow through it. Given its fluid nature, its architecture can be modified by manipulating the influx and efflux of material passing through its component cisternae. We now know that inhibition of traffic from the endoplasmic reticulum leads to the dispersion of the Golgi complex into small vesicles, whereas blocking the transport of vesicles that bud from it results in its enlargement. Although there is still considerable disagreement over how the actual traffic occurs—specifically over whether the Golgi cisternae themselves progress or mature along the pathway or if it is only their cargo that gets transported (see Glick and Luini, 2011)—what seems clear is that the Golgi complex is a self-organizing steady-state organelle (Tachikawa and Mochizuki, 2017). More broadly, live imaging techniques are unveiling the striking dynamicity that underlies the stability of intracellular

membrane compartments of both exocytotic and endocytotic pathways (Kerr and Teasdale, 2014).

Our understanding of the eukaryotic cell nucleus is also becoming radically transformed. Far from being the static, crowded, gel-like structure described in textbooks, the nucleus is extremely dynamic and surprisingly fluid. Most of its proteins are highly mobile, stochastically moving about the nucleoplasmic space contingently interacting with one another and participating in different nuclear functions, such as chromatin remodelling, transcriptional activation, ribosomal RNA processing, and DNA repair. The dynamic interplay between nuclear proteins results in an ever-changing, yet globally stable architecture within which nuclear processes take place (Misteli, 2001b; Janicki and Spector, 2003). The nuclear architecture includes a number of morphologically and functionally distinct compartments, such as nucleoli, Cajal bodies, and perinuclear specks, that are maintained in a state of "perpetual flux" (Misteli, 2001b, p. 844) by the constant exchange of their resident proteins, which also transiently associate with the chromatin. The latest research on these subnuclear, membraneless organelles strongly suggests that they are better conceived as liquid-like droplets than as solid, core-shell structures: they have a spherical shape, they fuse together, and their molecular constituents are constantly undergoing fluid internal rearrangements (Brangwynne et al., 2011; Shin and Brangwynne, 2017).

In addition to its instrumental role in generating and maintaining many organelles, recent studies suggest that self-organization is involved in some of the cell's most essential processes, including metabolism (De la Fuente et al., 2008), genome organization (Misteli, 2009), cell division (Loose et al., 2008), and cell differentiation (Woodford and Zandstra, 2012).

The self-organizing nature of the cellular architecture has far reaching theoretical consequences. Most fundamentally, it leads to a view of the cell that is completely at odds with the MCC. For one thing, it dispels the notion that the 'information' that specifies the spatial organization of the cell is somehow encoded in the genome. Strictly speaking, there is no genetic blueprint for the cellular architecture. Self-organization generates order in the absence of an external template or global plan. Genes specify only the primary sequence of macromolecules; the architecture of the cell, for the most part, arises from the interactions of numerous gene products with other cellular components. Genes are important, to be sure, but they do not set in motion a unique chain of events that produces the organization of the cell, as the use of the term 'information' sometimes misleadingly suggests. Rather, gene products are released into a cellular milieu that already possesses spatial structure, and they exert their influence under the physical constraints of the existing order—much of which is shaped by pre-existing self-organizing processes (Harold, 2005; Rafelski and Marshall, 2008).

In contrast to a machine, in which a fixed architecture performs a predetermined function, a cell is continuously transforming its internal architecture (by modifying the exquisitely regulated balance between the inflow and outflow of its molecular constituents) in order to keep up with its ever-changing functional needs. Cellular structures showcase what Dumont and Prakash (2014) appropriately refer to as 'emergent mechanics', which cannot be predicted from knowledge of their parts. The disparity with the mechanics of machines is all too evident, as the authors themselves explicitly acknowledge:

> Unlike the engineered macroscopic structures that we commonly build, biological structures are dynamic and self-organize: they sculpt themselves and change their own architecture, and they have structural building blocks that [...] constantly come on and off. A description of such structures defies current traditional mechanical frameworks. (Dumont and Prakash, 2014, p. 3461)

Indeed, no machine self-organizes by autonomously exchanging its material constitution in order to maintain its architecture in a dynamic steady state, yet this is precisely what happens in every cell. But why do cells favour self-organization over self-assembly as the main mechanism for creating their architecture? Would it not make more sense for a cell to build static, equilibrium structures that do not require a constant expenditure of energy to maintain them? Although self-assembly is a more economical and efficient means of producing durable macromolecular structures of great complexity (the viral capsid is a conspicuous example), the resulting structures lack morphological flexibility and do not lend themselves easily to modifications. The advantage of a self-organizing architecture, despite its huge energetic cost, is that it confers a great deal of plasticity without compromising on stability. It allows cells to respond rapidly and adaptively to external perturbations and other critical events that would otherwise jeopardize their systemic integrity.

Overall, recent research on the cellular architecture demands that we look more carefully at what we have previously assumed were well-defined structures and reconsider them as stabilized *processes*. Because processes are temporally extended, it follows that they can only be understood by giving time due consideration. And herein lies the problem: the methods traditionally used to probe the interior of the cell conceal the dynamic nature of its architecture because they have to incapacitate it in order to render it visible. Yet to study a cell frozen in time is already to approach it artificially as a static, machine-like object, rather than as the fluid system that it is in reality (Nicholson, 2018). The structure of a machine, after all, can be grasped in abstraction from time (as it is not constantly changing), whereas the structure of, say, a whirlpool or a stream cannot. This explains why, when we have started using techniques that allow us to examine the cellular architecture in real time, we have found that many of the cell's compartments and organelles are not fixed machineries at all, but stable macromolecular fluxes.

More broadly, the transition from a structural to a processual conception of the cellular architecture implies shifting our attention from matter to form. Due to its dynamic nature, what persists in a cell over time is its form, not its matter: the individual molecules that make up a cell come and go, but its overarching organization remains. Accordingly, if we are to grasp how a cell operates, mapping out the network of spatial and temporal relations that exist between its parts is as, if not more, important than characterizing the parts themselves. The need to adopt a non-reductionist stance is further intensified when we bear in mind that self-organizing processes—which, as I have shown, underlie much of the cellular architecture—force us to focus on systemic patterns and collective behaviours, rather than on the properties and structures of single molecules (which would suffice as an approach if the cell was primarily self-assembling and its order was ultimately encoded in the DNA).

The main differences between the two conceptions of the cellular architecture I have discussed in this section are summarized in Table 1.

Table 1
Key differences between the two conceptions of cellular architecture. On the left, the standard view derived from the MCC. On the right, the alternative view suggested by recent research.

Static structure	Stabilized process
Self-assembling	Self-organizing
Closed system	Open system
At equilibrium	Far from equilibrium
Genetically encoded	Emergently generated
Economical but inflexible	Costly but flexible
Temporally abstractable	Temporally non-abstractable
Amenable to reductionism	Not amenable to reductionism

3. Protein Complexes: Molecular Machines or Pleomorphic Ensembles?

The mechanicist foundations of molecular biology have not only guided our inquiries into the cell's internal organization, they have also shaped our theoretical understanding of its basic molecular components, especially proteins. As well as championing the MCC, Monod also declared in his *Chance and Necessity* that "[w]ith the globular protein we already have, at the molecular level, a veritable machine" (Monod, 1972, p. 98). In subsequent years, as it became apparent that most proteins in the cell associate with one another to form larger complexes comprised of different subunits, a new concept began to acquire currency, namely that of a *molecular machine*. In 1998, Alberts (then president of the National Academy of Sciences) published a brief but highly influential manifesto titled 'The Cell as a Collection of Protein Machines: Preparing the Next Generation of Molecular Biologists' (Alberts, 1998), in which he urged aspiring molecular biologists to embrace the MCC and learn to view the cell as "a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines" (ibid., p. 291). At the end of the article, Alberts prophesized that "much of the great future in biology lies in gaining a detailed understanding of the inner workings of the cell's many marvelous protein machines" (ibid., p. 293).

It is difficult to overestimate the impact that Alberts' paper has had on molecular biology. It has been so successful in popularizing the molecular machine concept that this term is now used to describe virtually any functionally specialized macromolecular complex found in the cell (e.g. Nogales and Grigorieff, 2001; Neupert, 2005: Frank, 2011), as the following quotation illustrates:

> Molecular machines are the basis of life. […] The cell's nanometer-scale machines are mostly protein molecules, although a few are made from RNA, and they are capable of surprisingly complex manipulations. They perform almost all the important active tasks in the cell: metabolism, reproduction, response to changes in the environment, and so forth. They are incredibly sophisticated, and they, not their manmade counterparts, represent the pinnacle of nanotechnology. (Phillips and Quake, 2006, p. 38)

Some have gone as far as to regard the molecular machine conception of protein complexes as "one of the most important contributions that biology has made to our understanding of how the living cell works" (Ji, 2012, p. 86). The reason this idea has been so successful, as I will argue below, is because it addresses in a unified way the two classical concerns of molecular biology research, namely *structure* and *specificity*.

Historically, molecular biology represents the confluence of two largely autonomous research programs, both of which can be traced back to the 1930s: one focusing on structure and another focusing on specificity, or information (cf. Kendrew, 1967; Stent, 1968; Hess, 1970). The structural school of molecular biology (promoted by the likes of Astbury, Bernal, and Pauling) employed methods such as X-ray crystallography to determine the atomic configuration of key biological molecules, and used those findings to make sense of their physiological role. The informational school of molecular biology (led by the so-called 'phage group' of Delbrück, Luria, and Hershey) used bacteriophages as model systems to investigate the molecular basis of heredity and its likely mode of transmission. The former had ties with biochemistry, while the latter had ties with genetics. The two schools came together in spectacular fashion in 1953 with the famous elucidation by Watson and Crick of the double-helical structure of DNA—a momentous discovery which notably combined structural determination with genetic reasoning (Watson and Crick, 1953). Nevertheless, during the latter half of the twentieth century, the structural and informational strands of molecular biology continued to develop more or less independently of one other, and it is in the context of this schism, I believe, that one can understand the appeal and success of the molecular machine concept, serving as it does to reconcile the distinct explanatory concerns of each school.

Conceiving of protein complexes as molecular machines draws our attention to their structure. When a mechanic or an engineer studies a machine, they examine its structure carefully because they know that this will enable them to understand its operation. Function is a direct consequence of structure, and so they elucidate the former by scrutinizing the latter. Accordingly, if as molecular biologists we want to work out what a particular protein assembly does, modelling it as a molecular machine gives us a clear plan of action. It tells us that "we must foremost know the structure of the static molecular machine at the atomic level as a precondition for making sense of its behaviour and going beyond mere phenomenological description" (Frank, 2011, p. 1). For many researchers, it is this privileging of structure when investigating cellular components that justifies seeing them as molecular machines. As Piccolino puts it, "*[g]iven the importance of structure*, modern biological pathways fully deserve the names 'molecular and supramolecular machines'" (Piccolino, 2000, p. 152, emphasis added).

In addition to emphasizing its structure, viewing a protein complex as a molecular machine serves to highlight the specificity of its operation. It leads us to view it as an intricately ordered assembly of subunits—each with a clearly defined role—which mechanically interlock with one another in a particular temporal sequence into a unique configuration that allows it to perform its function in an effective and predictable way. Reading Alberts' manifesto, it is clear that this concern with specificity is one of his main motivations for embracing the machine metaphor:

> Why do we call the large protein assemblies that underlie cell function protein *machines*? Precisely because, like the machines invented by humans to deal efficiently with the macroscopic world, these protein assemblies contain highly coordinated moving parts. Within each protein assembly, intermolecular collisions are not only restricted to a small set of possibilities, but reaction C depends on reaction B, which in turn depends on reaction A—just as it would in a machine of our common experience. (Alberts, 1998, p. 291)

Despite the popularity of the molecular machine concept, recent research is casting serious doubts on the theoretical adequacy of this notion. Some of its problems pertain to its undue emphasis on structure, and others pertain to its undue emphasis on specificity. I shall examine each of these in turn.

With regards to structure, it has become apparent that the widespread use of X-ray crystallography has biased our view of proteins. In the last fifty years, crystallographers have deduced the tertiary structure of a very large number of proteins by purifying them into homogeneous, solid-state crystals and then examining the ordered array of their atoms. Although the inferred structural reconstructions are of an extremely high resolution, they represent only snapshots of incapacitated proteins frozen in time. Still, we have relied on this technique so much and for so long that it has come to shape the way we think about protein structure and its relation to protein function. Specifically, it has led to the view that each protein has a unique three-dimensional conformation—corresponding to its most thermodynamically stable configuration—that it must adopt in order to carry out its intended function. This deeply-entrenched assumption has been called "the central dogma of structural biology" (Wright and Dyson, 1999, p. 322), and, as I have noted above, it reflects precisely how we think about the relationship between structure and function in a machine.

The problem, of course, is that proteins do not naturally exist in crystallized form. In fact, in their native environments, they behave more like liquids than like solids. Proteins are really "dense liquids", or "melted-solids", consisting of a "near-solid interior" and a "full-liquid exterior" (Rueda et al., 2007, p. 798; see also Zhou et al., 1999). In this context, the investigation of protein structure using nuclear magnetic resonance (NMR) spectroscopy, which probes proteins as they twist and turn in solution, has proved more revealing. Although this technique is almost as old as X-ray crystallography, for decades its applicability was severely limited. Only recently has it become possible to employ it in large-scale studies of protein structure determination[3]. What we have found by introducing the temporal dimension into our study of protein structure is that proteins are highly dynamic entities that display very high degrees of flexibility, ranging from simple side chain rotations to complete rearrangements of their secondary structure (Henzler-Wildman and Kern, 2007; Teilum et al., 2009). The structure of a protein is soft and fluid, not hard and rigid—like that of a machine.

Another important discovery, prompted by the introduction of single-molecule methods, is that proteins in vivo seldom exhibit a single ordered conformation. What is commonly referred to as *the* conformation of a protein actually comprises a range of well-defined configurations separated by low-energy barriers that a protein molecule continuously samples by means of stochastic fluctuations (Yang et al., 2003). Any population of seemingly identical proteins is really a heterogeneous mixture of molecules with slightly different conformations in equilibrium. It is just that classical structure determination methods identify only the predominant conformation in the population, averaging out the differences that exist between individual molecules (a problem that I shall discuss in more detail later on). This difficulty is compounded when faced with proteins that do not have a predominant conformation. For example, the globular protein lymphotactin adopts two completely distinct alternative conformations, and it undergoes major structural changes as it flickers from one to the other (Tuinstra et al., 2008; Murzin, 2008). More generally, it is important to realize that the conformational landscape of a protein is not fixed. The binding of ligands, post-translational modifications, temperature, pressure, and solvent concentration can all alter a protein's conformational landscape by changing the heights of the energy barriers that separate its alternative conformational states.

Lastly, and perhaps most surprisingly, many proteins do not have an ordered conformation *at all*, but instead roam the cell as unfolded polypeptide chains. This major class of proteins, which have only recently come to the attention of researchers, are called *intrinsically disordered proteins* (IDPs), and they are found in all domains of life. In mammals, it has been estimated that approximately 75% of signalling proteins and about 50% of all proteins contain at least one disordered region of more than 30 amino acids, and as many as 25% of all proteins are completely disordered (Dunker et al., 2008). It is possible to make such calculations because IDPs differ from ordered proteins already at the level of their amino acid sequence; peculiarities in this sequence define both the ability of ordered proteins to fold, and the ability of IDPs to stay unfolded. While the former have funnel-like conformational landscapes with a well-defined energy minimum, the latter have flatter conformational landscapes with numerous local energy minima, allowing IDPs to adopt a far wider spectrum of conformations—albeit less energetically stable ones (Uversky, 2013). Ordered and disordered proteins play different roles in the cell. While some functions (e.g. enzyme catalysis, immunological recognition, molecular discrimination by receptors, etc.) require proteins to have clearly-defined three-dimensional structures, other functions (e.g. cell signalling and regulation) can be accomplished by unfolded chains, sequence patterns, or isolated secondary structural motifs (Wright and Dyson, 2015).

From a structural perspective, IDPs pose an interesting challenge. The old idea that a protein binds to its substrate because their shapes match like a lock and a key seems totally inadequate. As Chouard (2011, p. 152) playfully remarks, "[y]ou might as well try to open the door with cooked spaghetti". In the case of an IDP, "the spaghetti uses the lock to mould itself into the shape of the key, rather than forming the key beforehand" (ibid.). IDPs only acquire stable functional conformations when they bind to appropriate targets. Some, however, remain disordered *even after* binding, such as the signalling protein Sic1, which stays unfolded upon binding to Cfc4 (Mittag et al., 2010). IDPs thus disprove the central dogma of structural biology, as they empirically demonstrate that an ordered conformation is not, in fact, required for protein function. It appears, moreover, that this lack of a definite structure confers a considerable functional advantage on IDPs, as it enables them to interact with a broad range of binding partners (including other proteins, membranes, nucleic acids, and various smaller molecules) by adopting different configurations. Macromolecular aggregates containing IDPs also tend to display high degrees of conformational ambiguity—a phenomenon which has been termed 'fuzziness' (Fuxreiter, 2012; Fuxreiter and Tompa, 2012). Here we are once again far away from how we think about structure and its relation to function in a machine. Indeed, the more we learn about the structure of proteins in their native state, the harder it is to uphold the mechanicist notion that protein complexes can be conceptualized, and effectively studied, as molecular machines.

Turning now to specificity, it is also becoming apparent that earlier generations of molecular biologists grossly overestimated the specificity of proteins (cf. Kupiec, 2010). This is partially due to the fact that for most of the twentieth century, methodological limitations required proteins to be studied in isolation from the cellular milieu in which they are embedded. Undoubtedly, one of the great appeals of the molecular machine concept is that it justifies ignoring this context, allowing researchers to focus their attention on the structure of the mechanical device and the 'mechanism' of its operation. The problem is that, when it comes to understanding what happens in a cell, context is everything! What a particular protein does—we now know—is largely defined by the environment it finds itself in and the interactions it has with the molecules around it; trying to acquire a complete picture of its behaviour while overlooking these factors is a futile exercise (Barabási and Oltvai, 2004; Gierasch and Gershenson, 2009).

I have already indicated that in the case of IDPs, function is determined not by structure, but by context. This lesson can be generalized to all proteins. Functional promiscuity seems to be the rule rather than the exception for proteins (Nobeli et al., 2009). Even enzymes, which have traditionally been regarded as remarkably specific catalysts, exhibit varying degrees of catalytic promiscuity owing to the inherent conformational flexibility of their active sites, among other factors (Babtie et al., 2010; Khersonsky and Tawfik, 2010). A rather extreme case is methane monooxygenase, which can hydroxylate 150 substrates in addition to methane (Copley, 2003). Not only are enzymes catalytically promiscuous, but many of them also perform a range of non-catalytic functions, such as cell motility, membrane trafficking, chaperoning, activation and inhibition of metabolic pathways, and chromatin organization. This exciting discovery has come as such a surprise (given that it conflicts with the mechanicist expectation of

[3] Increasingly, NMR is used in tandem with X-ray crystallography (see, e.g., Fenwick et al., 2014).

specificity) that the phenomenon has been called 'moonlighting' (Jeffery, 1999, 2003). A protein can have very different functions—even if it does not undergo any post-translational modifications—depending on where it is located in the cell, on the cell type in which it is expressed, on the nature and number of proteins it binds to, and on the amount of ligand, substrate, cofactor, or product available to it. As the multifunctional (or moonlighting) capacities of proteins are not coded in their genomic sequences, it is very difficult to predict them. Indeed, it is likely that many of the proteins that we think we know quite well actually perform additional functions that have not yet been experimentally identified.

It is also worth mentioning that large-scale studies of protein-protein interactions have revealed that the typical number of interactors for a given protein is far greater than was previously assumed (Cusick et al., 2005). This discovery becomes less surprising when we remember that the interior of a cell is a highly dynamic environment: most proteins within it are rapidly moving about, continuously interacting with ever-changing partners. Associations among proteins tend to be stochastic and short-lived, and are usually characterized by relatively low binding affinities (Misteli, 2001b). Clearly, the ambiguity, contingency, and context-dependence of protein-protein interactions are hard to reconcile with the exquisite specificity and tightly constrained operation that we would come to expect from a genuine molecular machine. Similarly, the transient nature of protein associations conflicts with the fixity and durability that we intuitively associate with the arrangement of parts in a machine.

Overall, the various findings I have discussed in this section regarding the structure and specificity of proteins (or lack thereof) are prompting a basic shift in how protein complexes are conceptualized. The potentially innumerable ways in which proteins can come together to form functional aggregates, the extraordinarily wide range of factors that can change their conformational state, and the dynamic and ephemeral nature of these associations has led some researchers to argue that many of the protein complexes found in the cell are better understood as *pleomorphic ensembles* than as molecular machines (Mayer et al., 2009; Suderman and Deeds, 2013; Falkenberg et al., 2013). Drawing especially on studies of protein complexes involved in intracellular signalling, these authors draw attention to the fact that these complexes are extremely diverse in size and composition, and undergo numerous reversible post-translational modifications (e.g. phosphorylations) in ways that drastically alter their conformation and activity. Receptor complexes, adhesion complexes, mRNA splicing complexes, trafficking intermediates, and many other kinds of protein associations do not exist in the cell as clearly delineated, structurally stable assemblies of fixed and highly coordinated subunits exhibiting a discrete number of conformations (in accordance with the molecular machine model), but as fuzzy and transient ensembles—with half-lives in the order of seconds or less—composed of weakly interacting and ever-changing subunits constantly flickering between alternative conformational states.

As Mayer et al. put it, after considering the vast range of potential configurations that a single transmembrane receptor complex for platelet-derived growth factor (PDGF) can adopt,

> the activated receptor looks less like a machine and more like a pleiomorphic ensemble or probability cloud of an almost infinite number of possible states, each of which may differ in its biological activity. (Mayer, 2009, p. 81.2)

Rejecting the molecular machine model has wide ranging implications for how we study, represent, and explain protein associations and interactions. Importantly, it compels us to call into question the widespread appeal to wiring diagrams and design charts (akin to those found in mechanical and electronic engineering) in schematic representations of metabolic, regulatory, and signalling pathways. An emblematic example is shown in Fig. 2. Such engineering-based diagrams present compact summaries of protein-protein interactions, and by deliberately imitating the design of electronic circuit boards, they convey the impression of understanding and control. Visualizing cellular pathways in this way gives us confidence and it emboldens us to speak optimistically about the current state of research in our particular fields.

A good illustration of this last point can be found in a well-known paper by Hanahan and Weinberg (2000), which made use of circuit-like representations of the cell to reflect on the state of cancer biology and define its agenda for the twenty-first century:

> Progress in dissecting signaling pathways has begun to lay out a circuitry that will likely mimic electronic integrated circuits in complexity and finesse, where transistors are replaced by proteins (e.g., kinases and phosphatases) and the electrons by phosphates and lipids. […] Two decades from now, having fully charted the wiring diagrams of every cellular signaling pathway, it will be possible to lay out the complete 'integrated circuit of the cell' upon its current outline. We will then be able to apply the tools of mathematical modeling to explain how specific genetic lesions serve to reprogram this integrated circuit in each of the constituent cell types so as to manifest cancer. (Hanahan and Weinberg, 2000, p. 59, 67)

The problem with these engineering-based descriptions and representations is that they do not accurately reflect biological reality. For wiring diagrams such as Fig. 2 to be as useful as the wiring diagrams of electronic engineering, they need to assume a very high degree of specificity in the molecular interactions and chemical conversions that are depicted as links in the circuits. And although this assumption of extreme specificity is perfectly consistent with the molecular machine model, it is not well supported empirically, as I have shown in this section. Recent research strongly suggests that most protein-protein interactions are contingent and opportunistic, and do not reflect a pre-determined (genetic) design. It is important to realize that wiring diagrams like Fig. 2 illustrate only one of the many—potentially innumerable—ways in which a given set of proteins may interact with one another in the cell depending on an eclectic range of factors and circumstances. Engineering-based representations of this sort are undoubtedly interesting to look at, but they are of limited explanatory value.

All things considered, such representations probably do more harm than good, as they wrongly imply that the proteins featured in them reliably and predictably form the same exact networks of interactions, which are envisaged (again, misleadingly) as fixed, solid-state, molecular circuit boards. In doing so, these diagrams prevent us from appreciating the vast spectrum of alternative interaction networks that the same set of proteins can and do form in different cells, and even in the same cell at different times (Kurakin, 2010; Talbott, 2013). Hence, when it is claimed, say, that "[s]caffold proteins are analogous to circuit boards—modular platforms that wire together components and direct the flow of information—and can program complex signaling behaviors" (Good et al., 2011, p. 682), one should take such assertions, and the representations from which they derive, with a generous dose of scepticism.

The main differences between the two conceptions of protein complexes I have discussed in this section are summarized in Table 2.

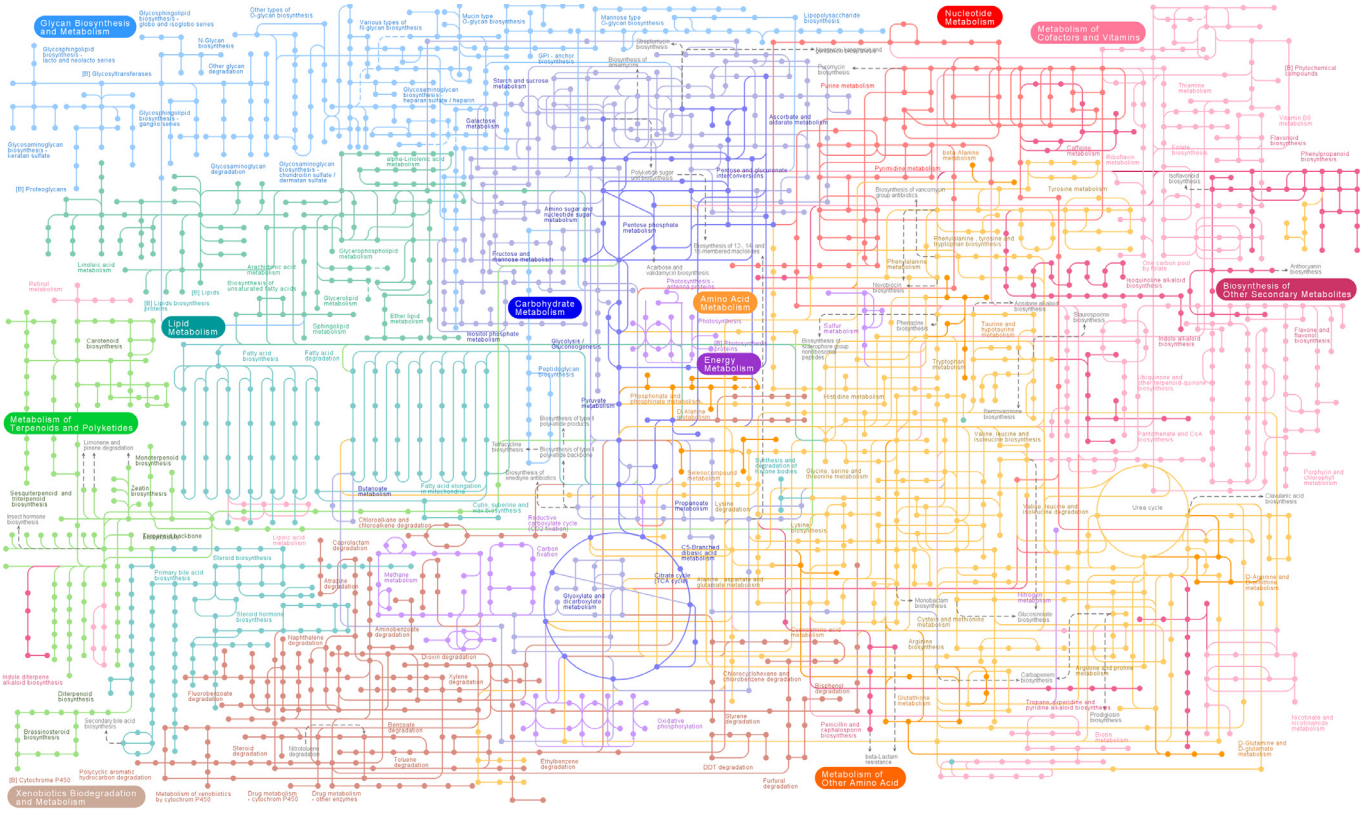

Fig. 2. Engineering-based wring diagram depicting the metabolic pathways included in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (see Kanehisa and Goto, 2000). Each node in the circuit corresponds to a protein (*source:* http://rest.kegg.jp/get/map01100/image; reproduced with permission).

Table 2
Key differences between the two conceptions of protein complexes. On the left, the standard view derived from the MCC. On the right, the alternative view suggested by recent research.

Molecular machine	Pleomorphic ensemble
Hard and rigid subunits	Soft and fluid subunits
Fixed size and composition	Variable size and composition
Few conformational states	Multiple conformational states
Functional specificity	Functional promiscuity
Context-insensitive behaviour	Context-sensitive behaviour
Stable, predefined interactions	Transient, opportunistic interactions
Amenable to crystallization	Not amenable to crystallization

4. Intracellular Transport: Power-Stroke or Brownian Ratchet?

In addition to grounding our theoretical understanding of the organization and constitution of the cell, the MCC has also influenced our view of the processes that take place inside it. To illustrate this, consider again the image of the cell as a highly intricate chemical factory—probably the most popular formulation of the MCC. The following extract from a recent textbook offers a typical articulation of this view:

> Cells act as chemical factories, taking in materials from the environment, processing them, and producing 'finished goods' to be used for the cell's own maintenance and for that of the larger organism of which they may be part. In a complex cell, materials are taken in through specialized receptors ('loading docks'), processed by chemical reactions governed by a central information system ('the front office'), carried around to various locations ('assembly lines') as the work progresses, and finally sent back via those same receptors into the larger organism. The cell is a highly organized, busy place, whose many different parts must work together to keep the whole functioning. (Hazen and Trefil, 2009, p. 252)

Descriptions of this kind make clear that the cell must possess highly effective means of sorting, packaging, and transporting cargo to different destinations. The efficient delivery of molecular products to their intended cellular location is known as *intracellular transport*, and it is of vital importance for the cell's normal functioning—when it is disrupted, complications and pathologies inevitably ensue. Prima facie, the orderliness and efficacy that is imagined in the targeted mobilization of essential cargo inside a miniature factory contrasts rather starkly with the physical reality of the cellular milieu. At the microscopic scale, all entities exhibit constant stochastic movements as a consequence of thermal agitation. This phenomenon, traditionally referred to as 'Brownian motion', causes molecules in solution to perform 'random walks' that result in *diffusion*. Although diffusion is a passive process, it nevertheless plays an indispensable role in the intracellular transport of small molecules, especially over short distances. For example, it serves as the primary basis for connectivity in signal transduction networks. However, diffusion becomes inefficient in the transportation of large vesicles and macromolecules. In such situations, the cell makes use of active and directional modes of transport, which are made possible by so-called 'motor proteins' that carry cargo quickly and efficiently across cytoskeletal tracks. The discovery of motor proteins has long been assumed to have provided empirical support for the MCC (see, e.g., Pollard, 1992; Urry, 1993; Block, 1997).

Motor proteins convert chemical energy—usually obtained by the hydrolysis of adenosine triphosphate (ATP)—into directional motion and the performance of work. There are many different kinds of motor protein, each of which performs a distinct motile function (Schliwa, 2003). Those that utilize the cytoskeleton for movement fall into two categories based on their binding partners: actin motors, such as myosin, move along microfilaments through interaction with actin, whereas microtubule motors, such

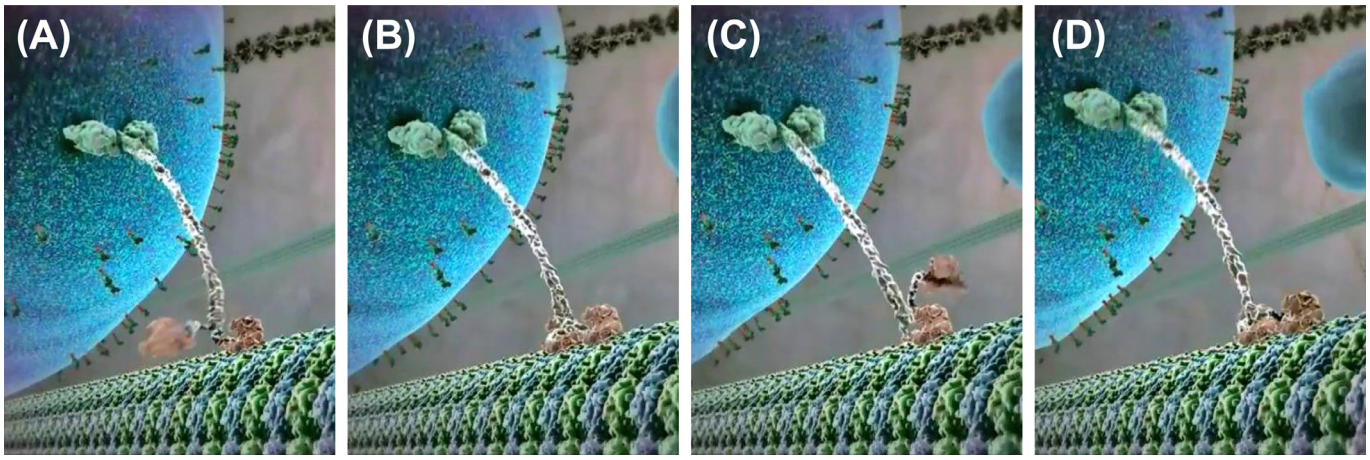

Fig. 3. Cropped snapshots of the acclaimed computer animation *The Inner Life of the Cell*, created by XVIVO for Harvard University's Department of Molecular and Cellular Biology. The four consecutive snapshots depict the cycle of orchestrated movements by which a cargo-carrying kinesin 'walks' along a microtubule. (**A**) ATP-binding to the motor domain of the left leg triggers a change in its conformation which generates a power-stroke in the linker region that throws the motor domain of the right leg overhead of the left leg. (**B**) The motor domain of the right leg re-attaches to the microtubule and the products of ATP hydrolysis are released. (**C**) Binding of ATP to the motor domain of the right leg in turn induces a rearrangement of its structure which generates a further power-stroke in the linker region that pushes the motor domain of the left leg above the right leg. (**D**) The motor domain of the left leg re-attaches to the microtubule and the products of ATP hydrolysis are again released, thus completing the cycle (*source:* http://www.artofthecell.com/the-inner-life-of-the-cell; © 2006 The President and Fellows of Harvard College).

as kinesin and dynein, move along microtubules through interaction with tubulin. These proteins also differ in the type of cargo they transport and in their direction of travel. In accordance with the MCC, motor proteins are conceptualized as miniature versions of macroscopic motors. They are often described as "tiny nanomachines [that] work in many ways just like an automobile on the highway" (Shi and Ha, 2011, p. 4), as they consume fuel to power their motion, and they move steadily in a directional manner at variable speeds along 'molecular highways'. Structural studies by X-ray crystallography have also been invoked to suggest that these proteins are reminiscent of large-scale machines (Rayment, 1996; Vale and Milligan, 2000). But how do motor proteins actually move?

The orthodox approach to explaining the directional movement of motor proteins is to appeal to the mechanical principles that govern the motion of man-made motors. In an internal combustion engine, for instance, the energy input (delivered by the ignition of a combustible gas under pressure) is tightly coupled to the performance of mechanical work, which manifests itself as a 'power-stroke' that results in the movement of the piston. In the same way, in motor-driven intracellular transport it is argued that the energy input (delivered by the hydrolysis of a high-energy compound like ATP) induces a large-amplitude conformational change in the motor protein which generates a mechanical force—a power-stroke—that drives the molecule forward relative to a polymeric track. Appropriately, this is called the *power-stroke model*, and it has dominated our theoretical understanding of how motor proteins work for decades (Cooke, 1986; Howard, 2001; Tyska and Warshaw, 2002). Sometimes, the chemically-induced conformational change in the motor protein that produces the power-stroke is compared to the mechanical release of a viscoelastic spring, which thrusts the molecule forward (e.g. Howard, 2006). In the case of kinesin, which forms dimeric 'legs' that alternatively attach to tubulin, the repetitive power-strokes result in a 'hand-over-hand' motion that makes the protein appear like it is 'walking' along the microtubule (Yildiz et al., 2004; Asbury, 2005). Fig. 3 illustrates how the kinesin walk is commonly represented in the technical literature, as well as in textbooks and other educational materials.

Of course, animations such as the one shown in Fig. 3 conform perfectly to what we would expect to find if the cell was indeed a machine, as they portray motor proteins as tiny robotic bipeds performing sequential cycles of precisely-coordinated, mechanically-powered movements along cytoskeletal tracks. However, upon closer inspection, it becomes apparent that these models of intracellular transport are fraught with problems. For a start, the plasticity, fluidity, and dynamicity that most proteins exhibit in vivo—which I discussed at length in the previous section—are difficult to reconcile with the rigidity, solidity, and stability that motor proteins would need to possess for them to move by power-stroke mechanisms. Moreover, these models tend to overlook the fact that proteins operate in an environment that is drastically different from the macroscopic one in which we, and our machines, exist. Motor proteins, like all other molecules, are subject to constant thermal and quantum fluctuations that make carefully-synchronized movements along a desired path challenging in the extreme. In fact, the energy of ATP hydrolysis responsible for generating the power-strokes that allegedly propel motor proteins forward is only about an order of magnitude larger than the environmental stochastic forces that are permanently buffeting them. In such conditions, moving mechanically and deterministically is like trying to 'swim in molasses' or 'walk in a hurricane' (see Astumian, 2007).

Besides these general worries, a number of surprising empirical findings—made possible by the use of novel methods—have called into question the theoretical adequacy of power-stroke models such as the one illustrated in Fig. 3. For example, although the hand-over-hand mechanism that underlies the walking motion attributed to kinesin is dependent on the protein's dimeric form, monomeric kinesin motors have been reported which are equally capable of directional movement (Okada and Hirowaka, 1999). More broadly, there is no obvious correlation between the amount of chemical energy a motor protein consumes and the distance it travels. Single-molecule measurements of myosin-mediated transport have revealed that a single cycle of ATP hydrolysis can result in displacements of wildly variable lengths, ranging from 5 to 30 nm (Kitamura et al., 1999). Additional studies of myosin movement indicate that the structural geometry of a protein's motor domain is not correlated with its step size (Yu et al., 2012). These and other recent findings suggest that the structure of motor proteins may not be as crucial for their operation as one might have expected if these proteins were *bona fide* molecular machines performing precisely-coordinated, mechanically-powered movements. Motor proteins also lack the functional specificity that is typically associated with machines, as many of them have been found to be

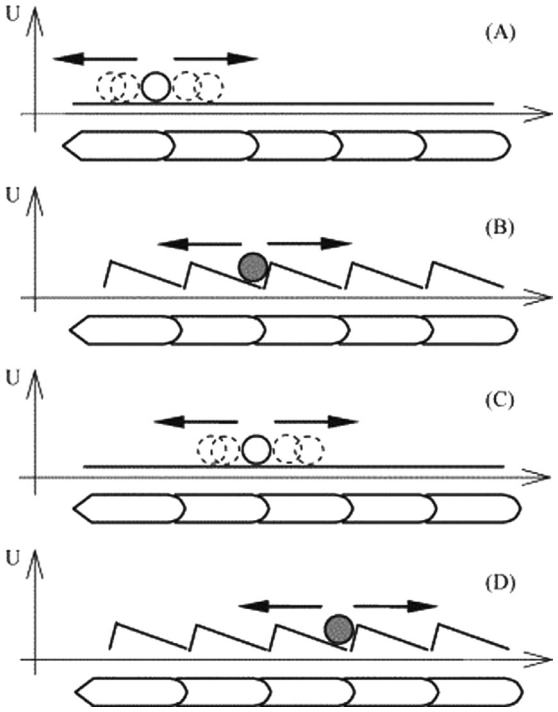

Fig. 4. Schematic representation of the Brownian ratchet model of intracellular transport. The motor protein attached to a cytoskeletal track is hypothesized to display two distinct potential energy landscapes depending on its conformational state. In the 'flip' conformation—(**A**) and (**C**), white ball—the energy landscape is flat, so the motor protein slides freely along the track, buffeted by stochastic fluctuations. In the 'flop' conformation—(**B**) and (**D**), grey ball—the energy landscape has a saw-tooth shape, so the motor protein drifts to the closest energy minimum where it remains until it acquires the 'flip' conformation. By periodically switching between these two conformations upon repeated cycles of ATP hydrolysis, the motor protein is driven by thermal fluctuations to the right. (Figure adapted from Kurakin, 2006; reproduced with permission.)

involved in a number of additional, non-motor cellular functions (Schliwa and Woehlke, 2003).

In light of these problems, in the last few years a completely different account of motor-driven transport has started to receive widespread attention known as the *Brownian ratchet model*. Although it is almost as old as the power-stroke model, it has remained relatively unknown until fairly recently—presumably because it does not appeal to our mechanical intuitions in the way that the comfortingly familiar idea of a power-stroke does. The basic contention of the Brownian ratchet model is that the directional motion of a motor protein is primarily driven by stochastic fluctuations and rectified (or biased) by chemical reactions, such as the hydrolysis of ATP. Empirical studies have established that motor proteins use the energy of ATP hydrolysis to flip–flop between two alternative conformations. What the Brownian ratchet model postulates is that the 'flip' and 'flop' conformations of a motor protein are characterized by different potential energy landscapes, as shown in Fig. 4. In the 'flip' conformation (A and C, white ball), the energy landscape has a flat shape. This means that the motor protein performs a random walk on its track as a result of thermal agitation, exhibiting equal probabilities of moving to the left or to the right of its initial position. In the 'flop' conformation (B and D, grey ball), the energy landscape has a jagged, saw-tooth shape. Consequently, random collisions jostle the motor protein overwhelmingly to the right, where it gets trapped in the nearest potential energy minimum trough. In this way, by stochastically switching between two distinct conformational states as a result of repeated cycles of ATP hydrolysis, the motor protein is able to harness the perturbations of Brownian motion to move in a specific direction along a cytoskeletal track (Astumian, 1997; Ait-Haddou and Herzog, 2003; Kurakin, 2006)[4].

A fundamental difference between the power-stroke model and the Brownian ratchet model is that the former takes chemical reactions (such as the hydrolysis of ATP) to be responsible for generating the mechanical forces that drive the motor protein forward, while the latter assumes that chemical reactions serve to bias the existing Brownian motion in a particular direction. In other words, a power stroke motor moves *despite* stochastic fluctuations; a Brownian ratchet motor moves *because* of them. In this respect, a very attractive feature of the Brownian ratchet model is that it explicitly factors in the counterintuitive physical conditions of the cellular milieu in its explanation of how proteins move directionally, instead of conveniently ignoring them or dismissing them as inconsequential.

A further advantage of the Brownian ratchet model is that it is able to make sense of experimental findings that appear perplexing from the perspective of the power-stroke model. The aforementioned reports of monomeric motors, the lack of correlation between the chemical energy used by the motor and the distance it travels, and the independence of step size from the structural geometry of the motor domain can all be straightforwardly accommodated within the Brownian ratchet model. In this model, 'structure' and 'specificity' do not play the same critical role in determining how the protein moves as they do in the MCC-derived power-stroke model. Once it is adopted, the discovery that motor proteins are also involved in other cellular processes ceases to be baffling or surprising.

Although the Brownian ratchet model is less intuitive and harder to initially grasp than the power-stroke model, it is in many respects the simpler model of the two. As there is no specific reference to the topological or geometrical configuration of the motor protein (other than to its alternative energy profiles, which do have a structural basis), there is no need to speculate about how its various structural domains interact with one another in a perfectly synchronized fashion to generate motion. Similarly, because there is no crucial mechanical step—no power-stroke—that can be identified as the specific moment at which chemical energy is transformed into work, it becomes unnecessary to invoke 'violent kicks' (Liphardt, 2012), 'judo throws' (Vale and Milligan, 2000), or any other anthropomorphic actions to explain how motor proteins move directionally.

But perhaps the greatest theoretical virtue of the Brownian ratchet model is that it elegantly demonstrates how stochasticity can be put to good use in the cell. Whereas the power-stroke model considers motor-driven transport to be an example of what Schrödinger (1944) called the 'order-from-order' principle, given that the complexity of the mechanism it postulates is assumed to derive from a pre-existing genetic design, the Brownian ratchet model regards it instead as an instance of the 'order-from-disorder' principle, which Schrödinger claimed (incorrectly, as it turns out) plays no role in biology. This is because it shows how the coupling of two random (or disordered) processes—namely Brownian motion and the binding of ATP—can result in a non-random (or ordered) outcome: directional movement. In this way, by providing a non-deterministic, design-free conceptualization of intracellular

[4] A ratchet is, of course, a machine, so it might seem odd—even contradictory—to criticize the invocation of mechanical principles in the explanation of intracellular transport and then propose an alternative explanation that explicitly refers to a mechanical device. But there is, in fact, no contradiction involved. The use of the term 'ratchet' in the Brownian ratchet model is merely intended to describe the spiky, non-sinusoidal shape of the energy profile of the motor protein when it adopts a 'flop' conformation (see Fig. 4). It does not imply or suggest that the protein structurally resembles a ratchet, or that it works mechanically like a ratchet. In fact, the operation of a Brownian ratchet is decidedly *non*-mechanical, as I will explain in the remainder of this section.

transport, the Brownian ratchet model strikingly illustrates how order can be generated out of chaos (cf. Prigogine and Stengers, 1984; Hoffmann, 2012).

It is undeniable that the growing use of single-molecule technology in experimental studies of motor proteins has greatly contributed to raising the profile of the Brownian ratchet model (Yanagida et al., 2007; Karagiannis et al., 2014). However, there is still no consensus as to whether motor-driven transport is best understood in terms of the increasingly popular Brownian ratchet model or the more traditional power-stroke model. Advocates of the former maintain that power-strokes are irrelevant in determining the directionality, stepping force, and optimal efficiency of motor proteins (Astumian, 2015), while supporters of the latter insist that motor proteins would not be as fast and powerful as we know them to be if they operated by a Brownian ratchet mechanism (Wagoner and Dill, 2016). Some claim that the two models sit at opposite ends of a continuum within which most motor proteins actually operate. For example, Oster and Wang (2003, p. 208) assert that "[t]here are only a few motors that can be regarded as being pure power stroke motors or pure ratchets; most protein motors employ a combination of the two strategies". If this is true, then the two models are not necessarily mutually exclusive. At present it is not yet possible to settle this theoretical dispute. The two models, we should not forget, constitute different attempts to interpret the same empirical data; they are extrapolations from experimental studies—no one has actually *seen* a kinesin literally walking along a microtubule, as portrayed in Fig. 3. Nevertheless, what can be asserted with a reasonable degree of certainty is that the engineering-based power-stroke model, at least when conceptualized in analogy with the power-stroke mechanism of an internal combustion engine, if not irretrievably flawed, at best offers only an extremely idealized interpretation of motor-driven transport as it occurs in the cell.

Still, even if the Brownian ratchet model becomes unanimously accepted as the preferred explanation of how motor proteins work, it could be argued that recognizing that the cell contains real motors is already one concession too many to the MCC. This, however, does not follow. 'Molecular motors'—if that is how one is to refer to motor proteins—are *not* miniature versions of macroscopic motors. In fact, they differ from macroscopic motors in almost every important respect (cf. Astumian, 2001; Linke et al., 2005; Wang, 2008). For one thing, they lack rotors, armatures, and all the other trappings of conventional motors. They are made of soft, flexible materials which exhibit high degrees of freedom, unlike the hard levers, cranks, and hooks that make up most mechanical devices. Moreover, due to their minuscule size, the influence of gravity and inertia on their operation is insignificant compared to that of the raging 'Brownian storm' that permanently engulfs them. This turbulence, combined with the high viscous drag of their fluid environment, makes the long-range transmission of precise mechanical forces physically impossible. In addition, as I have already indicated, whereas in man-made motors energy is used to drive motion, in molecular motors energy is used to restrain motion. The former move directionally by overcoming stochastic perturbations; the latter do so by exploiting them. A further difference is that molecular motors convert chemical energy directly into work without using heat or electrical energy as intermediates, which is why their efficiency is much higher than that of macroscopic motors. Overall, because of all of these crucial differences, although it may seem tempting to draw analogies between molecular motors and macroscopic ones, we should keep firmly in mind that "in answering fundamental questions regarding problems associated with friction, wear, transmission, efficiency, fuel, motion and work, such facile comparisons often serve to cloud rather than simplify issues" (Browne and Feringa, 2006, p. 26).

Table 3
Key differences between the two conceptions of motor-driven intracellular transport. On the left, the standard view derived from the MCC. On the right, the alternative view suggested by recent research.

Power-stroke	Brownian ratchet
Continuous forward movement	Discontinuous forward movement
Energy input generates motion	Energy input rectifies motion
Overpowers stochastic fluctuations	Harnesses stochastic fluctuations
Motor structure plays critical role	Motor structure is secondary
Coordinated motor movements	No coordinated motor movements
Includes crucial mechanical step	Lacks crucial mechanical step
'Order-from-order' mechanism	'Order-from-disorder' mechanism

Ultimately, the issue boils down to how we choose to define the term 'motor'. We tend to assume that motors constitute a class of machine—hence the objection I have just considered. But perhaps we should reverse this relation and consider machines to be a class of motor. After all, a motor can be defined very generally as an entity that imparts motion (in fact, this is the first definition of 'motor' that is listed in the *Oxford English Dictionary*). Given that machines are not the only entities capable of imparting motion, it follows that not all motors are machines. As I noted in the introduction, the word 'machine' tends to carry a number of additional connotations, such as a pre-existing design, a tightly constrained operation, and a deterministic outcome. It is therefore possible to conclude that motor proteins are indeed genuine motors, even though they are not machines. It is interesting to observe that some authors are starting to display an awareness of this important distinction, as the following passage suggests:

> Because they operate inside a cell, [molecular motors] are tiny and operate on a physical scale that makes them very different from the manmade, macroscopic objects we normally imagine when we hear the word 'machine'. Further, their size and soft structure allows them to be much more dynamic and robust than artificial machines. They work needing very little input, as energy levels not far from average thermal energy are sufficient for a given task. This property too contrasts with artificial machines, which work much more rapidly, accurately, and deterministically, but with higher energy demands and less adaptability. (Karagiannis et al., 2014, p. 3318)

The main differences between the two conceptions of motor-driven intracellular transport I have discussed in this section are summarized in Table 3.

5. Cellular Behaviour: Deterministic or Probabilistic?

So far, I have shown how the MCC has provided the theoretical foundation for our traditional understanding of the cell's internal architecture, of the macromolecular complexes that compose it, and of the transport processes that take place within it. But its influence does not end there. The MCC has also shaped the way we think about how the cell behaves, as I will argue in this section.

What a cell does is largely determined by its internal makeup. This makeup is constituted by a complex network of metabolic, regulatory, and signalling pathways that—as I have already discussed—are often misleadingly conceptualized in analogy with electronic circuit boards. These pathways, when prompted by internal or external cues, generate specific behavioural outputs that allow the cell to perform its various functions and respond to changes in its environment. As these pathways have a genetic basis, the behavioural outputs they specify are themselves dependent on how and when the pertinent genes become activated and transcribed in the cell. Consequently, in order to understand the basis of cellular behaviour, it is necessary to consider how the all-important process of *gene expression* is initiated.

Basically, an intracellular or extracellular signal—an 'inducer'—triggers a cascade of biochemical reactions that causes proteins called 'activators' (which are a class of transcription factor) to bind to specific sites in the DNA known as 'enhancers'. Upon binding, the activators interact with other proteins that recruit RNA polymerase and its associated transcription factors, collectively referred to as the 'preinitiation complex', to the 'promoter' region of the target gene, where it begins the process of transcription. Thousands of transcription factors have been identified in the past few decades, as have the enhancer and promoter regions of countless genes. But despite this wealth of information, there has been considerable debate regarding the precise way in which transcription is regulated and modulated.

It has long been known from numerous experimental studies that when cells are treated with varying intensities of an inducer and the gene product—mRNA or protein—corresponding to a specific gene is assayed, the level of gene product changes in a smooth, dose-dependent manner. Specifically, a gradual increase in the concentration of the inducer usually results in a proportional increase in the expression of the gene. In order to make sense of this observation, it was generally assumed that cells adjust the rate of expression of a responsive gene progressively and linearly from zero to its maximum output in direct proportion to a rising concentration of an inducer. This came to be known as the *graded model* of gene expression (it is sometimes alternatively referred to as the 'rate', 'analogue', or 'rheostat' model), and it remained the dominant view of gene expression until the end of the last century (Ross et al., 1994; Rossi et al., 2000; Pirone and Elston, 2004). It is a model that clearly exemplifies the MCC, as it construes the modulation of gene expression as a continuous, linear, mechanical process, "akin to depressing the accelerator on a car" (Hume, 2000, p. 2323). It is also a thoroughly *deterministic* model, as it suggests that gene expression, and by implication most cellular behaviour, can be anticipated, computed, and predicted—in principle, at least—from knowledge of the 'initial conditions' (such as the concentration of the inducer). It therefore fits well with the classic mechanicist idea that the cell is endowed with a genetic program, analogous to a computer program, which controls and reliably executes its operations in a predetermined way (Jacob, 1973; Bray, 2009; Nicholson, 2014).

When considering this model, it is important to bear in mind that until recently, gene expression—and cellular behaviour more generally—could only be studied by looking at large populations of cells. If one uses conventional molecular biology techniques such as Northern blots, microarrays, and reverse transcriptase-polymerase chain reaction, the only way to gather together enough gene product to reach a detectable threshold is to grind up vast numbers of isogenic (i.e. genetically identical) cells grown under the same conditions and then measure the amounts of the relevant mRNA or protein in the homogenate. What this means is that, although the goal is to understand the behaviour of an individual cell, one proceeds by studying the behaviour of a population of cells. The consequence of doing so is that the specific behavioural patterns of individual cells are averaged out across the entire population, and this can mask differences between members of the population.

In the past, this methodological limitation was not considered a problem because "molecular biologists habitually assume uniformity of the cell populations that serve as starting material for experimental analysis" (Huang, 2009, p. 3853). If all cells are presumed to be identical and one is therefore dealing with a *homogeneous* population, then one can confidently infer that the average behaviour of the population as a whole accurately reflects the individual behaviour of each cell in that population. Notice that this assumption—like so much else I have discussed in this paper—is grounded in the MCC: just as cars manufactured in an assembly line according to the same model design will behave almost identically, cells endowed with the same genetic program and grown in the same conditions are expected to behave almost identically.

The situation has changed radically in recent years. Technological advances in the experimental use of fluorescent reporter proteins and the development of new microscopy techniques have granted us unprecedented access to the real-time dynamics of individual molecules in single cells (van Holde, 1999; Deniz et al., 2008). As we have begun to monitor biological processes on a cell-by-cell and molecule-by-molecule basis, it has become apparent that there exists "a hidden world beneath population averages" (Altschuler and Wu, 2010, p. 559). Being able to precisely measure the distribution of cellular behaviours across a population, as opposed to merely relying on the average behaviour of the whole population, has unexpectedly revealed that even isogenic cells subject to the same environmental conditions behave quite differently from one another. There is no such thing as a perfectly homogeneous population of cells. All cell populations exhibit some degree of *heterogeneity*.

Gene expression offers perhaps the clearest illustration of this heterogeneity. Single-cell studies strongly suggest that increasing the concentration of an inducer in an isogenic population does not lead to a gradual increase in the rate of transcription in every cell in the population (as hypothesized by the graded model), but rather results in the recruitment of a rising number of cells that respond in an all-or-nothing fashion once their particular activation thresholds have been reached. In other words, in each cell of the population the target gene is either maximally expressed, or it is not expressed at all, and the probability of its expression in every cell rises as the concentration of the inducer increases. In addition, once a cell begins to express the gene, the rate of its expression remains largely unaffected by further increases in the concentration of the inducer. With regards to each of the genes it contains, a cell appears to exist in one of two meta-stable functional states: it is either 'on' or 'off'. This is generally known as the *stochastic model* of gene expression (it is also referred to as the 'binary', 'digital', or 'threshold' model), and it has become very widely accepted in recent years (Walters et al., 1995; McAdams and Arkin, 1997; Elowitz et al., 2002). In direct contrast to the deterministic character of the graded model, the stochastic model is inherently *probabilistic*. Each cell in the population exhibits a specific and distinct probability to respond to a given concentration of inducer, and this probability can vary widely—even among members of the same isogenic population.

According to the stochastic model, the regulation of gene expression is accomplished by modifying the probability that the preinitiation complex will come together successfully and bind to the promoter of the target gene. Activators and enhancers are presumed to act by increasing the likelihood that the promoter will be transcriptionally active at a given moment, but do not affect the rate of mRNA production once transcription has begun (Fiering et al., 2000; Blake et al., 2003). The random switching of the transcriptional apparatus between active and inactive states is deemed to generate short and sharp 'bursts' or 'pulses' of transcriptional activity, which result in corresponding bursts of translational activity. The hypothesis that proteins are synthesized in bursts has been subsequently verified by single-molecule experiments (Cai et al., 2006; Yu et al., 2006). Bursting behaviour in gene expression has been reported not only in bacteria, but also in yeast (Zenklusen et al., 2008), mammalian cells (Raj et al., 2006), and developing embryos (Paré et al., 2009). Even different nuclei in a single multinucleated syncytium such as a muscle fibre, which share not only a common environment but also a common cytoplasm, have been found to display disparate bursts of transcriptional activity (Newlands et al., 1998).

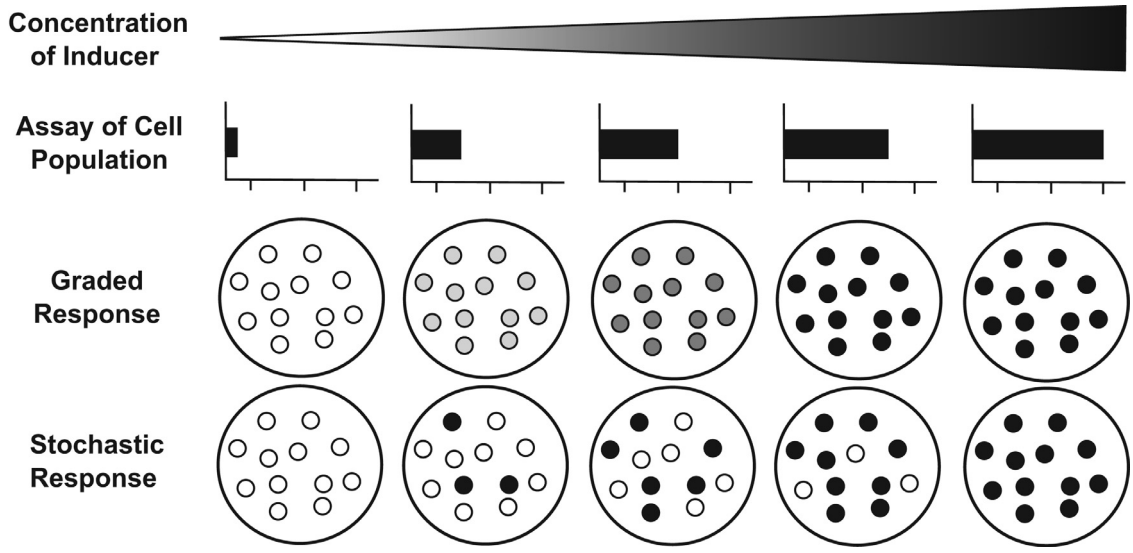

Fig. 5. Graded versus stochastic models of gene expression. Raising the concentration of an inducer results in a proportional increase in the expression of the relevant gene in an isogenic population of cells. If gene expression is measured by assaying the total amount of mRNA or protein produced by the population, it is not possible to distinguish between stochastic and graded transcriptional responses, as both are consistent with population-level observations. Single-molecule methods, however, have recently enabled gene expression to be studied on a cell-by-cell basis, and this has revealed that most cells exhibit an all-or-nothing stochastic expression pattern. (Figure adapted from Kringstein et al., 1998; © 1998 National Academy of Sciences.)

Given all this evidence in support of the stochastic model, it may seem difficult to understand why the graded model remained the orthodox view for as long as it did. But the answer is quite straightforward. When the only way to measure gene expression in a cell was to assay the total amount of mRNA or protein produced by an entire population of cells, it was simply not possible to discriminate between the two competing models. Indeed, the aforementioned observation that progressively raising the concentration of an inducer results in a proportional increase in the expression of the corresponding gene is perfectly consistent with both models, as Fig. 5 shows. The level of gene expression in the population as a whole could reflect similar levels of gene expression in all cells (as postulated by the graded model), or the statistical mean of different subsets of cells either expressing or not expressing the gene (as postulated by the stochastic model). It was not until single-molecule methods were developed that it became possible to examine gene expression on a cell-by-cell basis, and thereby distinguish the two models experimentally (Kringstein et al., 1998; Pirone and Elston, 2004; Kurakin, 2005). The study of gene expression illustrates rather dramatically how being forced to average out data across a biological population due to methodological limitations can lead to critical losses of information pertaining to the phenomenon under investigation.

Intriguingly, it has been suggested that the distinction between graded and stochastic models of gene expression "is conceptually similar to the difference between Newtonian and quantum mechanics, and it offers similar intellectual challenges" (Hume, 2000, p. 2324). This theoretical comparison is not as far-fetched as it may seem. The stochastic model regards cellular response patterns as state transitions, which are rather reminiscent of thermal or quantum phase transitions. Moreover, the precise timing and frequency of the switching between active and inactive transcriptional states is impossible to predict, as it is not a mechanical process but a stochastic one. In the absence of deterministic certainties, genes in a population cannot be considered to be either active or inactive at any given instant. Instead, they can only be attributed a *probability* of being active in a particular cell at a particular time, even if this probability can sometimes be very close to 0 or 1.

The variable flickering of transcriptional activity in different cells is one of the major causes of heterogeneity in isogenic populations. But where exactly does this cell-to-cell variability in transcriptional activity come from? The answer becomes apparent when we remember that gene expression is a *molecular* process, and like all molecular processes, it is inherently stochastic, given that it takes place in an environment that is subject to the chaotic dynamics of Brownian motion. Each step in the process relies on fortuitous encounters between molecules that are randomly moving about as a consequence of thermal agitation. Evidently, these molecules must be at the right place and at the right time—not to mention in the right vibrational state—for them to be able to participate in the appropriate reactions. The unpredictability of the whole process is further amplified by the fact that the participating molecules in each step are present in the cell in very low copy-numbers, as this decreases the chances of successful interactions between them. DNA is the most extreme example, since there are usually only one or two copies in a cell at any given time, but mRNA and most regulatory proteins and enzymes are present in remarkably small numbers as well (Xie et al., 2008). Other contributing factors to cell-to-cell variability in gene expression include variations in the topological configuration of the nuclear architecture (Cremer et al., 2006), and the uneven partitioning of cytoplasmic contents during cell division (Huh and Paulsson, 2011). Of course, gene expression is only one of many cellular processes that, due to the inherent stochasticity of the molecular interactions that underpin them, generate heterogeneous responses in isogenic populations.

From a theoretical perspective, the discovery of non-genetic heterogeneity in isogenic cell populations came as a huge surprise. At first, molecular biologists struggled to make sense of it, as it is a difficult phenomenon to accommodate within the mechanicist interpretive framework of the MCC. After all, as Kurakin (2005, p. 60) vividly puts it, "[n]o computers, no aircrafts, no automobiles, 'isogenic' as they are built, acquire spontaneously personality of their own and respond in a probabilistic manner to environmental cues by all-or-none functional and/or structural transitions". Because it conflicts with the deterministic assumptions of the MCC, non-genetic heterogeneity was initially viewed with suspicion, as

a consequence of 'rogue' cell behaviour resulting from so-called 'illegitimate transcription' (Chelly et al., 1989). But as instances of heterogeneity became more widely reported, researchers could no longer afford to dismiss them. As a result, an engineering term began to be used to designate this phenomenon in order to render it theoretically compatible with the MCC. This is the concept of *noise*, which has since become widely adopted by the community as whole (e.g. Elowitz et al., 2002; Rao et al., 2002; Raser and O'Shea, 2005). It is worth reflecting for a moment on the consequences of the biological appropriation of this concept.

In engineering contexts, noise refers to an unwanted random disturbance that hampers the perception of a transmitted signal. Noise is therefore regarded as a nuisance which engineers strive to overcome by designing machines that avoid or filter out its detrimental effects. Interestingly, when stochastic cell-to-cell variability began to be referred to as noise in the literature, those very same negative connotations from engineering became associated with this phenomenon. Accordingly, it was claimed that biological "[n]oise is often harmful, as it garbles cell signals, corrupts circadian clocks, and disrupts the fine-tuned process of development" (Ozbudak et al., 2002, p. 71). Similarly, it was frequently assumed that "[c]ell signalling pathways and developmental switches have evolved so as to minimize the disruptive effect of such fluctuations" (ibid.). Note that these negative assessments of the effects of stochasticity make perfect sense from the theoretical perspective of the MCC. Stochasticity is perceived to thwart the capacity of biologists to totally control cellular behaviour in the exact same way that noise thwarts the capacity of engineers to design perfectly efficient and predictable machines.

More recently, however, there has been a noticeable shift in how biologists speak about noise. Discussions about how cells *tolerate* noise are gradually giving way to discussions about how cells *exploit* noise (see, e.g., Huang, 2009; Eldar and Elowitz, 2010; Balázsi et al., 2011). The reason for this is that as research into the non-genetic heterogeneity of cells continues, evidence for the biological importance of this phenomenon is mounting ever-rapidly. We now know that non-genetic heterogeneity plays key roles in both microbial and eukaryotic cells, in embryonic development, and in evolution. For one thing, it is a crucial generator of phenotypic diversity, which enables cell populations to adapt rapidly to changing environmental conditions. It does so by permitting the implementation of probabilistic diversification strategies within a population, such as bet-hedging and divisions of labour, which can confer considerable fitness advantages. It also influences cell fate decisions, which facilitates the regulation of differentiation during development. Non-genetic heterogeneity has even been suggested to allow tumours to counteract the effects of chemotherapy, thereby limiting the efficacy of target-selective drugs (Brock et al., 2009). In general, it is clear that highly heterogeneous cell populations are more robust and they adapt, grow, and evolve faster than more homogeneous cell populations. Far from being a nuisance, 'noise', it turns out, is central to many cellular functions.

One very important theoretical implication of the probabilistic nature of cellular behaviour and the observed heterogeneity of cell populations is that, quite literally, every cell (in an organism and elsewhere) is a *unique* entity. No two cells are identical, given that no two cells respond to a stimulus in the exact same way—even if they are genetically the same. As this simple yet profound observation becomes more widely recognized, cell individuality is likely to become an ever-more important area of research. Our traditional dependence on methods that average out responses across populations has inadvertently driven us to rely on what Levsky and Singer (2003) fittingly call the 'average cell': a statistical contrivance for representing biological knowledge beyond the limits of detection. But as these authors point out, the advent of single-molecule methods has demonstrated that the average cell is a myth. Variability is everywhere in the cellular world. In fact, as a cellular phenomenon, variability is less difficult to explain than similarity. Looking to the future, as cell biology progressively morphs into 'single-cell biology' and we devote increasing attention to carefully characterizing not just individual cells, but also individual molecules in individual cells, we may soon find ourselves in the position of having to reconsider our understanding of even the most basic biological processes.

Table 4
Key differences between the two conceptions of cellular behaviour. On the left, the standard view derived from the MCC. On the right, the alternative view suggested by recent research.

Deterministic	Probabilistic
Individually predictable	Collectively predictable
Graded response patterns	Stochastic response patterns
Subject to linear dynamics	Subject to nonlinear dynamics
Produces homogeneous populations	Produces heterogeneous populations
Population averages are accurate	Population averages are misleading
Noise is a nuisance that is tolerated	Noise is an asset that is exploited
Every isogenic cell is the same	Every isogenic cell is unique

The following excerpt, co-authored by one of the leading figures in the field, effectively summarizes the two very different views of cellular behaviour that I have examined:

> As biologists, we must grapple with, and reconcile, two very different views of cellular behaviour. On the one hand, we frequently think of cellular functions as being determined by 'circuits' of interacting genes and proteins. In a loosely analogous way to electronic circuits, these chemical circuits encode genetic programmes that underlie differentiation, the cell cycle and other behaviours. They accurately respond to stimuli and generate precise behavioural programmes in individual cells. On the other hand, there is the 'noisy' view of the cell we get *when we actually look at cells*: they exist in squishy, dynamic and heterogeneous populations, the morphologies, gene-expression patterns and differentiated states of which differ from one another, even when environment and genotype are fixed. (Locke and Elowitz, 2009, p. 383; emphasis added)

The main differences between the two conceptions of cellular behaviour I have discussed in this section are summarized in Table 4.

6. Conclusions: Towards a New View of the Cell

I have argued in this paper that molecular biology is currently undergoing a fundamental shift in its theoretical conceptualization of the cell. The conventional mechanical, reductionistic, and deterministic view is gradually giving way to an understanding of the cell that emphasizes its fluidity, plasticity, and stochasticity. Faced with the formidable task of interpreting the vast and ever-growing amount of experimental data that continues to get published, explanatory appeals to engineering notions of design, programs, and circuits are increasingly being replaced by recourses to the physical principles of non-equilibrium thermodynamics and complexity theory. Cells are empirically revealing themselves to be inherently dynamic, self-organizing systems that respond stochastically and nonlinearly to environmental stimuli.

The inescapable conclusion that follows from the analysis I have presented is that the cell can no longer be unproblematically conceptualized as a machine[5]. Over the course of the paper, it has

[5] Note that this conclusion does not imply that every entity or process within the cell is being (or needs to be) reconceptualized. To be clear, the thesis I have sought to defend is not that *all* organelles exist as irreversible steady states, that *every* protein complex is a pleomorphic ensemble, and so on. It is rather that a

become apparent that cells lack all four characteristic properties of machines that were identified in the introduction. First, once the crucial role that self-organization plays in shaping the cellular architecture is acknowledged, it is difficult to uphold the idea that the spatiotemporal arrangement of the parts of a cell obeys a predetermined blueprint or design, as it does in a machine. Second, the conformational flexibility of most cellular constituents and the functional promiscuity they exhibit shows that a cell's operation is not as tightly constrained by its structural configuration as it is in a machine. Third, whereas a machine performs its function by precisely following a predefined sequence of steps, a cell can arrive at a particular end in a variety of ways: it can recruit different kinds of molecules to the same function—or the same kind of molecule to different functions—depending on the conditions it finds itself in. And fourth, a cell cannot be broken down into parts without jeopardizing its structural integrity in the way that every machine can. Cellular components form deeply intertwined, ever-changing networks of interactions that cannot be individually dissected without sacrificing the organization of the whole. "Cells are not engineered systems of discrete, interacting computational components, naturally yielding to compositional analysis" (Melham, 2013, p. 134), which is why they cannot be fully explained reductionistically; and neither do they operate deterministically, which is why their behaviour cannot be perfectly predicted.

Monod was wrong. The cell is *not* a machine, but something altogether different—something more interesting yet also more unruly. It is a bounded, self-maintaining, steady-state organization of interconnected and interdependent processes; an integrated, dynamically stable, multi-scale system of conjugated fluxes collectively displaced from thermodynamic equilibrium. Given its precarious nature, the cell is constantly having to negotiate a trade-off between structural stability and functional flexibility: too much rigidity compromises physiological adaptability, and too much promiscuity compromises metabolic efficiency. The cell accomplishes this by continuously turning over and reorganizing its constituents into different macromolecular complexes with diverse functional capabilities, which assemble and disassemble in order to meet the ever-changing demands of the environment. The permanent stochastic shuffling of molecules inside the cell and their opportunistic associations to form transient functional ensembles in response to intracellular and extracellular cues provides fast and robust solutions to the adaptive problems faced by the cell in a way that strikes an optimal balance between efficacy and plasticity (Misteli, 2001b; Kurakin, 2009).

Although this view of the cell has only come to the fore very recently, it is rather surprising to find that the theoretical principles that underlie it, as well as the empirical findings that support it, are not new at all. General denunciations of the MCC go back well over a century (e.g. Haldane, 1884), and even the recent empirical discoveries in each of the four domains I have examined in this paper have unmistakeable historical precedents. For instance, in the first half of the twentieth century it was not unusual for biochemists to describe the cell and its ostensibly solid and rigid contents in terms of streams, fluxes, and other processes (see Gilbert, 1982; Nicholson, 2018). A particularly visionary characterization of the dynamicity of the cellular architecture was offered by Bertalanffy, who was one of the first theoretical biologists (though today he is better known as the founder of general systems theory):

> Formations such as the nuclear spindle, the Golgi apparatus, and the like appear as structures when we have them before us in a fixed and stained microscopic preparation. However, if we consider them in their changes in time, they are a manifestation of processes at the chemical and colloidal levels, quasi-stationary states that last for a while but soon undergo changes or disappear. (Bertalanffy, 1952, p. 136)

Challenges to the undue emphasis on the structure and specificity of proteins are likewise nothing new. The first reports of proteins with disordered structural domains date back to the 1950s (Karush, 1950; Jirgensons, 1958), and some hypotheses regarding the substrate ambiguity and catalytic promiscuity of metabolic enzymes are over forty years old (Jensen, 1976). Similarly, the suggestion that a microscopic ratchet might be able to harness the energy of Brownian motion to generate directed movement was carefully explored by Feynman in his physics lectures more than half a century ago (Feynman et al., 1963). Moreover, experimental evidence for the stochastic nature of cellular behaviour goes back six decades (Novick and Weiner, 1957), and the heterogeneity of isogenic cell populations was already noticed in the 1970s (Spudich and Koshland, 1976). Nevertheless, all of these ideas and observations remained severely neglected for many years. Only in the last two decades have they begun to receive widespread attention—mostly because the adoption of novel experimental methods has served to empirically substantiate them, making them impossible to ignore.

But what is perhaps most surprising of all is that even though one would be hard-pressed to find a molecular biologist today that would dispute the fact that the cell is an open system far from equilibrium, or that because of its microscopic size the effects of stochastic fluctuations on its operation cannot be overlooked, many continue to explain cellular and molecular phenomena in the terms of classical mechanics, equilibrium thermodynamics, and mechanical and electronic engineering—that is to say, in terms of principles and concepts that are fundamentally at odds with the physical nature of the cell. This curious refusal of many researchers to accept, or even seriously consider, the new view of the cell that is arising is likely to be due to several factors. One might be that the new view is less intuitive than the MCC. The MCC, after all, draws on our everyday familiarity with machines. It is almost 'natural' for us to interpret everything in mechanical or engineering terms because such interpretations accord well with our experience of the familiar macroscopic physical world that we (and our machines) inhabit. Consequently, confronted with a microscopic entity such as a cell, "[t]he challenge for researchers is to look beyond our usual engineering principles and to appreciate the less familiar logic of biological organization." (Glick, 2007, p. 132).

Another factor that may help account for the reluctance of some researchers to endorse the new view is that it appears to make the cell a harder object to study than the MCC. Viewing the cell as a machine allows us to think of its organization in terms of modular, solid-state circuits that can be approached reductionistically, and it also gives us the confidence to expect that when we eventually work out how all of the cell's parts fit together, we will be able to completely predict its behaviour. If, on the other hand, we view the cell as a highly integrated, self-organizing, fluid system composed of densely interconnected processes ever-subject to stochastic fluctuations, we no longer have reasons to suppose that achieving such epistemic goals is even possible, let alone feasible. The stark contrast between these two outlooks is exemplified by their strikingly different ways of understanding causation in the cell (Bizzarri et al., 2019), and it serves to explain why some

very large number of cellular and molecular phenomena that were traditionally interpreted in terms that support the MCC are now being explained in terms that directly oppose it. It is also worth mentioning that the various MCC-derived characterizations and their alternatives I have considered (and which I summarized for contrastive purposes in the tables included at the end of each section) may in some cases represent idealizations: two opposite extremes of a spectrum of actual positions. I already hinted that this might be the case for the ongoing dispute between power-stroke and Brownian ratchet models of intracellular transport.

researchers find it easier than others to obtain funding and publish their work. As Mayer et al. point out:

> It is much easier to write and publish a paper suggesting Protein X is necessary for transmitting a signal from A to B, than one showing that Protein X is one of many potential components of a heterogeneous ensemble of signaling complexes that together couple A to B. (Mayer et al., 2009, p. 81.6)

A further factor could be that accepting the new view of the cell requires us to adopt, and maybe also develop, concepts that fall outside the remit of the conventional molecular biology toolbox. It requires us—among other things—to seriously consider how the ideas of non-equilibrium thermodynamics and complexity theory, and even those of condensed matter physics and quantum mechanics, may be brought to bear on the interpretation and explanation of the phenomena we investigate, and this might not be agreeable to all researchers, many of whom appear to show little appetite for theoretical considerations—or, worse still, assume that they can proceed in the absence of theory altogether.

Despite all of this, the advantages of embracing the new view of the cell are legion. Most importantly, the new view gives us a systematic and internally consistent interpretive framework capable of making theoretical sense of a multitude of empirical findings that appear paradoxical and almost inexplicable when viewed through the traditional lens of the MCC. Reports of self-organizing organelles, liquid-like macromolecular assemblies, fuzzy signalling complexes, moonlighting proteins, non-mechanical motors, order-from-disorder processes, non-genetic heterogeneity, and cell individuality seem totally baffling from the perspective of the MCC, but they can all be perfectly accommodated within the interpretive framework that is currently emerging. Findings which are confusing and unexpected within the old view become natural and expected within the new one. Ultimately, the current practice of overlooking some of the principles that govern the internal operation of the cell because they are unfamiliar, and of dismissing many of the cell's distinctive properties because they are difficult to study, is likely to be a mistake. Only by confronting these head on can we hope one day to arrive at a theoretically satisfying understanding of what the cell is and how it functions as an integrated unit.

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