Theoretical aspects of Systems Biology

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Review

Theoretical aspects of Systems Biology

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ABSTRACT

The natural world consists of hierarchical levels of complexity that range from subatomic particles and molecules to ecosystems and beyond. This implies that, in order to explain the features and behavior of a whole system, a theory might be required that would operate at the corresponding hierarchical level, i.e. where self-organization processes take place. In the past, biological research has focused on questions that could be answered by a reductionist program of genetics. The organism (and its development) was considered an epiphenomenona of its genes. However, a profound rethinking of the biological paradigm is now underway and it is likely that such a process will lead to a conceptual revolution emerging from the ashes of reductionism. This revolution implies the search for general principles on which a cogent theory of biology might rely. Because much of the logic of living systems is located at higher levels, it is imperative to focus on them. Indeed, both evolution and physiology work on these levels. Thus, by no means Systems Biology could be considered a 'simple' 'gradual' extension of Molecular Biology.

Contents

"Every part of nature agrees with the whole, and is associated with all other parts' and 'by the association of parts, then, I merely mean that the laws or nature of one part adapt themselves to the laws or nature of another part, so as to cause the least possible inconsistency" B. de Spinoza

1. Introduction

According to the paradigm inherited from Galileo and Newton, later philosophically theorized by Descartes, every phenomenon we observe can be 'reduced' to a collection of particles whose movement is governed by linear dynamics rules that drive the overall system toward a deterministic, predictable 'fate'. This approach was proven to be mistaken, even for apparently 'simple' situations characterized by linear dynamics, like the 'three body problem', sharply addressed by Henri Poincaré (Barrow-Green, 1997). Reductionism hardly allows us to understand the world's

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complexity, as was recognized by modern physics at the beginning of the last century (Laughlin, 2005): complex systems exhibit properties and behavior that cannot be understood from laws governing the microscopic parts given that such systems cannot be easily 'reduced' or explained by simple deterministic rules (Anderson, 1972).

To date, however, the positivist theoretical framework has survived in Biology under the spoils of "genetic determinism", which consider genes alone able to drive and determine the development as well as the characteristics of an organism. This is paradoxical when keeping in mind that "it seems odd […] that just when physics is moving away from mechanism, biology and psychology are moving closer to it. If this trend continues […] scientists will be regarding living and intelligent beings as mechanical, while they suppose that inanimate matter is too complex and subtle to fit into the limited categories of mechanism" (Bohm, 1969). In other words, Molecular Biology tries to explain the mysteries of the living being by exclusively considering it as a consequence of a linear translation of the 'DNA code'. As originally formulated (Crick, 1970), the 'central dogma' posits that 'information' flows unidirectionally from DNA to proteins, and not the other way around. However, environmental factors do change the genome, by both genetic as well as epigenetic mechanisms (Goldenfeld and Woese, 2007), and many types of molecules participate in 'information' transfer from one molecule to another (Barnes and Dupré, 2008). Genomic functions are inherently interactive (isolated DNA is virtually inert) (Shapiro, 2009), and biological processes flow along complex circuits, involving RNA, proteins and context-dependent factors (extracellular matrix, stroma, chemical gradients, biophysical forces) within which vital processes occur (Keller, 2000). Indeed, no simple, one-to-one correspondence between genes and phenotypes can be made (Noble, 2008a, b). Therefore, "the collapse of the doctrine of one gene for one protein, and one direction of causal flow from basic codes to elaborate totally marks the failure of reductionism for the complex system that we call biology" (Gould, 2001).

The concept of "gene" inherited by molecular biology has therefore been broadly revised (Moss, 2006; Pichot, 1999), taking into consideration that gene function is in fact "distributed" along a connection of corporate bodies that interact among them according to a not-linear dynamics (Siegelmann, 1998). Eventually, gene functional expression has lost a lot of its deterministic character after the demonstration of the fundamental stochasticity of gene expression at the single cell level (Elowitz et al., 2002).

The discovery of an irreducible level of stochasticity in single cell gene expression coupled by the substantial invariance of transcriptome profile at the tissue level emphasizes a fundamental question: how to reconcile the existence of stochastic phenomena at the microscopic level with the orderly process finalized observed at the macroscopic level. This situation is somewhat analogous to the behavior of gases, resolved by the classical thermodynamics for equilibrium systems, and further, by the non-equilibrium thermodynamics for dissipative processes (Nicolis and Prigogine, 1989). The theoretical framework provided by non-equilibrium theory contradicts the paradigm proposed by Schrödinger (1944) which was enthusiastically adopted by molecular biology. According to such an approach, "order originated from order", through the decoding of the information flux from DNA into proteins and, thereby, into tri-dimensional structures: each level of organization was produced by 'specific' interactions at the lower level. Thus, cell differentiation and organism development are traditionally described in deterministic terms of program and design, echoing a conventional clockwork perception of the cell at another scale. Accordingly, this conceptualization manifests itself in an all-pervasive vocabulary of "locks", "keys", "machineries", "power", "signals", that populate the past and current biological and medical literature. These exchanges consider valid all the familiar implications, consequences and interrelations between concepts used as metaphors. Thereby, methodologies as well as intellectual approaches are coherently shaped according to the aforementioned framework. Little doubt is left about adherence to such a mechanistic view significantly handicaps our ability to adequately comprehend and model biological phenomena (Kurakin, 2005).

Those statements and the widely used concept of "genetic program" are currently challenged by an alternative view for which the order "emerges" at the macroscopic level (cell, tissues) as a consequence of the microscopic stochastic behavior (Kauffman, 1995).

According to the classical deterministic, "instructive" model, cells differentiate and activate functional programs depending on "specific" signals. Every signal is thought to correspond to a "command" of the genetic "program". According to this deterministic model, all cells answer to the stimulus in the same way. Variability is not contemplated other than for correlated variance (externally imposed variability) or in the form of instrumental variability due to the uncertainty of the measures. On the contrary, the "selective" model posits that variability occurs on a larger scale and cells differentiate as a result of stochastic genetic events (Laforge et al., 2005).

The stochasticity of gene expression, originally proposed in 1983 (Kupiec, 1983), is today supported by a body of experimental data. Stochasticity is an inherent property of the non-linear dynamics of gene expression, which, in turn, can lead to bi-stable states in gene network activity (Becksei and Serrano, 2000): as such, it underlies the behavior of isogenic macromolecules (Xie and Lu, 1999), cells (Hume, 2000; Blake et al., 2003) and organisms (Herndon et al., 2002). Moreover, proteins are less specific than previously thought, and they can interact with different molecular components: in other words, protein interactions are also intrinsically stochastic and are not 'directed' by their 'genetic information' (Kupiec, 2010). This implies that, notwithstanding that differentiation is a highly precise and reproducible phenomenon, a deterministic mechanism supporting it is not really needed. Indeed, biophysical as well as biochemical interactions between cells and the surrounding microenvironment (stroma, extracellular matrix) converge in sorting and subsequently stabilizing the cellular phenotype, henceforth addressing its differentiation fate (Till, 1981; Balazsi et al., 2011) according to a Darwinian (selective) model of cell differentiation (Kupiec, 1997). Thus, the genome should not be considered a deterministic execution program (Coen, 1976), but rather a 'database' from which the dynamics of intra- and intercellular biophysical networks actively choose the desired inputs according the current needs of the system (Atlan and Koppel, 1990). Those features challenge expectations and assumptions of linear causality and reductionism that characterize the current molecular paradigm (Moss, 2006; Kurakin, 2005).

Consequently, scientific research was legitimate to give up models based on linear dynamics that are being substituted by approaches based on far-from-equilibrium systems and upon non-linear mathematical approaches (Kellenberger, 2004; Longo et al., 2012a, b).

A system characterized by non-linear dynamics is confined within a discrete number of configurations (stable states), represented by attractors in a phase-space landscape. Non-linear dynamics lead to symmetry breaking, hence allowing the system to choose among different fates, i.e. stable states or eventually chaotic regimens. Symmetry breaking confers irreversibility to the system, positioning it within the "arrow of the time", previously "omitted" in classical physics: that is to say the system has now a history and its further evolution shall depend from choices undertaken at the bifurcation points. Moreover, such 'complex' systems may display

the property of self-organization, characterized by the 'spontaneous' emergence of properties and ordered structures in time and space that confer to the system novelty and adaptation to a changing environment. These features are 'uncommon' for classical physical objects, characterized by stable symmetries and invariance, whereas in biological systems theoretical symmetries change and they become specified along (and by) their history (Longo et al., 2012a, b). Newtonian physics as well as molecular biology are clearly unfit to address these problems. However, around the middle of last century, researchers of different disciplines provided theories, concepts and methods in order to cope with complexity. Their contributions are coalescing into a new approach: Systems Biology.

2. Systems Biology: in search of a meaning

Efforts to define Systems Biology (a term coined by Mesarovic in 1968) (Mesarovic, 1968) through a rational path toward the integration of multidisciplinary, multi-hierarchical levels of analysis have been disappointing. As a result, the concept of "Systems Biology" remains as a somewhat nebulous idea (Boogerd et al., 2007). As pointed out by O'Malley and Duprè (2005), two principal streams can be recognized within Systems Biology: 1) Pragmatic Systems Biology, which emphasizes the use of large-scale molecular interactions ('omic' approach), aimed at building complex signaling networks by applying mathematical modeling and thus showing how cells make decisions based on the 'information' that flows through their networks (Brent, 2004; Melham, 2012); and 2) Theoretic Systems Biology, according to which both theoretical as well as methodological approaches in biological research must be radically changed. That statement has recently been underscored by Noble (Bard et al., 2012).

Pragmatic Systems Biology relies principally on high-throughput technologies and on massive data integration through mathematical modeling (Kitano, 2002). The advent of whole-genomic sequencing and other high-throughput technologies has transformed biological research from a data-poor discipline into a data rich one. However, as already pointed out by Poincarè, "Science is built of facts the way a house is built of bricks, but an accumulation of facts is no more science than a pile of bricks is a house" (Poincaré, 1902). Indeed, the massive acquisition of biological data has broadened the gap between the available information and the amount of actual, truly new knowledge, e.g. the comprehension of biological organizing principles. This accumulation of facts is unlikely to explain a system's behavior and cannot be a replacement for a robust theoretical framework (Joyce and Palsson, 2006; Assmus et al., 2006). Indeed, the 'pragmatic' approach has yet to produce a clear account of what "biological systems" are, because its philosophical underpinning have neither been stated nor addressed (Vidal, 2009). Furthermore, this approach still relies on a molecular level rationale as the privileged level of explanation.

In contrast, "theoretical" Systems Biology recognizes that complex physiological and adaptive phenomena take place at biological levels of organization higher than the subcellular one. This stream of thought posits that ad-hoc approaches are insufficient and proposes instead to consider emergent properties within a de novo theoretical framework (Saetzler et al., 2011; Morange, 2005). We may not completely understand biology until we fully embrace a new perspective: gene products do not act alone, individual cells separated from their neighbors lose most of their functional and structural attributes, macro-molecules and metabolites are intimately linked to each other. Importantly, evolution rarely acts on individual cells or on distinct species, but rather, impinges upon complex multi-scale systems in which these components are intricately interconnected according to a non-linear dynamics (Noble, 2011). The latter statement has practical as well as strategic relevance in implementing Systems Biology and it is the only reliable approach that would allow to cope with the intrinsic 'disorder' of living processes (Auffray et al., 2003). We are therefore facing a significant intellectual challenge: how to include chaotic and nonlinear, unpredictable processes into our comprehension of Biology. This task will likely improve our understanding of complexity of the real world, no longer confined to simplified and idealized phenomena (Prigogine, 1996).

Systems Biology entails investigating phenomena in terms of how the objects are related, rather than what their compositions are. Indeed, this is an old idea that can be traced back to the aftermath of the quantum physics, who stated that an elementary particle is not an entity that exists independently, but rather it is a set of relationships that reach out to other things (Stapp, 1971; Heisemberg, 1969). Therefore, at the core of the challenge is the need for a shift from reductionism to an "integrated", "holistic" (from the Greek: "wholeness") view. This perspective implies that the behavior of the basic bricks of life (i.e. the molecules) should be re-interpreted, tacking into consideration that biological processes did not happen in an ideal, linear, virtual milieu. Instead, cells are not a homogenous colloidal soup in which processes behave according to classical diffusion and kinetics laws, and cytosol never could be considered a "simple Newtonian fluid" (Clegg, 1984). Indeed cytoplasm is compartmentalized by spatial and temporal variation of its internal organization, quantitatively described as fractals of the type of percolation clusters (Rabouille et al., 1992). Processes structured in percolation clusters and belonging to a fractal milieu display astonishing properties: below a percolation threshold value a process behaves as locally connected while above that value the connection extend indefinitely: "Near the critical probability pc […] the percolation process undergoes a transition from a state of local connectedness to one where the connections extend indefinitely" thus, "local cytoplasmic behavior when subjected to fluctuations or perturbations may extend and globally impose that behavior to far remote regions in the cellular cytoplasm" (Aon and Cortassa, 1994). Enzymatic reactions can be influenced by topological segregation of the reactants, or because a volume may fractally evolve into an area by fractal folding. Thus a biological system can greatly enhance the targeting of a molecule through modification of its dimensionality (Dewey, 1997). That modulation, by regulating the geometry or architecture of cell's cytoskeleton, may in turn regulate the level of its percolation threshold and, as a consequence, the local level of a messenger or the product of an enzymatic reaction (Aon et al., 2000).

This shift highlights how profound the difference between the two aforementioned approaches is. The divergence is rather philosophical than technical, given that philosophy is central to all scientific endeavors, including experimental and Systems Biology (Saetzler et al., 2011).

However, more than just a pronouncement of a new approach is required. If Systems Biology is to become a true discipline, some conceptual hurdles will have to be addressed; they cannot be "reduced" to "data and software" problems, as it has been repeatedly claimed (Cassman et al., 2005). What is needed is to provide a conceptual framework able to integrate some entrenched aspects, such as complexity, hierarchical structured levels of observation, geometrical relationships, non-linear dynamics, network modeling, influence of biophysical constraints, operating on different scales, rather than solely focusing on building numerical mathematical or computer models (Auffray and Nottale, 2008). Those aspects must be collectively considered in order to find organizing principles that exactly outline the evolution of systems in space and time (Mesarovic et al., 2004).

Noble (2002) has keenly investigated a paradigmatic example of such approach. The construction of a mathematical model for the understanding of the generation and propagation of the heart rhythm required a multi-scale approach that included the tissue structure as well as the gross anatomy of the heart, without which the model could not work. This example implies that understanding the logic of living systems requires knowledge of the mechanisms involved at the levels at which functionality is expressed. This information does not reside in the genome, or even in the individual proteins that genes code for: it emerges as the result of interactions between many proteins relating to each other in multiple cascades and in interactions with the cellular environment. The cell machinery does not just read the genome, but it imposes extensive patterns of expression to the genes. These results call into question the concept of "genetic information" (Werner, 2007), given that transferring concepts from informatics into biology could be misleading without providing biology a pertinent observable for understanding and measuring organization (Longo et al., 2012a, b).

Explanations in biology should rather be pursued through an explicit search for a proper biological observable, present at the right level of organization (Bailly and Longo, 2009). The search for that level is indeed the primary aim of Systems Biology (Noble, 2008a, b).

2.1. A definition of "biological system"

Living systems acquire only a limited number of configurations (forms) as a consequence of the constraints exerted on its parts by the system as a whole. As suggested by Paul Weiss, biological components and processes have many degrees of freedom, but they are constrained to an "ordered pattern" by the integral activity of the whole system, which integrates the functions of its parts (Rosslenboich, 2001). This feature unravels the existence of different hierarchical levels of causality in living matter and outlines the relevance of the "supra-molecular" order.

A living complex system is thermodynamically open and is characterized by a non-linear dynamics, allowing it to have a history: this means that the present behavior of the system is in part determined by its past behavior. Such a system displays both sensitivity and resilience (robustness) with respect to the perturbations exerted by internal and/or external stimuli. In addition, living systems are characterized by both local and long-range interactions (non-locality), as well as by complex interactions between molecules and structures that make their determination "non-separable" (i.e. "entangled"), according to an analogy remnant of quantum mechanics (Longo and Montevil, 2011; Soto et al., 2008).

Biology deals with emergent properties arising from the non-linear interplay between different structures – intra-cellular organelles, epithelial and stromal cells, extracellular matrix components. This implies that the "observable" parameters cannot be "reduced" to intracellular biochemical pathways only. Some complex biological functions – like differentiation or pathological states – take place within tissues. It is therefore mandatory to consider the integrated interplay between epithelium and stroma as the proper level of investigation; that is, an active object (a cell, a biological function) must be described in its context, dealing with what it does, and not only with what it is. Overall, these factors determine the shape (or form) the system acquires.

Indeed, a complex network of non-linear interactions between the stroma, the extracellular matrix (ECM) and the epithelium drives tissue development and function (Müller and Newman, 2003). This is also true in carcinogenesis, where the relevance of cell–tissue relationships indicates that carcinogenesis is a tissue-based disease (Soto and Sonnenschein, 2005; Kenny and Bissell, 2003). Compelling evidence suggests that cancer is a consequence of the disruption of the reciprocal interactions between cells and the microenvironment, leading to unexpected and complex modifications in cell morphology, signaling pathways and genomic functions (Maffini et al., 2004; Bizzarri et al., 2008).

2.2. Biophysical constraints

It is quite difficult to accept that a biological form is dictated in every detail by a genetic code (Newman, 2002). Diffusible chemical factors alone, as well as genes products, are not sufficient to fully explain cell fate regulation, and even gradients of morphogenetic molecules cannot entirely explain morphogenesis as firstly proposed by Turing (1952). Rather than being the result of a mere genetic "adaptation", morphological plasticity reflects the influence of external physico-chemical parameters on any material system and is therefore an inherent, inevitable property of organisms (Newman et al., 2006). The physical milieu integrating through long-range correlations different chemical as well as physical components is recognized as the "morphogenetic field" (Belousov et al., 1997). Morphogenesis and phenotypic differentiation are therefore time and space-dependent processes (Nelson and Bissell, 2006). The forces generated by, and acting on, tissues influence the way tumors start, develop and metastasize. These forces precede and may even be more influential than molecular changes (given that "cancer is not strictly a disease of genetic mutations"), as it has recently been recognized by a special issue of Nature (2012).

Physical stimuli converge on common integrative sites where cells are physically anchored to extracellular matrix or to other cells. Cells dynamically adapt to force (shear and tensile stress, compressive forces, hydrostatic pressure) by modifying their behavior and remodeling their shape; through actomyosin- and cytoskeletal-dependent modifications, cells can in turn exert a reciprocal influence on their microenvironment (mechano-reciprocity), as well as on gene expression (Kirson et al., 2007; Hammond et al., 2000; Levin, 2003; Butcher et al., 2009; Ingber, 1997).

Living cells generate active tension in their cytoskeleton, thus any exogenous mechanical stress is imposed on a pre-existing force balance. By altering the balance of forces transmitted across the adhesion site, the signaling machinery can be altered, thereby producing different functional outputs (Chicurel et al., 1998). Given the multiple role of forces in tissue function, it is not surprising that several diseases, including cancer, are characterized by compromised tensional homeostasis (Tracqui, 2009). On the contrary, by normalizing the tissue tensional state of a tumor, cells can be reverted toward a non-malignant phenotype (Paszek et al., 2005). So far, changes in ECM and/or in the tensional balance of forces transmitted across focal adhesion, together with change in cell shape, might account for the complex phenotypic and functional transformations occurring during tissue development or neoplastic "transformation" (Ingber, 2005; Soto and Sonnenschein, 2011).

2.3. The proper level of observation

The genetic paradigm has largely privileged a specific level of observation, while reducing the complexity of a living system only to its molecular components. The integrated vision of biological process, able to involve a plurality of levels in the biological episteme, enacted among others by Claude Bernard, has been lost (Noble, 2008a, b). This is due to the fact that the gene paradigm has been "illegitimately extended as a paradigm of life" (Strohman, 1997). Understanding the logic of organisms implies to perform strict correlations between the 'local' processes and the 'global'

structure of the living beings, connecting every level with each other. The existence of levels means that molecules, components and structures belonging to the system are constrained to cooperate in the functionality of the whole. These constraints lie in the boundary and initial conditions, so that "the organization becomes cause in the matter" (Strohman, 2000). Every high-level function depends on effects attributable to the (non-linear) dynamical interaction between those factors and the 'internal' molecular (proteins, genes, lipids) 'circuits' (Neuman, 2007). Moreover, higher levels of matter aggregation display "emerging" properties that cannot be anticipated by "fundamental laws" or by analyzing the single parts" (Laughlin et al., 2000). Each level is both characterized and governed by emergent laws that do not appear at the lower levels of organization (Mazzocchi, 2008). In turn, hierarchical organization in between different levels creates 'downward causation' (Soto et al., 2008; De Haan, 2006; Barabasi, 2007) (Fig. 1). Yet, the middle-way-based approach doesn't exhaust the assignment of the biologist. The integration of the relationships must be extended to the level of organ and apparatus, promoting the "rebirth" of the time honored science of Physiology (Strange, 2004), which is built on the notion of scale hierarchy.

2.4. The morphogenetic field

The motion of one element – and latu sensu a biological function – is dominated by a field – a function of space-time producing force – which is a common rule, and, at the same time, a common product of a group of elements. Interactions between particles produce the field, and, in turn, some characteristics of the single particle are transferred into the field. In Biology, we are dealing with a special kind of field: the 'morphogenetic field' (Bolker, 2000), that, like a magnetic field, can maintain its pattern when its mass is either reduced or increased (Needham, 1950).

The morphogenetic field can be seen as a major unit of ontogenetic and phylogenetic change (Gilbert et al., 1996), thus explaining its current "rediscovery" (Gilbert, 1997). Within that framework, the relevance of genetic factors is not in any way denied, but their effects are significantly amplified, modulated or hindered by the field in which they are operating. Changes in these fields change the ways that tissues, organisms or animals develop. Recently, this concept had a spectacular challenge with the demonstration of the effect exerted by cell microenvironment on the expression of so-called oncogenes (Leung and Brugge, 2012).

Indeed, the concept of the morphogenetic field could helping establishing how self-organization processes take place in living organisms. One may think that complete disorder or chaos is the only natural state, as learned from the thermodynamics of open systems. However, the real world displays a variety of highly organized structures, able to counteract the "thermodynamic death", finding a self-consistent "solution" without any program or a priori aim. As demonstrated by Prigogine (Glansdorff and Prigogine, 1971), non-linear processes are at the root of the diversity of structures and phenomena: dissipative structures self-organize through fluctuations and instabilities, which lead to

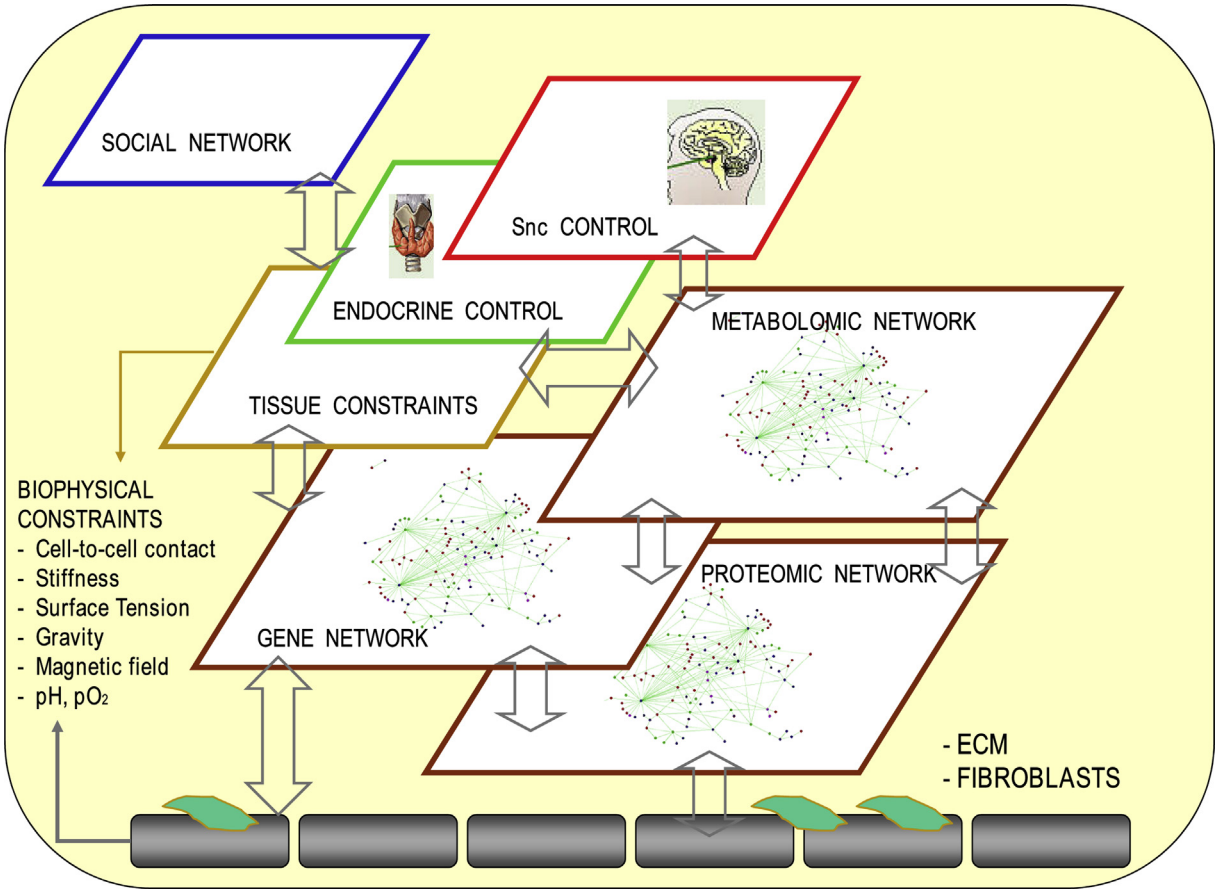

Fig. 1. Local processes involve the interplay between cells and stroma (the tissue level), where biophysical forces and molecular networks (genomic, proteomic, metabolomic) interact according to a non-linear dynamics. Reciprocal relationships are settled between tissues and higher level of organization. It must be outlined that each level is both characterized and governed by emergent laws that do not appear at the lower levels of organization. By this way, hierarchical organization in between different levels creates both bottom-up and downward causation.

irreversible bifurcations and new stable states. This approach is likely to be the one able to solve an old paradox: namely, how cans the increasing ordering that occurs in developmental biology - where self-organizing processes 'decrease' the system's disorder – be explained while the overall entropy increases? A solution may be gleaned by coupling a process producing order (negentropy), with another leading to increased disorder (entropy). Close to equilibrium, the dependence of the one process rate on the driving force of the other should equal the dependence of the other process rate on the former driving force (Onsager, 1931). These approaches have contributed to the non-equilibrium thermodynamics development (Westerhoff and Van Dam, 1987) and constituted a prelude to Systems Biology in that they dealt with quantitative integration, while providing general principles of organization (Westerhoff and Palsson, 2004).

2.5. Non-linear dynamics

The aforementioned questions are fully addressed through non-linearity (Yoshida, 2010). The term 'non-linear' dynamics roughly refers to changes whose entity does not linearly scale with their cause. One should not expect that any law or principle would hold unrestrictedly: indeed, a proportionality relation distorts when the magnitudes (the scale) of the intervening variables goes out of specific boundaries. A system moves from a linear regimen to a non-linear when one or more state parameters undergo a fluctuation above a threshold value, reaching thereafter a bifurcation point where it experiences a symmetry breaking. The symmetry breaking discloses different solutions for the same parameters values (hysteresis and bistability), therefore opening the system evolution toward novelty and variability. The system drives along different trajectories, thus converging into one or more 'attractors'.

An attractor is a stable solution to the set of mathematical equations that describe a dynamical system, representing the state of equilibrium to which the system will tend to move. Attractors are distributed along a complex landscape, in which stable (valleys), as well as metastable or unstable (hills) states are depicted (Huang and Ingber, 2007).

The rupture of symmetry gives the system a historical dimension, a sort of memory of an event that took place at a critical point and which will affect the next evolution, leading to relevant consequences addressed by the physics of criticality (Binney et al., 1992). Transition to the critical point allows the system to acquire relevant features, such as long-range correlations and scale invariance. The presence of long range correlations implies the determination of the system must be global and not only local. However, contrary to what happens in physics (where critical transitions are analyzed as isolated points), in biological processes symmetries breaking should be considered as "extended critical transitions". By 'extended' is meant that biological objects experience a continual transition between different symmetry groups (Bailly and Longo, 2008).

In this way, the system acquires a 'structure of coherence': local process is 'globally' determined and they display a fractal pattern. In turn, significant changes in fractal dimensions indicate that the system's parameters have overreached a threshold value and the system is undergoing a transition beyond the critical point, i.e. it is experiencing a symmetry breaking (Yoshida, 2010). Therefore, fractal analysis promises to be of strategic relevance in analyzing system's behavior.

According to this model, a system can be described by a phase-space diagram, by means of parameters ("observables") still largely unknown, since only few attempts have been performed to carefully recognize them (Dinicola et al., 2011; Guo et al., 2006). This model enable us to move from 'local' systems properties to more 'global' complex networks, as firstly guessed by Waddington (1957), when he proposed the concept of 'epigenetic landscape', conceived as a metaphor for the trajectory that a complex biological system might be traveling in response to genetic, physical, and environmental cues. Within such landscape even mild, gradual variations in a single control parameter can significantly affect non-linear processes, thus switching cells between distinct phenotypes, by analogy with phase-transition observed in physical systems (Kauffman, 1993).

The ability of attractors to integrate distributed signals could explain why physical perturbations can trigger a particular cell behavior, switching between proliferation, apoptosis, differentiation or neoplastic transformation (Huang et al., 2009; Blackiston et al., 2009), while involving a hundreds of genes in a collective, coherent transition from one attractor to another (Censi et al., 2010; Huang and Ingber, 2000; Zhang and Moriguchi, 2011). Thus, a discrete finite number of attractor classes can be singled out, corresponding to configurations allowed by their genetic and biophysical constraints (Guerroui et al., 2005; Lloyd and Lloyd, 1995; Huang, 1999). Within each attractor the expression of a huge number of genes is stationary, even if it is subjected to stochastic large fluctuations. It is worth noting that distinct genotypes can converge into the same phenotype, while keeping stable the attractor to which the system is embedded. These data favor a non-univocal genotype–phenotype relationship, suggesting that the 'robustness' of the phenotypic state cannot be linearly ascribed to the gene's configuration (Felli et al., 2010; Reuveni and Giuliani, 2011).

This implies a meaningful link between the multiplicity of microscopic states and the relative paucity of the corresponding macroscopic states that is at the basis of the impossibility of a one-to-one correspondence between molecular and tissue level representations. These features are mirrored by the shape (the phenotype) a cell acquires, and they emerge at the mesoscopic level of observation. A cell type proceeds through a discrete number of morphotypes along its differentiating pathway, and every morphotype could be considered as a quasi-stable state (Chang et al., 2008; Toussaint and Schneider, 1998).

How to describe these phenotypic switches? Functional states have been usually represented by gene-regulatory networks. Regulatory circuits are embedded into interconnected and complex networks, and they operate according to a non-linear dynamics (Chang et al., 2008). This task requires a huge amount of data from which statistical analysis can be based upon (Kitano, 2002). To overcome those limitations high-throughput techniques (functional genomic, metabolomic, proteomic and fractal analysis) are currently needed to obtain a reliable and understandable picture, and to allow further simulation by means of in silico models. The resulting models can be tested either by 'synthetic biology' or by systematic perturbations, or both (Alberghina et al., 2009). However, even such an approach is likely to be insufficient, given that the stability of functional states is largely dependent on external cues, as well as on system-level feedback controls (Kapuy et al., 2009). Thus, the system's dynamics in the phase space cannot be "reduced" either to a genetic wiring diagram or, even to the integrated functioning of a genome–proteome–metabolome network. Changes in shape and functions could be ascribed to the overall system and not to a single component, as important as it might be (Bizzarri et al., 2011a, b).

A unified theory of the multi-scale dynamic complex systems constituted by interacting molecules, physical cues and organized intra- and extra-cellular structures has recently been proposed under the name of 'interactome networks' (Stumpf et al., 2008; Vidal et al., 2011). The interactome model outlines those complex interconnections between molecules and physical factors, and

might be able to generate systems properties, recovering an old notion, firstly expressed by Kant. Organisms are organized natural products in which every part is reciprocally both end and means.

2.6. Putting genes in context

The Human Genome Project was initially conceived to provide a complete 'catalogue' of all the genes in a human being, with the explicit assumption that this collection of data "constitutes the complete set of instructions for development, determining the timing and details of the formation of the heart, the central nervous system, the immune system, and every other organ and tissue required for life" (DeLisi, 1988). Within this framework, organisms became nothing but the vehicles for genes (Noble, 2008a, b). However, the gene-driven "causal" role in biology cannot be separated from the context in which it is actually thought to "operate". Indeed, it could be envisaged that a relevant role for "genes", emerges only when the systems is experiencing a phase-transition, like those occurring during differentiation and/or when cells acquire a new phenotype. These instances may explain why mutated genes are per se ineffective in resting tissues, and why the relevance of differentiating gene-related pathways (like the p53 system) can only be appreciated during certain developmental phases: these pathways may react differently according to the tissue-context (Lane and Benchimol, 1990; de Keizer et al., 2010). Tissue context is indeed critical in addressing cell differentiation and behavior. The seminal experiment made by Mc Kinnell demonstrated how a strong morphogenetic field (i.e. the cytoplasm obtained from a toti-potent frog's egg) might successfully counteract any nuclear (DNA) "abnormality": nuclei obtained from kidney tumors after transplantation into the egg were eventually able to induce the development of a "normal" frog (Mc Kinnell, 1972). Several reports have later confirmed that the microenvironmental field can revert the neoplastic phenotype in both in vitro and in vivo experiments (Gerschenson et al., 1986; Hendrix et al., 2007; Krause et al., 2010; Bizzarri et al., 2011a, b). These experiments point out how relevant is the biochemical-biophysical context within which genes are embedded and how gene's function might be "constrained" and "driven" by the morphogenetic field. Biophysical constraints select and stabilize one of the alternative gene configurations 'offered' by the genome (Fig. 2). In turn, this selection provides a strong 'canalization' of gene expression, thus limiting the inherently wide stochastic activity and triggering a deterministic-like process. This kind of model is, in a way, analogous to that proposed by Noble, according to which genes are deemed to be 'physiological prisoners' (Noble, 2006).

In this way, novelty is acquired as a consequence of a local selecting process in between different states, allowing the system to reach new ordered configurations (Heylighen, 2002). Indeed, genome-wide correlations of transcriptome profiles relative to independent samples of the same tissue during phenotypic transition display extremely high values, indicating a strong common order parameter influencing the expression level of the entire genome (Kauffman, 1993; Guerroui et al., 2005). The presence of such an invariant order spanning more than twenty thousand elements (single genes) and around four orders of magnitude of expression levels is a signature of general order parameters organizing the entire cell regulation network. This character indicates that molecules are constrained by the physico-chemical milieu to behave according to a coherent behavior leading to an ordered "group coexistence" (Weiss, 1947). Such an astonishing property is generally recognized as "coherence" (Gershenson and Heylighen, 2005),

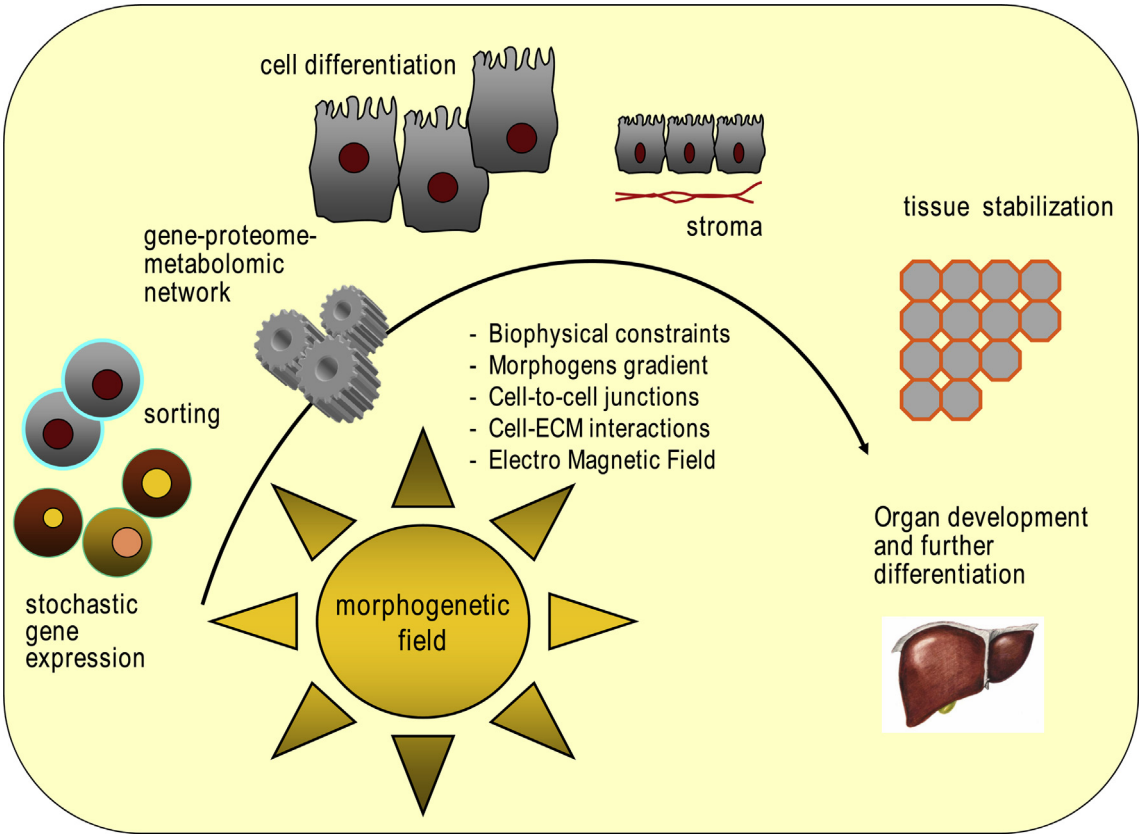

Fig. 2. Cell differentiation is driven by the interplay between the morphogenetic field and the gene expression pattern. Biophysical forces are acting within and throughout the field in selecting phenotypes that arise according to a stochastic process.

i.e. a synchronized behavior of coupled elements within a biological system, acting as a self-organizing force (Plankar et al., 2011).

2.7. Fractals and shape

One of the more astonishing properties of a self-organizing process is how they induce recognizable changes in the form the system acquire as a result of a phase-transition (Goodwin, 2000).

Why and how a living system acquires a specific conformation, selecting it among an array of an almost infinite number of possibilities, is still an unsolved problem, largely debated by contemporary morphologists (Day and Lawrence, 2000; Schock and Perrimon, 2002), since the outstanding book of D'Arcy Thompson (1917) was published. Both molecular and biophysical cues act in a non-separable way to generate form. For instance, epithelial cells (like normal hepatocytes) are roughly polyhedral when they are 'entangled' in tissues, but they become gradually spherical when they are dissociated and cultured in vitro (Kanamura et al., 1990). Many other processes, like crowding phenomena, surface tension, cell-to-cell adhesion, substrate interactions and cytoskeleton architecture, all converge in shaping the form a cell acquire (Goldmann, 2002; Jamora and Fuchs, 2002; Knust, 2000). Therefore, to explain how macroscopic form is generated, it is necessary to include a description of the spatial pattern of forces displayed by the morphogenetic field. Indeed, increasing evidence suggests that a substantial amount of order is given for free by merely physical factors, even though, so far, physical cues have been generally considered to play a very trivial role in evolution (even if with some relevant exceptions) (Kenny and Bissell, 2003), and namely in the generation of biological form.

Turing (1952) first described how simple non-equilibrium reactions could spontaneously cause patterns to in time and space, a finding further substantiated, among others, by the Belousov–Zhabotinsky experiment (Zaikin and Zabothinski, 1970), which suggests that the geometric form a system acquires – its shape – represents the integrated end point of the morphogenetic cues acting on the living system (Chen et al., 1997). Taking spatial relations into account is hence mandatory because signal transduction can be switched off and on, depending on cell shape (Gibson and Gibson, 2009). Therefore, it is not really surprising that several cellular parameters have been found to be determined by cellular geometry and shape-cytoskeleton dependent architecture (Zhu and Assoian, 1995; McBeath et al., 2004; Bissell et al., 1977; Singhvi et al., 1994). Thereby, cell shape should be considered a critical determinant of cell function, given that it appears to govern how individual cells will respond to physico-chemical cues in their local microenvironment (Ingber, 1999). Consequently, measurable parameters describing shape could be considered "omics" descriptors of the specific level of observation represented by the cell–stroma system (Huang and Ingber, 2007).

Fractals may quantify the irregularity of objects with a measurable value (fractal dimension), characterized by self-similarity or scale-invariance (Mandelbrot, 1985). In addition, fractal dimension can be viewed as a descriptor of cell morphologic complexity (Cutting and Garvin, 1987) and, as such, it can be thought in much the same way that thermodynamics look at intensive measures as temperature (Smith et al., 1996); thus, shape changes could be considered like 'phase-transitions', proceeding through qualitatively and- quantitatively different stable states. In other words, fractal values can be considered a system property, and together with one or more independent variables, they could draw a diagram of phase transitions aimed at describing the evolution of a living system (Huang and Ingber, 2007; Chang et al., 2008).

A theoretical approach to correlate spatial form to dynamics in order to provide a general model of morphogenesis has to consider how global cues contributes to the emergence of order, integrating positional data and local interactions into a harmonized patterning control (Bizzarri et al., 2013). Indeed, a compelling set of experimental data highlights how the control of local regions fate is embedded and coordinated into a 'global' morphologic 'plan' (Levin, 2009), thought to drive the morphogenetic process toward the form the organism will acquire. For instance, consider the astonishing fate of a tail blastema grafted into a host amphibian. The tail develops at first, but after few months the tail is 'reshaped' (correctly) into a limb. This illustrates how strong the 'global' control on morphogenesis is and how it dictates the more appropriate fate for organogenesis during structure remodeling (Farinella-Ferruzza, 1956). The mechanisms underlying such processes could arise, among others, from interactions with neighboring cells (Farhadifar et al., 2007; Blankenship et al., 2006) or extracellular matrix constituents (Théry, 2010; Théry et al., 2006). In turn, spatial patterning of the behavior of individual cells generates global changes in tissue architecture that drive morphogenesis and the pattern of localized proliferation (Nelson et al., 2005). Overall, these results provide a tantalizing hint that there is a fundamental tendency for a tissue to form a particular overall structure, and that the same structure will tend to be formed regardless of how its living material is partitioned into cells (Marshall, 2011; Fankhauser, 1945).

A paramount role in shape acquisition during developmental processes is sustained by biophysical forces, which determine the direction in which symmetry is broken, by analogy with ferromagnetism, which has been proposed as an analogy for understanding biological structure (Goldenfeld and Woese, 2010). Studies performed on cells growing on a microgravity field provide interesting insights on the matter. The disruption of the normal equilibrium of physical forces acting on a tissue may easily produce mutations and/or induce relevant changes in genes function, which is what happens when cells and tissue are exposed to microgravity (Han et al., 1999). It is noteworthy that such modifications are anticipated by dramatic changes in cell morphology, so that cell shape changes are currently considered paramount parameters of gravity response (Bizzarri, 2012; personal communication; Qian et al., 2012). Furthermore, microgravity affects microtubule self-assembly and thus hinders the right organization of intermediate filaments and cell's adhesion sites (Papaseit et al., 2000). Since cells rely on microtubule for their shape, and for many other functions (including maintenance of cell polarity), shape modifications might significantly change the way the cell behaves.

The aforementioned considerations are supported by the relevance the cell shape has in pathology and histopathology. There is a significant relationship between cell shape and several diseases, including cancer (Lelièvre et al., 1998; Rosai, 2001). In this regard, neoplastic transformation and malignant progression are characterized by a progressive increase in cell fractality (Pasqualato et al., 2012), whereas the reversion of tumor phenotype is followed by an impressive change in both the form and the fractal dimension of the cell (D'Anselmi et al., 2010).

3. Conclusion

Molecular biology, embedded into the reductionist paradigm, has removed from consideration those aspects of biology that it could not effectively deal with (Woese, 2004). By extension, the nature of the complex organization of the living matter was shortchanged.

Living objects consists of hierarchical levels of organization that range from subatomic particles and molecules, to organisms, ecosystems and beyond. Each level is characterized and governed by emergent laws that do not appear at the lower levels of organization. This implies that, in order to explain the behavior of a whole system,

a theory that operates at the corresponding hierarchical level is required. Hence, a profound rethinking of the biological paradigm is now underway and it is likely that such a process will lead to a 'conceptual revolution' emerging "from the ashes of reductionism" (Van Regenmortel, 2004). This revolution implies that a search for general principles on which a reliable theory of biology might rely is underway (Mesarovic et al., 2004). Because much of the logic of living systems is located at the higher levels, it is imperative to focus on those general principles, briefly outlined herein.

Systems Biology is frequently misunderstood as a mere procedure developed exclusively to manage the huge amount of new data obtained by omics and high-throughput procedures. However, by no means Systems Biology could be considered a 'simple' 'gradual' extension of Molecular Biology (Medina, 2013), despite efforts leaning in such direction (De Backer et al., 2010). Systems Biology ought to promote an integration of a different kind of knowledge, not a simple collation of disciplines, but a true multidisciplinary synergy (Kohl and Noble, 2009). That enterprise is likely to lead toward a "new revolution" in biological science.

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