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Critical Review

Stochastic Cell

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Summary

Accumulating experimental evidence of stochasticity, self-organization and abrupt non-linear transitions underlying the dynamics of cellular structure and function is increasingly more consistent with the concepts and models of phase transitions, critical phenomena and non-linear thermodynamics rather than with the conventional clockwork description of the cell. The novel emerging image of the stochastic cell suggests that familiar and convenient classico-mechanical interpretations may be limiting our ability to understand the behavior of biological systems and calls for active exploration of alternative interpretational frameworks.

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Keywords stochasticity, self-organization, gene expression, phase transitions, self-organized criticality, networks biology, nonequilibrium thermodynamics

The conventional framework, which is commonly used today for interpretation of experimental reality in molecular and cell biology, is the Newtonian-Cartesian paradigm of the world. It is inbuilt and maintained by education and general culture as a sub-conscious default in the mind of the biological observer, who tends to design, perform and interpret his or her experiments in accord with deterministic assumptions and terms of classical mechanics. The Newtonian interpretational framework combined with reductionism resulted over time in a clockwork image of the cell. The complexity of the cell is generally perceived to be different from the complexity of clockwork only in terms of quantity, but not in quality, and the design charts of modern automobile, aircraft and computer are routinely used as metaphors to illustrate the complexity and design principles of cellular organization. However, recent advances in analytical techniques, detection methods and computer-aided modeling and analysis are leading to the accumulation of experimental evidence inconsistent with the clockwork image of the cell. Specifically, inherent stochasticity and non-linear response patterns are emerging as general principles underlying cellular organization and function. As they are poorly consistent with the assumptions of design, purpose, linearity and determinism often implied by the mechanistic paradigm of the cell, the active search for adequate interpretational frameworks becomes the trend and the challenge of the time.

Stochasticity of cellular responses.

The physical dimensions and nature of cells and their molecular components in times of relative weakness of detection technology necessary led in the past to the usage of large populations of cells for quantitation of a particular parameter pertaining to the individual cell such as, for instance, a specific mRNA or protein product. Therefore the parameter measured was averaged over the population, while individual cells in the "homogeneous" population were commonly, in harmony with the clockwork intuition, assumed to be identical. In case of inducible gene expression it was often observed that a gradual increase in the concentration of an activating stimulus led to a proportional increase in gene expression measured as a total mRNA or protein product of a cell population. As a logical consequence of these observations, the rheostat or graded model of gene expression was put forward (1). This model assumes that each cell in a given population adjusts the rate of expression of a responsive gene gradually from zero to its maximum in proportion to a raising concentration of activating stimulus (Fig. 1).

Relatively recently, the technology became widely available and routinely used that allowed researchers to analyze specific gene expression and other cellular parameters on a cell-by-cell basis. As a result, a digital or stochastic model of gene expression is becoming predominant (see (2) for review and Table I). According to the stochastic model of gene expression, every cell in a population has a certain probability to respond to a given concentration of activating stimulus within a given time window by transcription of a responsive gene. This

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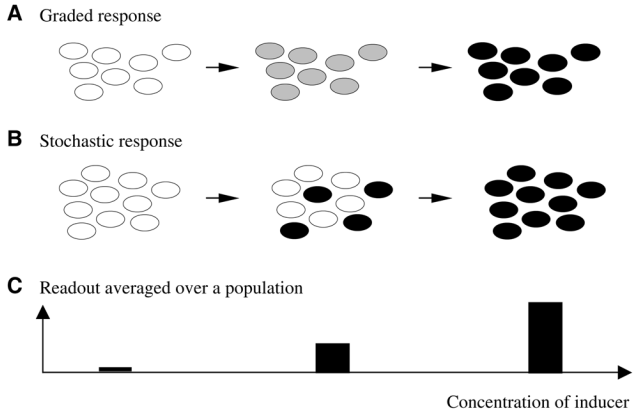

Figure 1. Cellular responses: graded versus stochastic. It is generally assumed that cells in isogenic populations display a graded response (A) to a rising concentration of an inducer. However, the studies, in which cellular responses are followed on a cell-by-cell basis, often reveal a stochastic response (B) – a rise in the concentration of an inducer results in a recruitment of increasing number of cells that respond in all-or-none fashion reminiscent of a phase transition. Measurements that average the response over a cell population (C) are unable to discriminate between the graded and the stochastic cellular responses. The common practice to perform averaging measurements on cell population but interpret the results in terms pertaining to individual cells has its roots in a clockwork perception of the cell, may lead to erroneous conclusions and mask a ubiquity of stochastic responses (see more in the text).

probability may vary widely among individual cells even within an isogenic population and the ensuing transcription follows all-or-none response pattern. The responsive gene is either maximally expressed in a given cell or not at all. An increase in the concentration of the activating stimulus results in the recruitment of increasing numbers of cells that switch in a digital manner from silence to gene expression (Fig. 1). The rate of gene expression in recruited cells remains largely unaffected by further increase in the concentration of activating stimulus (2,3). Within this model the transcriptional response is therefore perceived as a transition between two metastable functional states of the cell. An important corollary of the stochastic model is that every cell in an organism and elsewhere may be a unique entity, for every gene has a low, but larger than zero, probability to be expressed at any given time even in the absence of an activating stimulus. As the un-induced genes are indeed expressed at low frequencies (4,5), even a highly differentiated and specialized group of cells sharing similar expression profiles is always stochastic in the sense that it is composed of unique individuals at any given time due to the inherently probabilistic nature of gene expression.

The continuing advance and increasingly frequent use of technology and approaches addressing the behavior of individual living cells in large populations are leading gradually to realization that stochasticity and digital, switch-like functional state transitions are not a peculiar phenomenology specific for gene expression only. On the contrary, they emerge as general principles underlying the dynamics of cellular organization and function. Studies performed on isogenic cell populations and cell culture lines, where population dynamics was analyzed on a cell-by-cell basis, revealed an inherently stochastic nature and all-or-none response pattern in a wide range of disparate cellular processes such as gene expression, cell division, commitment to apoptosis (13), execution of apoptosis (14), response to oxidative stress (15), differentiation and lineage commitment (16,17), entry into replicative senescence (18), T cell activation (11), cell integrity in an ageing animal (19) etc.

It should be noted that the accumulating examples of stochasticity and digital behavior observed in various cellular processes are not predicted or systematically sought after within the conventional paradigm of the cell. On the contrary, they are counter-intuitive and perceived as surprises if one sub-consciously pictures the cell designed, organized and functioning like an aircraft or a computer. No computers, no aircrafts, no automobiles, "isogenic" as they are built, acquire spontaneously personality of their own and respond in a probabilistic manner to environmental cues by all-or-none functional and/or structural transitions to adjust their metabolism or to enter senescence, division or self-destruction. It is therefore reasonable to suggest that the design charts of mechanical and electrical engineering that are frequently exploited for representation and conceptualization of cellular organization are hardly meaningful, if not outright misleading. On the other hand, the same observations that are seen as paradoxes and surprises within the conventional paradigm become less confusing, sometimes even expected and predicted, if one discards the clockwork image of the cell altogether and considers the cell as a metastable self-organized system of conjugated fluxes, or, using a different vocabulary, as a dynamic integrated system of interacting, interconnected and interdependent steady-state macromolecular organizations, reminiscent in its dynamics and behaviour of the integrated system of human social and business organizations (see (20) for review).

Stochastic dynamics and self-organization of sub-cellular compartments and macromolecular complexes.

Recent studies addressing the real-time dynamics of fluorescently tagged individual molecules in living cells strongly suggest that the cytoskeleton, chromatin, sub-cellular and sub-nuclear compartments, as well as macro-molecular complexes mediating basic cellular processes, are

more adequately described in terms of dynamic steady-state molecular organizations, rather than as the pre-determined structures and ''machineries'' that are assembled and dissembled according to a pre-conceived design (of Mother Nature) (21). The molecular constituents of the cell appear to self-organize themselves spontaneously and transiently into metastable, morphologically and functionally distinct macromolecular organizations through dynamic, transient and inherently stochastic molecular interactions. The morphological appearance and function of these organizations seem to be defined by the balance between influx and efflux of their respective resident molecular components, and by the transient specific associations and activity of these components within the steady-state organizations (21). The coordination of their activity is suggested to take place through continuous exchange of shared molecular components (20). Experimental evidence indicates that an increasing number of phenomena in cell and molecular biology, which were previously considered to be static structures, are in fact dynamic processes realized in a probabilistic manner through stochastic molecular interactions. The cytoskeleton (22), heterochromatin (23), chromatin (24), sub-cellular and sub-nuclear compartments (21,25), as well as specialized macromolecular ''machines'' mediating transcription, DNA repair and splicing (see (20) for review) are among the most recent examples of steady-state molecular processes that seem more appropriate to describe in terms of metastable attractor states and probabilistic state transitions. The very abundance of these examples suggests that it may be fruitful to look more closely at what is traditionally and/or subconsciously treated as ''structures'' in biology and reconsider them as processes.

The novel emerging image of the dynamic cell appears to be more reminiscent of an integrated system of interacting and interdependent human social and business organizations and much less reminds the human-built machines and clockworks. In consistency with this, the topologies of both protein interaction and metabolic networks of the cell have been found to obey a power-law scaling, which is indicative of their self-organizing nature (26,27). The power-law scaling, as a signature of self-organized complexity, is shared by many physical, biological and social phenomena, but is not normally found in engineered structures and machines built according to a pre-conceived design (28).

In a search for an alternative framework: phase transitions, networks biology, self-organized criticality and dissipative systems.

As more and more of what was previously considered to be structures and pre-assembled macromolecular ''machines'' turn upon closer examination into dynamic processes and steady-state molecular fluxes, the development and application of novel and adequate concepts and models becomes a matter of outmost importance. Cellular responses are increasingly often perceived and modeled as state transitions, analogous to phase transitions in physical systems (29). While the respective conceptualization and statistical description seem to be well consistent with the experimental reality of the stochastic cell, the conventional notions of linear signaling ''pathways'', mechanistic ''switches'' and deterministic ''programs'' appear to provide a poor framework to account for stochasticity and non-linearity in cellular responses. The models and concepts of condensed matter physics and nonequilibrium thermodynamics (30) are becoming popular and increasingly often seen as more adequate. The self-organized criticality and percolation theory have been applied to analyze mitochondrial network responses to oxidative stress (31), extracellular matrix formation (32), metabolite concentration dynamics (33) and protein interaction networks (34). Cell locomotion (35), cytoskeletal self-organization (22,36) and metabolic waves and oscillations (37,38) are treated within the theoretical framework of chemical dissipative systems. The alliance of the phase transition formalism and networks biology appears to be an especially powerful combination that holds a great promise of imminent breakthroughs in conceptualization of biological complexity (39–41).

Concluding remarks.

Although the amount of information that has been obtained, analyzed and structured in various biological databases for the last 50 years is phenomenal and continues to grow at explosive rates, the progress in the actualization of this knowledge into an understanding of life systems and their control appears to be disproportionately slow.

One may argue that the problem as to why there is so much information and so little comprehension is rooted in the complexity of biological systems, that once the cell is finally disassembled to its basic components and all its constituent molecules and interconnections between them are listed, then the properly applied math and computers will uncover the underlying design and engineering charts of life systems become widely available - to reproduce, to control and to repair. The alternative opinion is that the problem may be not so much in the complexity *per se*, but in the interpretational defaults, concepts and models used to comprehend this complexity. If the cell is more adequately described as a metastable system of conjugated fluxes, then the inadequacy of classico-mechanistic interpretations should be acknowledged and models and concepts of condensed matter physics and nonlinear thermodynamics need to be brought more actively into and used more widely within the biological research mainstream. Shifting from models of mechanical and electrical engineering to models of phase transitions and nonlinear thermodynamics may prove to be more fruitful and rewarding in terms of both conceptual biological insights and practical treatments and drugs.

Table I.
Examples of systems and methods used to demonstrate stochasticity in gene expression.

Stimulus	Cells	Promoter/Gene	Method	Reference
Tat transactivator	RAW 264	HIV1-LTR/lacZ	A	[6]
IL-2	Jurkat	NF-AT/lacZ	A	[7]
dexamethasone	mouse Ltk^-	MTV/lacZ	A,B	[8]
sodium butyrate	HeLa	hCMV, HIV-LTR/luciferase	C	[9]
forscolin, TRH, bFGF	rat pituitary GH3 cells	hPRL pituitary promoter/luciferase	C	[10]
APC	T cells 5C.C7	Ca^{2+} release	D	[11]
regeneration	nuclei in mouse myofibers	α-skeletal actin troponin I slow	E	[12]

Methods:
A – flow cytometry;
B – histochemistry.
C – luminescence, single cell imaging;
D – fluorescence, single cell imaging;
E – *in situ* hybridization.

PS. Conceptual shift in a historical perspective.

It may be argued that the biological relevance of stochasticity and non-linear, all-or-none type responses is not a novel development and has been well appreciated for a long time. Indeed, as an example, the first experimental observations of stochastic gene expression in bacterial cells date back to 50's (42) with grounding work in this field done in 90's (see for review (43) and Table I). The realization that abrupt non-linear transitions play a key role in life phenomena can be traced even further back to the "clinamen" of Lucretius, the first century BC (44). However it is reasonable to suggest that these observations and their appreciation have remained confined within relatively few isolated domains of experimental and theoretical research, away from the mainstream, as they are poorly compatible with the clockwork interpretation of life phenomena that is tacitly implied in the dominating Newtonian paradigm of the world. Being confined and isolated, the earlier studies and insights played nevertheless a crucial role degrading the grasp of deterministic thinking, spreading the sentiment and preparing the ground for a conceptual shift. It is the development and the introduction of novel technologies and methods, in particular fluorescent probes and advanced imaging techniques, as well as the proliferation of single cell analysis studies what is bringing about an explosive accumulation of experimental evidence implicating self-organization, stochasticity and phase transition-like responses as basic principles underlying the dynamics of cellular structure and function. The new image of the cell as self-organizing molecular system, emerging as a result of these observations, has far-reaching implications that necessitate the re-conceptualization of many other widely accepted dogmas rooted in the mechanistic interpretation such as deterministic notions of cell differentiation and organism development, as an example (45). As noticed earlier by T. Kuhn (46), only the systematic and widespread appearance of experimental observations inconsistent with the dominating paradigm, which is sooner or later unavoidably precipitated by the continuing technological advance, is able to cause a crisis of that paradigm, stimulating dogma-independent thought and a search for alternative interpretational frameworks, a development that is hopefully taking place today.

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