From Molecules to Organisms: Towards Multiscale Integrated Models of Biological Systems

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Abstract: A consensus has recently emerged that further progress in understanding human physiopathology will demand integrative views of biological systems. In this context, complex systems and related interdisciplinary approaches of biology are expected to help. The aim of this collective paper is basically to provide a starting point for further discussions and interactions within the community of complex systems biologists. After briefly introducing some general concepts, we present four major challenges that should be tackled in the next years. These represent future directions that we isolated as priority concerns for modern biology. Suggestions of how to reach these destinations are provided, with the hope that they will soon lead to concrete advances towards fully consistent multiscale models of biological systems and a better understanding of physiopathology.

Keywords: regulation, modeling, multiscale, network, fluctuation, systems biology, integrative biology, physiopathology

Introduction

(Complex) systems biology

Recent advances in functional genomics and in the study of complex diseases (such as cancer, immunodeficiency, autoimmunity, mitochondrial diseases or metabolic syndromes) have shown the necessity of a new way of thinking in biology, which is to consider pathology and physiology as resulting from interactions between many processes at various scales. Systems biology emerged from this need (Ideker et al. 2001; Kitano, 2002). This scientific field addresses the study of gene (expression, evolution), protein interactions, biochemical reaction networks, cell populations and tissues in organisms considered as dynamical systems (Maynard Smith, 1998). It aims at studying the biological properties that result from the interaction of many components, investigating processes at different scales and achieving their integration. Complex systems conveniently provide a conceptual framework and effective tools to unravel emergent and immergent features from molecules to organisms and back. The latter, described as immergence, microemergence or downward causation (first defined in (Campbell, 1974) as the necessity for all processes at the lower level of a hierarchy to act in conformity to the laws of the higher level), means that some macro-level constraints are expected to cascade back onto micro-levels, the macro-level being itself an emergence. In a complex systems perspective, both emergent and immergent properties should be derived from the multiscale reconstruction of data recorded at the appropriate spatial and temporal scales to be defined through new types of protocols, involving high-throughput measures, data mining and analysis, modeling and manipulation of the system (Ideker et al. 2006). We expect to find generic processes or "design patterns" (a concept introduced by the architect Christopher Alexander (Alexander, 1977) as a formal way of documenting a solution to a design problem, further adapted for computer science (Gamma et al. 1995)). This concerns upper to lower organisational level and

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vice versa and allow their coupling (synchronisation, reinforcement, amplification, inhibition) achieved through basic processes such as signalling through molecular interactions, diffusion, vesicular transport, ionic transport, electric coupling or biomechanical coupling. These molecular features both influence and are the results of the dynamics and regulation at cell population levels in tissues and finally in the whole organism. As an example, in fluid cell populations such as lymphocytes with complex dynamics and turnover (Thomas-Vaslin et al. 2008), complex regulation processes at cellular and molecular levels determine tolerance versus reactivity of the immune system, i.e. health or immunological related diseases (Coutinho et al. 2005).

The scale problem

Complex systems are almost always multiscaled both in time (typically femtoseconds in chemical reactions, seconds in metabolism processes, days/weeks in cell populations, months/years in an organism life) and space (typically nanometers for molecular structures, micrometers for supramolecular assemblies, organelles and cells, millimeters to meters for tissues, organs and organisms). Finding the pertinent space and time scales for experimentation and modeling is a major issue. As a result of evolutionary opportunism (biological tinkering), space and time multiscale correlation is not a priori given. Classical approaches (biochemistry, cellular and molecular biology, behavioral and cognitive studies) usually have their "preferred" scale by default, mainly due to the fact that protocols and experiments are often designed to work only at a specific scale. This makes back and forth interactions between different scales in observations, experimentations, models and simulations a very challenging transdisciplinary issue.

The variation problem

Variation in biological systems raises the issue of an average, typical or representative behavior. Addressing this point requires characterizing and measuring variability and fluctuations at the molecular, single cell, cell population and physiological levels. The origin, time and space scales, control and functional significance of fluctuations in biological systems are largely unknown. Their functional significance might be approached through their multiscale transmission and possible amplification or damping. Variation includes fluctuations and noise but also participates to the global stability or response of the system. The meaning of usual averaging processes in experimental biology should be questioned. For instance, in the case of biochemical networks, one may wonder if data gathered on cell populations can be used to infer the actual network in a given single cell.

Towards an enlarged integration of biological systems

Obviously, understanding will not arise from a one-to-one description and modeling of organisms (virtual cell, virtual organism) but rather from the correct identification of which components (and systems properties emerging from these components) are relevant for a given problem and the reconstruction of the mechanisms involved. Such a reconstruction should use computational, mathematical and physical tools, some borrowed from out-of-equilibrium thermodynamics and dynamical systems. New tools will also be required to answer specific questions of biology. Complementary to bottom-up or top-down approaches, a middle-out approach starting from the cell should also be an efficient modeling strategy to analyse biological systems (Brenner, 2003; Noble, 2002). Ultimately, injecting a system vision and using complex systems principles and conceptual frameworks for a better understanding of human physiopathology could lead to novel differential diagnosis and improved medical care. Some future prospects are discussed below. They only represent a small set that we picked up out of the numerous challenging issues that should be addressed in the following years.

First Challenge: Building and Handling Multiscale Models

The scale issue

Biological processes involve events occurring at many different time and space scales. The hierarchy of these scales enters the scene only because it corresponds to our subjective views on the system usually based on our various discrete experimental accesses. Multiscale approaches inspired from theoretical physics have been

developed essentially in a unidirectional (bottom-up) way to integrate parameters and mechanisms at a given scale into effective, and hopefully reduced, descriptions at higher scales. However, lower-scale properties are directly coupled with properties of the higher scales; for instance, 3D chromosome distribution in the nucleus partly governs gene expression which itself participate in nuclear architecture (Misteli, 2005; Misteli, 2007). The very complexity of living systems and biological functions lies partly in the presence of this bidirectional feedbacks between upper scales and smaller scales that have settled in the course of evolution. Self-consistent or iterative "up-and-down" approaches are therefore to be introduced to account for the strong interconnections between the levels and ensuing circular causal schemes.

Multiscaling vs. selfscaling

To properly account for the behavior of a biological system, a multi-scale approach should tackle jointly all the scales, with no way to a priori neglect some microscopic details or macroscopic assemblies. Obviously, such proper modeling would rapidly reach high level of complexity, and would ultimately be intractable. This limitation about multiscale descriptions imposes a drastic change in the paradigm underlying the modeling of biological systems. To reduce the complexity level, it has been proposed to devise models taking the biological function as a starting point and continued guideline, driving both integrated modeling and supervised data analysis to parallel the biological functional logic (Lavelle and Benecke, 2006; Lesne et al. 2006). Decomposition is achieved by dissecting its logic and implementation into basic processes. These elementary processes involve features at different scales and are already integrated in their formulation. More generally, such a decomposition results in "self-scaled" functional modules, independent of the arbitrary description or observation scale. As function-dependent representations are inherently multiscale in nature, and the function cannot be discontinuous, this paradigm-transition consequently requires a scale-continuous model. Scale-continuous descriptions may at first sight look prohibitively complex and non-realistic; however, when such a scale-continuous model is constructed in the context of a function-dependent representation, the dimensionality of the to-be-considered variable-vector collapses (Lavelle et al. in progress).

Emergence vs. immergence

Modeling of biological systems required to develop formalisms in order to rebuild the complete system by integration of its hierarchical multi-scale levels. It can be achieved by defining micro to macro functions (emergence), macro to micro functions (immergence, microemergence or downward causation; see discussion above) and integrating intra-level (horizontal) and inter-level (vertical) couplings. The definition of pertinent variables at each level of organization and their relationships is necessary to obtain emergence (resp. immergence) functions that would allow analysis to jump from a microscopic (resp. macroscopic) level to a macroscopic (resp. microscopic) level. Emergence and immergence phenomenon are well known in biology such as the links between the topology of tissues and the cell behavior. Still these causal relationships are difficult to decipher mainly because scales at which they occurred are not necessary those at which observations and experiments are done.

Some specific issues to be addressed

1. How to select relevant space and time scales in our experiments/models/theories, i.e. how to achieve selfscaling when exhaustive multiscaling is unreachable? Can we correlate multiscale in time and space (at least in some instances) in this sorting?
2. How can we carry multiscale reconstruction from data recorded at different scales? To which spatial and temporal scales the obtained model/simulation will be valid?
3. Regarding multiscale variability (see second challenge below), how can we improve the technology for quantitative measurements of noise and fluctuations in single cells, cell populations, tissues, organs and individuals. In particular, it will be necessary to identify the characteristic times at each level of organization and the most appropriate experimental indicators. What are the modalities of multiscale transmission of fluctuations? Are fluctuations amplified or reduced/damped from one scale to the others? Are they important with respect to bifurcations in the organism/cell fate?

Significance of this challenge

Experimental as well as modeling approaches usually scrutinize/describe phenomenon at a defined and limited time and space scale. However, only interconnected multiscale models will allow us to reach a comprehensive understanding of biological processes. To characterize and integrate interactions between different scales in experimentation and simulation is thus a very important challenge.

Second Challenge: Characterizing/ Quantifying Fluctuations and their Role(S)

Fluctuations and noise in biological systems

Modern biology has developed with the idea of average behaviors or individuals. Yet, this conceptual framework has recently been challenged by various observations. Quantitative measurements within living single cells have indeed revealed extensive variability and fluctuation of cellular dynamics among different cells or among different times within the same cell (Arkin et al. 1998; Blake et al. 2003; Kaern et al. 2005; Ozbudak et al. 2002; Swain et al. 2002). Moreover, recent methodologies that enable the reconstitution of the population mean from individual cell measurements (rather than the standard direct measurement of this mean) showed that even for fixed genotypes and environments, a large variability of phenotypes can occur across a population (Kaern et al. 2005; Smits et al. 2006). In addition, monitoring biochemical reactions as a function of time within a single cell has also evidenced strong and unexpected stochasticity (Elowitz et al. 2002). These observations open a new conceptual framework in biology, in which noise must be fully considered in order to understand biological systems, while the classical framework tended to consider it as a mere measurement error or as "simple" thermodynamic fluctuations that have to be reduced by cells. This new point of view raises lots of questions and practical as well as theoretical issues that are likely to deeply modify our understanding of biological systems.

The function(s) of fluctuations

A stimulating proposal is that biological systems could take advantage of fluctuations i.e. that noise may play a real functional role in biological systems. Although we are still far from understanding the possible functional significance of fluctuations in the different biological systems (Samoilov et al. 2006), several studies have proposed general mechanisms by which noise could regulate biological systems. For instance, it has been proposed that fluctuations—sometimes related to regulations—can enhance the robustness of living beings (Furusawa and Kaneko, 2008), alter their tolerance to environment variations (Kussell and Leibler, 2005; Thattai and van Oudenaarden, 2004; Veening et al. 2008) or species evolution (Fraser et al. 2004). But we also need a better understanding of the effects of molecular fluctuations at the level of cells or organisms. Some researchers have started to work in this direction and showed, for example, how noise can be expected to alter intracellular signalling rates (Bhalla, 2004) or cell differentiation (Suel et al. 2007). Such studies can be considered the building blocks on which the effects of noise at larger scales can be estimated, in a classical bottom-up approach. This will then allow us to tackle issues such as: are noise/fluctuations important with respect to changes (bifurcations) in the organism/cell fate? What is the influence of molecular noise on the development of pathologies? To answer these questions, we need a better insight into the modalities of multiscale transmission of fluctuations, i.e. to understand in which conditions or systems are fluctuations amplified or reduced/damped from one scale to the others.

Integrating multiscale fluctuations

To this aim, we will as well have to indentify the precise mechanisms by which noise and fluctuations arise in biological systems. At the molecular level, the low copy number of some enzymes or proteins is generally considered the main source of stochasticity but other mechanisms such as spatial heterogeneity or geometrical complexity (molecular crowding) have been proposed. It is likely that other sources still have to be identified. Answering these questions will enable us to delineate possible mechanisms by which biological systems may *control* their level of fluctuation at different levels. At the molecular scale (i.e. in biochemical or gene regulation networks), possible regulating mechanisms include negative/positive feedback loops (Becskei and Serrano, 2000). At a multicellular scale, neuronal

adaptation in cortical networks (Wang et al. 2003) may be a good example of noise regulation. At an evolutionary scale, adaptive mutations and mutation hotspots (Moxon et al. 2006; Taddei et al. 1997) can also contribute. On a technical point of view, we need to improve the technology for quantitative measurements of noise and fluctuations in single cells, cell populations, tissues, organs and individuals. Noise in biological systems is classically measured by the normalized variance but this measure only gives a very rough and static view. It will probably be necessary to identify more appropriate experimental indicators to measure e.g. characteristic times and spatio-temporal correlations at each level of organization.

Some specific issues to be addressed

1. It is now clear that the transcriptional activity of the cell is highly stochastic (Ozbudak et al. 2002; Yu et al. 2006). Some of the molecular causes of this stochasticity have been identified (e.g. low copy numbers, spatial heterogeneity, 3D diffusion) (Kaern et al. 2005; van Zon et al. 2006). However, the precise origin and regulation mechanisms of this stochasticity are still to be uncovered. This will first require developing adequate measurement methodologies to be able to quantify these fluctuations at different time scales in single cells.
2. The on-going activity of cortical circuits is a spontaneous activity generated by the recurrent nature of these networks (Tsodyks et al. 1999). It has long been considered a mere noise added to the environmental signals. However, more recent studies have proposed a real functional role in which on-going activity could facilitate signal spreading and be implicated in adaptive processes (Goldberg et al. 2004). Inhibitory effects have been shown to reduce variability at both the single-cell and population level (Mar et al. 1999). Indeed, the effect of noise on networks can be a general question that links different biological domains (Swain and Longtin, 2006).
3. Uncontrolled variability is often accused to be a source of major perturbations in the fate of organisms (Samoilov et al. 2006). Examples can be found in the process of ageing, cancer, infectious, autoimmune or degenerative diseases. Yet the precise influence of noise is still a question of debate. In particular, one important point is to determine to what extent degenerative processes are a consequence of noise accumulation, a consequence of a variation of the noise properties or a consequence of rare stochastic events. This applies also for the evolution of lymphocyte repertoire diversity and occurrence of immunological memory in response to antigenic stimulations, where aging contributes to the collapse of diversity (Goronzy and Weyand, 2005). The immune system is characterized by diversity at different levels. Lymphocyte receptor diversity, cell-populations dynamics and turnover, emergence of memory, cell selection and competition, efficiency and control of effector cells by regulatory cells and migration through the whole organism are the result of somewhat stochastic or programmed mechanisms. This self-organization impacts in the overall efficiency of the system (Atlan and Cohen, 1998; Langman and Cohn, 1999) and needs to be further characterized. Variation in lymphocyte antigen-receptor repertoire diversity and regulation of immune repertoires and functions could furthermore be addressed in physiological and pathological situations (Kedzierska et al. 2008).
4. Variability at the genetic level is the major engine of evolution. Yet, the genetic variability may be indirectly regulated depending on the spatio-temporal characteristics of the environment (selection for robustness, selection for evolvability) (Kirschner and Gerhart, 1998; Knibbe et al. 2007a; Knibbe et al. 2007b; Lenski et al. 2006). For instance, the somatic diversity of lymphocyte receptors is acquired through somatic DNA rearrangement together with clonal selection of lymphocytes related to environment influences, increasing the diversity and variability. Moreover, clonal individuals may be very different one to the other due to intrinsic and extrinsic phenotypic variability. The mechanisms by which heritable and non-heritable variability are regulated, as well as their influence on the evolutionary process, still need to be characterized.

Some ways to improve available tools for a better modeling of fluctuations

1. Stochastic models are largely used in molecular systems biology. The simulation algorithms (Gillespie algorithm; (Gillespie, 1976; Gillespie, 2000; Gillespie, 2001)) use Delbrück-Bartholomay-Rényi representation (Bartholomay, 1957;

Delbrück, 1940; Rényi, 1954) of biochemical kinetics as jump Markov processes. In order to improve the performance of these time-consuming methods, several approximate schemes were proposed, for instance the approximation of Poisson variables by Gaussians (tau-leap method; (Gillespie, 2001)). Hybrid approximations are more appropriate when the processes are multi-scale and these approximations could be developed by combining averaging and the law of large numbers. Note that in certain simple cases, the master equation can be exactly solved.

2. Part of the biochemical noise in a cell is due to diffusion (of chemoattractants in signaling (Berg and Purcell, 1977), of transcription factors in gene expression (van Zon et al. 2006), etc). The internal geometrical complexity (complex geometry compartments, macromolecular crowding) impacts on diffusion and on mixing (Saxton, 1994), thus on noise and fluctuations. New or improved modeling methods are needed to delineate the influence of these effects on cell behavior/physiology and development (Gregor et al. 2005; Takahashi et al. 2005). We now need to go beyond classical simulations and models (e.g. laws of mass action) that consider the evolution of enzyme concentrations over time. News tools able to simulate cellular systems in 4D (space and time) are mandatory if we want to understand the complex influence of cellular structure on biochemical processes (Bork and Serrano, 2005; Lemerle et al. 2005).
3. It would also be interesting to transfer ideas from statistical physics to biology. For instance, fluctuation theorems (for a review, see (Rondoni and Monasterio, 2007) and references therein), which concern the occurrence of out-of-equilibrium fluctuations in heat exchanges with the surrounding environment and work theorems, concerning thermodynamic fluctuations in small systems close to equilibrium, could be applied to characterize fluctuations in gene networks, DNA transcription processes and biomolecules unfolding (Ritort et al. 2002). Cell population dynamics could also certainly benefit from physic, fluidics or ecological approaches and multi-agent simulations.

Significance of this challenge

Far from being an insignificant phenomenon, noise is now recognized as one of the fundamental property of biological systems. Noise is expected to have a role in many biological processes, either normal (e.g. robustness, differentiation) or pathological (e.g. cancer). Yet, we still need to quantify its actual contribution to these processes: is noise the main factor or is it simply a side effect? To what extent can noise be considered a reservoir of innovation? What is the influence of molecular noise on ageing process? Do biological systems use noise to improve their behaviour and how? To tackle these questions, it is necessary to develop a complete scientific program from precise measurements to analysis of the origin and functional role of stochasticity in biological systems at all of their time and space scales. Adopting a broader perspective could also yield greater insights and enhance our level of understanding of the averaging processes that are routinely employed in experimental biology. In the case of biochemical/gene regulation networks for instance, can data gathered on cell populations be used to infer the actual network in a given single cell? Similar issues arise in the case of connectivity structures of cortical neuronal networks, cell lineage reconstruction or lymphocyte repertoire selection.

Third Challenge: Understanding Stability in Biology

The concept of stability

Complex systems may be defined as systems where there is "structure with variation" (Goldenfeld and Kadanoff, 1999). Stability of complex systems is a much richer concept than stability of simple physical systems. In particular, the notion of biological stability has a long history. Thus, homeostasis was introduced by various authors as the property to keep parameters or variables values constant or within narrow intervals. One can find numerous examples of homeostatic variables in physiology (glucose concentration, blood pressure, etc.). Conrad Waddington then proposed in the 1930's the concept of homeorhesis (different from homeostasis and meaning resistance of epigenetic trajectory to being changed) to describe canalized development. Evolutionary biologists added a new dimension to stability, related to adaptation with respect to genetic or environmental changes. Biological systems must be able to bear a certain amount of variability in their structure without losing their stability. This can be done by various buffering

mechanisms or by adaptation. More recently, systemic approaches to biological systems brought in concepts coming from engineering, such as robustness (defined as insensitivity of systems properties with respect to perturbations). Interestingly, in many biological systems, aging seems to be related to loss of complexity and regulations lowering stability and robustness of the system (e.g. lower bone density, decrease of cell production and repertoire diversity of lymphocytes, less network connectivity, less co-gene expression) (Kirkwood, 2008).

Some specific issues to be addressed

1. New mathematical approaches to stability and robustness: several mathematical concepts could be applied to various forms of biological robustness. Thus, finite-time stability (Weiss and Infante, 1965) could be used to define stability in the case when the system is known to operate or to preserve its structure unchanged up to a finite horizon. Related to finite-time stability, meta-stability has been used to model robust cognitive transients in large networks simulating brain activity (Rabinovich et al. 2008) or alternating synchrony which can occur for instance as intermittent synchronization in ensembles of linearly and nonlinearly coupled nonlinear oscillators (Tyukin et al. 2008). Meta-stability and finite-time stability concepts could be applied to the dynamics of mechanistic models of genetic regulation and of signaling (gene and signaling networks), as more realistic approaches to stability than attractors and steady states.
2. The notion of resilience is another generalization of stability which is particularly appealing in the context of biological stability and robustness. Indeed, it focuses on the ability to restore or maintain important functions when submitted to perturbations. The formalizations of this concept, founded on dynamical system properties (measure of attraction basin sizes), or on viability theory (cost to return into a viability kernel (Aubin, 1991)) could be applied to biological systems. Last but not least one would like to understand the robustness proprieties of gene and signaling networks models. Thus, many of such models have robust behavior in spite of variability of the regulation mechanisms (pathways details and interaction strengths). This type of robustness has been illustrated numerically on a model of segment polarity network of Drosophila (von Dassow et al. 2000). Segment polarity gene patterns are insensitive to large variations in the model parameters. Related to this, sloppiness of sensitivities of systems biology models has been reported (Gutenkunst et al. 2007). Mathematical concepts such as Gromov/Talagrand concentration could provide a strikingly clear pictures of the origin of this type of robustness (Gorban and Radulescu, 2007).
3. Role of complex multi-scale organisation in maintaining stability: function of multicellular organisms occurs at the level of the population not of the individual cell. Furthermore, the stability of a cell population (tissue) is generally different from the one of the individual cell. For example, cells extracted from tumours can reverse to normal activity when injected in healthy tissue. In this framework, how to define and study the stability of a population in relation to the stability of individuals? In addition, the same relation should be considered in the context of a developing organism taking into account differentiation and organogenesis. These processes are examples of symmetry breaking and we would like to know if symmetry arguments can be used in the study of stability properties (Golubitsky et al. 2004; Pinto and Golubitsky, 2006).
4. Models of an organ and models relating several organs imply the collaborative representation of the components. Similarly, gene regulation models gather numerous molecular details. In the modelling process, we should be able to zoom in and out between various levels of complexity. Stable properties of the system could be those that are common to several levels of complexity. More generally, is there a connexion between stability and complexity?
5. Robustness and therapy: systems biology study robustness as an important organizing principle of biological systems. As pointed out by H. Kitano, cancer is a robust system with some points of fragility (Kitano, 2004). Thus, finding treatment and cure of diseases may consist in determining the fragility points of a robust system. In order to answer this question we need good models, new mathematical theories and computer tools to analyse properties of models and new experimental techniques to quantify robustness.

Significance of this challenge

Knowledge on the stability of the biological systems with definition of scale for its measurement, related to permanent sensing and adaptative responses, should be useful to better understand deregulations and pathologies that affect this equilibrium. Solving the above problems is crucial for fundamental science and for applications. The problem of how organisms preserve their stability and function reliably in spite of genetic and environmental variability is central to biology. Robustness is a systems-level property, presumably common to all complex biological systems. Major breakthroughs in the understanding of this property could come from new mathematical theories.

Fourth Challenge: Tackling Human Physiopathology

Characterizing pathology

Human physiopathology creates uncertainties with constantly moving frontiers between discipline fields, for example neurology, psychiatry, immunology, cancer, infections, and metabolism. Thus, the issue concerns the whole internal and general medicine, in intercrossing fiels as well as paediatrics, geriatrics, functional re-education and public health. Human physiopathology is characterized by progressive dysfunction and deterioration at multiple space and time scales with non-linear interactions between physiologic/biologic functions, cognitions, emotions, and social consequences. Problems can result initially from local conflict between internal and external signals (e.g. dizziness) but this conflict can expand diffuse and create additional loops with multiple pathogenic reciprocal interactions. Functional problems could be primary or secondary effects of spontaneous adaptive mechanisms aiming to counter primary insult and dysfunction, and is important to dissociate.

Some examples

In the field of neurology, among functional problems (some of them with no measurable organic basis) are vertigo (dizziness and equilibrium problems, fear to fall in elderly, isolated hearing loss, tinnitus), learning problems (dyslexia), and neurodegenerative diseases (types of dementia, Lewy-Body, Alzheimer). Major questions are the significance of instantaneous fluctuations of measures (physiologic, behavioral, e.g. in the case of dementia) in relation to physiopathology and progressive degeneration of cortical-subcortical circuits. Other examples could be given in immunology: investigations and global analysis over time and space (lymphoid tissues) have to be done to tackle antigen-receptor repertoire selection and functions of lymphocytes in a dynamic way under physiological (ontogeny to aging, gestation) and pathological conditions (cancer, autoimmunity, infections), and interactions with other biological systems like nervous, endocrine, metabolic systems. The global approach could be based on dynamics analysis of fluid lymphoid cell populations, identification and quantification of cell phenotype and functions (through imaging or flow cytometry) in relation with repertoires analysis, genomics and proteomics. In this line, deciphering the significance of immune repertoire diversity clearly requires to take into account their multiscale level from the molecule to cell populations as well as from the individual to species evolution (Boudinot et al. 2008).

Some specific issues to be addressed

1. To apply complex system principles and theoretical frameworks on designing experimental studies, and analysis of data at different scales (neurological, physiological, behavioral, neuro-psychological, immunological under physiological or pathological conditions) from individual or large patient populations.
2. To search for cross-correlations and interactions in order to get new insight about pathogenic primary or secondary mechanisms. This could lead to novel more sensitive differential diagnostic tools, but also for better medical care or functional re-adaptation. This requires developing new databases and global analysis tools, available for biologists/medical researchers, 1) to integrate billions of data issued from various technological approaches: not only from genomics and proteomics as currently developed, but also integrating clinical observations and reactions to treatments/stimulus, and other biological investigations like whole body/organ or microscopic imaging, individual phenotypic/functional cell analysis through multicolour flow-cytometry analysis, various functional tests, evolution with time…; 2) to generate

global analysis with new bioinformatics/statistics/mathematics and simulations tools.

Significance of this challenge

Most biological investigations are expected to provide knowledge transferred at some point to clinical research for handling human physiopathology. This means that we hope to cure better if we understand better. Now, as discussed before, a better understanding will only arise from an integrative view of biological systems. We thus need to develop further this integrated comprehension and to transfer the knowledge acquired in this framework toward clinical research and practice. This ambitious interdisciplinary approach should help in getting novel insights into pathology and ultimately innovative treatments.

Conclusion

How investigations should be driven in biology is a matter of debate. Should they be data-driven, object-driven or hypothesis-driven? Do we at least agree about the aim of deciphering the causality underlying biological processes? Do we expect models to bring insights and knowledge in biological systems behaviors through predictions? All these important questions and others are addressed by the complex systems science and its ever-growing biological interests. We can hope that new paradigms will soon arise that will enable us to more efficiently tackle human pathology and its treatments. Such a project will require going beyond a limited multi-disciplinarity of parallel different approaches and using complex systems tools to cross data and methods from different fields. Hence, by building a true "integrative global interdisciplinary multiscale approach", we will be able to go further in the comprehension of variation/stability in physiology and better characterize what give rise to pathologies. Merging various scientific communities' concepts and skills is obviously a first step towards this ambitious task.

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