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Self-organization versus watchmaker: Molecular motors and protein translocation

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Abstract

Generation of directional movement at the molecular scale is a phenomenon crucial for biological organization and dynamics. It is traditionally described in mechanistic terms, in consistency with the conventional machine-like image of the cell. The designated and highly specialized protein machines and molecular motors are presumed to bring about most of cellular motion. A review of experimental data suggests, however, that uncritical adherence to mechanistic interpretations may limit the ability of researchers to comprehend and model biology. Specifically, this article illustrates that the interpretation of molecular motors and protein translocation in terms of stochasticity and self-organization appears to provide a more adequate and fruitful conceptual framework for understanding of biological organization at the molecular scale.
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Keywords: Self-organization; Stochasticity; Determinism; Molecular motors; Brownian ratchet; Protein translocation

1. Introduction: molecular motors and conventional views

Motion is one of the defining characteristics of life. 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 (PS) produced by molecular motor are generated as a result of periodical conformational rearrangements of protein structure driven by the 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 elements (Schliwa and Woehlke, 2003). 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 physicists trained in the mechanistic tradition in the 1960s, who most likely did their best to match their mechanistic world outlook and the electron

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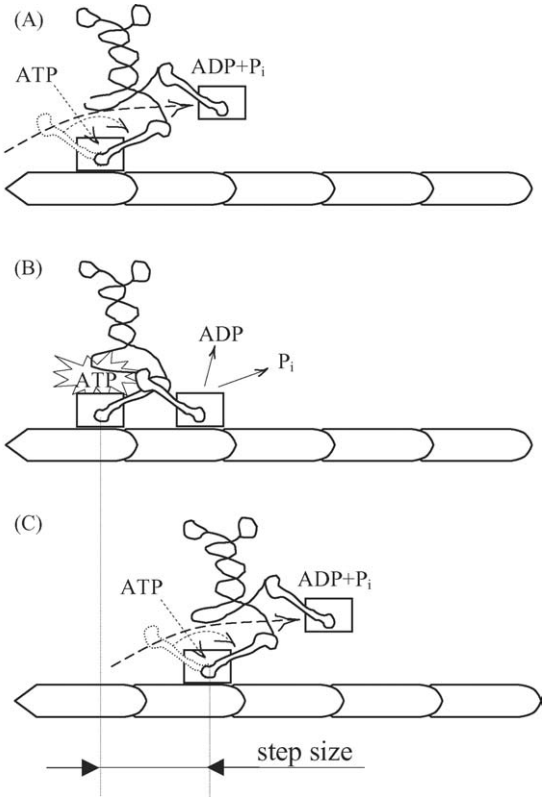

Fig. 1. Generic model of the "walking" protein. Molecular motors, such as kinesin form dimers with motor domains acting as "feet" that step along a cytoskeletal track, such as microtubules. (A) Binding of ATP to the motor domain of the leading leg causes its structural rearrangement that moves the trailing motor domain "over head" of the leading domain, which now becomes the trailing domain, (B) the former trailing and now leading motor domain binds to the microtubule and releases the products of ATP hydrolysis, ADP and P_i. The former leading and now trailing motor domain hydrolyses ATP and (C) binding of ATP to the motor domain of the leading leg triggers its structural rearrangement that throws the trailing motor domain "over head" of the leading domain, thus completing the cycle.

microscopy images of actomyosin complex. The model was later reinforced by biochemical data on actomyosin's enzymatic cycle of ATP hydrolysis and, relatively recently, in the 1990s, by structural data illustrating fine details of different conformational states of molecular motor proteins (Huxley, 1969; Lymn and Taylor, 1971; Rayment et al., 1993; Rice et al., 1999).

(ii) It is a very appealing interpretation for our intuition originating from our human scale physical experience and is in harmony with the mechanistic world perception shared by the members of technology-driven society. It is natural for us to interpret everything as mechanical devices. It is an "easy sell" for our mind.

(iii) It is a 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.

The reductionist method, addressing properties of parts in isolation, normally disregards the environment. In the case of molecular motors, the mechanistic models ignore the fact that molecules in the cell operate in an environment that is drastically different from our scale, familiar conditions. Our physical intuition therefore is more often inappropriate for the interpretation of events on the microscale than it is not. The molecules in the cell operate in conditions of continuous stochastic thermal fluctuations. This fact is traditionally visualized as Brownian motion. The energy of ATP hydrolysis allegedly responsible for the generation of the 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 an even smaller amount of energy for work (Rice et al., 1999; Vale and Milligan, 2000). Next, the energy of ATP hydrolysis is said to be amplified through the angular motion of "mechanical elements" of molecular motors, such as the "lever arm" or the "relay helix" (Vale and Milligan, 2000). At the same time, it is not discussed that the strength of non-covalent bonds responsible for the very existence of the 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 (Dill, 1999; Frauenfelder et al., 1988; Kumar et al., 2000; Volkman et al., 2001). Even the core of a tightly folded protein displays a liquid-like behavior (Lindorff-Larsen et al., 2005). It is difficult to reconcile the dynamics and plasticity of proteins in solution with the presumed ability of molecular motors to store, transduce and amplify mechanical energy. The low inertia of macromolecules, internal thermal fluctuations and "breathing" of a polypeptide chain in conditions of constant bombardment by surrounding molecules is expected to lead to the dissipation of any form of mechanical energy in picosecond scale time intervals (Spirin, 2002a,b). Keeping this in mind, the estimated 50–60%

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efficiencies of molecular motors when compared to the 10–15% efficiencies of our human scale motors are staggering (Astumian, 2001; Vale and Milligan, 2000).

The staggering and surprise pertaining to experimental outcomes are indications of failed anticipations, and are suggestive of inadequacy of the interpretational model chosen and its underlying paradigm. The surprises in experimental research on molecular motors are meanwhile abundant:

(a) 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 movements (Schliwa and Woehlke, 2003).
(b) Due to mechanistic considerations, the processive movement, defined as the advance of a motor protein bound to the cytoskeletal track over a long distance before its dissociation was believed to require dimeric motors. The surprise came when monomeric KIF1A kinesin (Okada and Hirokawa, 1999), monomeric class IXb myosins (Inoue et al., 2002) and monomeric inner arm dynein (Sakakibara et al., 1999) were found to move processively.
(c) Surprisingly, there is no obvious correlation between structural geometry of swinging legs and step size in different molecular motors (Rock et al., 2001; Tanaka et al., 2002; Veigel et al., 2002).
(d) Another example of poorly understood phenomena are related motors, such as conventional kinesin and non-claret disjunctional (ncd) protein that move in opposite directions even though they have similar structures and are positioned in the same orientation relative to the microtubule track (Astumian and Derenyi, 1999; Schliwa and Woehlke, 2003).
(e) Single molecule measurements revealed that the single myosin head moved 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 molecule (Kitamura et al., 1999).

To summarize, the mechanistic conceptualization of molecular motors often leads to “surprises” in experimental outcomes rather than provides a unifying interpretational framework of reasonable predictive power. Perhaps due to problems with self-consistency between different motor models, the current reviews on molecular motors give the impression of a chaotic mosaic of individual case micromodels, often of staggering complexity, where one would expect to see a self-consistent, systemic and structured description of the phenomenon.

2. Brownian ratchet (BR)

There exists an alternative model of molecular motors based on the Brownian ratchet principle (Astumian, 1997; Feynman et al., 1963; Huxley, 1957). It is counter-intuitive and takes an effort of mind to grasp. Probably for this reason, although it is as old as the mechanistic interpretation, it has never been as popular, despite its sound physical background.

First, let us consider the 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 when explaining non-mechanical phenomena. This is yet another indication of the power of the 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 the sawtooth shape of the paddle wheel combined with the stochastic disengagement and re-engagement of the pawl, the gear will have a tendency to turn clockwise. In this

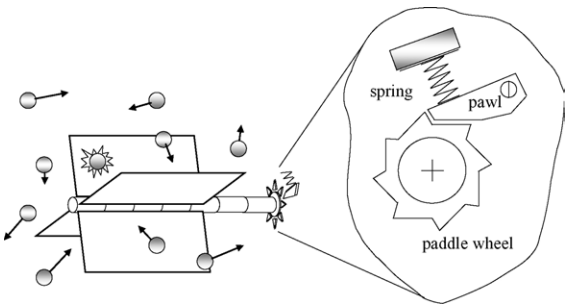

Fig. 2. Brownian ratchet, see description in the text. Adapted from Astumian (2001).

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

Next, let us consider kinesin as an example of a molecular motor in the framework of the Brownian ratchet model. A large body of evidence suggests that molecular motors, using the 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 (Fig. 3A and C, white ball) the energy profile is flat, so that bound kinesin is free to slide along the microtubule in both directions upon the influence of thermal fluctuations. In the "flop" conformation (Fig. 3B and D, 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. However, they are not prohibited.

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 the 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 the motor molecule driven by the energy of thermal fluctuations. The ATP hydrolysis cycle maintains the system in non-equilibrium conditions and biases the random walk of kinesin in one direction.

Consider the rich ramifications of the Brownian ratchet model of molecular motors and its possible evolutionary underpinnings. There is no design and 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 the kinesin molecule. If the kinesin's flip and flop conformations happen by chance to have similar energy profiles on a polymer other than microtubules, kinesin will work as a molecular motor using that other polymer as a track as well. If that other polymer happens to be, for instance, DNA, and the movement of kinesin along DNA would happen somehow to facilitate removal of oxidated bases, then

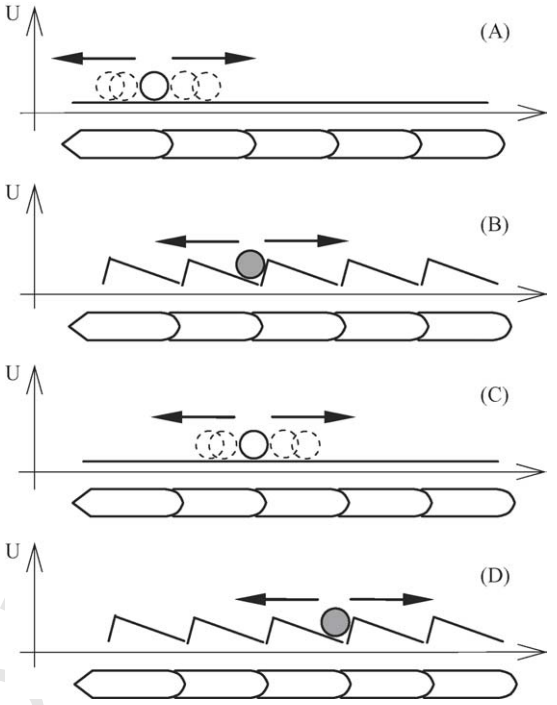

Fig. 3. Brownian ratchet model of a molecular motor. The motor molecule bound to a cytoskeletal track is hypothesized to have two different potential energy profiles depending on its conformational state. In one conformation, referred in the text to as the "flip" conformation (A and C, white ball), the 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.

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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 the 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, non-motor proteins that can generate directional movement. This is exactly what recent experimental data suggest. Examples include the kinesin-related family of MCAK proteins that are not motile, but act as microtubule depolymerases (Hunter et al., 2003), G-proteins that generate mechanical force (Kosztin et al., 2002), ribosomes (Spirin, 2002a) and RNA polymerases described as molecular motors (Gelles and Landick, 1998; Spirin, 2002b).

The Brownian model of molecular motors resolves what is perceived as inconsistencies and surprises within the power stroke model (Nishiyama et al., 2002; Okada and Hirokawa, 1999; Yanagida and Ishii, 2003). Multiple functions of molecular motors, stochastic movement along tracks, independence of step size from geometry of a motor, the processivity of monomeric motors, the absence of general correlation between step size of a motor and the energy spent to make this step are often self-explanatory when molecular motors are considered within the Brownian ratchet framework. Importantly, the Brownian ratchet provides a unifying principle of rectification of directional movement from the thermal chaos at microscale (Hanggi and Bartussek, 1996). In other words, it illustrates how order can be generated out of chaos (Prigogine and Stengers, 1984). This principle is believed to underlie the functioning of such "molecular machines" as RNA polymerases (Gelles and Landick, 1998), ATP synthases (Ait-Haddou and Herzog, 2003), ion pumps (Astumian and Derenyi, 1998), ribosomes (Spirin, 2002a) and others (Astumian, 2001). It is also considered to be responsible for many types of biological transport driven by non-equilibrium chemical reactions. One relevant example is protein translocation across lipid membranes to which we now turn.

3. Protein translocation

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 the 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 mitochondria (Bauer et al., 2000) and the Sec complex in the endoplasmic reticulum (Deshaies et al., 1991; Van den Berg et al., 2004). Two functionally distinct parts of these protein translocases are recognized, the protein channel (Matlack et al., 1998; Simon and Blobel, 1991) 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. Import is achieved by unfolding and threading of the passenger polypeptide chain through the import channel. Energy-coupled translocation motors are thought to play a critical role in the unfolding and unidirectional transport of the preproteins across membranes. The molecular chaperones of the heat shock protein 70 (HSP70) family, which reside in the lumen of ER (Vogel et al., 1990) and in the matrix of mitochondria (Strub et al., 2000), 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 controversial (Neupert and Brunner, 2002). Two models of translocation motors have been proposed, the power stroke model (Glick, 1995; Matouschek et al., 2000; Voisine et al., 1999; Voos et al., 1996) and the Brownian ratchet model (Matlack et al., 1999; Okamoto et al., 2002; Schneider et al., 1994).

According to the PS model, mitochondrial HSP70 (mtHSP70) molecules associate with the outlet of the import channel inside mitohondria and use the energy of ATP hydrolysis to produce a pulling force applied to the passenger protein. The power stroke generated by the 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. 4A). 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 chaperone molecule needs to be precisely and steadily positioned at the outlet of the import channel in order to generate a force perpendicular to the plane of the membrane using the channel as a fulcrum. Following the generation of the power stroke,

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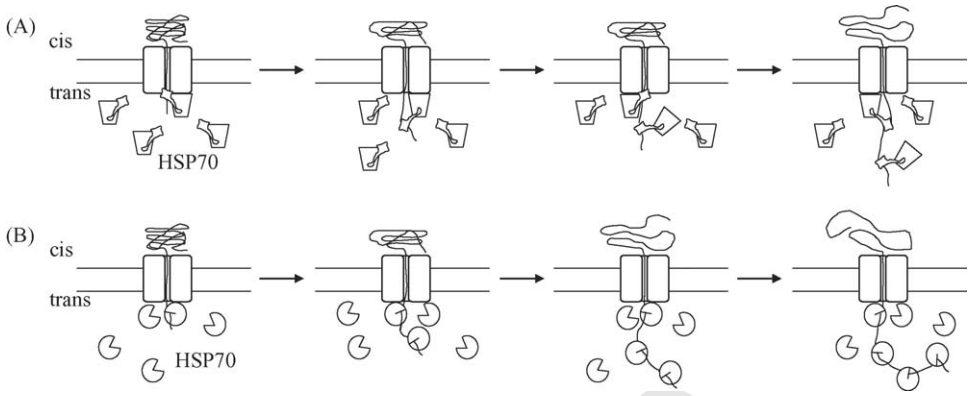

Fig. 4. The power stroke and Brownian ratchet models of import motors. The HSP70 family proteins residing in the lumen of ER (BiP) and in the matrix of mitochondria are recruited and bind to the polypeptide chain translocating through import channel and to the channel itself to serve as import motors. (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 the 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 the passenger protein and random sliding of the incoming polypeptide chain within the import channel are driven by random thermal fluctuations.

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 (Glick, 1995; Lim et al., 2001; Neupert and Brunner, 2002). Characteristically, the PS model largely disregards the environment in which import motors operate. However, the energy of thermal fluctuations cannot be possibly ignored and should either be used by molecular motors or worked against.

A significant body of experimental data is inconsistent with the PS model of translocation motor. To mention a few examples, peptides composed of glutamic acids (polyE) or glycine residues (polyG) were shown to exhibit no or very poor binding to 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 sequences (Okamoto et al., 2002).

Tightly folded immunoglobulin (Ig)-like domains, which require a mechanical force of approximately 200 pN for their unfolding, as judged by atomic force microscopy measurements (Carrion-Vazquez et al., 1999) were efficiently imported into mitochondrial matrix, even if they were preceded by a 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 in the order of 3–10 pN and it is very unlikely that the putative mtHSP70-based motor would generate a force of >14 pN (Okamoto et al., 2002).

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 stability (Gaume et al., 1998).

Strikingly, 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 ATP in a reconstituted in vitro import system (Matlack et al., 1999).

Although the experimental observations mentioned and others are poorly consistent with the "pulling" model of the translocation motor, they can be readily explained within 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 the energy of random thermal fluctuations. In this model, the HSP70 family molecular chaperones, residing in the ER lumen or in the mitochondrial matrix act as molecular ratchets preventing the backsliding of incoming polypeptide chain as it appears at the channel outlet and progresses inside the compartment (see Fig. 4B). According to the BR model, the signal sequence of a preprotein targets it to and initiates the threading of the preprotein through the import channel. The local reversible unfolding of the passenger protein accompanied by the random diffusion of unfolded polypeptide segments inside the channel are both driven by the energy of thermal

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fluctuations. The HSP70 molecules "harvest" the local unfolding and make the sliding of passenger polypeptide statistically unidirectional by the stochastic binding and release of the incoming polypeptide chain on the *trans* side of the membrane ("trapping") (Neupert and Brunner, 2002). The action of molecular ratchets therefore biases the otherwise reversible and chaotic processes, such as polypeptide unfolding and sliding. Notice that 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 the passenger protein, the random diffusion of its unfolded segments within the 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 the statistical biasing of chaotic processes.

Protein import into mitochondria and into the ER has become a general model for post-translational protein translocation. The detailed elucidation of the mechanisms of protein import to other cellular compartments awaits focused experimental efforts. Meanwhile, it is becoming clear that the mechanistic interpretations may constitute a poor framework for the modeling and comprehension of the phenomenon. The mechanistic reasoning would necessary require the existence of distinct molecular machineries 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 the mitochondrial matrix and the lysosome and peroxisome interiors, as examples. In addition, it is also difficult to contemplate a plausible evolutionary scenario of emergence of distinct protein import machineries well-adapted for each specific cellular compartment in the conditions of inherent unpredictability of evolutionary process.

Conversely, the Brownian ratchet principle provides an evolutionary-conscious, design- and determinism-free conceptualization of protein translocation. The harvesting of local spontaneous unfolding of passenger protein and the biasing of the random walk of the translocating polypeptide inside the import channel can be potentially realized in many different ways, thanks to a variety of asymmetries normally existing between the *cis* and *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 (Simon et al., 1992). Thus, the Brownian ratchet principle provides a broad and general theoretical framework for the explanation and modeling of protein translocation across biological membranes. It should be noted that both protein translocation and the gradient X causing that translocation are continuous and dynamic processes and therefore can be considered as conjugate fluxes, a conjecture that is more appropriate to treat in terms of non-equilibrium thermodynamics, rather than mechanical engineering. The power stroke model, on the other hand, does not permit to entertain and to explore alternative lines of thought and restrict researchers to the image of clockworks, determinism and the logic of linear causation.

4. Concluding remarks

Two qualitatively different perceptions of the same molecular phenomena, molecular motors and protein transclocation are presented here in the context of recent experimental data to illustrate the relative deficiency of the mechanistic interpretation at the molecular scale. The examples of inadequacy of the Cartesian–Newtonian mechanistic framework, which is broadly and often uncritically used for interpretation of biological phenomena are by no means limited to problems of generation of directional movement, nor they are restricted to the molecular scale. They are widespread at the molecular, cellular, organismal and higher levels of description and are often apparent whenever the explanation of emergence of order in biological organization is attempted in mechanistic terms of design and determinism, see Kurakin (2004) for review. Defying the ideas of design and clockwork determinism, a leitmotiv of the latest experimental research are the ubiquitous observations of self-organization and stochasticity that appear to emerge as general principles underlying the dynamics and organization of life systems at all scales. Stochastic molecular motors (Astumian, 2001; Yanagida and Ishii, 2003), stochastic enzymes (Xie and Lu, 1999), stochastic self-organization of cytoskeleton structures (Nedelec et al., 2003), sub-cellular and sub-nuclear compartments (Misteli, 2001), stochastic self-organization of macromolecular complexes mediating transcription (Dundr et al., 2002; Kimura et al., 2002), DNA repair (Essers et

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al., 2002; Hoogstraten et al., 2002) and chromatin structure/function (Cheutin et al., 2004; Misteli et al., 2000), see Kurakin (2005a) for review, stochastic gene expression (Kurakin, 2005b) and stochastic cellular responses (Kurakin, 2005c) are poorly compatible with the familiar notions of design, programs, instructions and codes, and their systematic appearance is a call for active efforts to loosen the grip of the conventional mechanistic models and concepts in a search for an alternative and more adequate description of life systems.

Stochasticity has been long acknowledged to be at the heart of biology and its appreciation can be traced back to the first century B.C. and the "clinamen" of Lucretius (Prigogine and Stengers, 1984). However this appreciation and acknowledgement have remained isolated within few specialized fields of research, away from the biological mainstream dominated by clockwork interpretations and mechanistic mindset. It is the progress in research technology, promoted and supported, ironically, by the mechanistic paradigm, what brings about the accumulation of experimental data inconsistent with the mechanistic interpretation and precipitates a widespread crisis of the dominating paradigm much in the way described by Thomas Kuhn in his classics "The Structure of Scientific Revolutions" (Kuhn, 1996).

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