Emergent Properties and Stability in Hierarchical Biosystems: There Is no Privileged Level of Causation

Gustavo M. Souza and Marcelo N. do Amaral

Abstract The main question herein is about the extent that reductionist approach can reach higher-level explanation as well as predict behavior from lower level of systems organization when concerning stability. Starting from a theoretical discussion on the complex hierarchical organization of biological systems, we offer a variety of examples showing the interchanging between bottom-up and top-down events underlying the regulation of the system. Moreover, we discuss and illustrate with different examples, how system stability under externally changing conditions are reached from many strategies without a specific level of causation, highlighting the role of downward causation with upward causation. Finally, we would like to demonstrate that there should be no dogmas when it comes to causation in biological systems.

Introduction

The basis of the classical paradigm of biological sciences, which supposes that the understanding of the parts building up an organism could allow to comprehend the whole system, has been challenged by an emergent antireductionist perspective (Baetu 2012). The classical reductionist view is founded on the assumption that the parts integrating a system are mechanically connected (in a linear straightforward way) allowing reliable and predictable explanation towards a higher-level organization (e.g., from set of molecules to whole metabolism). One of the fundamental aspects of biological systems is their evident nested hierarchical organization, in which lower levels are contained in higher levels (e.g., from a set to whole metabolism), such as Russian dolls (Souza et al. 2016a). Noble (2011) has argued that this approach can reinforce a metaphor on the scientific thinking with a biased view to represent the lower levels as more concrete, supporting a reductionist perspective that the micro-scale components (e.g., molecules) are the cause of the higher-level properties (a bottom-up causation).

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On the other hand, the systemic approach (Alcocer-Cuarón et al. 2014; Souza and Lüttge 2015; Souza et al. 2016b) starts from the assumption that biological systems constitute of a set of self-organized components interacting with each other in a non-linear way far from thermodynamical equilibrium, engendering emergent properties at higher levels of organization. The concept of emergence, as a set of phenomena that cannot be deduced from underlying laws, in an epistemic sense, presupposes the existence of levels of organization. Ontologically, emergence presupposes new higher levels of entities arising and having casual powers not possessed by the parts (Kauffman and Clayton 2006). Therefore, the real meaning of the possible effects from lower-scale organizations (e.g., molecular level) should be considered at the proper higher-scale context (e.g., metabolic) (Kohl et al. 2010). In other words, the context, in which a given biological process takes place, matters for its own regulation as well as for its role in the system organization as a whole as well (Amaral and Souza 2017).

The main question herein is about the extent that reductionist approach can reach higher-level explanation as well as predict behavior from below. In the middle of the twentieth century, Ludwig von Bertalanffy published the seminal book General System Theory (von Bertallanffy 1968), in which some principles of the systemic thinking were formalized, specially taking into account the biological systems. Recently, system biolog is gaining renewed interest due to the progress in molecular biology, particularly in genome sequencing and high-throughput measurements, inaugurating the "OMICS" age (Amaral and Souza 2017). The focus is on the understanding of the system's structure and dynamics, because a system is not just an assembly of genes and proteins, its properties cannot be fully understood merely by drawing diagrams of their interconnections, such as static "road maps." The knowledge of how changes to one part of a system may affect other parts, or how its control is performed, is not satisfactorily provided by road maps (Kitano 2002).

In the following sections, we explore the systemic hierarchical view in biological thinking and some consequences, specially, toward the concepts of downward causation and stability.

Hierarchy as the Fundamental Shape of Biological Systems Organization: Upward X Downward Causation

The concept of hierarchical levels and its implications for the biological organization have been early recognized (Novikoff 1945), and different hierarchical models of biological organization have been proposed (MacMahon et al. 1978; Korn 1999; Rojdestvenski et al. 1999; Ravasz et al. 2002; Souza et al. 2016a). However, the trend, starting at the end of twentieth century, to examine biological systems mostly from the molecular perspective has overshadowed the integrated view of living beings. On the other hand, recent discoveries have led to the identification and characterization

of the systemic aspects of biological organization, identifying network hierarchical modularity at cellular level (Ravasz et al. 2002; Dietz et al. 2010; Lucas et al. 2011).

The bases for complexity are modular hierarchical structures, leading to emerging levels of structures and functions based on lower-level networks. These three aspects, modularity, hierarchy, and structure, are crucial in the emergence of complexity through interactions between simpler units (Booch 1994; Ellis 2008). The basic principle is that a complex system is divided into ever simpler subsystems, and assigning these systems to specific modules. Each module is again divided into submodules until it reaches a base level, where the required tasks are simple operations that can be performed by simple mechanisms. This is the level where the actual work is done, each of these components feeding their results into the next higher-level components and interacting with each other until the desired result emerges at the appropriate top level. The result is a highly structured hierarchy of interacting entities (Ellis 2012).

Beyond a pure epistemological meaning, the importance of studying integration of different levels of biological organization relies on an ontological base. Schneider (1998) has summarized the main arguments: 1—spatial and temporal patterns are dependent on the scale of analysis, 2—there is more than one characteristic scale for the research, 3—experimental results cannot be directly transferred to larger scales, 4—biological interactions with the environment occur in multiple scales, and 5—environmental problems are created by the propagation of effects on different scales in the biosystem. In this context, for instance, experimental evidence provided by Vítolo et al. (2012) and Bertolli et al. (2014) showed that, in a particular experiment, in which plants are exposed to environmental changes, different variables measured at different scales of plant organization exhibited different levels of homeostatic capacity, impairing a unique diagnosis by a single variable. In other words, there was no specific representative scale of organization accounting for the whole system.

Each level of the hierarchy consists of modules that interact with other modules through weaker connections and lower-frequency interaction dynamics, so each module can be composed of an interactive network of lower-level modules. The emergence of a top-level system from these lower-level modules occurs when a reliable top-level behavior arises from the lower-level actions that occur in the context of that structure. Each of these different levels of hierarchical function can only be described and behave appropriately only at that level (for example, the basic concepts of molecular biology cannot be described in the language of quantum physics) (Ellis 2012). In other words, while the links among components of a specific subsystem are mostly strong (supporting a local pattern of organization), the links among different subsystems cross scale are mostly weaker. Ultimately, all types of links can be taken as informational insofar as the links act as "signals" among different parts of the system, enabling integration, i.e., hierarchical organized networks. Inasmuch as natural networks are not ideal, because there are often reversible feedback relationships, the downstream flow of information is ambiguous, bringing uncertainty to the system, making the accesses to the higher-scale traits of the system from its basic components unsettled. This is a strong limitation for the reductionist approach (Corominas-Murtra et al. 2013; Souza et al. 2016a). Therefore, ideas concerning lower-level causality

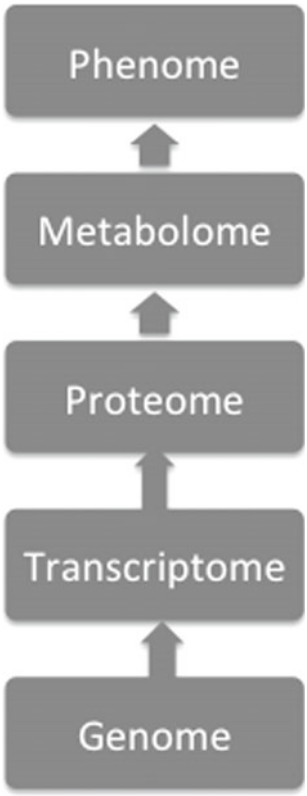

Fig. 1 Scheme of the traditional view of a bottom-up hierarchical system exhibiting upward causation

alone cannot explain higher-level behavior, since the concepts employed are simply not appropriate to the above-mentioned types of causality. Higher-level entities have causal power in their own right, which partially determine what happens at lower levels in the hierarchy (Ellis 2012). Thus, the upper levels of the system organization work as a framework for entities of the lower levels (Ahl and Allen 1996).

As discussed above, regardless the epistemological perspective, it is very common in biological sciences to think in terms of hierarchy of levels, with genes occupying the lowest level, and the networks of proteins and metabolites, organelles, cells, tissues, organs representing different intermediate levels, and the organism as a whole occupying the highest level of an individual organization. The reductionist causal chain is, then, represented in a single bottom-up direction (Fig. 1). However, there is no literal sense in which genes are below cells, for example. In fact, the only reason for DNA sequences to be at the bottom of the hierarchy is that they exist on a smaller (i.e., molecular) scale in biological systems. The formation of networks, cells, tissues, and organs should be seen as the creation of processes at different scales (Noble 2012).

The central dogma of molecular biology itself (Crick 1970) leads us to a bottom-up view of systems biology by stating that information flows from DNA to RNA, RNA to proteins, which can then form protein networks, and so on through biological levels until it reaches the whole organism. That is, from the knowledge about genes and proteins, we could reconstruct all other levels (Noble 2008a). Actually, the simple idea of a "dogma" is a contradiction, since in science there is no "truths" and the knowledge is in continuous movement of construction.

Contrary, many evidences seem to lead to the idea that the development of very complex systems, such as those in biology, requires downward causation in order to construct the necessary biological information (Kuppers 1994; Roederer 2005). This information cannot be originated in an ascending way because it implicitly incorporates information about environmental niches. It would be different in a different environment. Thus, upper-level conditions influence what happens on the lower scales, even if those lower scales do the work. This is what Ellis (2012) characterizes as top-down causation, in a similar way as downward causation (Noble 2008a).

For instance, DNA sequences form a list of proteins and RNAs that can be made in a given organism, as well as regulatory elements incorporated within it. These regulatory elements allow the regulation of gene group expression to be coordinated (Jacob 1960). Therefore, the genome behaves as a database that can be organized in response to cellular and environmental signals (Shapiro 2011). However, what proteins and RNAs will be produced at what time and place is not fully specified. DNA sequences do absolutely nothing until they are triggered to do so by a variety of transcription factors, which activate and deactivate genes, and various other forms of epigenetic control. All of these, and the processes of cells, tissues, and organs that determine when they are produced and used, "control" the genome. That is why it is possible for several different cell types to originate using exactly the same genome. A heart cell is made using precisely the same genome at its core as the various cells in the human body (Noble 2008a).

This does not imply that this bottom-up approach is mostly wrong. It is conceivable that it may function in some cases in simpler organisms, such as prokaryotes. This simply means that this approach and the vast amount of data coming from genomic, transcriptomic, and proteomic techniques need to be complemented by other approaches. In the case of eukaryotes, many of the interactions among the components are restricted by the complex cellular structure, including a system of membranes and organelles. The direct approach would necessarily include such structures, in which case it is no longer purely from the bottom up because, as we have already seen, many of these structural features are inherited independently of the DNA sequences. Higher levels of DNA and proteins would be required for successful modeling (Kohl et al. 2010).

One of the most classic examples to report the importance of non-genomic elements in biological processes was demonstrated and discussed by Noble (2012) using heart rate. Heart rate is generated by the electrical potential in nerve cells and is constituted by the so-called Hodgkin cycle of the oscillating ion current through a membrane. The oscillations occur through channels of proteins that cross the cell membrane. However, the control of these channels is, in turn, controlled by cell

potential (a cell-level parameter) that is influenced by intercellular coupling and the dynamics of other processes across the membrane. Consequently, the behavior of the system cannot be understood from an analysis of the constituents alone or on the lower scale. This is a multi-level loop. Potential is a cell-level parameter; the openings and closures of the ion channel are protein level parameters. With rupture of feedback (downward causality) the cellular potential, ion channel gating, and cellular rhythm are eliminated (Hodgkin and Huxley 1952; Gross and Green 2017). The sequence of events, including feedback between cell potential and protein activity, is simply heart rate. It is a property of the interactions between all components of the system. It does not make sense to talk about heart rate at the protein and DNA levels, and it makes no sense to assume there is a separate program that "runs" the rhythm. Without the restrictions imposed by the cellular structure itself, mainly of the membranes and their compartments, besides other processes that maintain the ionic concentrations, the rhythm would not occur. There is, therefore, a complex and continuous feedback between processes at different levels (Noble 2012).

There are several other examples of biological processes regulated by feedbacks between two or more levels. In *Arabidopsis thaliana*, the circadian rhythm is regulated by three interlocking feedback loops, involving several transcription factors that, according to the time of day, repress or activate other genes, functioning as links between environmental factors and the regulation of gene expression (Alabadí et al. 2001; McClung 2006; Nakamichi et al. 2010; Nusinow et al. 2011; Sanchez and Kay 2016). In addition to the transcription–translation feedback cycles, the circadian rhythm is regulated by many other mechanisms, including hormones, epigenetics, and post-transcriptional and post-translational modifications (Seo and Mas 2014; Nolte and Staiger 2015; Romanowski et al. 2015), in addition to the communication between organelles (nucleus–chloroplast) (Hassidim et al. 2007). Both phytochromes and cryptochromes provide light input to the clock, although the signal transduction pathways are incompletely defined. Interestingly, photoreceptor expression is itself rhythmic, indicating that the clock gates its sensitivity to light (e.g., Tóth et al. 2001), although bulk phytochrome protein levels do not oscillate (Sharrock and Clack 2002). However, the interaction and hierarchical structures between levels of regulation still need to be clarified (Sanchez and Kay 2016).

In addition, there are several other mechanisms that demonstrate the importance of downward causation (top-down regulation). These include triggers of cell signaling (via hormones and transmitters), control of gene expression (via transcription factors), and the protein machinery that reads genes and continually repairs copying errors and makes the genome reliable and epigenetic control (Noble 2012).

Genes store hereditary information, but their actual expression usually depends on other factors, both physical and chemical, external to DNA. The term epigenetic was coined to denote this higher level, extra-DNA, activity level, and control. Epigenetic changes can occur due to chemical signals received from other genes, or from chemical and physical signals originating from other cells, organs or the external environment. These signals can serve to activate or deactivate genes using molecular markers or the physical rearrangement of DNA (Davies 2012).

Additionally, genes can be switched off, not only by proteins that bind to DNA, but also by several small molecules attached to specific points in the DNA. For example, the methyl group is often used to effectively mute a gene by being attached to the GC sites. The methylation pattern in a genome can be copied and inherited by the daughter cell, ensuring that the same genes are active in stem cells and daughters of the same type. This is an example of epigenetic heritage. Methylation of DNA may itself physically prevent binding of transcriptional regulators to the gene, and more importantly, methylated DNA participates in the formation of chromatin through interactions with various other epigenetic modifications such as the histone code, polycomb complexes, nucleosome positioning, non-coding RNA, and ATP-dependent chromatin remodeling proteins (Wade 2001; Suzuki and Bird 2008; Jin et al. 2011). Other epigenetic mechanisms involve several post-translational modifications of proteins. For example, histones, which will form nucleosomes, have small attached tails, which can be modified in this way, leading to thousands of different variations in the tail pattern of each nucleosome. Modification of specific histone tails is associated (though not necessarily) with altered chromatin structure and behavior (Henikoff and Shilatifard 2011).

For genetic instructions to be read from DNA, the reading machine must have physical access to the gene in question. This is difficult if the gene is physically inaccessible within a highly compacted structure. Chromatin is therefore incessantly compacted and uncompressed to expose specific genes that need to be transcribed at a given time. The genetic signals originating from DNA and their biological consequences depend, therefore, on the three-dimensional organization of the chromatin (Davies 2012). An important example of the physical structure of chromatin is given by cancer cells, in which chromatin can become drastically and visibly reorganized in the nucleus, a gross abnormality that correlates with the oncogenes being expressed and the tumor suppressor genes being switched off (Misteli 2010). Some chromatin architecture can also be inherited, providing yet another form of epigenetic cell memory. The silencing of genes due to this physical factor seems to be a crucial feature of the regulation and control of eukaryotic genes. Chromatin marks the intersection of causality up and down, because its structure and behavior are influenced by both the genes it contains and by the macroscopic forces acting on it from the rest of the cell and the cell environment (Davies 2012). Recently, evidence has also emerged that the dynamics of nucleosomes along the chromatin chains have functional importance for epigenome maintenance, gene regulation, and control of DNA replication (Deal et al. 2010). Davies (2012) defines this genome–epigenome relationship brilliantly: "In a nutshell, genetics deals with what genomes are and epigenetics deals with what they do."

However, unlike the genome, which has a "physical headquarters" in DNA molecules, in the case of epigenetics, there is no physical "seat," no physical place from which epigenomic instructions emerge. The epigenome should not be found in one location and the final source of epigenetic information cannot be localized specifically; instead, it is distributed throughout the cell. To be sure, the epigenome is manifested in particular structures (histone tails, nucleosome patterns, methylation patterns, and chromatin packing), but it does not originate there. The epigenome is

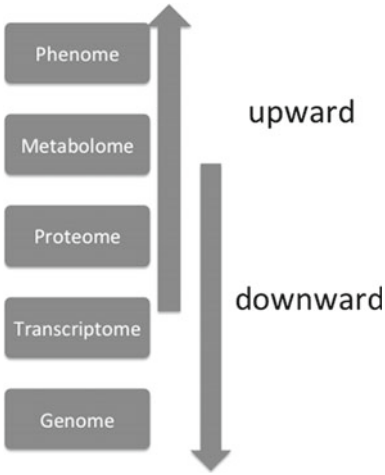

Fig. 2 Scheme showing the perspective of a hierarchical system exhibiting no privilege level of causation. Both upward and downward causation events regulate the whole systems organization altogether

everywhere and nowhere; it is a global, systemic entity. Expressed more starkly, the epigenome is a "virtual object" (Davies 2012).

As we have seen so far, top-down causality occurs because of the crucial role of context in influencing the lowest level organization. Higher levels of organization restrict and regulate lower-level interactions. A key feature here is the multilevel capability of performing higher-level functions and the consequent existence of lower-level variable equivalence classes with respect to higher-level actions. An equivalence class identifies all lower-level states that correspond to the same top-level state. A European team (Wegscheid et al. 2006) performed in vivo complementation experiments on the bacteria *Escherichia coli* and *Bacillus subtilis*, which have significantly different conformational structures that are associated with important biophysical and biochemical differences in vitro. However, in spite of these structural and functional differences in vitro, it has been shown that they can be substituted in vivo without loss of functionality, at least under standard growth conditions, demonstrating functional equivalence. This shows that the traditional reductionist view is insufficient to explain complex biological phenomena that involve different scales of organization, and downward causation (Fig. 2) plays an essential role on biological organization.

Summarizing, Noble (2008b) proposed some principles, among others, of the system biology approach: 1—biological functionality is multi-level; 2—transmission of information in not one way; and 3—theory of biological relativity: there is no privileged level of causality.

Therefore, according to Noble (2008b) "*it is simply a prejudice that inclines us to give some causal priority to lower-level, molecular events. The concept of level in biology is itself metaphorical. There is not literal sense in which genes and proteins lie underneath cells, tissues and organs. It is a convenient form of biological classification to refer to different levels, and we would find it very hard to do without the concept. But we should not be fooled by the metaphor into thinking that 'high'*

and 'low' here have their normal meanings. From the metaphor itself, we can derive no justification for referring to one level of causality as privileged over others. That would be a misuse of the metaphor of level."

Moreover, there is a relationship between decreasing restrictions among different organization scales in a biological system insofar the highest levels, somehow, restrict the organization of the lowest levels (Korn 1999). This relationship can be correlated to system regulation, which creates greater global robustness (general stability system) and a buffer effect between the superior levels and the inferior ones in relation to the potential destructive effects of environmental disturbances (Ahl and Allen 1996). For instance, the study of Vítolo et al. (2012) showed that the deleterious effects of high temperature on plants were expressed most on growth level, indicating that, somehow, the effects on plant growth acted as a buffer allowing photosynthetic maintenance. The understanding of the precise processes that allow such buffer effects needs future attention, but Souza et al. (2009) suggest that such buffer control could be related to changes in the links among plant subsystems. Robustness of the system is related to a spatial–temporal disconnection between the different levels of organization (Rojdestvenski et al. 1999), which confines the environmental disturbances into certain modules (subsystems) of the system that are directly influenced by the external environment. This could be reached by increasing the number of weak links among network motifs (Csermely 2006).

Stability as a Trade-off Between Plasticity and Robustness: There Is no Privileged Level of Causation

Biological stability has been defined as a global emergent property from complexity, diversity, and plasticity according to the "quadruped" model of Souza and Lüttge (2015). In this sense, stability is a dynamical property allowing biological systems to face different environmental conditions by interchanging strategies to acclimate. There is no unique solution to deal with external disturbances, regardless of the specific situation; plasticity (flexibility) of metabolic networks or resistance to change by homeostatic mechanisms (robustness) could work properly well for different types of organisms. For instance, different plant species can show different strategies to face water shortage, which is one of the most limiting factors for plants life. In the same environment, while some species maintain their water status relatively constant controlling water loss by an efficient stomatal dynamics of closing and opening (*isohydric* species), other species support significant water content fluctuations without constrains on their physiological functioning (*anisohydric* species) (Tardieu and Simonneau 1998; Braga et al. 2016). However, on the other hand, different species can show a convergence of strategies to maintain stability, as observed by Souza et al. (2009). In a study with different tropical tree species (early and late successional), growing in environments significantly different (understory and gap conditions), the same pattern of physiological network connectance was observed, indicating that

there is a conservative pattern of photosynthesis regulation based on network modulation and environmental coupling, regardless of plant species. This suggests that changes in network connectance may not be specific of a functional group but rather a more general response to environmental fluctuations, strongly related to system stability (Souza et al. 2009).

The ability of a system to maintain its organization stable when subjected to external changes relies on the capacity to control networks properties. The system simply "absorbs" the effects of the perturbation, as a network, by compensating it through internal reciprocal adjustments between tightly coupled constraints, together with the metabolites and reaction processes involved, while the whole dynamics is maintained in the initial attractor or shifts to a new available one (metastability) (Souza and Lüttge 2015; Bich et al. 2016). In the literature, this kind of response is usually referred to as dynamic stability (Rosen 1970), the capability to counterbalance the displacement of the system from a certain initial state, provoked by a perturbation, and end up in the same final state. On the other hand, regulatory control requires a subsystem that is sufficiently independent of the dynamics of the controlled processes, and which can be altered without disrupting these processes (Bechtel 2007; Bich et al. 2016). A regulatory control is that the former, in order to reach a compensatory response, the changes from many local processes may need to cross the entire system, thus the time taken to implement a response can be proportional to the size of the system. On the other hand, regulatory control allows a decoupled subsystem to induce the appropriate collective pattern of behavior in a more rapid and efficient way. Its compensatory action is more efficient and robust because, instead of involving each time the progressive accommodation of the constitutive organization to the change, it can rapidly switch between available regimes through dedicated mechanisms, acting in relation to specific perturbations. The efficiency of this kind of response is not negatively affected by the size of the system (Bich et al. 2016).

The stability of the constitutive regime against disturbances can also be improved by the contribution of additional interactions or mechanisms that are still part of the basic metabolic network and self-maintenance through feedback events, which are therefore considered as the essence of regulation. Thus, feedbacks are especially relevant to the stability of a system because they perform circular causal relationships between the output of the system and one or more of its inputs, such that the functioning of the system becomes dependent on the effects of its own actions. This type of response depends only on the interaction between the components, reactions, and control subsystems that already participate in the constitutive regime, without resorting to additional and dedicated mechanisms. When biological systems are found in dynamically stable regimes, they present a basic form of robustness as an inherent ability to respond to perturbations through highly distributed endogenous compensation patterns to remain within their viability region (Bich et al. 2016).

Because organisms constantly face changing environmental conditions, they have developed systems that allow phenotypic changes or prevent them. At one extreme, as quoted above, there are some traits that exhibit little or no phenotypic change despite an environmental challenge. These traits are robust (Waddington 1961; Lempe et al. 2012). At the other extreme, there are traits that exhibit a high degree of plastic-

ity—programmed changes in the phenotype in response to different environments (Pigliucci 2001), which are important for physical fitness.

Within the biological networks, there are at least two components that influence the position of a trait in the robust-to-plastic spectrum: redundancy and topology of the interactions. Redundancy influences phenotypic robustness and plasticity. For example, duplication of a gene may provide robustness through a redundant function or may promote plasticity through its subfunctionalization. In the same way, the network topology also influences phenotypic robustness and plasticity. In most of the existing literature, the modulation of robustness and plasticity has been attributed to specific mechanisms. Ultimately, traits are shaped by a blend of robustness and plasticity acting at different times (Lachowiec et al. 2015).

In many species, the robustness in several traits has been attributed to the function of genes that play crucial cellular functions and are involved in cellular homeostasis (Queitsch et al. 2002; Levy and Siegal 2008). Examples include chaperone proteins (Rutherford and Lindquist 1998; Queitsch et al. 2002; Yeyati et al. 2007), genes that function in proper maintenance of the genome, and genes that modify the expression of many others (Lehner et al. 2006; Levy and Siegal 2008). Importantly, these genes may affect several networks and specific regulatory motifs without being an integral component of these networks (Whitacre 2012; Lachowiec et al. 2015).

Hsp90 (Heat shock protein 90) is a highly connected and evolutionarily conserved protein chaperone that facilitates the maturation of certain proteins (clients) and keeps them poised for activation (Zhao et al. 2005; McClellan et al. 2007; Jarosz and Lindquist 2010; Gopinath et al. 2014). Hsp90 clients, among them many kinases, TFs and receptors, are conformationally flexible, which is the property that the chaperone is thought to recognize (Taipale et al. 2010). The role of Hsp90 in maintaining phenotypic robustness (Rutherford and Lindquist 1998; Queitsch et al. 2002; Sangster et al. 2007; Yeyati et al. 2007; Jarosz and Lindquist 2010) is fundamentally an epistasis phenomenon on a network scale. The interaction of Hsp90 with its many clients maintains robustness, yet the chaperone's property of enabling signal transduction proteins to perceive and transduce signals is also of fundamental importance for phenotypic plasticity, in particular programmed plasticity. Environmental stresses such as increased temperature, drought, oxidative stress, and altered conductivity compromise protein folding and increase demands for Hsp90. Under these conditions, Hsp90 clients will be less functional and the genetic network will be perturbed at many nodes. Hence, it is not surprising that Hsp90 function is environmentally responsive. Hsp90 levels are strongly upregulated in conditions that compromise protein folding to bolster protein homeostasis (Gerspacher et al. 2009; Cid et al. 2010; Park et al. 2010; Pratt et al. 2010; Sekimoto et al. 2010; Stensløkken et al. 2010).

The classical response to thermal shock (stress) (HSR) was attributed to denaturation of proteins, including membrane proteins. However, induction of HSPs occurs in many circumstances where protein denaturation is not observed (de Marco et al. 2005). Recently, considerable evidence has been accumulated in favor of the "Membrane Sensor Hypothesis," which predicts that the level of HSPs can be altered as a result of changes in the plasma membrane. Small temperature stresses can modu-

late significantly transient receptor potential (TRP), an important group of cationic channels that play an important role in sensory physiology and some members are very sensitive to temperature, and are also influenced by lipids. In addition, stress hormones often modify plasma membrane structure and function and thus initiate a cascade of events, which may affect HSR. The major transactivator heat shock factor-1 (HSF) integrates the signals originating from the plasma membrane and orchestrates the expression of individual heat shock genes (Török et al. 2014).

Members of the HSF family control the expression of HSPs. Under optimal growth conditions, HSF1 shuttles as a latent monomer between the cytoplasm and the nucleus in complex with Hsp90, p23 and immunophilin. Upon elevated temperatures, this complex disintegrates; HSF1 accumulates in the nucleus, quickly undergoes several post translational modifications, trimerizes as a homo- or heterotrimer together with HSF2 and binds to its recognition site (heat shock element, HSE) in the HSP-promoter region and ultimately drives HSP expression (Sandqvist et al. 2009; Anckar and Sistonen 2011). Understanding of how the signal from heat perception at the plasma membrane is transduced toward HSF1 activation, ultimately driving the expression of HSPs, is still incomplete and fragmentary. However, over the last decades a complex interplay of multiple pathways emerged in which derivatives of plasma membrane phospholipids, sphingolipids, and cholesterol all play important roles (Török et al. 2014).

The biochemical process underlying cells organization, in particular the role of the enzymes in biosynthesis, results in a complex non-linear dynamics, since a single enzyme may function in many interconnected enzymatic pathways. Moreover, the diffusion and the number of signaling molecules (e.g., plant hormones) among developing cells follows a complex self-organized non-homogenous dynamics, inducing minimal differences among cells (Choi et al. 2016). Nonlinear complex systems, such as cells whole metabolism, are sensitive to small perturbation, mainly under suboptimal conditions. Thereupon, random perturbations tend to become magnified, increasing the differentiation among groups of cells and creating independence from each other (Møller and Swaddle 1997). Moreover, the complex electrical network, named "electrome," underpinning cellular activities, shall display a major role on the whole system organization (De Loof 2016). For instance, recent analyses of plant electrome (Saraiva et al. 2017; Souza et al. 2017) have uncovered the high complexity involved in plant electrophysiological dynamics. The electrical signaling flowing through plant's body exhibits a range of color noises, with short time correlation, varying from reddened to black noise, showing that the temporal dynamics of the micro-volted electrical signals are not random, with some traits similar to human brain EEG. Furthermore, such complexity can be affected by environmental stimuli (Saraiva et al. 2017), for instance, decreasing complexity under stressful conditions. Moreover, under some specific constraining conditions (for instance, under drought), plant electrome can be pushed to a self-organized critical state, showing bursts of electrical "spikes" without a characteristic size (Souza et al. 2017).

Overall, as aforementioned, such "epigenetic noise" (including epi-regulation of gene expression, short and long-distance signaling regulating different scales within the system) allows a variation of responses, from the cell to the whole plant level,

to a plethora of environmental cues. Such plasticity and complexity, as well as the diversity of individual components, are the bases of system stability, conferring multi-functional regulatory capacity for plants, without a unique direction of causation (Noble 2008b; Souza and Lüttge 2015).

Concluding Remarks

As exemplified above, notwithstanding the environment triggering responses in the organism, the pattern of such responses is determined by the internal dynamics of the system itself. This internal dynamics is integrated in a complex metabolic network that operates out of some rules of interactions. The rules that specify the interactions among the system components are performed using local information, without reference to a pre-existing global pattern (Camazine et al. 2001), which can be spreading throughout the whole system by different processes of signaling (Choi et al. 2016). In complex systems, such interactions are typically non-linear processes based on negative and positive feedback loops. The negative feedback plays a crucial role in maintaining homeostasis of the system, whereas the positive feedback operates propagating and amplifying signals throughout the system. Both processes work together in the formation and stabilization of new patterns of organization, which makes the prediction of their global behavior difficult (Camazine et al. 2001; Lüttge 2012). There are multiple feedbacks from higher levels to lower levels in addition to those from lower to higher levels. Through the existence of multiple back-up mechanisms, many DNA changes, such as knockouts, do not have a phenotypic effect on their own (Noble 2011). For instance, about 80 per cent of the knockouts in yeast are normally "silent" by alternative metabolic pathways (Hillenmeyer et al. 2008). Such a dynamical process of organization, operating through the different scales of the organization of the system, produces stable emergent properties and, hence, makes the system as whole non-reducible to its components at smaller scales of organization (Souza et al. 2016b).

The limitations of the reductionist molecular approach have become increasingly evident. It became clear that biological systems cannot be explained only at the genetic level. Instead, they should be understood as complex systems resulting from dynamic interactions of different components at different levels, each individually functioning as wholes, which eventually control the phenotype. Complex systems exist at different levels of biological organization ranging from the subatomic realm to individual organisms to whole populations and beyond. Hence, a need arose for an integrative framework, which provides a holistic understanding of the biological systems (Sheth and Thaker 2014).

Finally, according to Noble (2011) a downward form of causation is not a simple reverse form of upward causation; it is better seen as completing a feedback circuit. "*There should be no dogmas when it comes to causation in biological systems.*"

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