

Lecture #7

Self-Organization versus Watchmaker: stochasticity and determinism in molecular and cell biology

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Introduction

The paradigm can be defined as a self-consistent system of interrelated and interconnected concepts, principles, theories and postulates, which forms an interpretational framework for the description, modeling and comprehension of reality. Neither the minds of individual people, nor science or any branch of it can operate without a paradigm. The Cartesian-Newtonian paradigm underlying modern thought has dominated the minds of scientists and general public for more than three hundred years. This mechanistic, reductionist and deterministic paradigm states that a whole can and should be understood only through a study of its individual isolated parts. Philip Handler wrote in his book “Biology and the future of man”: “One of the acid tests of understanding an object is the ability to put it together from its component parts. Ultimately, molecular biologists will attempt to subject their understanding of cell structure and function to this sort of test by trying to synthesize a cell”¹.

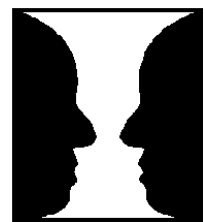
We all have been brought up and educated in the tradition of Newtonian science. And we continue to live and to see the world through Newtonian glasses. It means that, consciously or subconsciously, we are trying to see mechanical devices and deterministic processes in everything around us. Our descriptions of the cell are full of mechanistic analogies. We see proteins produced like cars on assemblage lines according to programs encoded in the DNA, motors moving molecular complexes pre-assembled for specific tasks to defined destinations along microtubule tracks. Power stations producing and supplying energy where and when it is needed, recycling factories of proteosomal machines etc. It is very illustrative and helpful to look at the titles taken from our most respectful bioscience textbooks, scientific presentations and publications, to realize the all-pervasiveness of the mechanistic interpretation.

What is the main idea underlying the most fashionable research today in molecular and cell biology? It is to make a comprehensive list of all components of the cell, see how they are connected and interlocked with each other and draw comprehensive engineering-looking charts as if the cell was clockwork and the molecules were gears and springs of a watch-like mechanism. The hope is that those charts will allow us to infer “the design” of the cell as soon as

we have learned the functions and properties of its individual components. In other words, in biomedical sciences we sub-consciously perpetuate the image of the cell as clockwork and follow today a traditional reductionist approach disassembling the cell to individual basic units in order to understand the whole through the study of its isolated individual parts. Remarkably, while using mechanistic analogies and interpretations, we commonly ignore the idea that any mechanical device implies pre-existence of its design, and, therefore, a designer. The question “who is the designer?” is normally omitted from consideration by life scientists, probably in an attempt to mask from themselves the disturbing realization that science itself is not an isolated unity, but is always influenced by and is an inseparable part of the evolving social, political, cultural and economical context.

The inadequacy of the mechanistic interpretation of life is becoming increasingly obvious. Despite decades of intense biomedical research, over 25 billion dollars-yearly budgets of the National Institutes of Health only, and tera-bytes of fragmented experimental information we do not have any reasonably articulated mechanistic model of any human disease, be it a common cold or more complex ailments such as cancer, obesity or degenerative disorders. The modern high-throughput molecular technologies generate enormous and rapidly increasing amount of data opening novel research fields such as genomics, structural and functional proteomics, pharmaco-genomics, chemical genomics, metabolomics, etc. Unfortunately, most of the newly generated data cannot be integrated and comprehended with any reasonable degree of self-consistency within the interpretational framework of the current mechanistic paradigm.

The crisis of a dominating paradigm normally leads to the exploration and development of alternative interpretational systems. Just as the same pattern on the picture shown here can be perceived either as two faces or a vase, the same set of experimental data viewed from different paradigms gives rise to distinct perceptions of the same phenomena. I would like to discuss in this review examples of the same molecular phenomena that are considered from two different points of view: traditional mechanistic standpoint, and an alternative one that is based on a novel emerging view of biological systems treated as self-organizing fluxes or ever-evolving and dynamic organizations of interacting components.



It should be pointed out that the following chapters, which consider competing models and alternative perceptions of the same phenomena, are not intended to provide a comprehensive

analysis of experimental data in order to decide in favor of one or another model or viewpoint. They are meant only to illustrate the idea that the mechanistic world perception and reductionism, which currently dominate our subconsciousness and dictate the choice of the questions we ask, the models we study and the interpretations we accept as scientifically sound, are becoming increasingly inadequate for description and comprehension of biological phenomena. It is the mechanistic paradigm and its underlying assumptions what causes confusion and inconsistencies in the studies discussed below and in many others left outside the scope of this review. The very progress in technology and methodology that allows us to probe the biological phenomena more accurately and to analyze them more precisely highlights and sharpens the inadequacy of mechanistic interpretations. The crisis of the old paradigm is concomitant with an emergence of the new interpretational framework that is being shaped today. Patterns of emerging paradigm are discussed throughout the review and summarized in the last chapter.

Molecular motors and Brownian ratchet

The motion is one of the defining characteristics of life. The special protein molecules, called molecular motors, are believed to bring about most of the directed movement in the cellular world. The traditional textbook interpretation of molecular motors such as kinesin, myosin and dynein portrays these proteins as micromotors functioning much like their would-be macroanalogs. They are often described as “ingenious” nanotechnological devices that convert chemical energy into mechanical work. The repetitive “power strokes” produced by molecular motor are generated as a result of periodical conformational rearrangements of protein structure driven by enzymatic cycle of ATP hydrolysis. According to the conventional view, a small conformational change in the globular motor domain of molecular motors caused by ATP binding or hydrolysis is amplified and translated into movement of the motor with the aid of additional structural elements². The generalized model of how the power stroke of a kinesin-type motor leads to its directional movement is shown in Fig. 1. According to this model, molecular motors move themselves and the attached cargo by “walking” along cytoskeleton elements such as microtubules or actin filaments. Notice, please, the following:

i) It is an interpretation of experimental data – no one has ever seen a “walking” protein. The “power stroke” model of molecular motors originated, one is tempted to say naturally, as an

interpretation of mechanistically trained physicists in the 60s, who were trying to match their mechanistic world outlook and the electron microscopy images of actomyosin complex. The model later was reinforced by biochemical data on actomyosin's enzymatic cycle of ATP hydrolysis and, relatively recently, in the 90s, by the structural data illustrating fine details of different conformational states of molecular motor proteins.³⁻⁶

ii) It is a very appealing interpretation. Why? Because it appeals our physical intuition that originates from our human scale physical experience and is in harmony with our mechanistic paradigm of the world. It is natural for us to interpret everything as mechanical devices or walking robots. It is an easy sell for our mind.

iii) It is deeply deterministic, clockwork-like interpretation. So many molecular events are precisely coordinated and synchronized in this model, that the impression of “ingenious” design is difficult to avoid. There is no place in this model for fluctuations, mistakes and evolution.

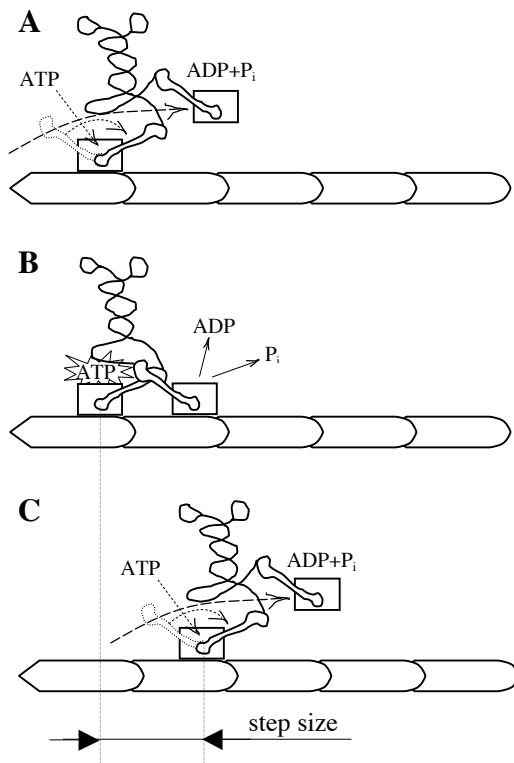


Figure 1. Generic model of the “walking” protein.

Molecular motors such as kinesin form dimers with motor domains acting as “feet” that step along cytoskeletal track such as microtubule. **A.** Binding of ATP to motor domain of the leading leg causes its structural rearrangement that move the trailing motor domain “over head” of the leading domain; **B.** The former trailing and now leading motor domain bind to microtubule and release products of ATP hydrolysis, ADP and P_i . The former leading and now trailing motor domain hydrolyses ATP; **C.** Binding of ATP to motor domain of the leading leg causes its structural rearrangement that move the trailing motor domain “over head” of the leading domain, thus completing the cycle.

Any paradigm makes people blind to everything that does not fit the paradigm. The mechanistic paradigm is not exception from this rule. It blinds us from obvious questions and

facts. One obvious question, which remains tacitly ignored in case of every mechanistic interpretation – Who is the designer? Who has designed this ingenious nanotechnological device, the “walking” protein? One is posed before two alternatives as answers. Either we should be consistent like Newton and acknowledge the existence of God and his omnipotent intelligence, or, as a second alternative, we can hypothesize that the molecular motor has evolved naturally. But then the model of the walking protein should incorporate at least hints on a plausible mechanism of its natural emergence and evolution. The conventional model fails to provide or even address any evolutionary scenario explaining the appearance of molecular motors.

The reductionist method addressing properties of parts in isolation normally disregards their environment or the context. In the case of molecular motors, mechanistic models ignore the fact that molecules in the cell operate in the environment that is drastically different from our scale familiar conditions. Our physical intuition therefore is more often inappropriate for interpretation of events on a microscale than it is not. The molecules in the cell operate in conditions of continuous, violent and chaotic turmoil, caused by stochastic thermal fluctuations. This fact is traditionally visualized as Brownian motion. The energy of ATP hydrolysis allegedly responsible for generation of power stroke in molecular motors is only about one order of magnitude larger than the average energy of thermal fluctuations. In addition, some variants of the power stroke model claim that force generation occurs in conventional kinesin upon ATP binding, which presumably provides even smaller amount of energy for work ^{6,7}. Next, the energy of ATP hydrolysis is said to be amplified through the angular motion of “mechanical elements” of molecular motors such as “lever arm” or “relay helix” ⁷. At the same time it is not discussed that the strength of noncovalent bonds responsible for the very existence of those molecular “levers” is of the same order of magnitude as the average energy of thermal fluctuations of the environment they operate in. Protein dynamics studies indicate that folded proteins in aqueous solutions at room temperature are far from being rigid structures. The protein molecule is more appropriately described as an ensemble of conformational substates. The protein structure constantly fluctuates sampling different subconformations ⁸⁻¹¹. It is difficult to reconcile the dynamics and plasticity of proteins in solution with the presumed ability of molecular motors to store, to transduce and to amplify mechanical energy. The low inertia of macromolecules, internal thermal fluctuations and “breathing” of a polypeptide chain in conditions of a constant bombardment by surrounding molecules is expected to lead to a

dissipation of any form of mechanical energy in picosecond-scale time intervals^{12,13}. Keeping this in mind the estimated 50-60% efficiencies of molecular motors when compared to 10-15% efficiencies of our human scale motors are simply staggering^{7,14}.

The staggering and surprise pertaining to outcomes of experimental results are indications of failed anticipations and may signify a crisis of interpretational model and its underlying paradigm. Staggering and surprise simply mean that something very different was anticipated by the model. So different that even the wishful thinking of an experimenter fails to convince itself. The surprises in the experimental research on molecular motors are meanwhile abundant.

Three known types of molecular motors were originally believed to be involved in clearly separate functions, i.e. kinesin in organelle transport, myosin in contraction and movement and dynein in ciliary beating. Further research demonstrated that these anticipations based on the mechanistic intuition were unfounded. Kinesins have been implicated in ciliary function, myosins in organelle transport and dyneins in vesicle and cell movements².

Due to mechanistic considerations, the processive movement, defined as the advance of motor protein bound to cytoskeletal track over a long distance before its dissociation, was believed to require dimeric motors. The surprise came when monomeric KIF1A kinesin¹⁵, monomeric class IXb myosins¹⁶ and monomeric inner arm dynein¹⁷ were found to move processively.

Surprisingly, there is no obvious correlation between structural geometry of swinging legs and step size in different molecular motors¹⁸⁻²⁰.

Another example of poorly understood phenomena are related motors such as conventional kinesin and nonclaret disjunctional protein (ncd) that move in opposite directions even though they have similar structures and are positioned in the same orientation relative to the microtubule track^{2,21}.

Single-molecule measurements revealed that the single myosin head moves stochastically in steps ranging from 5.5 to 27.5 nm long, sometimes even stepping backwards. Surprisingly, each step, independent of its size and direction, required only one ATP molecule²².

To summarize, the mechanistic interpretation of molecular motors leads increasingly often to “surprises” in experimental outcomes rather than provides a unifying interpretational framework of reasonable predictive power. The current reviews on molecular motors give

impression of a chaotic mosaic of individual case micro-models, often of staggering complexity, where one expect to see a self-consistent, systemic and structured description of the phenomenon.

An alternative model of molecular motors based on the Brownian ratchet principle has been proposed²³⁻²⁵. It is counter-intuitive and takes an effort of mind to grasp. Probably for this reason, though it is as old as mechanistic interpretation, it has never been as popular, except for may be very lately.

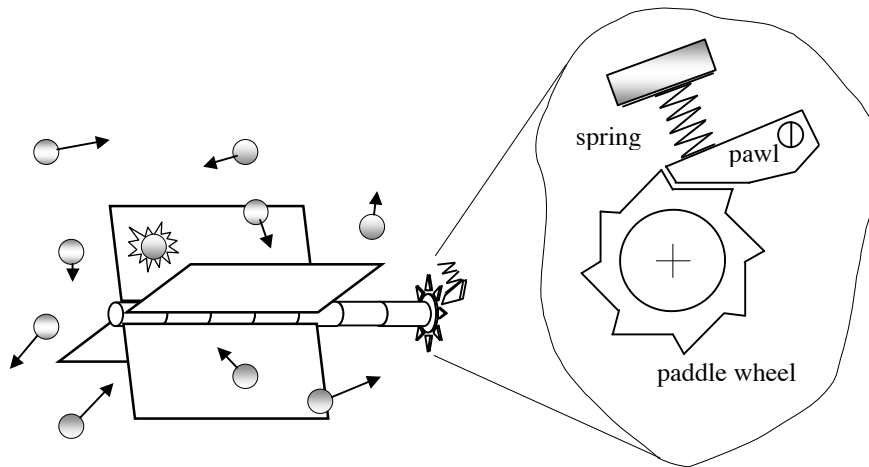


Figure 2. Brownian ratchet. See description in the text. Idea of the representation is taken from Astumian R.D.¹⁴

First, let us consider principle of the Brownian ratchet itself. Imagine a miniature mechanical device like the one shown in Fig. 2. It is rather ironic that in order to be convincing we prefer to use mechanical analogies even if explaining non-mechanical phenomena. This is yet another indication of the power of current paradigm over our habitual way of reasoning and perception. The balls chaotically bouncing around the ratchet mechanism symbolize thermal fluctuations. Driven by some especially strong fluctuations, the paddle wheel shown in Fig. 2 will be turning counter-clockwise, because the clockwise movement is prohibited by the structure of the ratchet. Since the spring pressing on the pawl is an outside source of energy, there is no contradiction with the second law of thermodynamics. Next, imagine another situation when the spring is engaged and disengaged chaotically allowing the pawl to go “on” and “off” the paddle wheel. During the time interval when the pawl is disengaged the gear can turn clockwise or counter-clockwise with equal probability upon impact of thermal fluctuations. However, due to sawtooth shape of the paddle wheel combined with random disengagement and

re-engagement of the pawl, the gear will have tendency to turn clockwise. In this second scenario, a superposition of two random processes, the thermal noise and the chaotic engagement-disengagement of the spring, results in generation of a directional clockwise movement of the gear. The system is maintained in non-equilibrium conditions by energy of the spring. The Brownian ratchet principle illustrates how directional movement can be rectified from the chaotic thermal fluctuations at microscale.

Next, let us consider kinesin as an example of molecular motor in the framework of the Brownian ratchet model. A large body of evidence suggests that molecular motors, using energy of ATP hydrolysis, flip-flop between two alternative conformations. It is postulated in the Brownian ratchet model that the “flip” and the “flop” conformations of kinesin have respectively two different potential energy profiles when the motor molecule is bound to a microtubule (see Fig. 3). In the “flip” conformation (A and C in Fig. 3, white ball) the energy profile is flat, so that bound kinesin is free to slide along the microtubule in both directions upon influence of thermal fluctuations. In the “flop” conformation (B and D in Fig. 3, gray ball) the energy profile of bound kinesin has a sawtooth shape and the kinesin molecule gets trapped in the potential energy minimum troughs. Only especially strong, and therefore very rare, thermal fluctuations can displace the motor molecule in the “flop” conformation from one energy trough to another, or, in other words, to move kinesin away from its dynamic equilibrium position on a microtubule. But they are not prohibited.

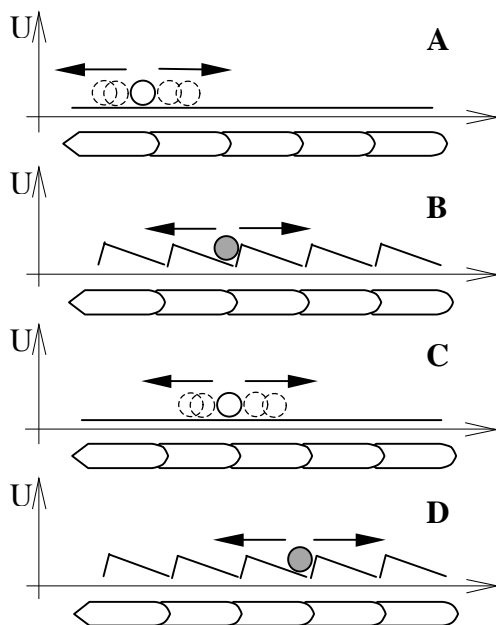


Figure 3. Brownian ratchet model of a molecular motor.

The motor molecule bound to a cytoskeletal track is hypothesized to have two different potential energy profiles depending on its conformational state. In one conformation, referred in the text to as the “flip” conformation (A and C, white ball), the energy profile is flat and the molecule is free to slide stochastically along the track upon influence of thermal fluctuations. In another conformation, referred to as “flop” (B and D, gray ball), the energy profile of the motor molecule has a sawtooth shape, so that the molecule will tend to drift accordingly to a nearest energy minimum and remain there unless it acquires the “flip” conformation or is misplaced by unusually strong thermal fluctuation to a neighboring energy trough. Chaotically switching between its “flip” and “flop” conformations upon ATP hydrolysis, the motor molecule will be driven by thermal fluctuations to the right. The movement is inherently stochastic with occasional “stepping back” and “jumps” forward.

According to this scheme, the microtubule-bound kinesin chaotically flip-flops between its two distinct structural conformations. It is a random, chaotic process. When kinesin is in its “flip” conformation, the motor molecule is propelled by thermal fluctuations with equal probability either to the left or to the right of its initial position. When it is in the “flop” conformation, the kinesin molecule equilibrates at the nearest energy minimum. Because of the stochasticity of conformational switches, and due to the sawtooth-shaped energy profile of kinesin’s “flop” conformation, the thermal noise will drive the motor molecule along microtubule to the right in the example shown in Fig. 3. In this model, a superposition of two chaotic processes, the conformational flip-flop of kinesin and the thermal environmental noise, results in the directional movement of motor molecule driven by energy of thermal fluctuations. The ATP hydrolysis cycle maintains the system in nonequilibrium conditions and biases the random walk of kinesin in one direction.

Consider rich ramifications of the Brownian ratchet model of molecular motors and its possible evolutionary underpinnings. There is no design, no determinism in this model. All the processes are inherently stochastic. Outcomes are statistical. The overall effect, a directional movement of kinesin to the right, is only statistically the same. But each molecule performs its own unique “dance” while moving to the right. Importantly, in this interpretation of molecular motors, there is no pre-designed function inbuilt into kinesin molecule. If the kinesin’s flip and flop conformations happen by chance to have similar energy profiles on the polymer other than microtubules, kinesin will work as a molecular motor using that other polymer as a track. If that other polymer happens to be, for instance, the DNA, and the movement of kinesin along DNA would happen somehow to facilitate removal of oxidated bases, then kinesin would function and be known to researchers as a part of the DNA repair system. On the other hand, if the conformational cyclical rearrangements of kinesin molecule happen by chance to facilitate transformations of yet another molecule, then kinesin will be known as an enzyme as well. The functions of kinesin therefore are not pre-designed and inbuilt into it, but rather they are selected to exist because of a competitive advantage they may confer to a higher-level system such as the cell, for instance. Following this logic, one would expect to find the motor proteins that do not function as motors and, conversely, the non-motor proteins that can generate directional movement. That is exactly what recent experimental data suggest. The examples include the kinesin-related family of MCAK proteins that are not motile, but act as microtubule

depolymerases²⁶, the G-proteins generating mechanical force²⁷, the ribosomes¹³ and the RNA polymerases described as molecular motors^{12,28}.

The Brownian model of molecular motors resolves what is perceived as inconsistencies and surprises within the power stroke model^{15,29,30}. The multiple functions of molecular motors, the stochastic movement along tracks, the independence of step size from geometry of a motor, the processivity of monomeric motors, the absence of general correlation between size of a step and the energy spent to make this step, the unusually high efficiency of molecular motors are almost self-explanatory when molecular motors are considered within the Brownian ratchet framework. Importantly, the Brownian ratchet provides a unifying principle of rectifying directional movement from a thermal chaos at microscale³¹. In other words, it illustrates how the order can be generated out of chaos³². This principle is believed to underlie functioning of such “molecular machines” as RNA polymerases²⁸, ATP synthases³³, ion pumps³⁴, ribosomes¹³ and others¹⁴. It is considered to be responsible for many types of the biological transport driven by nonequilibrium chemical reactions. One of the examples is protein translocation across lipid membranes to which we now turn.

Protein translocation

The introduction of electron microscopy and other advanced imaging techniques led to appreciation of the compartmentalization as one of the basic principles of cellular organization early on. Multiple and diverse sub-cellular compartments such as cytoplasm, nucleus, lysosomes, peroxisomes, mitochondria, endosomes, chloroplasts, endoplasmic reticulum (ER), secretory vesicles etc. are commonly recognized and studied as distinct structural and functional entities. The borders of individual compartments are usually delineated by lipid membranes. The specific set of membrane-associated proteins is constantly engaged in maintenance of the typically unique intracompartamental milieu. In a simplified way, the cell can be viewed as an organization of functionally interlinked and distinct microenvironments that are created, separated and maintained by specific membranes and their associated proteins. As a part of constantly on-going protein turnover and renewal of cellular compartments, new proteins are continuously synthesized in the cytoplasm and delivered inside various compartments through specific mechanisms that often involve protein translocation across lipid membranes. Several proteinaceous machineries mediating protein import have been identified, such as the

TOM/TIM23 complex in mitochondria³⁵ and the Sec complex in endoplasmic reticulum^{36,37}. Two functionally distinct parts of these protein translocases are recognized, the protein channel^{38,39} and the import motor.

The newly synthesized polypeptides are translocated across mitochondrial membranes as preproteins that are later converted into mature proteins by the mitochondrial processing peptidase (MPP) residing in the matrix of mitochondria. The import is achieved by unfolding and threading of the passenger polypeptide chain through the import channel. The energy-coupled translocation motors are thought to play a critical role in the unfolding and unidirectional transport of the preproteins across membranes. The molecular chaperons of heat shock protein 70 (HSP70) family, residing in the lumen of ER⁴⁰ and in the matrix of mitochondria⁴¹, constitute core elements of translocation motors. However, the mechanism by which these molecular chaperones unfold translocating preproteins and drive their unidirectional movement across membrane remains somewhat controversial⁴². Two models of translocation motors have been proposed, the power stroke (PS) model⁴³⁻⁴⁶ and the Brownian ratchet (BR) model⁴⁷⁻⁴⁹.

According to the PS model, mitochondrial HSP70 (mtHSP70) molecules associate with the outlet of import channel inside mitochondria and use the energy of ATP-hydrolysis to produce a pulling force applied to passenger protein. The power stroke generated by mtHSP70 structural switch is hypothesized to actively unfold the passenger protein on the *cis* side of the membrane and to drive its unidirectional movement inside the compartment (Fig. 4, A). This clockwork-like interpretation implies an exquisite complexity in organization and coordination of the protein translocation machinery and consequently invokes a feeling of an “ingenious design”. To assure a proper performance, the chaperon molecule needs to be precisely and steadily positioned at the outlet of import channel in order to generate a force perpendicular to the plane of the membrane using the channel as a fulcrum. Following generation of the power stroke, the chaperon molecule is required to dissociate from the channel and later from the incoming polypeptide. These dissociation events need to be synchronized with the binding and proper positioning at the channel outlet of another chaperon molecule in order to complete the cycle and to prevent backsliding of the passenger polypeptide^{42,43,50}.

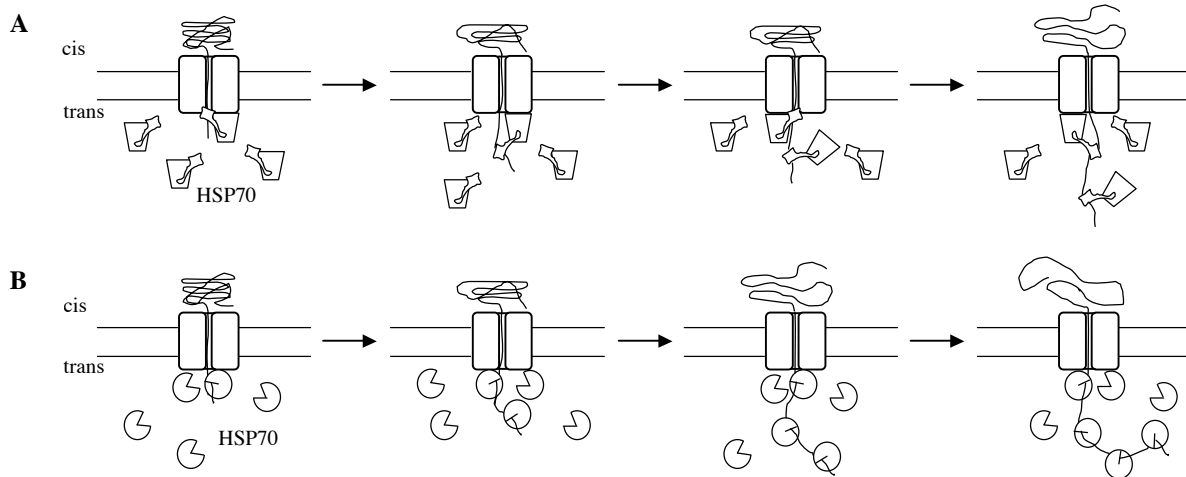


Figure 4. The power stroke and Brownian ratchet models of import motors.

The HSP70 family proteins residing in the lumen of ER (BiP) and in the matrix of mitochondria (mtHSP70) are recruited and bind to the polypeptide chain translocating through import channel and to the channel itself to serve as import motors. **A.** The power stroke model assumes that HSP70 molecules use the channel outlet as a fulcrum “to pull” incoming polypeptides inside the compartment. It is hypothesized in this model that the HSP70 molecules are able to generate a mechanical pulling force upon ATP hydrolysis, caused by their cyclical conformational rearrangements. **B.** According to Brownian ratchet model, HSP70 chaperons, through stochastic binding and release of the incoming polypeptide chain inside the destination compartment, act as molecular ratchets preventing backsliding of the passenger polypeptide. The local spontaneous unfolding of passenger protein and random sliding of incoming polypeptide chain within the import channel are driven by random thermal fluctuations.

Once again, little, if at all, attention is paid in this model to the environment in which import motors operate. The energy of thermal fluctuations cannot be ignored and should be either used by molecular motors or worked against.

A significant body of experimental data is inconsistent with the PS model of translocation motor. For instance, the peptides composed of glutamic acids (polyE) or glycine residues (polyG) were shown to exhibit no or very poor binding to the mtHSP70, respectively. However, the introduction of long polyE or polyG stretches in front of folded domains did not prevent their efficient import into the mitochondrial matrix, even though the mtHSP70 molecules could not possibly “pull” the introduced leading sequences⁴⁹.

The tightly folded immunoglobulin (Ig)-like domains, which require mechanical force for their unfolding of approximately 200 pN as judged by atomic force microscopy measurements⁵¹, were efficiently imported into mitochondrial matrix, even if they were preceded by the 50 amino acids long polyE leading sequence. It should be mentioned that conventional motors such as

kinesin or myosin are able to generate forces only on the order of 3-10 pN and it is very unlikely that the putative mtHSP70-based motor would generate a force more than 14 pN⁴⁹.

Unexpectedly, the efficiency of protein import was shown to correlate with the rates of local thermal breathing of passenger proteins, rather than with their overall thermodynamic stability⁵².

Strikingly, the antibodies raised to several different parts along the length of a passenger protein successfully mediated the protein import in the absence of any motor proteins and the ATP in a reconstituted *in vitro* import system⁴⁸. These and other data are poorly consistent with the “pulling” model of a translocation motor, but are readily explained by an alternative model based on the Brownian ratchet principle.

The BR model assumes that both the unfolding of proteins and their vectorial movement through the import channel are driven by energy of random thermal fluctuations. The HSP70 family molecular chaperons residing in the ER lumen or in the mitochondrial matrix act in this model as molecular ratchets preventing the backsliding of incoming polypeptide chain as it appears at the channel outlet and progresses inside the compartment (see Fig. 4, B). According to the BR model, the signal sequence of a preprotein targets it to and initiates a threading of the preprotein through import channel. The local reversible unfolding of passenger protein accompanied by random diffusion of unfolded polypeptide segments inside the channel are both driven by energy of thermal fluctuations. The HSP70 molecules “harvest” the local unfolding and make the sliding of passenger polypeptide statistically unidirectional by stochastic binding and release of the incoming polypeptide chain on the *trans* side of the membrane (“trapping”)⁴². The action of molecular ratchets therefore biases the otherwise reversible and chaotic processes such as polypeptide unfolding and sliding. Notice, that the protein translocation, according to the BR model, does not require any design and is simply the result of a superposition of several stochastic processes such as the reversible local unfolding of passenger protein, the random diffusion of its unfolded segments within import channel and the stochastic binding and release of chaperon molecules trapping the incoming passenger protein sequences inside the destination compartment. The outcome of translocation of individual molecules across the membrane is only statistically the same, but each individual molecule performs its unique “dance” of folding/unfolding and translocation events. The energy for translocation and unfolding is taken

from the environment, i.e. from the thermal bath in which the molecular system resides. The energy of ATP hydrolysis is used only for “ratcheting” or statistical biasing of chaotic processes.

The comprehensive review and analysis of experimental data put forward to support either the PS or the BR models of mitochondrial translocation motor can be found in Ref ⁴². The authors of this analysis conclude that though no decisive judgment can be made at present to prefer one model over another, all the existing experimental data on the structure and function of mitochondrial protein import motor can be explained by the Brownian ratchet model and in a more simple way as compared to the power stroke model. The devout followers of Occam will probably take this conclusion itself as a final judgment.

Protein import to mitochondria and to ER has become a general model for post-translational protein translocation. The detailed elucidation of mechanisms of protein import to other cellular compartments awaits focused experimental efforts. Meanwhile, it is becoming clear that the clockwork interpretations may provide a poor framework for modeling and comprehension of the phenomenon. The mechanistic reasoning would necessary require the distinct molecular machineries existing for each distinct compartmentalized microenvironment, for it is difficult to imagine that the same import apparatus can operate equally well inside such different milieus as mitochondrial matrix, lysosome and peroxisome interiors, as examples. In addition, evolutionary emergence of a new and unique cellular microenvironment would necessary require the intervention of an external designer and the creation of a respective protein import machine, since the evolution is not practiced as explanatory principle by clockwork interpretational paradigm.

Conversely, the Brownian ratchet principle provides evolutionary-conscious, design- and determinism-free mechanism of protein translocation. The harvesting of local spontaneous unfolding of passenger protein and the biasing of random walk of translocating polypeptide inside import channel can be potentially realized in many different ways due to a variety of asymmetries normally existing between the *cis* and the *trans* sides of cellular membranes. Disulfide bond formation, binding of ligands or chaperons, glycosylation or other types of post-translational modification inside the destination compartment, electrochemical, pH, ionic and other gradients across membranes, may all serve as ratcheting mechanisms to bias the otherwise chaotic movement of translocating polypeptide chains ⁵³. Thus, the Brownian ratchet principle provides a broad and general theoretical framework for explanation and modeling of protein

translocation across biological membranes. It should be noted that both the protein translocation and the whatever gradient causing that translocation are continuous and dynamic processes and therefore can be considered as conjugate fluxes, the conjecture that is more appropriate to treat in terms of nonequilibrium thermodynamics, rather than mechanical engineering. The power stroke model, on the other hand, does not permit to entertain and to explore these fascinating lines of thought and restrict us to the image of clockworks, determinism and logic of linear causation.

Sub-cellular organization

The proverb “better one time to see, than three times to hear” is indicative of our tendency to attribute an ultimate importance to the information received through our eyes. The same holds true for the information received through the technological extensions of our eyes such as magnifying glass and microscopy. Most of our visual knowledge about sub-cellular architecture originated from the images representing snapshots of fixed, desiccated and stained biological structures, which were collected by conventional electron and light microscopy. The interpretation of these images within mechanistic paradigm naturally led to the perception of sub-cellular organization in terms of static architectures resembling our human scale constructions. Exquisite structural details combined with interlinked and well-orchestrated functionalities of sub-cellular compartments rarely fail to invoke a feeling of awe and nifty design. Not surprisingly, as all the power stations, transport machinery, highways, storage deposits, recycling factories etc. have been designed on our scale by human intellect.

Structural and functional organization of macromolecular systems mediating basic biological processes, such as cytoskeleton, chromatin, transcription apparatus, splicing and translation machineries, DNA replication and repair systems etc., have been traditionally studied in a reductionist manner, in other words: i) separated from each other and ii) by means of isolation and characterization of their individual components. Combined with the habitual mechanistic interpretation of biochemical and imaging data, the reductionist agenda resulted over time in a clockwork image of the cell. The organization of the cell is generally perceived to be different from clockwork only in terms of quantity, but not quality, and the design charts of car, aircraft and computer are routinely used today to illustrate complexity of the cell and to point out how cleverly the cell exploits the advanced design principles of modern mechanical and electrical engineering.

Meanwhile, the introduction and ingenious use of new approaches allowing researchers to follow, to quantitate and to model movements and distribution of specific molecules, proteins, nucleic acids and their macromolecular complexes in real time in living cells is leading to a rapid accumulation of experimental data that are inconsistent with the clockwork interpretation of the cell^{54,55}. Instead they favor a new image of the cell as a dynamic complex of interlinked and interdependent steady-state molecular organizations. The modeling and analysis of cell behavior in accordance with this new image requires introduction, development and usage of the concepts, principles and descriptions that are qualitatively different from the mechanistic ones.

The cytoskeleton is composed of actin filaments, microtubules and intermediate filaments. It underlies structural integrity, spatial organization and morphological appearance of the cell, orchestrates cellular directional movements and interactions with other cells and substratum. It was appreciated early on that the actin filaments and microtubules, the polymers made of evolutionary conserved protein monomers, actin and tubulin, are highly dynamic self-organizing macromolecular structures⁵⁶. In contrast to the protein assemblies representing near-equilibrium molecular complexes, such as bacteriophage particles, the actin filaments and microtubules are steady-state structures that exist in far-from-equilibrium conditions and are maintained by the energy and matter flowing through these structures (Fig. 5).

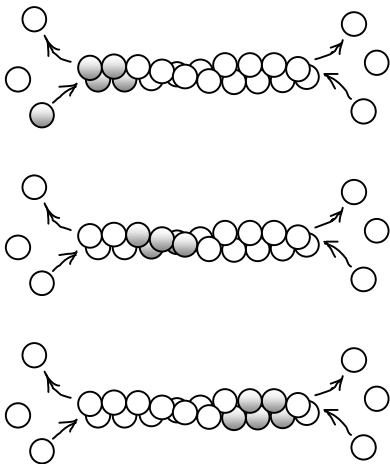


Figure 5. Steady-state organization.

Actin filaments and microtubules are dynamic polymers that constantly exchange their subunits with a free pool in the cytoplasm. Matter and energy continuously flow through the steady-state cytoskeletal organization. The structure shown can be dynamically and quickly extended or shrunk by adjusting rates of association and dissociation at either of the polymer ends.

The elegant experiments with purified tubulin, molecular motors, ATP and GTP have revealed that even simple molecular mixtures can give rise *in vitro* to a rich variety of different macromolecular structures resembling those that are observed in live cells, such as meshworks,

vortices and asters. Three observations are especially relevant to the discussion: i) the structures observed in these studies represented self-organized steady-state molecular organizations feeding on flow of energy and matter; ii) emergence of a particular structure was dependent on biophysical properties of mixed components and their relative concentrations; iii) the model that faithfully reproduced experimentally observed structures in computer simulations assumed stochastic interactions underlying self-organization of macromolecular complexes composed of tubulin and motor molecules^{57,58}. An important implication of these *in vitro* experiments is that all the dynamic complexity and variety of cytoskeletal structures observed in living cells can be controlled by spatio-temporal differential distribution of a limited number of molecular components. Or, putting aside externally imposed purpose and control, cytoskeleton structures might simply be *reflections* of differential spatio-temporal distribution of its basic constituents within the cell.

A rapidly growing body of experimental evidence, obtained *in vitro* and in the studies performed in living cells, strongly suggests that both the spatial organization and the directional movement of the cell are mediated by steady-state dynamic macromolecular cytoskeletal structures^{55,59,60}. In the light of these data it seems more appropriate to consider the cytoskeletal structures as self-organized molecular fluxes, rather than deterministic assemblies of clockwork gears. The increasingly prevailing view of dynamic cytoskeleton defies mechanistic intuition and linear causation and renders the clockwork interpretations and assumptions inadequate and obsolete.

The nucleus contains multiple morphologically and functionally distinct compartments such as nucleoli, Cajal bodies, perinuclear compartment (PNC), promyelocytic leukemia (PML) bodies, splicing compartments etc. In contrast to typical cytoplasmic compartments their sub-nuclear counterparts are not delineated by membranes, though they are readily visualized under microscope and some of them have been isolated and studied biochemically. How these sub-nuclear compartments are formed and maintained has remained unclear. Recently, quantitative analysis of real-time movement of fluorescently tagged molecules in living cells dethroned several mechanistic conceptions and assumptions pertaining to structural and functional organization of the nucleus and brought about a new image of the sub-nuclear compartments as steady-state molecular organizations that are formed through stochastic molecular interactions and maintained by the balance between influx and efflux of their resident proteins^{54,61,62}.

Defying the previously widely held notion of nucleus as viscous gel-like environment, the mobility of non-physiological solutes in nucleoplasm was shown to be only about four times lower than in aqueous solutions^{63,64}. The fluorescently tagged dextran microinjections revealed that the nuclear space inaccessible for injected molecules constituted less than 15% of the total nuclear space, thus challenging presumed “crowdedness” of the nucleoplasm^{54,63}. Energy independent random diffusion appears to be efficient enough process to account alone for rapid translocations of proteins, RNAs and their complexes inside the nucleus⁶⁵⁻⁶⁷. The macromolecules roam the nucleus in a search of their interacting partners and transient “employment opportunities” within the steady-state macromolecular organizations. Using kinetic modeling of data obtained in the photobleaching experiments performed in living cells it was estimated that about 10’000 molecules of pre-mRNA splicing factor SF2/ASF and about 12’000 molecules of RNA processing protein fibrillarin were leaving each second their respective compartments in a single nucleus. The residence times of these proteins within splicing compartments and nucleoli were estimated to be less than 50 sec for SF2/ASF and less than 40 sec for fibrillarin, respectively⁶⁵. The transcription/repair factor TFIIH was shown to be rapidly and stochastically exchanged between at least four pools within the same nucleus, the RNA polymerase I (RNAP1) transcription sites, the RNA polymerase II (RNAP2) transcription sites, the DNA repair sites and a freely mobile unbound pool of TFIIH in the nucleoplasm. The translocation between these pools was suggested to be energy independent diffusion-driven stochastic process. The residence times of TFIIH molecules engaged in functional pools were estimated to be approximately 25 sec, 6 sec and 4 min for RNAP1, RNAP2 and DNA repair sites, respectively⁶⁸.

The architecture of sub-nuclear compartments appears to be tightly coupled to their function. The inhibition of ribosomal gene transcription results in disassembly of nucleolus⁶⁹. Conversely, addition of extrachromosomal ribosomal genes leads to appearance of micronucleoli^{70,71}. Expression of the Cajal body resident p80-coilin protein in p80-knockout cells is sufficient to regenerate Cajal bodies⁷². Blocking splicing factors efflux from the splicing compartments leads to their enlargement and reshaping⁵⁵. Nuclear sub-compartments are naturally lost and re-assembled during the course of each cell division^{73,74}.

Not readily explainable within clockwork paradigm, the recently collected experimental data are consistent with and support the image of sub-nuclear compartments as self-organized

dynamic macromolecular organizations that exchange their components incessantly. The specific interactions and activity of proteins within the steady-state macromolecular complexes appears to be the defining factor of their apparent mobility and transient immobilization events^{54,68}.

The gene expression is a complex set of coupled molecular processes such as chromatin remodeling, transcription, RNA processing, RNA transport and translation. These basic biological processes are presumably performed by specialized, pre-designed and often pre-assembled macromolecular “machines”^{56,75}. The measurements of mobility of protein components of the respective macromolecular complexes in living cells revealed a highly dynamic and inherently stochastic molecular behavior underlying formation and maintenance of these complexes. The residence times of chromatin-binding structural proteins such as histone H1 and high mobility group (HMG) proteins, previously believed to be stably associated with their binding sites on chromatin, was found to be unexpectedly short, on order of 1 or 2 minutes for H1 molecules and on order of seconds for HMG molecules^{65,76,77}. Though binding sites on chromatin remain occupied by respective proteins, the occupancy is characterized by rapid exchange rates and short residence times⁵⁴. These observations suggest that chromatin itself is not a near-equilibrium assembly of mechanistic type, but a steady-state molecular structure, in other words, a self-organized flux.

The same appears to be true for organization of transcription and DNA repair systems. Biochemical studies suggested that glucocorticoid receptor (GR) molecules remained bound to their responsive elements on DNA as long as their cognate ligand was present⁷⁸. Photobleaching experiments revealed that though GR molecules indeed were present on their responsive elements the association was transient with individual GR molecules being in continuous and rapid exchange between their DNA sites and a free pool⁷⁹. The similar observations have been made for estrogen receptor⁸⁰, co-activator GRIP-1⁸¹ and transcription factor TFIID⁶⁸. Two recent publications addressing dynamic of RNAP1⁸² and RNAP2⁸³ machineries and recruitment of their components to respective functional complexes have suggested that components of transcription complexes do not reside in pre-formed and stable holoenzymes, but rather transiently and dynamically associate through stochastic interactions into elongation-competent complexes. The stochastic assembly model was proposed to operate in the case of two different DNA repair pathways as well^{66,68,84}.

As could be envisioned, and has been actually shown for transcription of ribosomal genes, the stochastic assembly is highly inefficient and wasteful process. As an example, more than 90% of RNA polymerase I subunits are not engaged at any given moment in elongation complexes, but scavenge the nucleoplasm in a random search for binding partners⁸². This apparent inefficiency of stochastic assembly, as judged from mechanistic design perspective, is more than counterbalanced by invaluable properties it may endow cellular systems. The conditions of natural existence require constant adaptation of cells to their changing and unpredictable environments. Therefore, it is highly advantageous for the cell to keep transcription, DNA repair and other systems flexible and ready to respond to any unforeseen stimuli, damages and/or changes in extra- and/or intracellular milieu. Consider the transcriptional response as an example. It requires combined activities of both common and stimulus-specific factors to initiate transcription from a wide variety of promoters in the genome. The same is true for the DNA repair system that is poised to mend diverse DNA lesions. The constant stochastic shuffling of molecular components and the transient self-organization of specific complexes in response to an activating stimulus or a particular DNA lesion provide robust just-in-time specific solutions, while continually maintaining systems flexibility and responsiveness. In addition, the coupling and coordination of different processes, such as transcription and DNA damage repair, may occur automatically, since some of the participating molecules, such as TFIIF mentioned above, are shared by different functional systems. None of the most ingenious deterministic models of transcription or DNA repair “machines” can outperform this solution in conditions of inherent unpredictability of cell environment and cell fate.

To generalize, the phenomena of stochastic assembly and self-organization lead to a new image of the cell as an ever-evolving multi-scale system of interconnected and interdependent molecular organizations. The steady-state macromolecular organizations are realized through transient and specific molecular associations and coupled by fluxes of the molecular components that are shared between different functional systems. From this point of view, the “surprising” discovery of so-called moonlighting proteins⁸⁵, i.e. proteins involved in two or more unrelated functions, seems natural and predictable. Consider examples of moonlighting proteins that may potentially couple and coordinate different cellular functions: Clf1p splicing factor participating in DNA replication⁸⁶, proteosomal subunits participating in transcription⁸⁷, PutA proline dehydrogenase acting as transcription regulator⁸⁸, ribosomal proteins functioning in DNA repair

⁸⁹, enzyme of phenylalanine metabolism, DcoH, dimerizing homeodomain transcription factors ⁹⁰ etc.

The Golgi complex is an example of intracellular compartment delineated by membranes that displays clear hallmarks of self-organization ⁵⁵. Following synthesis and translocation to ER, new proteins are transported to the Golgi complex for their further maturation, sorting and dispatching to other cellular compartments, secretory pathway, or the cell surface. Multiple vesicles mediate vectorial traffic of lipids and proteins from ER to the *cis* Golgi network (CGN) through the Golgi stack to the *trans* Golgi network (TGN). The proteins and membranes leaving the TGN are sorted further to other destinations. The tracking of fluorescently labeled proteins in living cells revealed that the Golgi complex, previously regarded to be a static structure, is in fact a dynamic steady-state organelle with continuous and rapid exchange of lipid membranes and proteins between the Golgi compartments, the secretory pathway and the ER ⁹¹. The morphology of Golgi complex is dependent on its functional status and can be modified by manipulating influx and efflux of the material passing through the compartment. Inhibition of traffic from the ER leads to a dispersion of the Golgi complex into small vesicles ⁹², while blocking vesicle transport from the TGN results in an enlargement of the last ⁹³. It is speculated that in a cell entering mitosis the continuous shedding of budding vesicles concomitant with the block of their fusion results in disintegration of the Golgi complex ^{94,95}. The reassembly of Golgi in telophase is thought to occur by self-sorting and fusion of the dispersed vesicles through specific protein-protein interactions. The Golgi complex reassembly from mitotic Golgi fragments can be achieved in a cell-free system, indicating on a self-organizing character of this organelle ^{96,97}.

To summarize, the sub-cellular compartments and specialized macromolecular complexes emerge as extremely dynamic, yet overall stable structures with their architecture representing the balance between binding and release of a specific set of molecular components. Stochastic self-assembly is proposed to be the mechanism of formation and maintenance of the steady-state compartments and specialized molecular complexes. The morphological appearance of self-organized macromolecular organizations is defined by their functional status.

Gene expression and cell differentiation

The mechanistic interpretational paradigm residing in and governing the subconsciousness of a modern researcher leaves him/her no alternatives but to treat the cell as clockwork with all the ensuing panoply of logical inferences and assumptions. For instance, cells in a typical cell culture experiment are usually assumed to be identical like clockworks of a certain type. The same assumption is held for distinct cell lineages in context of the organism. As a logical consequence, measurements of inducible gene expression, performed on “homogeneous” cell populations rather than on individual cells, naturally led to a “rheostat” or “graded” model of regulation of gene expression, since in many cases a linear dependence was observed between the concentration of external activating stimulus and the corresponding gene expression measured as a total protein or mRNA specific product of a large cell population. Within the framework of graded response, the relationship between rate of transcription and concentration of activating stimulus is generally expressed in deterministic terms and linear causation. The appearance of activating stimulus and increase in its concentration are assumed to cause the corresponding and proportional rise in the rate of transcription of a responsive gene gradually from zero to its maximum in every cell of a population (Fig. 6).

Relatively recently the methods and technology were introduced and became readily available that allowed researchers to analyze gene expression and other parameters in cell populations routinely on a cell-by-cell basis. As a result, the “digital” stochastic model of gene expression is becoming widely accepted. According to this model individual cells in any cell population, including a clonal one, have a certain and distinct probability to respond to a given concentration of activating stimulus by transcription of a responsive gene. This probability may vary widely among individual cells in a population and the ensuing gene expression follows all-or-none response pattern. The responsive gene is either maximally expressed within a certain time window in a given cell or not at all (Fig. 6).

Quantitative experiments performed in different model systems, including animals, cultured cells and purified DNA templates, indicate that the increase in concentration of activating stimulus usually initiates a “recruitment” of increasing numbers of cells or templates in a given population that switch from silence to expression of the stimulus-responsive gene. At the same time the level of expression in the recruited cells remains largely unaffected by changes in the concentration of stimulus⁹⁸⁻¹⁰⁵.

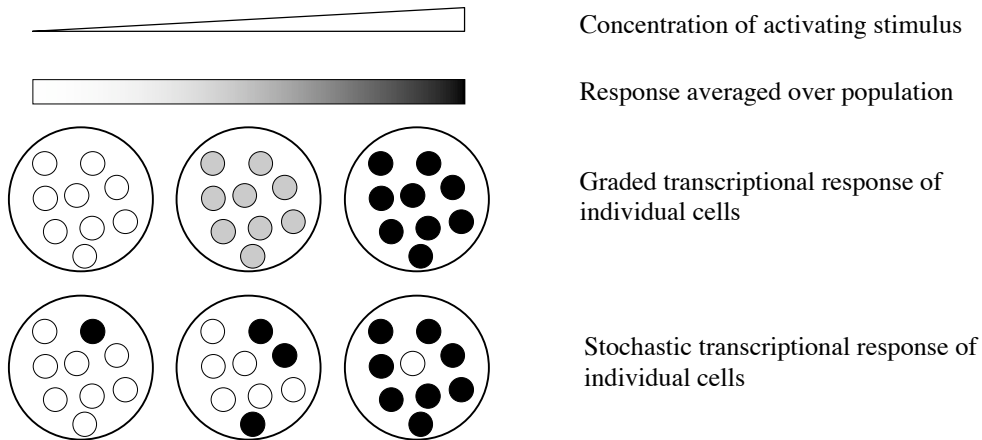


Figure 6. Graded and stochastic transcriptional responses.

As concentration of activating stimulus increases in the culture medium, the total specific mRNA or protein product of a responsive gene rises proportionally. However, at the level of individual cells the same overall increase of the product may result either from gradual increase of transcriptional rate from zero to its maximum in each cell of the population (graded response), or from recruitment of increasing number of the cells that switch from silence to maximum expression of the responsive gene once the concentration of activating stimulus exceeds their individual response thresholds (stochastic response).

Transcription is proposed to be a stochastically determined event that occurs in short pulses. The number of active templates in a population defines its overall transcriptional output at any given time. The probability of a particular template to be active within a certain time window, rather than the rate of transcription from this template is subject to regulation^{105,106}. Transcriptional regulatory elements such as enhancers and activators, according to the probabilistic model of gene expression, simply increase the likelihood that their cognate promoter will be transcriptionally active within a certain time window, but do not affect rate of transcription from their promoters^{106,107}. In a number of studies it was suggested that transcriptional activators might act by modifying the probability of successful formation of pre-initiation complexes¹⁰⁷⁻¹¹⁰. The stochastic self-assembly of macromolecular complexes discussed above is consistent with the probabilistic model of gene expression. The specific molecular mechanisms that account for a binary response in inducible gene expression have been proposed. As an example, Rossi et al. argued that a competition of transcriptional factors with opposing functions, such as repressors and activators, for the same target promoter might be necessary and sufficient for establishment of an all-or-none transcriptional switch¹¹¹.

The quantitative analysis of transcriptionally active sites within nuclei of individual cells suggested that only insignificant fraction, approximately 6-8%, of protein-encoding genes may be expressed in each cell at any given time ^{112,113}. It is tempting to speculate that due to inherently stochastic nature of gene expression while each cell in a population expresses small fraction of a genome, the large enough population may express all or almost all genes within a certain time window. The cell population therefore represents a large “receptive field” for any possible environmental challenge as opposed to narrow “receptive fields” of individual cells. This view is supported by recent statistical analysis and modeling of the large-scale gene expression data ¹¹⁴ and experimental observation of promiscuous gene expression in differentiated cell populations ¹¹⁵.

Consider the same molecular phenomenon, this time the gene expression, from the point of view of two alternative paradigms. The deterministic paradigm, which brought to life the rheostat model of transcriptional regulation, implies that the specific pattern of gene expression in individual cell is instructed to this cell by extracellular clues in its environment. Which, in its turn, implies the pre-existence of specified schemes for cell fate determination and organism development. “How else?” – some readers would most likely shrug. Indeed, the cell differentiation is presented today in textbooks as a unidirectional hierarchically structured program, where a molecular signal triggers sequential expression and silencing of defined sets of specific genes in a cascade fashion driving the cell to lineage commitment and differentiation. The subconscious mechanistic world perception makes us to see the cell itself as a gear inside clockwork of a larger scale system, the organism. The specific expression profile of each cell in a mature organism is therefore pre-determined, according to mechanistic intuition, to fit specifications of respective gear in the context of organism design.

The stochastic model of gene expression on the other hand does not require any specifications or design. And the designer to this end. It suggests that any cell population is highly heterogeneous in at least two respects. First, genes are expressed stochastically though infrequently in the population ^{105,114}. Second, each cell in the population has a different threshold or a different probability to respond by specific gene expression to a given activating stimulus at any given time ¹⁰⁶. Appearance of an external activating clue normally selects the sub-population of cells that happen by chance to be most responsive to this particular stimulus in that specific moment. These recruited cells switch then to expression of the responsive gene. The expression

of responsive gene presumably leads to re-arrangements in individual transcriptional networks of the recruited cells shaping these networks towards more similar yet distinct patterns of gene expression. The gene expression in the recruited cells becomes in part synchronized by appearance and presence of activating stimulus. The activating stimulus may provide a selective advantage to the recruited sub-population on a local scale, but at the same time the synchronization of the recruited cells should be consistent with and, most probably, provides a selective advantage to the whole of which this recruited population is a part. In the context of organism any recruited sub-population is always embedded into a larger matrix of cell-cell interactions. In the case of hematopoiesis a considerable body of experimental evidence suggests that lineage commitment occurs probabilistically and regulatory factors select sub-population of cells in which the commitment has already occurred, rather than dictate cell fate to target cells

106,116

The inherent stochasticity underlying gene expression of individual cells combined with interactions between the cells turns the cell population into the whole that is more than a sum of its parts. This whole becomes sensitive and discriminative to a much wider variety of changes in its environment than individual cells. It is reasonable to expect that virtually any new lasting environmental change will bias the “chaos” of individual expression profiles by giving selective advantage to certain profiles in the population. At the same time, indirectly, through cell-cell interactions, it will affect and shape the global structure of the population’s transcriptional network. As the spectrum of interdependent expression profiles of a cell population is molded and maintained by the interaction with environment, the population as a whole *reflects*, models or, in other words, becomes cognizant of its environment.

It is illustrative and useful to draw analogy between cell differentiation and professional specialization in developed human societies. Let us take as an example the military service in a country and times where and when this service is not obligatory. At any given moment there is a very large civil population of highly heterogeneous personalities. Consider the opportunity and attraction of army service as an activating stimulus. Each civil individual has its own and very different threshold to be attracted to become military. The decision to enroll is inherently probabilistic at the scale of individuals, but statistically the flux of new recruits is maintained constant or regulated by changes in the activating stimulus. The individuals who are finally recruited switch in all-or-none fashion to those certain and similar patterns of behavior, habits,

moral norms, and even thoughts that differentiate the military from the rest of population. To a certain degree individual psychosomatic networks of the recruits are re-arranged, made more uniform and synchronized by being military, yet each of servicemen is still distinct individual. Notice that an individual is not born, or designed from the birth to become, as an example, the air force officer. His/her specialization as an air force officer is selected in a probabilistic manner and is the product of individual psychosomatic development and needs of a larger scale system, the society, of which this person is a part. In fact if needs of society in army or air force suddenly were to disappear or to diminish, our air force officer might be attracted to other society's needs and would become a businessman or restaurant owner, for instance. The particular outcome again would depend on the interaction between individual psychosomatic network of the retiring officer at the moment of retirement, the current needs of society and a chance. In this sense functions or specializations of a protein, a cell or a human individual are not pre-determined by unspecified designer, but results from the interaction and mutually beneficial compromise between development and interests of a part and needs of a whole. The very concept "function" pertains to a whole and is meaningful only in its context. It is not the inherent property of a part, which can be deduced from physical characteristics of a part alone.

The remarkable parallels between outlined models of cell and professional specialization and the response threshold models of division of labor in social insects are again unlikely to be coincidental and most probably reflect common patterns in dynamic and evolution of self-organizing complex systems^{117,118}. The response threshold models assume that individual insects in a swarm will start performing a particular task only when corresponding stimulus from their environment exceeds a certain value. The response threshold varies among members of the group and individuals with lowest response thresholds are recruited first to the task. By performing the task, the recruits diminish the stimulus, and thus reduce the probability that other individuals will be recruited to the same task. Since insects in the group are characterized by different individual thresholds to distinct environmental stimuli, division of labor occurs in a self-organized fashion benefiting both the group as a whole and the individual members as its parts.

Analysis

The debates over adequacy of mechanistic interpretations of life have been interminable throughout history. But the issue has never before been of such urgency and verve, as today when the life sciences are entering their Renaissance.

The molecular and cellular phenomena discussed above were chosen to illustrate a relative deficiency of the Cartesian-Newtonian paradigm to serve as a unified and self-consistent framework for comprehension and modeling of life systems. The analysis of common patterns underlying alternative interpretations of life phenomena is expected to facilitate the development and maturation of the interpretational paradigm that is more suitable for the purpose. The patterns and principles of novel emerging paradigm uncovered in one research field may be instrumental to gain insights in another. Conversely, the analysis of common patterns of thought and assumptions by which the mechanistic paradigm manifests itself in experimental research may help to avoid unnecessary outlays in time and efforts and to facilitate eradication of errors of self-perpetuating dogmatism in biomedical sciences.

Mechanistic interpretation and reductionist analysis as subconscious defaults. Watchmaker.

The works of Descartes and Newton placed firm foundations for emergence, development and subsequent reign of both the mechanistic and deterministic world perception and the reductionist scientific method. The economic success of the technological revolution ignited and fueled by Newtonian science has been matched by constantly increasing share of mechanistic indoctrination in professional training, education and general culture. We are programmed to interpret reality in mechanistic terms and to approach analysis of any phenomenon by reductionist method. The mechanistic paradigm and reductionism have rarely failed Western mind and technology, economically speaking, and have eventually become our subconscious operational defaults. Their applicability is tacitly assumed unlimited unless proven to the contrary.

According to Newtonian tradition, any phenomenon can be reduced to the motion of elementary material particles in the void. God created the particles and established the immutable fundamental laws of motion. The whole universe has been running ever since the creation in ways specified by divine design. Consequently, any biological system implies purpose and design behind it within the interpretational framework of the mechanistic paradigm.

William Paley in his *Natural Theology*¹¹⁹ presented the watchmaker argument as a "proof" of God's existence. He argued that since "every indication of contrivance, every manifestation of design, which exist[s] in the watch, exists in the works of nature..." then we have to acknowledge existence of "The Watchmaker" who designed and created the Nature.

Watchmaker patterns

Pattern 1. Tacitly assumed design and negligence of evolutionary argument.

The power stroke models of molecular motors and protein import, the deterministic views of sub-cellular organization and gene expression invoke the idea of design behind them, but do not address the question "who is the designer?" The mechanistic interpretations always imply externally imposed purpose, design and determinism. They do not require and normally omit evolutionary reasoning.

Pattern 2. Assumed linear causation.

As it is often true for our scale physical world, the linear causation is assumed to be a rule rather than exception in the molecular world as well, and large effects are perceived to originate from proportionally large causes. For this reason the independence of step size from geometry of a motor, the absence of correlation between size of a step and the energy spent to make this step, the movement of similar motors in opposite directions etc. are perceived as "surprises". The self-organization of functional nucleoli, which consist of dozens of different functional components highly organized in time and space, by simply introducing the ribosomal gene copies into nucleoplasm and the stochastic nature of transcriptional response appear surprising as well, because they are not consistent with linear causation.

Pattern 3. Assumed determinism.

Determinism is intimately linked to the ideas of design and external purpose, as any design is meant to specify behavior of a system according to the purposes of designer. Functional promiscuity of molecular motors, antibodies performing as import motors, stochastic and transient self-organization of steady-state macromolecular complexes, moonlighting proteins, probabilistic nature of gene expression are all "striking surprises", as they fail anticipations of assumed determinism.

Pattern 4. Tacitly assumed independence of the context.

Reductionist agenda and mechanistic intuition result in a habit of ignoring the context from which parts are isolated. The considerations of environmental thermal noise and dynamic behavior of macromolecular polymers are typically absent from the discussions of power stroke models of molecular motors. Furthermore, the assumption that an isolated part, such as molecule, cell or organism, is not changed, or is not significantly changed, by the fact of isolation remains beyond the burden of proof within the mechanistic paradigm despite accumulating evidence to the contrary (see self-organization patterns E and F).

Pattern 5. Chaos of specialization.

The reductionist method of disassembling a whole and scrutinizing its components in isolation naturally leads to rapid diversification and subspecialization in sciences. “We know more and more about less and less”. The current level of this fragmentation and its rate are beyond any rational reasoning. To follow all publications in even narrowly specialized areas of research has become physically unfeasible. The biomedical science is being increasingly split into smaller and smaller subdivisions with less and less interaction and understanding between them. This state of affairs is not a problem if there is a pre-existing design. Consider the case of automobile production, as an example. The fragmentation and separation between specialists of production enterprise is typically an advantage and not a drawback, as long as their work is determined and organized by pre-existing production plans and automobile design. To solve growing confusion of specialization, therefore, the biomedical science either needs to call the designer and ask for specifications, or, alternatively, it is in a dire need to develop and to formulate the novel general principles of organization and behavior of life systems that would transcend current idiosyncrasies of specialized subdomains of knowledge and unite them by one conceptual framework and description language. Whatever framework of the future paradigm might be it is clearly not the mechanistic one, because the latter requires knowledge of the design of life that we do not possess.

The systematic appearance of the experimental data challenging the mechanistic intuition is brought about by advance in technology and is a sign of the paradigm in crisis.

It takes an inquiring mind and a formidable amount of perseverance to publish the counter examples or the results that challenge conventional views. However manifold may be the uncovered inconsistencies between theory and reality, they do not disturb the paradigm in power unless they become systematic. The systematic appearance of results inconsistent with conventional interpretations is normally brought about by progress in technology, indicates the paradigm crisis and becomes the harbinger of scientific revolution ¹²⁰.

The introduction of novel fluorescence-based imaging methods of enhanced spatial and temporal resolution, confocal and time-lapsed microscopy, photobleaching techniques, genetically encoded fluorescent tags and computer-aided image processing and analysis has allowed researchers to follow individual macromolecules quantitatively in real time in living cells and are responsible for the radical shift occurring in our perception of sub-cellular organization ^{54,55}.

The introduction of fluorescent probes, expression reporter systems, fluorescence-activated cytometry, and advanced imaging and detection methods has allowed quantitative analysis of cell populations on cell-by-cell basis. The previously fragmented reports indicating on the probabilistic nature of gene expression became more systematic following acceptance and broad use of the novel technologies ^{106,121}.

The introduction and development of fluorescent probes, optical trapping nanometry and nanomanipulation are rendering the single molecule analysis more reliable and routine. The data on chemomechanical properties of different molecular motors, which accumulate as a result of application of these novel technologies, appear to result in a progressive build up of inconsistencies within the deterministic framework of power stroke models and to favor instead the Brownian ratchet principle as a unifying theoretical framework of directional movement generation at microscale ³⁰.

According to Thomas Kuhn, the progress in technology and methods aiming initially to extent scope and precision of the scientific knowledge that can be generated by paradigm in power, inevitably results in undermining the paradigm itself, as it leads sooner or later to a systematic accumulation of facts that are unexpected, inconsistent with and resist assimilation within the old paradigm ¹²⁰. The examples of inadequacy of clockwork interpretations by no

means are limited to the phenomena discussed in this review. They are widespread and can be recognized as the “surprises” in experimental outcomes that become difficult to ignore due to their systematic appearance and accumulation.

On the molecular scale consider example of moonlighting proteins⁸⁵ that have multiple and surprisingly unrelated functions, such as, for instance, glycolytic pathway enzyme phosphoglucose isomerase functioning as well as neuroleukin¹²², as autocrine motility factor¹²³ and as differentiation factor¹²⁴. Consider examples of proteins, such as L-aspartate aminotransferase and D-amino acid aminotransferase, which share no significant sequence homology, have totally different 3D structures, but perform the same chemistry¹²⁵. Consider the surprising discovery of a whole class of so-called “natively unfolded” proteins, which do not appear to possess any rigid structure in solution. Their very existence disputes the validity of one of the mechanistic foundations of protein science, the structure-function paradigm^{126,127}.

On another scale the deterministic doctrine of cell differentiation is being shattered by multiple studies reporting such examples of unexpected cell plasticity as neuronal stem cells turning into hematopoietic cells¹²⁸, bone marrow cells engrafting as liver and neuronal cells¹²⁹⁻¹³¹ and hematopoietic stem cells differentiating in cells of endodermal and ectodermal lineages such as epithelial cells of the liver, lung, stomach, small and large intestine, and skin¹³². Recently Theise and Krause reviewed the experimental evidence challenging unidirectional and hierarchical lineage commitment and suggested the new paradigm of cell plasticity, which states that i) any cell with intact genome can potentially become any other cell type under appropriate treatment of the cell and its microenvironment; ii) any attempt to isolate a cell from its natural context alters the cell at the time of characterization and introduces inherent uncertainty in respect to the cell’s origin and fate; iii) the nature of cell differentiation and lineage commitment should be treated as probabilistic¹³³. This new outlook on cell differentiation lines up well with the discussed above stochastic phenomena and is a far cry from determinism.

On the scale of whole organisms the surprises coming from knockout studies in mice are increasingly difficult to ignore. Deletion of supposedly key elements of cell physiology, such as cyclin E¹³⁴, Cdk2¹³⁵, p53¹³⁶, cholesterol¹³⁷ etc. results in no or mild phenotypes suggesting that they are dispensable for animal development and life, while knockout of a pseudogene causes severe developmental abnormalities and premature death¹³⁸.

These and multitude of other examples add to a growing confusion. But they are and will remain inconsistencies only within the framework of clockwork interpretations. They are less surprising and many of them become predictable if the cell and the organism are treated not as machines, but as self-organizing fluxes or ever-evolving interconnected and interdependent multi-scale organizations of interacting molecules.

Patterns of self-organization

Pattern A. Assume no design, but self-organization.

All non-mechanistic interpretations of the phenomena discussed in this review assume no design but invoke principle of self-organization to explain the emergence of molecular fluxes and steady-state macromolecular structures. The concept of self-organization originated from the studies performed on relatively simple open chemical and physical systems maintained in far-from-equilibrium conditions. The formalism and quantitative models of nonequilibrium thermodynamics hold a promise for development of novel analytical approaches to study life systems^{139,140}.

The self-organization principle indicates that open physicochemical systems, which are maintained in far-from-equilibrium conditions by flux of energy and matter passing through them, may spontaneously generate ordered steady-state macrostructures through coordinated action of their microcomponents. The emergence of structures, their co-existence, development and organization into higher order structures are more appropriately described in Darwinian-like terms of random variation, competition, selection, adaptation and evolution. The principle of self-organization is inconsistent with the idea of externally imposed purpose and design.

Pattern B. Assume no determinism, but evolutionary robustness.

One may definitely argue that the biological systems we study, such as biomolecules, cells and organisms, display a great deal of seemingly deterministic behavior. The cells in culture, for instance, faithfully repeat cycles of cell division duplicating their genomes, packing them into chromosomes, assembling mitotic spindle, aligning their chromosomes along metaphase plate before segregating them into daughter cells etc. Each newborn organism repeats the same and, therefore, predictable stages of development and so forth. It is important to realize that these are examples of evolutionary robustness and not of determinism. The coordinated

behaviors of large populations of interacting molecules or cells, which we observe as condensation of chromosomes or formation of a neural tube, represent statistically similar evolutionary outcomes of molecular and cellular interactions robustly reproduced every time under certain conditions. Regrettably, the mechanistic paradigm, operating as subconscious default, makes us to perceive and to interpret the experimental results in terms of clockwork determinism. For this reason we do not ask questions “Why is this particular evolutionary outcome of molecular interactions, such as chromosome condensation, faithfully reproduced over and over again?”, “What are possible alternative evolutionary outcomes of interactions between the same set of molecular components?” or “What are the causes of transitions between different steady-state organizations of genomic DNA?” and so forth. Instead the typical questions asked today are “What is the program of chromosome condensation?”, “How does the design chart of chromosome condensation look like?”, “How do the input elements A, B ... [on the design chart] transfer a signal to the converters J, K ... , which transform that signal into another form, and, through the amplifiers X, Y ..., impinge on the motors W and O that are hypothesized to compact DNA?”

Pattern C. Selection for function.

The causes bringing about directional movement of motor protein, expression profile of differentiated cell and formation of a certain macromolecular complex are assumed to be selective, not instructive according to non-mechanistic interpretations of the phenomena discussed above. Therefore, the function of a part, consider the protein or the cell, is not inbuilt in and inseparable from the part, but emerges as a compromise between capabilities of a part and “needs” of a whole, a larger scale system, such as the cell or the organism. The particular protein or cell is selected to perform “needed” function as appropriate candidate for this function under conditions of selection. The situation is more adequately described by using social metaphor of hiring appropriate candidate for the vacant position representing certain “function” within a complex business organization. Conversely, the deterministic paradigm implies that properties of a part, such as the protein structure or the cell expression profile, determine its function within a larger scale system, much like physical structure of a gear determines its function within clockwork.

Pattern D. Look for a “biased chaos” and ask what flux keeps it biased.

In all the phenomena discussed above and many others one can find a “biased chaos” and the flux, or gradient, maintaining this bias. Chaotic movement of molecular motors along cytoskeleton track is biased by ATP/ADP gradient. The random sliding of passenger polypeptide within the protein import channel is biased by gradient of chaperon activity maintained between the *cis* and the *trans* sides of mitochondrial membrane. The nuclear compartments formed and maintained through stochastic molecular interactions are yet another example of a biased chaos, though what are the fluxes critical for their maintenance remains to be determined. The chaos of expression profiles of a cell population is biased by the flux of activating stimulus. The antigen biases stochastically expressed antibody repertoire according to the clonal selection theory. The bias of continuous and chaotic autocatalytic remodeling of the actin network is hypothesized to underlie directional cell crawling¹⁴¹.

Pattern E. Substitute structures by processes.

The mechanistic paradigm has a vested interest to interpret everything as structures. It has little handle on processes. The concept “structure” carries a static undertone. The structure can often be isolated and studied separately. It can be further separated into independent substructures. The concept “process” has more holistic connotations. It is difficult to imagine isolating a process without significantly affecting it. It is even more difficult to imagine studying sub-processes separately and inferring how would the whole process look like when the sub-processes are combined. Meanwhile, experimental data indicate that an increasing number of phenomena in cell and molecular biology considered previously as static structures are in fact dynamic processes. The cytoskeleton, chromatin, subcellular compartments, specialized macromolecular “machines” are most recent examples of steady-state molecular processes. It may be fruitful to look more closely on what is traditionally and/or subconsciously treated as “structures” in biology and reconsider them as processes.

Pattern F. Probabilistic nature of life systems. Role of chance. Nonlinearity.

The stochasticity rooted in the molecular level manifests itself throughout all larger scales of biological organization. While the measurements of certain characteristics or responses

averaged over a large population usually result in deterministic and linear interpretations, the analysis of the same population on individual-by-individual basis often reveals underlying chaos. The discovery of chaos appears to be a general outcome in studies performed on populations where molecules, cells or animals composing the population are followed individually.

The stochasticity of individual behaviors combined with nonlinear interactions between the individuals renders the behavior of a population itself inherently probabilistic. There is always a chance that the population as a system will respond in unexpected and unpredictable fashion and will be driven away from its most probable behavior trajectory by self-amplifying individual fluctuation. The nonlinearity and sensitivity to infinitesimal fluctuations in the population's dynamic is analogous to "butterfly effect" of the chaos theory and may provide rationale for "The American dream" if one assumes that the socioeconomic dynamic is simply an evolution of self-organizing complex system composed of interacting human individuals and their organizations.

Pattern G. Be environmentally conscious. Bear in mind the whole while analyzing its isolated parts.

The selection for function principle discussed above implicitly involves consideration of environment, or the context of a whole. Any biological system is a whole of its constituents and, at the same time, a part of a larger scale system. As the part, it is defined by its matrix of interactions with other components and aligned with the "needs" of a larger scale whole. The biological system is, therefore, re-defined every time when one matrix of its interactions is substituted by another one. The reductionist approach has severe limitations because it allows one to address in more or less adequate terms only those properties and processes in isolated molecules, cells or organisms that are relatively unperturbed by the fact of isolation. How many of them *are* perturbed by the isolation is difficult to judge, but certainly significantly more than conventional clockwork intuition may suggest. The conclusion of studies of adhesion complexes in fibroblasts cultured in three-dimensional (3D) matrices derived from tissues as opposed to standard culturing conditions stated that "current concepts of the biological and signaling roles of classical focal and fibrillar adhesions need to be reexamined in light of these findings on 3D-matrix adhesions" ¹⁴². The 3D culturing of the mammary epithelial cells profoundly affects both their biology and their resistance to anticancer drugs ¹⁴³. About two hundred functionally

important and often highly conserved proteins that lack any ordered structure when isolated from cell environment have been identified ¹²⁶. The genetic material isolated from cancer cell drives normal embryonic development when placed in the environment of a normal enucleated mouse oocyte ¹⁴⁴. These examples and others support the idea that biological systems are highly context-dependent and highlight the grave limitations of reductionist method applied to life systems.

Pattern H. Bear in mind interconnectedness of a whole and consider the whole to be a “small world”.

The important and successful breakthrough in the conceptualization of biological complexity is the description and analysis of biological systems as self-organizing networks ¹⁴⁵⁻¹⁴⁷. In particular it was demonstrated that the cellular metabolic and protein interaction networks belong to a class of so-called “scale-free” networks, and are topologically similar to other self-organizing networks such as the Internet, air traffic networks, neuronal system of *C. Elegans*, social networks etc. One of the properties of scale-free networks is their “small world” character ¹⁴⁵, meaning that any two arbitrary chosen nodes are separated in small world networks by surprisingly few intermediate nodes. Specifically, any metabolite in the metabolic network of *E.coli*, which consists of approximately 800 chemicals, may be connected to any other through only three metabolic reactions on average ¹⁴⁶. In a small world of the cell therefore everything is tightly interconnected and interdependent and any molecule and any process are only few steps away from any other molecule and a process.

Small world character and self-organizing properties of both the metabolic and protein regulatory networks, which share many common components, supports view of a cell as ever-evolving network of molecular interactions realized as self-organized steady-state transient macromolecular organizations physically connected and functionally coordinated by fluxes of shared elements.

Pattern I. Avoid mechanical metaphors, instead look for and use the social ones.

Consider the relatively simple transient organizations that are formed and maintained through inherently stochastic and probabilistic interactions, such as restaurants, for example. For alien observer coming from another planet, who is unaware of socioeconomic motives underlying behavior of humans, the formation and maintenance of restaurants would be a typical

example of stochastically self-organized steady-state structures. Though they seem to emerge independently, they have similar organizations of distinct functional components such as chefs, waitresses, accountants, dishwashers etc. involved in a similar set of interactions. The restaurant is a part of and is embedded into a larger socioeconomic and cultural matrix of human interactions in the respective societies. The restaurants are maintained and regulated by flows of money, food, personnel and customers passing through them. They are steady-state organizations with a characteristic “residence times” for different occupations, with unqualified workers having usually shortest and the owner having longest “residence times” within the organization. Consider a thought experiment. Let us “isolate” Italian restaurant from Italy and try “to culture” it in America of our days, in America of the 20s, and on uninhabited Pacific island. In America of our days, as one may have guessed, the overall organization of Italian restaurant will be preserved, though its Italian employees may sooner or later be replaced by local people. Whatever efforts and energy are spared to keep the restaurant authentic, it will soon faithfully reflect the environment it is placed in. It is difficult to find ashtray indoors in a typical Italian restaurant in America, but one can surely rely there on understanding while asking for diet coke or cheeseburger with fries. Commonly, the Italian dishes having the same name in Italy and America taste surprisingly different in different countries and so forth. It is reasonable to suggest that the modern Italian restaurant isolated from Italy and transferred to Chicago of the 20s would again faithfully reflect its environment and, most probably, soon after the transfer, evolve into a bootleggers establishment. It would be a fair guess that Italian restaurant isolated from its native environment and transferred to uninhabited Pacific island with no connections to civilization will not survive as organization, even for a short while, though the island conditions are more than compatible with life and functions of its constituents. Most probably, the restaurant employees on the island will reorganize themselves through stochastic socioeconomic interactions of probabilistic nature into other steady-state primitive organizations, which will grow, develop and evolve in accord with population growth and environmental conditions of the island.

The social metaphors may often be more appropriate and revealing when used to describe molecular and cellular phenomena as compared to the conventional mechanistic ones. Certainly they are more consistent with the accumulating experimental data and a new image of the cell as a dynamic multi-scale self-organized network of interacting molecules.

As a rule, molecular and cellular phenomena are “projected” on our scale physical reality and comprehended in our world’s concepts by our mind. This conceptualization involves and assumes as valid all the familiar logical implications, consequences and the interrelations between the concepts used as metaphors. The metaphors such as “motor”, “machinery”, “lever arm”, “relay”, “channel”, “pump” and so forth may be misleading and blinding. Because they appeal to our intuition and can be easily communicated to public they are convincing and convenient. But the advantage of being appealing, convincing and convenient often turns them into the mental blocks that prevent us to see and to communicate alternative perceptions of the same phenomena.

Future prospects

It would be naïve, impossible and dreadfully unthankful to suggest abandoning the Cartesian-Newtonian paradigm altogether. The mechanistic world interpretation combined with the reductionist method is perhaps the second highest achievement of human intelligence in modeling the world after introduction of religion, chronologically speaking. But, as I argued elsewhere the Newtonian paradigm is just another approximation of reality¹⁴⁸. It was, it is and it will remain to be the best one as a practically serviceable approximation for a limited range of phenomena. The point is to recognize dimensions of its limitations and to develop the more adequate and self-consistent non-mechanistic model(s) that would reflect better the reality we probe through our experimental research. The inadequacy of mechanistic paradigm has been recognized in subatomic physics and need to be acknowledged in life sciences as well if the aim is to progress in understanding of life.

It is a trend of our time to bring together specialists from very different backgrounds to work together on problems of biology. The systems biology term has been coined, the multidisciplinary research centers are built and multimillion-dollar initiatives are launched. It is intuitively perceived that only highly diverse interdisciplinary efforts are likely to be successful to understand complex biological systems. In other words, understanding of biosystems is believed to require the knowledge accumulated in physics, mathematics, meteorology, hydrodynamics, sociology, economics, ecology, environmental sciences and so forth. Which is essentially the same as to acknowledge that the evolution of ecosystems, the motion of large masses of gases in atmosphere, the dynamic of markets and social behavior, and the molecular

dynamic inside the cell share common patterns and properties and are described by similar concepts and models. Consequently, the notion of a life system is bound to undergo a radical transformation and expansion. It will, most probably, include within a unified framework many phenomena that are not traditionally treated as “alive”, such as economics, business and political organizations, social, ecological and informational systems, consciousness and a planet-wide integrated system of life.

What will be a future unifying framework of life systems? On one side, we have the ruling mechanistic paradigm and its proponents who, with help of appealing, convincing and convenient clockwork metaphors, are trying to suggest that once the engineering-like comprehensive charts of the cell are outlined, the computers and math will do the magic of turning robot into a living creature. They are consistent and aligned with their reductionist agenda - “One of the acid tests of understanding an object is the ability to put it together from its component parts. Ultimately, molecular biologists will attempt to subject their understanding of cell structure and function to this sort of test by trying to synthesize a cell”¹. But, putting aside faith in magic, the future prospects for the chaos of specialized knowledge that is being produced by reductionist agenda and mechanistic paradigm appears grim in the absence of explicit design specifications of life. On the other side, there is a panoply of separated theories of yet little practical significance, but of rapidly increasing influence in biological sciences, such as chaos theory, theory of complex systems, nonlinear thermodynamics, network theory, game theory etc. Some models and concepts of these theories represent certain aspects of life phenomena remarkably well, though at present they lack a unified conceptual framework. Given certain breakthroughs in conceptualization of biosystems, these theories hold a great promise to evolve into a unified paradigm of life that is radically different from the mechanistic one. The future prospects for adherents of self-organization paradigm are bright. Chaos is a source of order. The chaos of specialized knowledge will self-assemble itself into meaningful *reflections* or models of biological reality as a result of stochastic interactions between different specialists within the multidisciplinary research incubators, provided an adequate flux of capital and scientific talent is maintained. It is just another step forward in never ending process of self-organization and cognition.

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