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Review Article

The unforeseen challenge: from genotype-to-phenotype in cell populations

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Abstract

Biological cells present a paradox, in that they show simultaneous stability and flexibility, allowing them to adapt to new environments and to evolve over time. The emergence of stable cell states depends on genotype-to-phenotype associations, which essentially reflect the organization of gene regulatory modes. The view taken here is that cell-state organization is a dynamical process in which the molecular disorder manifests itself in a macroscopic order. The genome does not determine the ordered cell state; rather, it participates in this process by providing a set of constraints on the spectrum of regulatory modes, analogous to boundary conditions in physical dynamical systems. We have developed an experimental framework, in which cell populations are exposed to unforeseen challenges; novel perturbations they had not encountered before along their evolutionary history. This approach allows an unbiased view of cell dynamics, uncovering the potential of cells to evolve and develop adapted stable states. In the last decade, our experiments have revealed a coherent set of observations within this framework, painting a picture of the living cell that in many ways is not aligned with the conventional one. Of particular importance here, is our finding that adaptation of cell-state organization is essentially an efficient exploratory dynamical process rather than one founded on random mutations. Based on our framework, a set of concepts underlying cell-state organization—exploration evolving by global, non-specific, dynamics of gene activity—is presented here. These concepts have significant consequences for our understanding of the emergence and stabilization of a cell phenotype in diverse biological contexts. Their implications are discussed for three major areas of biological inquiry: evolution, cell differentiation and cancer. There is currently no unified theoretical framework encompassing the emergence of order, a stable state, in the living cell. Hopefully, the integrated picture described here will provide a modest contribution towards a physics theory of the cell.

Keywords: cell-state organization, adaptation, population dynamics, evolution, cell differentiation, cancer

(Some figures may appear in colour only in the online journal)

Contents

What was life, really? It was the existence of what… It was not matter, it was not spirit. It was something in between the two, a phenomenon borne by matter, like the rainbow above a waterfall, like a flame.

Thomas Mann, *The Magic Mountain,* 1924[1]

1. Cell-state organization: a phenomenological approach

1.1. The nature of living cells

Living cells, under specific conditions, exhibit well defined traits and long-term stability, even in fluctuating environments. However, these cells also present a broad spectrum of characteristics with flexibility that allows them to adapt to novel and severe challenges. Indeed, the development of a multicellular organism from the single-cell zygote, crucially depends on the process of cell differentiation; the stabilization within a lineage of specific cell states (types)—neuron, muscle, skin etc, at precise times and locations, forming the body-plan and functional tissues. This developmental process is highly reproducible and robust; so much so, that it is almost natural to regard development as an execution of a program, much like a computer program. However, the simultaneous existence of these seemingly contradicting capabilities, robustness and flexibility, is an essential property that enables evolution [1–3]. The roots of the organism's ability to evolve—the hallmark of the living world—to large extent may be traced to the level of the cell and manifested in cell plasticity, its ability to react to internal or external cues and constraints. Thus, the organization of cell states in development and the emergence of novel phenotypes in evolution are tightly connected complementary characteristics of the living cell and their coexistence is one of the greatest mysteries in biology.

The issue of an emergent order—stable cell states—in essence is that of the *genotype-to-phenotype* associations. The genotype of a cell is its genetic makeup while the phenotype encompasses its traits, such as morphology and function. Genotype and phenotype represent two separate cellular entities; while the former is the structure of the genome—the DNA sequence, the latter is the determination of the form, growth and interactions with the external world of the cell. The phenotype is a central concept in the description of a biological system. In principle, it can be any observable property of the living organism. However, in the context of the present article, we reserve this term to the composite of observables related to the growth, morphology, metabolism and functionality of the cell. The establishment of a phenotype, given a certain genotype, depends on the protein makeup of the cell. The set of expressed proteins, a subset of the entire genome potential, and their concentrations, are determined by regulatory systems at many levels. Thus, the emerging phenotype depends on the spectrum of *regulatory modes*—temporal profiles of expressed genes. However, a snapshot of the molecular content of a cell and the structure of its underlying interactions do not capture the spectrum of regulatory profiles (the intermolecular correlations and the stability of temporal modes) that define the *relevant* observables that determine the phenotype. As shown below, the protein content of each isolated gene is by itself not such a relevant observable. Therefore, inquiring into the genotype-to-phenotype associations requires a shift in focus from *structure to dynamics*, from the molecular *stuff* of the cell to its temporal *organization*.

The prevailing approach of modern molecular biology for studying the living cell relies on several tenets. First, a biological cell is the product of an evolutionary process; the neo-Darwinian framework regards existing genotypes as the result of the accumulation of random mutations in DNA sequences, shaped by selection processes. Second, the genotype dictates the phenotype. The Central Dogma of molecular biology [4] assumes a one-way mapping from DNA to protein through

[1] Translated from German by J E Woods 1995 (New York: Vintage International), 271

the processes of transcription (mRNA production from the DNA sequence) and translation (production of proteins from mRNA) without the possibility for proteins to affect the DNA sequence itself [5]. These molecular processes are regulated at different levels by protein–DNA and protein–protein interactions as well as by interactions of other molecules. The prevailing reductionist approach to molecular biology seeks the molecular cause underlying any cellular process, preferably due to a single or a few genes. The genotype-to-phenotype mapping is largely assumed to be deterministic in nature, accompanied by 'noise' by environmental influences and intracellular stochastic processes due to the small volume of the cell and the small number of molecules involved. The Darwinian evolution approach motivates the assumption of optimization; a sweeping view regarding the biological processes and cell functionality, including every molecular interaction, as optimized by strong selection towards a specific function.

There is currently ample evidence calling for an expansion of the prevailing molecular framework. Most importantly, this approach dictates the way biological cells are studied; seeking the molecular causation of a response following a specific and well-designed perturbation (either genomic or environmental), assumed to be weak enough to allow isolation of a specific molecular module from the rest of the cellular processes. This approach is highly successful in isolating molecular elements involved in specific responses and enabled molecular biology to become the leading avenue in almost all areas of biological inquiry and medical applications. However, this methodology does not allow the search for a wider understanding of the cell as a complex dynamical system. As shown in this article, the seemingly simplistic molecular picture is only part of the story. Applying a different methodology, studying the long-term dynamic response to a strong perturbation and diverting the cell far away from its relaxed state, leads to a very different picture of the genotype-to-phenotype associations and the processes underlying the stabilization of an adapted cell state. This methodology allows separation of the phenotype dynamics from the genotype structure. This approach to cell biology is in fact complementary to the molecular approach. While the latter allows the efficient construction of the catalogue of molecular processes, the former is essential in order to understand the underlying physical principles. Since the methodology presented here is unconventional, let us first expand on its underlying principles and then summarize to what extent it leads to deviations from the prevailing picture.

Studying the principles underlying *cell-state organization,* calls for an investigation of cells in their natural context. The lineages emerging from the zygote, as well as proliferating unicellular organisms, like bacteria or yeast, should be regarded as heterogeneous populations that can develop multiple coexisting phenotypes. In both types of cells, a developing embryo or unicellular asexual organisms, we are interested in the *potential* of a founder 'mother-cell' to produce a spectrum of phenotypes under constraining external and internal conditions. The living cell is a complex dynamical system, the underlying molecular interactions spanning a huge combinatorial space of possible temporal modes. The emphasis on studying the potential of the system, rather than merely its *end-state realizations*, enables us to expose the nature of the biological system that can support such seemingly contradictory behavior; the simultaneous capabilities of stability and evolvability [6]. However, this shift in attention towards the potential of the cell similarly demands a shift in emphasis, from *structure to dynamics*. Moreover, a biophysical understanding of proliferating cells requires us to link intracellular dynamics and gene regulation, via the process of cell division, to the level of the population. This is particularly essential since in many cases, the intracellular responses extend over time-scales longer than a cell generation, which is not well separated from the time-scales of protein production, degradation or metabolic processes. These in turn depend on the history of the population mainly through inheritance of molecules (e.g. proteins) and structures (e.g. DNA conformations or cellular organelles) for generations [7]. This article therefore is focused on the dynamics of cells in the context of a population, emphasizing the interrelations between these two levels of biological organization, the cell-and the population. Note that a population of cells is not a statistical ensemble of independent individuals; transgenerational inheritance and long-term dynamic modes imply that cells within a population are correlated. This important distinction between populations of living cells and physical ensembles requires the development of experimental methodologies to study cell-population dynamics, emphasizing the population aspects of living cells.

Central to our understanding of biological phenomena and diversity is the complex challenge of uncovering the potential of cells to evolve. Arguably, this avenue of research also sets a new frontier in the field of complex systems. Living cells are history-determined objects, whose precise evolutionary history is not known, so uncovering their intrinsic potential requires us to discriminate between necessity and contingency, between inevitable and accidentally instilled intracellular processes.[2] This is a non-trivial experimental challenge and often a source of confusion; the erroneous assignment of crucial functional roles to elements that have emerged in the system by mere historical accidents.[3] Thus, there is a need to develop a new experimental paradigm exposing the potential of cells and universal principles of organization. This is highly non-intuitive since the common motivation in biological research is usually the opposite; exposing specific mechanisms underlying a given cell state.

We took an experimental approach to bypass this obstacle of the cell's unknown history and tried to penetrate the heart of the ability of cells to evolve by measuring the dynamics of yeast cells facing an *unforeseen challenge*—a novel perturbation they had not encountered before in their evolutionary

[2] This notion that the living world presents both inevitable and accidentally instilled processes was also discussed by Gould and Lewontin (1979 *Proc. R. Soc. Lond.* B **205** 581), Jacob [94] and Koonin (2011 *The Logic of Chance* (Upper Saddle River: FT Press)).

[3] This feature of a biological cell is well captured by Rube Goldberg caricatures, where a functional machine is made out of what looks like a random pile of elements, each of which is nevertheless absolutely necessary for its functionality (see [129]). This is as far as it can get from optimal design of machines in the engineering world—a most commonly used and somewhat misleading metaphor in biology.

history. This experimental framework allows us to acquire an *unbiased* view of the cell dynamics. We let the experiments lead us, instead of testing hypotheses that reflect our prejudices. Evidence accumulated from our experiments revealed a coherent set of observations within this framework, painting a picture of the living cell that in many ways is not aligned with the conventional one. It is worth summarizing, even at this early stage, the main deviations from the prevailing picture. (i) Inherited adaptation of cells responding to a perturbation could result from processes other than mutations in DNA sequences and is not necessarily the result of selection. Thus, cellular response is consequently not an optimization process. (ii) The genotype does not directly dictate the phenotype and it is not possible to reduce the adaptation process to a simple set of molecular causes determining the stabilization of a cell state. (iii) There is a strong cross-talk between levels of organization, in particular between the intracellular and population processes mainly due to long-term correlations for generations. (iv) The environment plays an outstanding role by participating in the cellular dynamics rather than being a passive selection filter. (v) Population dynamics support the coexistence of a wide spectrum of metastable phenotypes, in contrast to the Darwinian picture in which an optimized phenotype takes over.

The consistency of our results has been verified in multiple ways, by different types of experiments and in laboratories other than ours. The somewhat surprising picture emerging from our endeavor can be easily dismissed as a mere case-study; *it is not!* The lessons learned from our experiments, open a wide vista on some fundamental issues in cell biology. Starting from experiments that aimed to study adaptation of cells to an unforeseen challenge, we have discovered that some basic concepts of cell biology need to be revisited. Of particular importance here is our finding that adapted cell-state organization is essentially an *exploratory dynamical process* [6, 8]. This observation has significant consequences for our understanding of the emergence and stabilization of a cell phenotype in diverse biological contexts.

This article is not a typical review summarizing the state of the art of a field. The first part presents an experimental framework, developed and elaborated by us over the last decade, focusing on adaptation of yeast populations to an unforeseen challenge. The discussion avoids technical details to make it accessible to non-experts. These technical details and other data omitted here for the sake of coherency and brevity can be found in the listed references. The lessons learned from the yeast experiments, motivate revisiting and re-interpreting published results from different branches of biology. The discussion in the second part of this article reflects on cell-state organization in three major areas of biological inquiry: *evolution, cell differentiation and cancer*. It does not mean in any way to be an exhaustive review of these broad fields, or even a representative overview of them. Rather, the discussion is focused on a limited set of examples that highlight essential principles and stimulates further inquiries. Finally, we summarize by noting that there is currently no unified theoretical framework encompassing the emergence of order, a stable phenotypic state, in the living cell. There are many beautiful pieces that until now have not provided the critical seeds for a collective effort towards such understanding. Hopefully the integrated picture painted in this article will provide a modest contribution towards a physics theory of the cell.

1.2. An experimental framework: genome rewiring and cell adaptation to unforeseen challenges

> *Organisms can do all types of things: they do fantastic things... Trying to make everything fit into set dogma won't work… So if the material tells you, 'It might be this', allow that. Don't turn it aside and call it an exception, an aberration, a contaminant…. That's what's happened all the way along the line with so many good clues.*
>
> Barbara McClintock[273]

We present now our experimental framework based on cell adaptation to an *unforeseen challenge*. It is important first to realize the significance of this concept by distinguishing two types of cellular responses to a perturbation. Cells react to a common perturbation by fast operation of existing 'hard-wired' functional modules. By contrast, they do not have such a 'pre-designed', specific response to a novel challenge—a perturbation leading to significant deviations from the current cell state and therefore requiring a very different type of operation. For example, a change in food ingredients (e.g. switching between two types of sugars) or an environmental stress (e.g. a reduction in oxygen level) familiar to the cell, usually result in a fast stereotypic response of dedicated genetic and protein networks having specifically determined functionalities. On the other hand, exposure to an unforeseen challenge in which no *a priori* response is 'instilled' in the cell, requires genuine adaptation. The two types of responses differ not only in their speed of reaction. As we shall see, adaptation to an unforeseen challenge is based on global reorganization of *regulatory modes*, determining the protein makeup of the cell.

Remodeling gene regulation has been recognized as playing an important role in evolution. The biodiversity observed in nature shows that the emergence of novelty is at the heart of the evolutionary process. But, how do such novelties emerge, and how are they utilized by organisms during evolution? These issues are still open and serve as subjects of inquiry at the forefront of evolutionary biology. Genomes are roughly composed of two functionally distinct parts; coding regions, dictating the amino-acid sequences of proteins (with the possibility of alternative splicing, combining exons—separated coding portions of the same gene—into different coding sequences) and regulatory regions. The latter are part of the genome 'dark-matter', classified in times misleadingly as 'junk' DNA, but lately revived as vastly containing functionally important regions [9, 10]. Many years ago, King and Wilson [11] suggested that developmental evolution involves changes in gene regulation rather than merely mutations in coding regions. Since their work, the significance of regulatory evolution has gained further support by numerous studies, relying on detailed comparative genomics enabled by the available genomic sequences across organisms and species.

metabolism; all aspects that are either missing or weak in the case of mutations. Aneuploidity might be a secondary effect due to an instability from the emergence of other processes. Other extensions, like transposons [251], illuminate additional mechanisms that broaden the range of intracellular responses. The tissue (population) organization field is conceptually different since it brings to the table the crosstalk between two levels of organization. Irrespective of details, the huge heterogeneity within a single tumor calls attention to the external organization field. Indeed, recent studies show that any measured aspect, reflects this heterogeneity. For example, gene expression profiles of different regions within a tumor lead simultaneously to 'good' and 'bad' prognosis [252]. The same study also found with 26 of 30 samples from four tumors had ploidy heterogeneity (different number of sets of chromosomes) and divergent allelic-imbalance. From another angle, there are strong indications for the field organization coming from cell transplant experiments. For example, tissue recombination of stroma (connective supporting tissue) exposed to a carcinogen with normal unexposed epithelial cells resulted in neoplasm (tumor) [253]. Interestingly, induction of neoplasm was also shown when embryonic cells were misplaced in adult tissues, and reverted to normalcy when placed into early embryo [254]. This point was already discussed above. Interrupting the normal developmental trajectory or misplacing cells developing along a certain differentiation trajectory into the wrong context (e.g. adult tissue) is enough to trigger a change in type leading to cancer. The opposite process leading to reversion of cancer is also observed. Indeed, reversion of the tumor phenotype was found when cells from rat mammary-gland tumor were inoculated into rats of different ages; in adult rats these tumor cells generated phenotypically normal mammary ducts [255, 256]. Similarly, when renal carcinoma cells in frog were transplanted into enucleated and activated ova, they developed and reached the swimming tadpole stage [238, 257]. The transplantation of tissues from these tadpoles into normal recipients generated normal tissues that were indistinguishable from those of the host [258]. Reversion of different neoplastic phenotypes when the cancer cells are transplanted into a normal tissue is a well documented phenomenon found in many studies (see for example, [259, 260]). The plasticity of the neoplastic phenotype shows the involvement of epigenetic processes [261] and great susceptibility to the tissue organization field—features not well aligned with of the concept of mutations as the basis of cancer. The reversion of malignant cells by embryonic environments has been suggested to be due to common regulatory signals of embryonic and tumor stem cells [260, 262]. Transplanted human melanocytes and metastatic melanoma cells into zebrafish blastula-stage embryos showed that these cells could survive and *participate in embryo development* without forming tumors. The melanoma cells lost their tumorigenic phenotype [263]. All these examples indicate that the embryo microenvironment is able to affect cancer cell and change their phenotypes, supporting the dynamic field-view presented above.

The above discussion suggests that we should think of cancer as an *adaptation* phenomenon. In other words, the emergence of cell states resulting from exploratory dynamics triggered by a perturbation. This suggests that the *adaptation of yeast cells to unforeseen challenge belongs to the same class of phenomena*. The dependency on context, history of the cells, their environment and all other constraints eventually specify the dynamic trajectory and the realization of various cell states [218]. In the case of yeast, the population level of organization was found to play a crucial role, similar to the tissue-level in the case of solid tumors. Recall that cells within a population respond by expressing genes in a coherent way while the correlation between responses of different populations is weak [40]. Thus, even though the profiles of gene expression are non-specific and irreproducible between populations, even between 'twin' populations, cells within the population exhibit a highly coherent response. The lesson from these experiments is that the population 'field' affects the gene expression response of individuals, which does not result solely from autonomous intracellular processes. There is no need for specific, intracellular molecular signaling to achieve coherence among cells in a population. Their common environment serves to converge their response through nonlinear dynamical processes, i.e. the growth of cells affects other cells by their uptake and extraction of ingredients changing their common environment. Under certain conditions, cell metabolism can become very sensitive to small environmental changes that are then amplified by the response of other exponentially growing cells. Similarly, in the case of cancer, there is no need for special signaling; the tissue provides the organizing field. Therefore, understanding cell-state organization and the associations of genotype and phenotype requires one to go beyond genetic networks and 'information' approaches. In short, one needs to understand the type of dynamic organization presented by the living cell.

3. Summary and outlook

> *A society that permits biology to become an engineering discipline, that allows that science to slip into the role of changing the living world without trying to understand it, is a danger to itself.*
>
> C R Woese, 2004 [274]

Starting from experiments aiming to explore the adaptation of yeast cell populations to an unforeseen challenge, a set of concepts at the basis of cell-state organization and the genotype-to-phenotype associations have been developed. Extending the discussion by revisiting three major branches of biological inquiry—evolution, cell differentiation and cancer—gives a wider vista of these concepts and demonstrates their generality. The common theme is the question: *How does phenotypic order emerge from molecular disorder in the living cell?* This is a shift of focus from the famous concept of '*order from order*' developed by Schrodinger in his influential book *What is Life*? [264]. The focus of this article is the emergence of phenotypic order, cell-state organization, from the disordered molecular makeup of the cell which at the same time maintains its flexibility to evolve. The genotype, an apparently

ordered entity, is not translated directly to a phenotypic order. Rather, cell-state organization is a dynamical process in which the molecular disorder manifests itself in macroscopic order. The genotype, notwithstanding its important role, participates in this process but does not fully determine it. The genome in this view provides a set of constraints on the spectrum of regulatory modes, analogous to boundary conditions in physical dynamical systems.

This article attempts to sketch an organizing framework for a reader interested in lifting the veil from this fascinating issue of cell organization in biology. It provides only an outline, necessarily bringing up more open questions than answers. We have a long way to go towards resolving this issue, but the emphasis on *dynamic organization* taken here, which I believe should occupy a central stage in modern biophysics, might help to crystallize it. Paraphrasing William James statement on the mind, *the emerging order in the living cell is a process not a stuff* [275]. This emphasis is hardly new. However, as this article attempts to show, accumulation of experimental evidence makes the time ripe to bring it back to center stage.

One should be aware of the deep difference between the analysis of the molecular makeup of a cell and the organization principles underlying the emergence of order. The contrast between process and stuff, in the context of organization, has been discussed in the past. It could be crudely illustrated in analogy to the insight leading Kepler to write his marvelous little book, *On The Six-Cornered Snow-Flake*; [276] attempting to explore the principles underlying organization of a snow-flake into well-defined patterns. This is perhaps one of the earliest scientific attempts to understand the emergence of natural patterns, beyond the specificity of their underlying material content. In the introduction Kepler writes: *I crossed over the bridge, mortified by my incivility in having appeared before you without a New Year's gift… Just then, by a happy occurrence, some of the vapor in the air was gathered into snow by the force of the cold, and a few scattered flakes fell on my coat, all six-cornered… Here, indeed, was a most desirable New Year's gift for the lover of* ***Nothing***. Kepler indeed emphasizes that organization principles are about '*Nothing*'. They are not stuff, and therefore are a proper gift to his mentor who is rich, famous and clever and so could not be easily impressed by material objects. At the end he remarks: *But I am getting carried away foolishly, and in attempting to give a gift of almost Nothing, I almost make Nothing of it all. For from this almost Nothing, I have very nearly recreated the entire universe, which contains everything*. Organization of patterned snow-flakes is an issue altogether detached from the catalog of underlying atoms forming a water molecule; the same combination of atoms, indeed the same water molecules, could just form a structureless fluid. Surely, there is no realization of a snow-flake without the water molecules. But the existence of these molecules, by itself, could not explain the highly symmetrical macroscopic six-cornered pattern of the snow-flake, which is an *emergent* phenomenon. Today, scientists are well aware of the issue of emergence, most notably when driven by *symmetry breaking* processes [265], either in space, time or space-time. The argument is that in trying to understand cell-state organization, we should put more emphasis on the 'nothing'—the principles of dynamic organization rather than relying solely on the material stuff—trying to build order from the specificity of molecules and their interactions.

Biological cells present a challenge for our quest to perceive and comprehend organization in natural phenomena, far beyond the ones presented by physical systems; in particular, due to their microscopic heterogeneity and the multiple types of interactions of their underlying constituents. Unfortunately, the complexity of biological systems seems to mask this conceptual separation between stuff and process, between the underlying molecular material objects and the dynamic process of organization. In principle, we know three ways in which systems can develop macroscopic order from their underlying molecular disordered constituents. Thermal equilibrium in thermodynamic systems is the most common way and stands at the basis of equilibrium statistical mechanics. Next, constraints can limit the number of possible modes in a non-equilibrium driven system. Examples are lasers in optics and patterns emerging in fluid dynamics. Finally, there are cases in which dynamical rules dictate order. This is the class of self-organized systems, manifested more recently also in examples in the form of random organization [266]. In biology, the first option is ruled out; the living cell is far from thermal equilibrium. There are certainly constraints applied on the cell, both physical [267] and genetic ones dictated by the composition of the genome and its organization. However, note that in a system spanning a microscopically combinatorial large phase-space, if constraints are responsible for the macroscopic order they need to eliminate a huge number of possible dynamic modes in a very effective way. As far as I know, there is no example for such a process in amorphous heterogeneous materials. At this stage, we cannot entirely rule out this possibility that in fact reflects the common picture in biology, based on selection in the evolutionary process. However, as demonstrated in our yeast experiments and further discussed in the examples from other branches of biology, this approach is not compatible with the ability of cells to efficiently adapt to arbitrary *unforeseen challenges*, nor it is compatible with the observed plasticity and evolvability that are the hallmarks of the biological cell. We are left with the concept of *dynamic organization* based on exploratory processes. Note however, that random exploration in a large combinatorial space spanned by the living cell is highly inefficient and can hardly lead to the emergence of order discussed in this article. Processes like random organization mentioned above [266] work away from thermal equilibrium and are not limited by exhaustive scans of a huge number of microscopic configurations. They rely on stabilization of a many-body system at an absorbing state, one out of many possible degenerate states available to the system. Thus, such processes can in principle serve as a basis for a dynamical theory of cell-state organization. Unfortunately, until now a theory connecting such processes to biology is lacking. In particular, a significant obstacle along the way is the gap in our ability to identify the *relevant* variables underlying the dynamics in the living

cell. As demonstrated by our work, as well as of others, the huge combinatorial phase-space spanned by the intracellular microscopic degrees of freedom, is highly degenerate. It has been recognized that many complex systems belong to the same class, roughly identified as 'sloppy systems', in which most directions of change in variable space do not affect the macroscopic behavior, while a small number of 'stiff' directions do affect it [86, 268, 269]. I strongly believe that this is not a problem of parameter estimation in multivariate complex models. Rather, it is a central property reflecting the nature of biological systems in general and the living cell in particular—enabling the duality of robustness and flexibility and underlying the exploration-exploitation dynamics. In other words, the biological cell itself is a *sloppy* system. Finding the 'stiff', *relevant* variables, is indeed one of the most urgent and important problems in our quest for a theory of the biological cell.

A plausible sketch of an *exploratory* adaptation process includes the following ingredients: (i) a driving force resulting from stress due to the mismatch between the inner state of the cell and the external and internal demands (e.g. inner metabolic fluxes not compatible with the environmental demands). This driving force is global, non-specific and works like 'heat', by causing large-scale changes in gene expression profiles. (ii) Exploratory dynamics, involving a plethora of emerging modes, which compete over the limited resources of the cell (e.g. modes of gene expression competing over limited numbers of polymerases, ribosomes, protein–DNA binding sites etc). These dynamics are based on the labile protein–DNA and protein–protein interactions due to their weak (compared to k_BT) intermolecular forces. (iii) Physiological selection of a set of compatible modes, which does not need to be unique, thanks to the degeneracy of the system. (iv) Finally, a drive-reduction mechanism [270] which alleviates the stress and leads to relaxation and stabilization of a cell state. A theoretical framework along these lines should go beyond the program formulated by D'arcy Thompson in his famous book *On Growth and Form* [271], which highlights respectable mathematical principles behind growth and form of biological systems, but remains relatively silent about the biology itself (not surprisingly, given the biology state of the art at his time). Unfortunately, without a specific theoretical framework, these ideas leave the concept of dynamic organization quite empty at this stage. A collective experimental and theoretical effort is required in order to crystallize such a framework.

In 1966, Waddington organized the first of four yearly meetings, aiming to discuss a sketch towards theoretical Biology [155]. Unfortunately, this effort to inquire into the principles underlying the organization of living matter did not proceed far beyond those meetings. It was soon overshadowed by the fast and swamping progress in molecular biology; the quest for universal principles was displaced by the 'hunting' of molecules. Notwithstanding the impressive advance in molecular biology, the last 50 years have taught us that progress in understanding biology, which is not synonymous with progress in medical applications, is severely impeded by the reductionist approach, focusing solely on cataloguing an ever increasing list of molecular processes, without a complementary effort in unraveling the system-level organization principles. What seems to be missing is indeed a unifying concept of organization. Regrettably, very little is left from Waddington's spirit [109]. The recent intense interest in living systems of people coming from disciplines outside of biology, in particular physicists and computer scientists has raised hope for reviving such a theoretical-based program. Indeed, the previous wave in the 1930s of physicists moving into biology, made a great impact [272]. The current multi-disciplinary effort is still on, so it might be too early to judge. However, until now it has not yet developed an original view, raising its own voice regarding the origin, evolution and development of biological systems as *natural phenomena,* independent of the tyranny of the molecular approach. I hope that the experimental framework and discussions presented in this article will stimulate readers to meet the challenge of developing a physics-based framework of cell-state organization.

For newcomers to biology, it helps to appreciate the formidable mission of facing the essential complexity of biology. This is echoed in the words of the famous physicists-becoming-biologist Max Delbruck[17] in describing his attempts to solve the '*riddle of life*' (virus replication), assuming initially that it '*... so simple a phenomenon that the answers cannot be hard to find; In a few months we will know.*' He later admits: '*Well, I made a slight mistake, I could not do it in a few months. Perhaps it will take a few decades, and perhaps it will take the help of a few dozen other people. But listen to what I have found, perhaps you will be interested to join me.*' We have to face the reality presented by the complexity in biology. It might take more than what Delbruck could have imagined to have a theory of the living cell. But whether it can be done at all, we will never know without trying. Physics can provide a fruitful framework and essential tools. There is no better way to state this than in the words of the great physicist Leo Szilard [277]: '*The mysteries of biology are no less deep than the mysteries of physics were one or two generations ago, and the tools are available to solve them provided only that we believe they can be solved*'. This article reflects my personal journey into the living cell, insisting on a physics approach. It has opened perspective on biology that has changed my view of some fundamental issues of biological organization. I invite the interested reader to join the effort. We are still far from solving the riddle of biology but the journey is certainly worth the effort, as the road itself is not less exciting than the target.

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[17] 1946, in a lecture to the Harvey Society. See [272] p33–4.