Spring 2018 – Systems Biology of Reproduction
Discussion Outline (Systems Biology)
Michael K. Skinner – Biol 475/575
Weeks 1 and 2 (January 18)

Systems Biology

Primary Papers

1. Westerhoff & Palsson (2004) Nat Biotech 22:1249-1252
2. Joyner (2011) J Appl Physiol 111:335-342
3. Bizzarri et al., (2013) Progress in Biophysics and Molec Biol 112:33-43

Discussion

Student 1 - Ref #1 above
-How does this support evolutionary systems biology?
-What was the convergence discussed?
-Give an example that supports this perspective.

Student 2 - Ref #2 above
-What is the problem with reductionism?
-What is the void?
-What is the solution?

Student 3 - Ref #3 above
-What local processes are involved in a system?
-What is the future fate of reductionism?
-Is systems biology a simple extension of molecular biology?

nature biotechnology

The evolution of molecular biology into systems biology

Hans V Westerhoff[1] & Bernhard O Palsson[2]

Systems analysis has historically been performed in many areas of biology, including ecology, developmental biology and immunology. More recently, the genomics revolution has catapulted molecular biology into the realm of systems biology. In unicellular organisms and well-defined cell lines of higher organisms, systems approaches are making definitive strides toward scientific understanding and biotechnological applications. We argue here that two distinct lines of inquiry in molecular biology have converged to form contemporary systems biology.

Whereas the foundations of systems biology-at-large are generally recognized as being as far apart as 19th century whole-organism embryology and network mathematics, there is a school of thought that systems biology of the living cell has its origin in the expansion of molecular biology to genome-wide analyses. From this perspective, the emergence of this 'new' field constitutes a 'paradigm shift' for molecular biology, which ironically has often focused on reductionist thinking. Systems thinking in molecular biology will likely be dominated by formal integrative analysis going forward rather than solely being driven by high-throughput technologies.

It is, however, incorrect to state that integrative thinking is new to molecular biology. The first molecular regulatory circuits were mapped out over 40 years ago. The feedback inhibition of amino acid biosynthetic pathways was discovered in 1957 (refs. 1,2), and the transcriptional regulation associated with the glucose-lactose diauxic shift led to the definition of the *lac* operon and the elucidation of its regulation[3]. With the study of these regulatory mechanisms, admittedly on a small scale, molecular biologists began to apply systems approaches to unravel the molecular components and logic that underlie cellular processes, often in parallel with the characterization of individual macromolecules. High-throughput technologies have made the scale of such inquiries much larger, enabling us to view the genome as the 'system' to study. Thus, the popular contemporary view of systems biology may be synonymous with 'genomic' biology.

This article discusses two historical roots of systems biology in molecular biology (**Fig. 1**). Although we briefly outline the more familiar first root—which stemmed from fundamental discoveries about the nature of genetic material, structural characterization of macromolecules and later developments in recombinant and high-throughput technologies—more emphasis is placed on the second root, which sprung from nonequilibrium thermodynamics theory in the 1940s, the elucidation of biochemical pathways and feedback controls in unicellular organisms and the emerging recognition of networks in biology. We conclude by discussing how these two lines of work are now merging in contemporary systems biology.

Scaling-up molecular biology

In the decades following its foundational discoveries of the structure and information coding of DNA and protein, molecular biology blossomed as a field, with a series of breathtaking discoveries (**Fig. 1**). The description of restriction enzymes and cloning were major breakthroughs in the 1970s, ushering in the era of genetic engineering and biotechnology. In the 1980s, we began to see the scale-up of some of the fundamental experimental approaches of molecular biology. Automated DNA sequencers began to appear and reached genome-scale sequencing in the mid-1990s[4,5]. Automation, miniaturization and multiplexing of various assays led to the generation of additional 'omics' data types[6,7].

The large volumes of data generated by these approaches led to rapid growth in the field of bioinformatics, again largely emanating from the reductionist perspective. Although this effort was mostly focused on statistical models and object classification approaches in the late 1990s, it was recognized that a more formal and mechanistic framework was needed to analyze multiple high-throughput data types systematically[8,9]. This need led to efforts toward genome-scale model building to analyze the systems properties of cellular function.

Molecular self-organization

Even before the first key events in the history of molecular biology, several lines of reasoning revealed that integration of multiple molecular processes is fundamental to the living cell. Biochemical processes necessitate the production of entropy (chaos in the thermodynamic sense) as driving force. The paradox felt by many, but expressed by Schrödinger in his war-time lectures[10], was how one could explain the progressive ordering that occurs in developmental biology (that is, the 'self-organization,' decrease in chaos) when entropy ('chaos') must be increased.

The answer was that one process could produce order (negative entropy or negentropy) provided it was coupled to a second process that produced more chaos (entropy): coupling, another word for integration of processes, is therefore essential for life. Onsager[11] provided the basis for this concept by stressing the significance of the coupling of dissimilar processes. He is also relevant because he discovered a law for such systems of coupled processes: close to equilibrium the dependence of the one process rate on the driving force of the other process should equal the dependence of the other process rate on the

[1]Departments of Molecular Cell Physiology and Mathematical Biochemistry, BioCentrum Amsterdam, De Boelelaan 1085, NL-108, HV Amsterdam, the Netherlands. [2]Department of Bioengineering, University of California-San Diego, 9500 Gilman Drive, La Jolla, California 92093-0412, USA. Correspondence should be addressed to H.V.W. (hw@bio.vu.nl) or B.O.P. (palsson@ucsd.edu).

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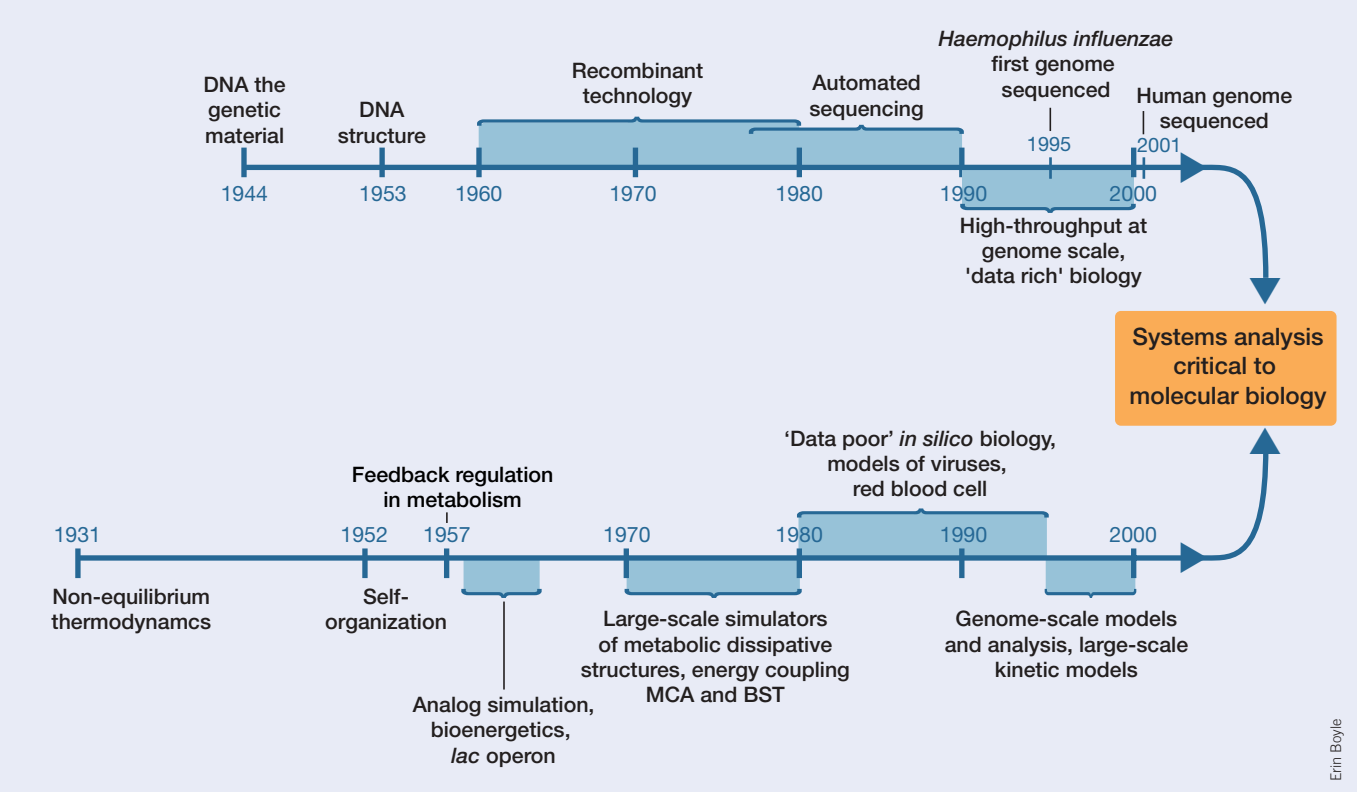

Figure 1 Two lines of inquiry led from the approximate onset of molecular biological thinking to present-day systems biology. The top timeline represents the root of systems biology in mainstream molecular biology, with its emphasis on individual macromolecules. Scaled-up versions of this effort then induced systems biology as a way to look at all those molecules simultaneously, and consider their interactions. The lower timeline represents the lesser-known effort that constantly focused on the formal analysis of new functional states that arise when multiple molecules interact simultaneously.

former driving force. Caplan, Essig and Rottenberg[12] later defined a coupling coefficient, which quantifies the extent to which two processes are coupled in a system and showed that this coefficient must range between 0 and 1.

These approaches were called nonequilibrium thermodynamics and constituted a prelude to systems biology at the cell and molecular levels in that they (i) dealt with integration quantitatively and (ii) aimed to discover general principles rather than just being descriptive. An improved procedure for describing ion movement and energy transduction in biological membranes, termed mosaic nonequilibrium thermodynamics, further progressed towards systems thinking in that it (iii) established a connection to molecular mechanisms and (iv) enabled the determination of the stoichiometry of membrane energy transduction from system data[13]. Peter Mitchell's[14] chemiosmotic coupling principle was another early case of systems analysis in cell and molecular biology. It stated that ATP synthesis was coupled in quite an indirect way to respiration, involving an entire intracellular system, including a volume surrounded by an ion-impermeable membrane and proton movement across it. Indeed, for eukaryotes, this provided much of the rationale for the organization of the mitochondrion. In his calculations verifying that that the proposed chemiosmotic mechanisms transferred sufficient free energy to empower ATP synthesis, Mitchell demonstrated the sort of quantitative thinking that would eventually prove crucial to the study of biochemical systems[14].

The problem of biological self-organization was to understand how structures, oscillations or waves arise in a steady and homogenous environment, a phenomenon called symmetry breaking. Turing[16] led the way, but the Prigogine school[17] and others developed the topic from the perspective of nonequilibrium thermodynamics in molecular contexts such as biochemical reactions involved in sugar metabolism (glycolysis). They demonstrated how having a sufficient number of nonlinearly interacting chemical processes in a single system such as the Zhabotinski reaction, a developing tissue, or glycolysis, could lead to symmetry-breaking as a result of self-amplification of random fluctuations. Of course, more recent molecular developmental biology studies have shown that reality is even more complicated; prespecification by external (maternally specified) gradients of morphogens may substitute for the random fluctuations, increasing the robustness of development[18]. Perhaps more importantly, Prigogine searched for and found a law (on minimum entropy production). Although it is strictly valid only in Onsager's near-equilibrium domain, it testified to the systems scientists' quest for the principles underlying systems, rather than just for their appearances.

Early on, oscillations in yeast glycolysis were the experimental systems of choice. Although intact cells were studied[19], more often measurements were made using cell extracts[20]. Reductionist biochemical thinking proclaimed that a single pacemaker enzyme should be responsible for the oscillations. Only relatively recently has systems-based analysis in one of our laboratories (H.V.W.) been used to reveal that the oscillations are simultaneously controlled by many steps in the intracellular network[21] and how the oscillations in the individual cells synchronize actively[22]. Of course, with the more recent experimental capability to inspect single cells dynamically, more and more cells are

seen to exhibit asynchronous oscillations of all sorts and some of these cases are up for systems biology analysis. Slime mold aggregation was another early case where a network of reactions was shown to be essential for systems biology reaching one step beyond cell biology, again by combining mathematical modeling with experimental molecular information[23].

Building large-scale models

Following the events of the late 1950s and early 1960s, researchers undertook efforts that were not well publicized and formulated mathematical models to simulate the functions of newly discovered regulatory circuits in cells. Even before digital computers became available, simulations of integrated molecular functions were performed on analog computers[24]. These efforts grew in scale to dynamic simulation of large metabolic networks in the 1970s[25–27]. Following the pathway-centered kinetic models in the seventies[28], cell-scale flux models of the human red cell were published by the late 1980s (ref. 29), and by the early 1990s genome-scale models of viruses and large-scale models of mitosis were formulated[30]. With the advent of genome-scale sequencing, the first genome-scale, constraint-based metabolic models for bacteria were constructed[31]. These models describe reconstructed networks and their possible functional states (phenotypes) and are now available at the genome-scale for a growing number of organisms. They treat the 'genome' as the 'system.'

Progress toward the development of detailed kinetic models at a large scale has proven to be slower. Some of these models approach computer replicas of pathways of metabolism, signal transduction and gene expression, and are active on the web, ready for experimentation and integration (compare http://www.siliconcell.net/). Obtaining *in vivo* numerical values for kinetic constants remains a key challenge.

Metabolic control analysis

We have agreed that contemporary systems biology has an historical root outside mainstream molecular biology, ranging from basic principles of self-organization in nonequilibrium thermodynamics, through large-scale flux and kinetic models to 'genetic circuit' thinking in molecular biology. 'Systems thinking' differs from 'component thinking' and requires the development of new conceptual frameworks.

Metabolic control analysis (MCA), developed in the early seventies[28,32], presented a key example of approaches to characterize properties of networks of interacting chemical reactions. At this time, thinking in biochemistry was dominated by the concept that there had to be a single 'rate-limiting' step at the beginning of all metabolic pathways. Criteria used to establish whether a given enzyme was rate-limiting referred to it as being far from equilibrium, strongly regulated by various metabolic factors or causing pathway flux to decrease when inhibited.

However, the application of these criteria to some metabolic pathways suggested that they contained more than a single rate-limiting step. Network thinking through MCA helped to resolve this paradox. First, mathematical models of metabolic pathways were developed both for inspiration and discovery, and subsequently used to check numerically the principles they conjectured[28,32]. Second, quantitative definitions were developed to describe the extent to which a step limited the flux through a pathway. This 'flux-control coefficient' of a particular step corresponded to the sensitivity coefficient of the pathway flux with respect to the activity of the particular enzyme. Third, these investigators looked for proof of the concept that there should be a single rate-limiting enzyme in a pathway that should have a flux-control coefficient of unity, with all others having flux control coefficients of zero. Instead, they found a theorem stating that all the flux-control coefficients must sum to unity[28,32]. This result then suggested that there need not be a single rate-limiting step to a pathway and that instead many enzymes can contribute simultaneously to the control of the network. Thus, control was not a component property but a network property. The network nature of regulation was shown experimentally to be the case for mitochondrial ATP generation, where control was indeed distributed over more than three steps, and quite notably not particularly strong, neither for the first nor for the irreversible step of the pathway[33].

An important aspect of systems biology is to relate the system properties to the molecular properties of components that comprise a network. The kinetics-based sensitivity analysis by MCA, and its close relative, biochemical systems theory proposed by H.V.W and Chen[34], showed that by focusing on the properties of an individual component, one cannot properly decipher its role in the context of a whole network. The connectivity laws proven by MCA[28,34] (see other references in ref. 35) pinpointed how that distribution of control relates to network structure and the kinetic properties of all network components simultaneously. Similarly, the topological analyses of network structure by our groups[31,36] have revealed the existence of network-based definitions of pathways that can be used mathematically to represent all possible functional states of reconstructed networks[37]. Thus, a growing number of methods now exist to analyze the properties mathematically of the large-scale networks that we are now able to reconstruct based on high-throughput data.

Convergence

Figure 1 presents our interpretation of the history of systems analysis in cell and molecular biology. Events in the upper timeline have been much more to the fore of scientific thinking than those in the lower timeline. In one sense, the dazzling stream of discoveries and exciting technologies (most recently with genome-wide data) provides the 'biology' root to contemporary systems biology. In contrast, scientific progress in the lower timeline has never gained much notoriety, although work in this area was much more prominent in European science throughout this period. This latter branch might be thought of as the 'systems' root of systems biology.

Systems modeling and simulation in molecular biology was once seen as purely theoretical and not particularly relevant to understanding 'real' biology. However, now that molecular biology has become such a data-rich field, the need for theory, model building and simulation has emerged. The systems-directed root always had the ambition of discovering fundamental principles and laws, such as those of nonequilibrium thermodynamics and MCA. This ambition should now extend to systems biology.

All too often, the field has been perceived as just pattern recognition and phenomenological modeling. Systems biology is a thorough science with its own quest for scientific principles at the interface of physics, chemistry and biology, with its remarkable mixture of functionality, hysteresis, optimization and physical chemical limitations. *In silico* analysis of complex cellular processes (whether for data description, genetic engineering or scientific discovery), with its focus on elucidating system mechanisms, has in fact become critical for progress in biology.

The historical dichotomy in approaches to molecular biology must now be reconciled with the need to corral resources and expertise in systems approaches. Although the reductionist molecular biological root has been the focus of a plethora of investigations, literature sources and curricula, the same is not true for the systems molecular biology root. There is now a need for development of theoretical and

analytical approaches, curricula and educational materials to advance understanding of the systems in cell and molecular biology. Unknown to many, the 'pre-online PDF' era contains answers to many of the current challenges and pitfalls facing the field. So although systems biology has an intellectually exciting future ahead of it, the leaders in the field should try to minimize rediscovery and focus on the newer challenges facing us, particularly those that come with the application of existing concepts to genome-scale problems and identification of the new issues that arise from the study of cellular functions on this scale.

Where has this history brought us? We now have the growing and general recognition that systems analysis is important to the future evolution of cell and molecular biology. Some reeducation of workers in the field may be in order (http://www.systembiology.net/). Over the near term, it is likely that successes with practical applications of systems biology will be confined to unicellular systems. We are now seeing successful applications of systems biology to microbes, including pathway engineering (e.g., see our recent publications[37,38]), network-based drug design (e.g., H.V.W. and colleagues[39]), and prediction of the outcome of complex biological processes, such as adaptive evolution (B.O.P and colleagues[40]). Although the mathematical modeling of whole-body human systems cannot yet be linked to genome-wide data and models, data analysis and modeling are likely to contribute to the success of realizing the goal of individualized medicine. Even if we have to rely on less precise models than the currently available genome-scale models of microorganisms, systems biology may soon lead to better diagnosis and dynamic therapies of human disease than the qualitative methodology presently in use.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Edward F. Adolph Distinguished Lectureship

Giant sucking sound: can physiology fill the intellectual void left by the reductionists?

Michael J. Joyner

Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota

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Joyner MJ. Giant sucking sound: can physiology fill the intellectual void left by the reductionists?. *J Appl Physiol* 111: 335–342, 2011. First published June 2, 2011; doi:10.1152/japplphysiol.00565.2011.—Molecular reductionism has so far failed to deliver the broad-based therapeutic insights that were initially hoped for. This form of reductionism is now being replaced by so-called "systems biology." This is a nebulously defined approach and/or discipline, with some versions of it relying excessively on hypothesis-neutral approaches and only minimally informed by key physiological concepts such as homeostasis and regulation. In this context, physiology is uniquely positioned to continue to provide impressive levels of both biological and therapeutic insight by using hypothesis-driven "classical" approaches and concepts to help frame what might be described as the "pieces of the puzzle" that emerge from molecular reductionism. The strength of physiology as a "bridge" between reductionism and epidemiology, along with its unparalleled ability to generate therapeutic insights and opportunities justifies increased attention and emphasis on our discipline into the future. Arguments relevant to this set of assertions are advanced and this paper, which was based on the 2011 Adolph Lecture, represents an effort to fill the intellectual void left by reductionism and improve scientific progress.

homeostasis; regulation; integrative

THIS PAPER REFLECTS IDEAS that were presented as part of the 2011 Adolph Lecture at the Experimental Biology meeting that was held in Washington, DC. The goal of the talk was to share a physiologist's perspective on what reductionism in general and the "omic" revolution in particular has or has not done for biomedical research and associated therapeutic insights or advances. The main ideas highlighted in the lecture were the following.

1) Reductionism via various flavors of molecular biology and "omics" has so far failed to deliver its self-promoted revolution in clinical medicine.

2) Systems biology has a cell-centric focus that is marked by a limited understanding of and application to biology beyond the cell.

3) The failure of systems biology to recognize and use key concepts from physiology about homeostasis, regulation, redundancy, feedback control, and acclimation/adaptation are major limitations to this poorly defined approach.

4) While all the attention has been focused on reductionism and more recently systems biology, physiology continues to provide important biomedical insights that lead to therapeutic advances.

Address for reprint requests and other correspondence: M. J. Joyner, 200 First St. SW, Dept. of Anesthesiology, Mayo Clinic, Rochester, MN 55905 (e-mail: joyner.michael@mayo.edu).

As the title demonstrates, my goal in the Adolph Lecture and in this paper was and is to be intentionally provocative and hopefully generate a dialogue with the reductionists. In this context, and because I am "taking sides", I have adopted what might be called a conversational approach to this paper.

BIOLOGICAL ORTHOPEDIC SURGERY

A key idea or theme that seems to underpin the impetus for reductionism and various flavors of "omics" as applied to biomedical problems might be described as biological orthopedic surgery: "the gene is broken → fix the broken gene → cure the patient." This thinking clearly seems to explain the enthusiasm about gene therapy that emerged after the discovery of the genetic defect responsible for the most common form of cystic fibrosis and more recently ideas about a limited number of common gene variants explaining the risk for common conditions like atherosclerosis and diabetes (10–12, 43, 51). The line of thinking described above flows from what Denis Noble has critically termed "Neo-Darwinian" thinking about the relationship between genes and phenotype (45, 46). It is exemplified by two quotes, the first from 1989 and second from Francis Collins (the current director of NIH), one of the people involved in the cystic fibrosis gene discovery.

> *The implications of this research are profound; there will be large spin offs in basic biology, especially cell physiology, but the largest impact will be biomedical (51).*

Here we are in 1997, eight years later, and the management of her disease has not changed. . . . .But I will predict that in the course of the next 10 years management of CF will change. . . . .The healthy form of the gene itself may even be used in so-called gene therapy (12).

What is interesting to note is that while gene therapy for cystic fibrosis has failed to materialize in the 20+ years since the gene defect was identified, there are traditional ion channel-based drugs that target the CFTR protein in clinical trials that show promise in cystic fibrosis (18, 66). At one level, the development of these drugs was likely facilitated by the genetic discoveries because they permitted the development of models that advanced the understanding of the biophysics and ultimately pharmacology of the defective channel. However, one is tempted to speculate, for cystic fibrosis and perhaps other diseases, that much faster therapeutic progress might have been made if traditional physiological and pharmacological approaches had been a bigger area of focus. Perhaps the optimism and drive for gene therapy was an example of what might be termed "silver bullet" thinking that I will discuss below.

REDUCTIONISM IS SEDUCTIVE

The type of reductionism that I have termed "biological orthopedic surgery" has a number of attractive features and is at some level very seductive. It is easy to understand, and when it delivers it is associated with a heroic narrative by a lone scientist or team of scientists making a fundamental discovery that solves a problem. This is the sort of silver bullet thinking mentioned above. However, it has been known for some time that both the easy to understand elements and heroic narratives associated with reductionism are mirages. In this context, when the factors that contribute to biomedical breakthroughs were subjected to analysis by Comroe and Drips (13) in the late 1960s and early 1970s via the "retrospectoscope," biomedical breakthroughs are in fact more nuanced, incremental, and associated with a more serendipitous view of progress vs. the heroic narrative of reductionism.

HEMOGLOBIN IS A SHIFTY MOLECULE

Homeostasis—the ability to regulate key bodily functions within a narrow range in response to either internal (e.g., exercise) or external (e.g., harsh environmental conditions)—is one of the fundamental (perhaps the fundamental) concept in physiology (7). Homeostasis is also subserved by ideas about regulated systems, feedback control, redundant control mechanisms, and adaptation and acclimation over time. These physiological concepts and mechanisms contribute to what might be described as emergent properties, so that the behavior of the system is far more complex and (and likely more robust) than might be predicted on the basis of a single reductionist property (35).

A good, and early, example of this concept comes from the textbook description about the right shift in the oxygen-hemoglobin dissociation curve that occurs at high altitude or during other forms of hypoxia. The standard teaching is that under these conditions there is a rise in 2–3 DPG that allosterically modifies oxygen-hemoglobin dissociation curve and creates a right shift that facilitates the unloading of oxygen at the tissues. However, when measurements of the oxygen-hemoglobin dissociation curve are made in humans who have traveled to high altitude (Fig. 1), under many circumstances there is in fact a net left shift in the oxygen hemoglobin dissociation curve. This left shift is facilitated by the rise in pH and fall in CO_2 caused by the hyperventilation driven by systemic hypoxia. Additionally, under some circumstances, it is driven further leftward by a fall in body temperature (68). Furthermore, it is of interest to note that all genetically adapted high altitude animals and the human fetus in the hypoxic intrauterine environment also have left shifted oxygen-hemoglobin dissociation curves, some with P50 values in the teens.

These observations make it seem likely that the main adaptive strategy is to shift the oxygen-hemoglobin dissociation curve to the left to facilitate the "loading" of oxygen at the lung in conditions (altitude) where oxygen availability is limited. This strategy also takes advantage of the fact that the mitochondria in the tissues can work efficiently at very low P_{O_2} values (and that under specific needs such as muscular exercise in hypoxia local increases in $[H^+]$ and temperature will reduce the leftward shift in muscle capillaries so that "unloading" of oxygen and tissue O_2 levels can be facilitated). It is also of note that the left shift in the oxygen-hemoglobin dissociation curve has been "known" since at least the 1920s. Along these lines, the sequencing of hemoglobin and the understanding of its biophysical properties was one of the earliest triumphs of what has come to be described as molecular biology (55). However, when the interpretation of such discoveries is too narrow, key physiological insights can be missed. The 2–3 DPG story is also an excellent and early example of how physiology trumps reductionist molecular biology as multiple systems and regulatory strategies interact to regulate homeostasis for the whole organism.

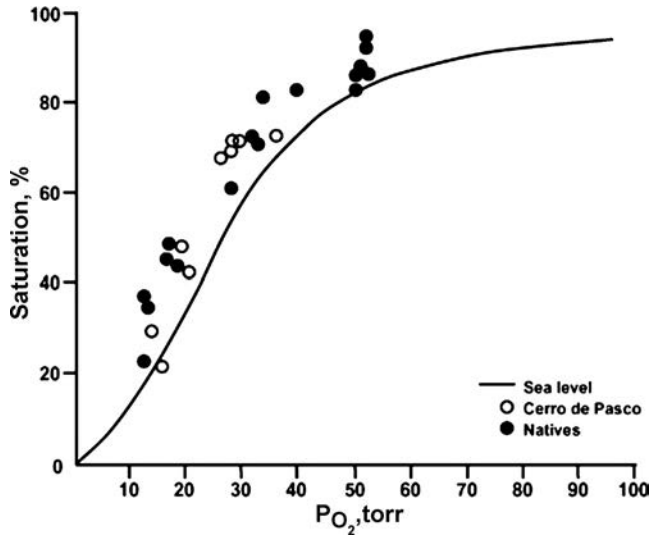

Fig. 1. Oxygen-hemoglobin dissociation curve demonstrating a left shift among sojourners (○) to high altitude and natives. The left shift in the oxygen-hemoglobin dissociation curve under these circumstances demonstrates that the combined effects of hypocapnia, increased pH, and cold override the simple effects of 2–3 DPG on the oxygen-hemoglobin dissociation curve. These data are an outstanding example of the limits of single mechanism reductionism. They are also consistent with the left shift seen in many genetically adapted animals that are native to high altitude. [Reprinted from Ref. 68, with permission from Elsevier.]

PREDICTIVE POWERS OF GENES?

In addition to gene therapy and other molecular treatments for rare diseases, reductionism also made promises about its ability to provide insight about who gets what complex disease like atherosclerosis, diabetes, hypertension, etc. As the quote below demonstrates, this idea became extremely popular after the sequencing of the human genome, and scientific funding agencies like the National Institutes of Health have invested huge sums of money in so-called "genome wide association studies" (GWAS) and other efforts to determine if a few genetic variants are harbingers of future disease in the population as a whole (10, 12, 43).

> *. . . because it been known all along that virtually every disease tends to track in families. What has changed is that. . . . .we are now beginning to see possible therapeutic approaches based on gene discoveries that will change the way medicine is practiced (12).*

One attractive element of this paradigm was that if a few common variants explained much of the risk for disease like diabetes, then it should be possible to identify those at risk and target them for early intervention. So far, the data from many, if not most or even all of these studies, have been underwhelming (43). First, a large number of variants seem to cause a significant increase in risk, but this increase is small compared with behavioral and environmental factors. An increased risk of several percent seems also likely to fall below what might be described as a phenotypic signal-to-noise ratio. Second, when the gene variants (single nucleotide polymorphisms, SNPs) that have been identified via GWAS or other experimental approaches are tested in large populations, the distribution of risk SNPs is typically strikingly similar in populations with and without disease (50, 63; Fig. 2). Third, when so-called genetic risk scores for disease are compared with predictive algorithms based on traditional risk factors (family history, lifestyle, age, etc.), the genetic risk scores are far less predictive than traditional phenotype-based risk scores. Furthermore, addition of genetic risk elements to phenotypically based scores adds little or no additional predictive power (50, 63). Finally, the idea that identifying prospective genetic risks for complex diseases that include a number of lifestyle and environmental factors (and increasingly even prenatal factors) is fundamentally wishful thinking, because behavioral health issues and culture play such a dominant role in determining who gets what disease when, and it is unclear if people will change their behavior in a positive way if they know prospectively they are at increased risk (24). Paradoxically, perhaps those at reduced genetic risk would pay less attention to behavioral risks.

SUCCESS IN PHARMACOGENOMICS AND ANTHROPOLOGY

So far, this paper has offered a sharp critique of the reductionists and taken the position that they over-sold what their technology had to offer on both the individual (gene therapy) basis and also in terms of population risk and intervention. However, there have been some notable successes stemming from application of this technology and two that seem especially worthy of comment. For example, there has been success in so-called pharmacogenomics. It has been well-known for some time that there are "responders" and "non-responders" to many forms of drug therapy. In many cases, this is related to how rapidly drugs are metabolized. In the case of tamoxifen, which had a dramatic effect on the recurrence of breast cancer, individuals with decreased drug metabolism appear to be at increased risk for recurrence. This is especially important for drugs like tamoxifen, which are ingested as pro-drugs with one or more metabolites that are active (56).

Another field where "omic" approaches have yielded dividends is anthropology. Two good examples include discoveries related to the independent development of lactase persistence into adulthood in areas of the world that were early adopters of herding (23, 34). In this context, one can imagine that the ability to digest lactose into adulthood provided the affected individuals a significant survival advantage and thus became the dominant genotype in only a few generations. Another good example that is perhaps counterintuitive relates to the individuals who migrated to the Tibetan plateau. These individuals do not develop chronic mountain sickness even with lifelong living at 3–4,000 m of elevation. These responses contrast to the high altitude natives in the Andes Mountains, who do develop chronic mountain sickness (58, 61, 70). Along these lines, those who migrated to the Tibetan plateau appear to have had selection pressure that favored a less functional variant of the hypoxia-inducible factor that, among other things, prevents them from developing excessive polycythemia, which plays a critical role in chronic mountain sickness.

INTERIM SUMMARY

So far, I have provided a general critique of what might broadly be termed "molecular reductionism". I have presented evidence that its failure to live up to its self-generated hype is in reality a failure to recognize larger ideas about homeostasis

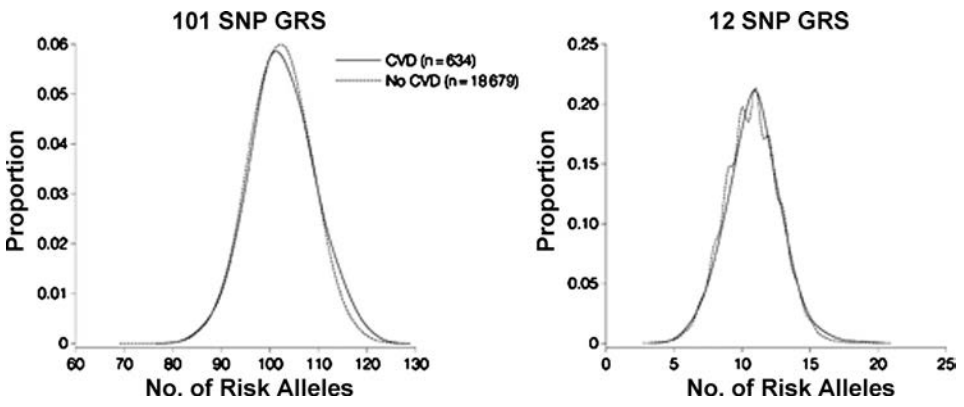

Fig. 2. Distribution of so-called high risk genes for cardiovascular disease in women with and without known coronary artery disease. The distribution of risk genes is similar, and construction of a genetic risk score for cardiovascular disease is thus problematic. This is just one example of the limited predictive power of "genomics" as it relates to the ability of relatively common gene variants to predict common diseases. [Borrowed with permission from Ref. 50. Copyright © 2010 American Medical Association. All rights reserved.]

and regulation that are central to physiology. This includes the specific example of the idea of gene therapy for relatively common genetic disorders like cystic fibrosis and also the limited predictive power of gene variants for common diseases. The question now is whether there is some way out of this problem and a better way to use potentially powerful technologies championed by the reductionists in a biomedical context.

IS SYSTEMS BIOLOGY THE ANSWER?

One idea to address the "failure" of molecular reductionism described above is to use a new approach called systems biology. The idea is that if powerful modeling tools and other data analysis techniques could be applied to the data generated via high throughput molecular reductionism, then somehow more meaningful insights would be generated and ultimately exploited for predictive or therapeutic purposes. The rationale for systems biology comes from a sampling of the comments on www.systemsbiology.org web site (34a).

> *Systems biology is the study of an organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions which give rise to life. Instead of analyzing individual components or aspects of the organism, such as sugar metabolism or a cell nucleus, systems biologists focus on all the components and the interactions among them, all as part of one system. These interactions are ultimately responsible for an organism's form and functions.*
>
> *Traditional biology—the kind most of us studied in high school and college, and that many generations of scientists before us have pursued—has focused on identifying individual genes, proteins and cells, and studying their specific functions. But that kind of biology can yield relatively limited insights about the human body.*
>
> *Biologists, geneticists, and doctors have had limited success in curing complex diseases such as . . . . diabetes because traditional biology generally looks at only a few aspects of an organism at a time.*

To a physiologist, there are obvious problems with systems biology. The problems start with the fact that physiology has been attempting for hundreds of years to understand the integrated function of organs and whole organisms that culminated in unifying big ideas about homeostasis and regulation discussed earlier. It is also clear that the type of biology that physiologists have been interested in starting with Harvey and the circulation has been about systems and has used modeling and computational techniques (1, 32, 57). Additionally, at this time the concept of systems biology and how it is defined remains very nebulous (52). Is systems biology a new discipline, an approach, a collection of tools, or merely a new name for integrative physiology generated by individuals who are generally unaware that our field exists (2, 28, 34a, 36, 40, 41, 45, 57)? Clearly physiology has provided and continues to provide insight about human disease, including insight that has led to vast therapeutic advances in recent years (37). Perhaps, the obvious question for the advocates of the cell-centric view of systems biology is did they skip physiology as part of their course work as students?

The concerns about systems biology outlined above at some level are about definitions and perhaps intellectual ownership. However, it also seems fair to ask what the long-term outlook for cell-centric systems biology is as an approach to making sense out of the vast amounts of data that can be generated using modern "omic" technology. In this context, there are key intellectual issues related to how data elements are generated, their spatial and temporal relationships, and how many ways they might interact (Fig. 3) that question the very fundamental assumptions about systems biology and its reliance on "bottom up" or "hypothesis neutral" modeling (2, 6, 15, 27, 35, 36, 38, 48, 67). It seems to me that without a narrative approach that includes hypothesis testing and key concepts like homeostasis, systems biology runs the risk of becoming scientific "Abstract Expressionism". Given the issues discussed earlier with gene therapy and GWAS approaches and the hype that surrounds systems biology, these concerns raise questions about what kind of science and scientific approaches deserve our future attention and funding (2, 24, 35).

REDUCTIONISM STALLS PHYSIOLOGY PROGRESSES

This is not the place for a comprehensive review of the contributions of physiology to biomedical research and therapeutic progress over the last 20–30 years. However, a few highlights that were initially seen as counterintuitive seem warranted. An obvious one is the discovery of EDRF and nitric oxide (25). This observation, which challenged the idea of the endothelium as merely a barrier, led to the discovery of gas-based signaling mechanisms and new therapeutic targets for conditions as diverse as erectile dysfunction and pulmonary hypertension. Would gas-based signaling mechanisms have been discovered by sequencing genes? Physiology has also helped redefine the optimal strategy used during mechanical ventilation in patients with adult respiratory distress syndrome (ARDS; 26). This has led to abandonment of strategies associated with high airway pressures and maintenance of arterial blood gases toward so-called permissive hypercapnia, alternate forms of mechanical ventilation and pressure support. Importantly, these new strategies that emphasize the avoidance of barotrauma have been associated with significant reductions in morbidity and mortality for ARDS. While part of the conventional wisdom now, this strategy was initially seen as counterintuitive.

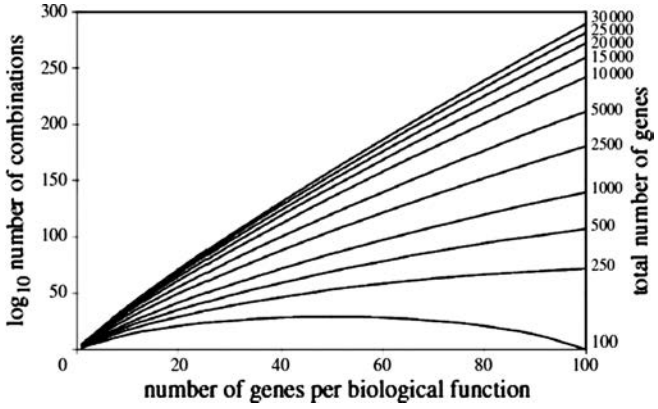

Fig. 3. Simulation of a number of possible combinations of genes gene interactions depending on the number of genes per biological function (*x*-axis) and the total number of genes in the organism. For biological functions with roughly 50 genes, $\sim 10^{150}$ possible combinations exist for most mammals. This figure shows the immense challenge associated with hypothesis-neutral systems biology and "bottom up" modeling. [Borrowed with permission from Ref. 46.]

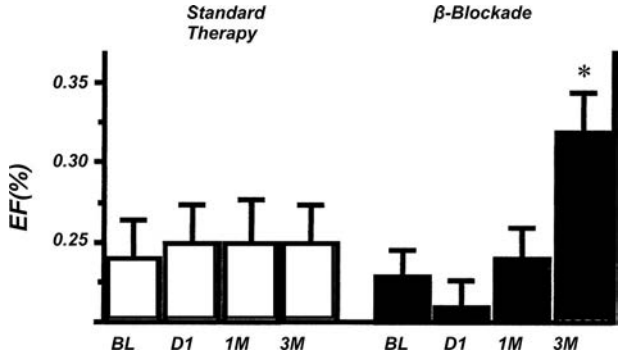

Fig. 4. Demonstration that beta-blockade can improve ventricular function (%EF) in humans with congestive heart failure over time. Standard therapy was associated with stable ventricular ejection fraction over 3 mo. By contrast, metoprolol (β-blockade) increased ventricular ejection fraction by >50% over 3 mo (*<0.05 vs. baseline). This finding, while initially counterintuitive, was based on sound physiological reasoning and along with other therapies has improved outcomes for patients with congestive heart failure. [Adapted from Ref. 20, with permission from Wolters Kluwer Health.]

Another example of a counterintuitive physiologically based clinical strategy was the use of beta-blockers in congestive heart failure. For many years these drugs were contraindicated in congestive heart failure (CHF) because it was felt that high sympathetic drive to the heart was required to maintain an adequate cardiac output in CHF. In reality, high sympathetic activity to the heart over time contributed to the progression of the disease and promoted a downward spiral of cardiac remodeling and reduced function (20). Thus the use of beta-blockers along with vasodilator therapy has been revolutionary and can interrupt or slow the downward spiral noted above in patients with congestive heart failure (Fig. 4). Again, the conventional wisdom was turned on its head and provided new insights that ultimately led to improved therapy. In the case of ARDS and congestive heart failure there has also been a two-way street between observations from clinical research conducted "at the bedside" to more fundamental observations in the laboratory.

Three other examples of more straight forward physiologically based therapeutic successes in recent years include the long story of improved outcomes for premature infants cared for in the neonatal ICU including altered ventilatory strategies, avoidance of oxygen toxicity, and surfactant therapy (9, 60). These improved outcomes, in the littlest ICU survivors, continue to seem miraculous to individuals who care for these patients and practiced medicine or nursing prior to their use. A second example has been oral rehydration solutions that are life saving in infants and children with diarrheal disease, especially in developing countries where it is a primary and frequent cause of death (8). Finally, in the developed world, where obesity and physical inactivity are leading to a pandemic of type 2 diabetes, physical activity (especially walking training in middle-aged people) has been proven to be highly effective in preventing, limiting, and in some cases reversing type 2 diabetes (16, 29). Each of these therapeutic successes is based on a foundation of physiologically based experimental evidence and insights.

REDUNDANCY, FEEDBACK, AND ACCLIMATION/ADAPTATION

Why has physiology continued to contribute in the era of reductionism? Physiologists are well versed in the overall concept of homeostasis, regulation, feedback, redundancy, and acclimation/adaptation. A classic example of redundancy comes from coronary circulation where coronary vasodilation is tightly linked to myocardial oxygen demand. In this context, a number of vasodilator systems likely contribute to this response. However, pharmacological blockade of one system, or in fact multiple systems, fails to alter this fundamental relationship between coronary vasodilation and myocardial oxygen demand in most species (19, 64; Fig. 5) This suggests that multiple redundant pathways contribute to this critical physiological response so that when one is blocked or absent, oxygen supply to the heart is not threatened when demand rises.

The fundamental relationship between coronary vasodilation and myocardial oxygen demand is also an observation that has had vast therapeutic implications and explains in large part why age specific death rates for cardiovascular disease have fallen dramatically over the last 30–40 years. There are drugs the reduce myocardial oxygen demand, mechanical therapy like stents, bypass surgery that improves myocardial oxygen delivery, and other drugs and lifestyle interventions that can affect both elements of the equation over time (30, 44). This physiological narrative and the progress that has flowed from it is in stark contrast to the relative lack of progress against cancer where there does not seem to be a unifying physiologically based story or model that can be exploited to address the general problem of cancer.

One of the classic feedback control mechanisms in physiology is the arterial baroreflex. While barodenervated animals have relatively normal blood pressure over a given 24 h period, their blood pressure becomes much more variable (14). The relative stability of blood pressure in the long run shows the power of redundant control via renal regulation of arterial pressure. However, for short-term adaptations, essential for things like exercise or changes in posture, feedback control

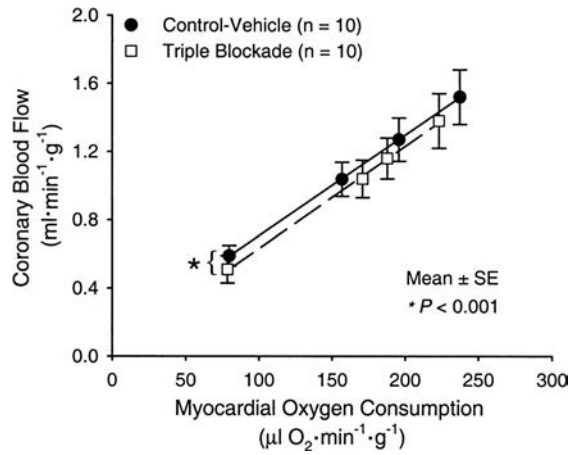

Fig. 5. Myocardial oxygen demand on the x-axis and coronary blood flow on the y-axis. Note that coronary blood flow rises in proportion to myocardial oxygen demand and that this rise is unaffected by triple inhibition of kATP+ channels, nitric oxide synthase, and adenosine receptor s This is a classic example of the concept of physiological redundancy. This well-known phenomenon may also explain why the absence of many so-called critical genes or proteins has limited impact on overall organ or organism function. This is because so-called redundant systems are able to alter their function and "upregulate" when one or more systems is blocked. [Borrowed with permission from Ref. 64.]

from arterial baroreflexes is essential for normal physiological responses.

An outstanding example of how humans acclimatize and adapt to physiological stress comes from studies that demonstrate that the ability of individuals to exercise in the heat can be remarkably improved by a few weeks of training in the heat (54). This improved exercise tolerance in the heat is associated with expanded plasma volume, increased sweating, and altered thermoregulatory skin blood flow. Another outstanding example is what might be called the adaptability of insulin sensitivity and glucose uptake in skeletal muscle. These variables are extremely sensitive to exercise and changes in daily activity and seem especially relevant in the era of the physical inactivity/obesity pandemic (29, 49, 53, 65).

Ideas about redundancy, feedback control, and acclimation/adaptation are also why physiologists are not that surprised by the ability of various gene knockout animals to survive and thrive (33). At some level this approach is conceptually similar to the classic denervation or high dose pharmacological blockade studies used by physiologists for generations and primarily show the power of the regulatory mechanisms highlighted above to preserve both long term phenotype and homeostasis despite the loss of one or more critical pathways or mechanisms (17). In this context, it is not surprising the yeast can survive without 80% of their genes and the function of these genes only becomes apparent when the organism is stressed (33). Is it too cynical to point out that knockout animals are essentially a "can't lose" experimental approach? If the knockout is lethal or leads to significant phenotypic dysfunction it is essential. If it survives then genetic or other compensatory mechanisms were upregulated to overcome the absence of the essential gene.

Physiology or physiologically based tests can also provide insight into the risk of future disease and/or predictive outcomes. For example, the blood pressure responses to common sympathoexcitatory stress can be used to define those at risk for future hypertension in a way that is potentially much more predictive than any current genetic test. Additionally, tests of autonomic function are strong predictors of outcomes in large populations of humans, and cardiorespiratory fitness is an especially good predictor of all-cause mortality.

TOOLS VS. BIG IDEAS

At some level molecular reductionism and systems biology are at existential cross roads. Are they in fact real disciplines informed by big ideas like homeostasis and regulation, or are they essentially tools and approaches that will facilitate the work of disciplines informed by bigger ideas and more importantly bigger questions and more comprehensive strategies? Based on the concepts and examples highlighted in this paper I would argue that until the vast amounts of data generated by modern "omic" techniques are put in a physiological context it will be an exercise in what Sydney Brenner has deemed "low input, high throughput, no output biology" (6). Along these lines, I want to end on an optimistic note with examples of how physiology is making a difference by applying reductionist tools as part of a more comprehensive approach to important questions. Because the Adolph lecture is sponsored by the Exercise and Environmental Physiology section of the American Physiological Society, relevant examples from related areas will be used. In each case there seems to be an overall hypothesis and a strategy that exploits what might be called responders and non-responders to an intervention.

Britton and Koch and colleagues (39, 69) have used selective breeding strategies to develop rats with vastly different inherent aerobic endurance capacities (Fig. 6). These animals have been used in a variety of studies to better understand the gene environment interactions. In many instances the animals selected for low intrinsic aerobic capacity seem to be at increased risk for complex diseases like diabetes, obesity, and heart disease. Additionally, studies using these animals have begun to identify genetic and transcriptional factors and networks that explain in part this increased risk (39).

Another example of how physiologists are using tools from the "new biology" is the HERITAGE study, which broadly seeks to understand the genetic basis for the differing physiological responses to exercise training in a large number of humans exposed to a standard protocol (3–5). This is an excellent example of how what might be called "high resolution" physiologically based phenotyping in conjunction with genetics. This hypothesis-driven approach also includes uses

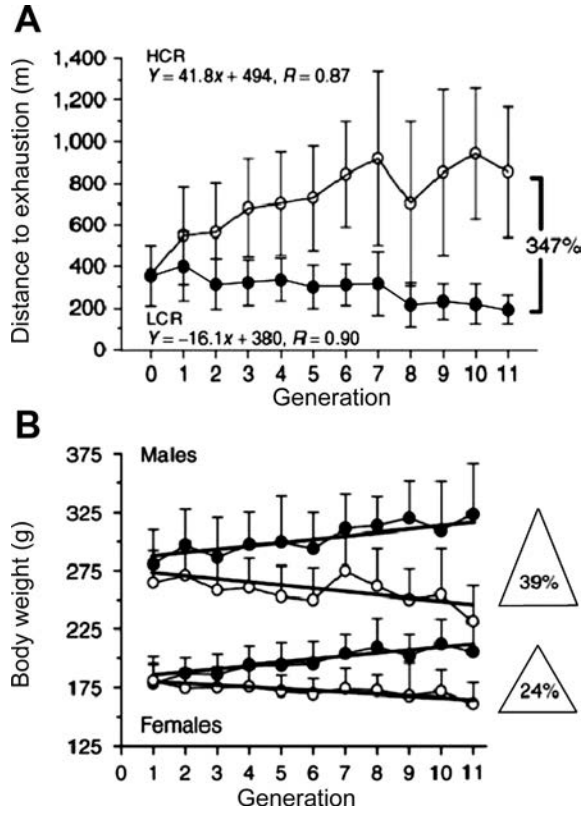

Fig. 6. Selective breeding of rats with divergent aerobic capacities. These data show that animals selected for their running capacity diverge dramatically after a few generations and is sustained for many generations. Importantly, at the same time body weight also began to diverge as did a number of risk factors for cardiometabolic disease. Phenotypic studies conducted on these animals in conjunction with more targeted forms of "omic" approaches and other types of molecular reductionism are providing new insights about gene environment interactions. These findings may also have applicability to physically active and inactive humans. The approach of Britton and Koch is a classic example of using reductionist tools in a physiological context to gain new insights with direct applicability to human health and disease. [Reprinted from Ref. 39 with permission from Macmillan Publishers Ltd. *Obesity Suppl*. copyright 2008.]

various "omic" and systems biology approaches and was initiated by physiologists before the terms genomics or systems biology existed. Additionally, like the examples from pharmacogenomics and anthropology discussed earlier, it takes advantage of the fact that there are responders and non-responders in response to a given intervention or environmental stressor.

Finally, my collaborator John Eisenach and I along with our colleagues have performed carefully controlled studies on how common genetic variants in the β_2-adrenergic receptor influence a number of physiological responses and how any genotype-based differences might be influenced by dietary sodium (21, 22, 31, 59). These studies were initiated because epidemiological evidence suggested that genetic variation in the β_2-adrenergic receptor influenced blood pressure in large populations. In our studies only homozygotes for the genetic variant of interest were recruited in an effort to see the maximum potential physiological effect of the variants. Using this approach, it appears that there are genotype-specific patterns associated with increased cardiac output responses to exercise that may interact with NO-mediated β_2-adrenergic receptor peripheral vasodilation. These responses clearly link and mechanistically define how a common gene variant in a key regulatory system can influence a physiological response in humans. They may also provide physiological explanations relevant to the original epidemiological observations on blood pressure and other outcomes, including those in patients with the acute coronary syndrome (42).

SUMMARY

In this paper and in the Adolph Lecture I have highlighted some of the claims associated with molecular reductionism and more recently systems biology. In both cases I have argued that the apparent inability and/or unwillingness of the advocates of these approaches to use key concepts from physiology and ultimately use their tools in a physiological context has limited the contribution of the approaches they advocate. By contrast physiology has continued to use new tools in the service of its big ideas and also continued to provide biomedical insight and therapeutic advances. As the final examples show, it is possible to incorporate reductionist tools in a physiological context to gain broader biomedical insights. Hopefully these insights will fuel the next wave of physiologically inspired therapeutic advances.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

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Review

Theoretical aspects of Systems Biology

Mariano Bizzarri [a,*], Alessandro Palombo [b], Alessandra Cucina [c]

[a] Department of Experimental Medicine, Systems Biology Group Lab, Sapienza University of Rome, via Scarpa 14-16, 00161 Rome, Italy
[b] Department of Clinical and Molecular Medicine, Sapienza University of Rome, Italy
[c] Department of Surgery "Pietro Valdoni", Sapienza University of Rome, Italy

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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

* Corresponding author. Tel.: +39(0)649766606; fax: +39(0)649766897.
E-mail addresses: mariano.bizzarri@uniroma1.it (M. Bizzarri), alessandro.palombo@uniroma1.it (A. Palombo).

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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 epistememe, 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

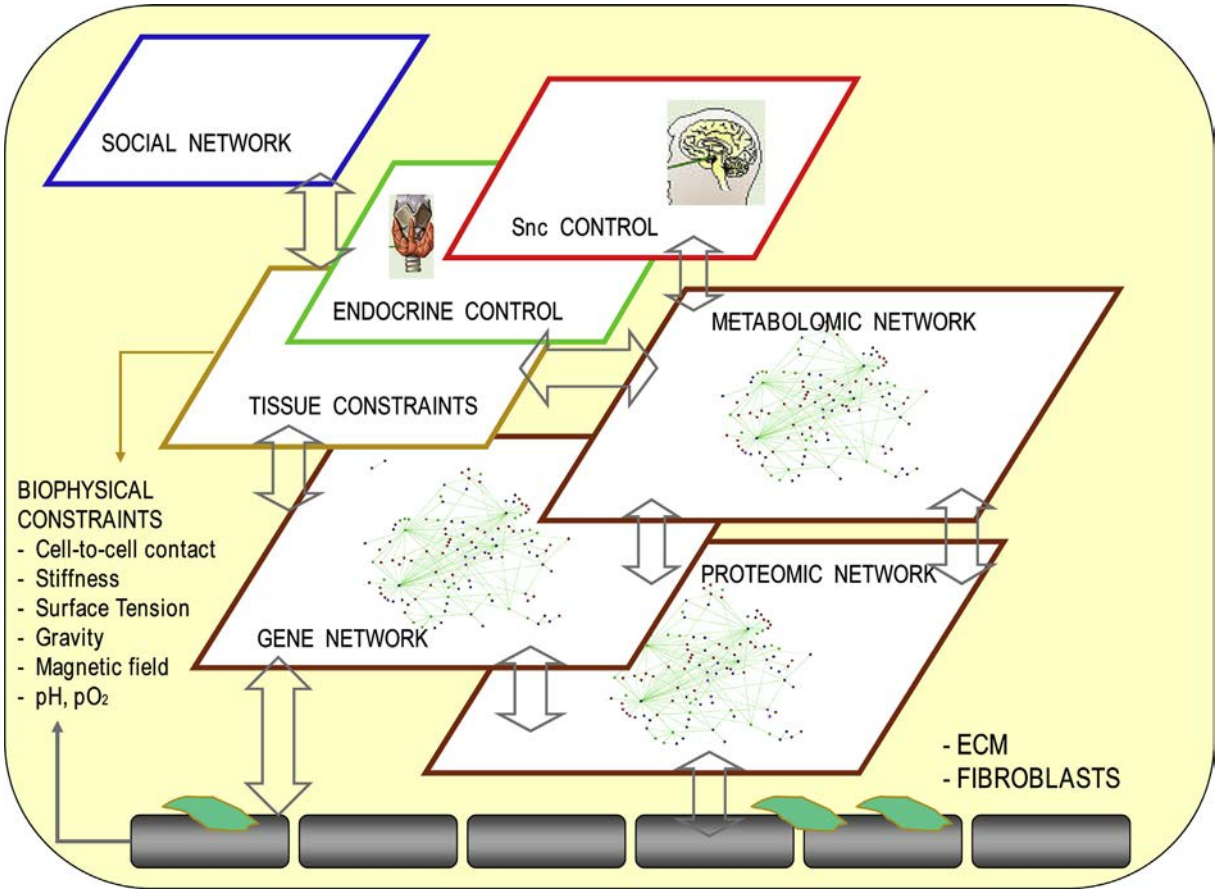

Fig. 1. Local processes involve the interplay between cells and stroma (the tissue level), where biophysical forces and molecular networks (genomic, proteomic, metabolomic) interact according to a non-linear dynamics. Reciprocal relationships are settled between tissues and higher level of organization. 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),

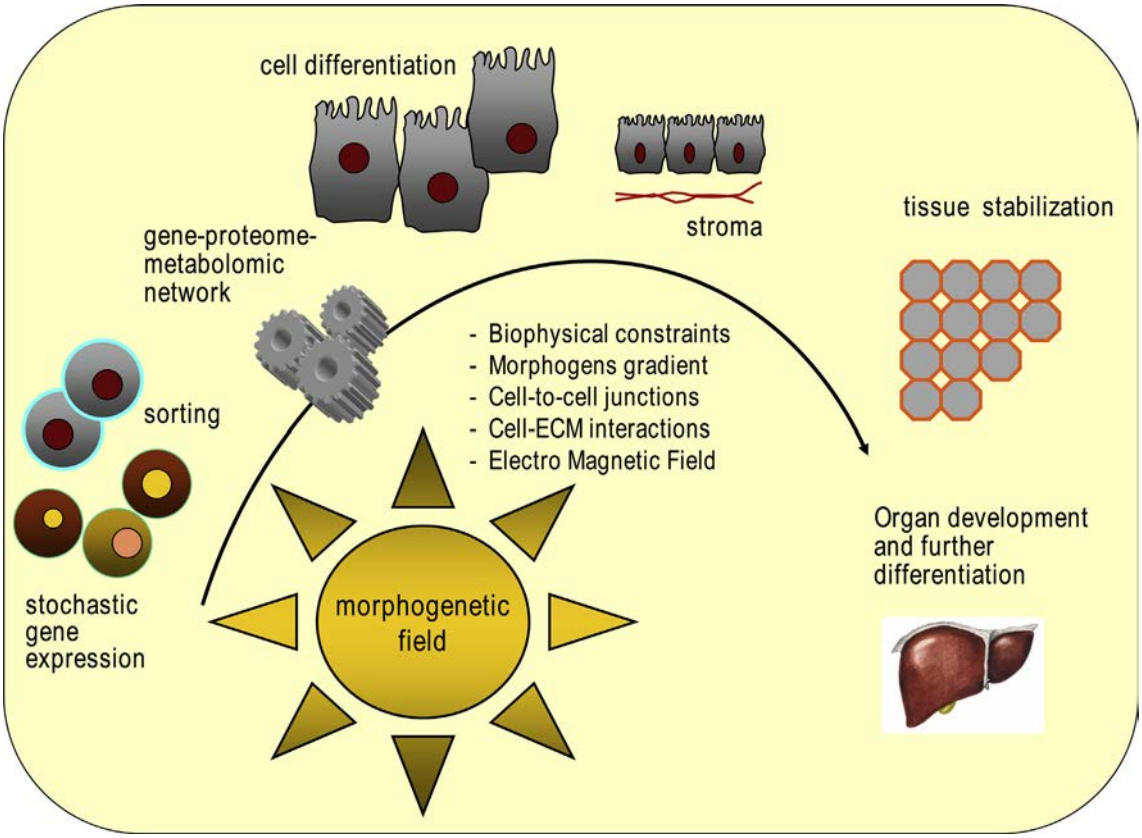

Fig. 2. Cell differentiation is driven by the interplay between the morphogenetic field and the gene expression pattern. Biophysical forces are acting within and throughout the field in selecting phenotypes that arise according to a stochastic process.

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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