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The nature of systems biology

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The advent of functional genomics has enabled the molecular biosciences to come a long way towards characterizing the molecular constituents of life. Yet, the challenge for biology overall is to understand how organisms function. By discovering how function arises in dynamic interactions, systems biology addresses the missing links between molecules and physiology. Top-down systems biology identifies molecular interaction networks on the basis of correlated molecular behavior observed in genome-wide 'omics' studies. Bottom-up systems biology examines the mechanisms through which functional properties arise in the interactions of known components. Here, we outline the challenges faced by systems biology and discuss limitations of the top-down and bottom-up approaches, which, despite these limitations, have already led to the discovery of mechanisms and principles that underlie cell function.

Early beginnings: molecular biosciences and systems sciences

The successes of the molecular biosciences – boosted by bioinformatics, genome-sequencing and high-throughput genome-wide experimentation ('omics') – have led to some characterization of most components of an appreciable number of organisms. This success has shifted the focus of research from molecules to networks [1]. Molecular bioscience studies molecules one by one with the aim of elucidating how molecules work, not to predict the consequences of particular biological mechanisms for the organism as a whole [2,3]. In characterizing the molecular basis of life, the molecular biosciences have become one of the most successful branches of science of the previous century, culminating in determination of the human genome sequence in 2001 [4,5]. However, the function of living organisms cannot be addressed satisfactorily by looking at molecules alone, not even if all molecules are studied. Such studies would not elucidate supramolecular functional properties such as the cell cycle, metabolic steady states and cell (dys-) function. They would not enable the understanding of multifactorial diseases nor would they empower white (industrial) and green (agricultural) biotechnology. To address the function and dysfunction of organisms, a systems approach is needed.

Until recently, systems analysis was not part of mainstream bioscience; most of the technology required for systems-level analysis of organisms was not yet available, mathematical equations were not used in molecular biology journals and experimental biology was frowned upon in the mathematical literature. In the past, other sciences developed systems perspectives, for example: (i) the development of nonlinear dynamical systems theory (bifurcation and chaos) in mathematics; (ii) understanding stochastic phenomena and self-organization using non-equilibrium thermodynamics and statistical physics; (iii) the use of biophysics and biochemistry in understanding cooperative enzyme kinetics, motor proteins and ion channels; and (iv) the use of engineering to develop control theory, system identification and metabolic engineering [6]. Systems perspectives taken in biology were mostly limited to ecology.

Systems biology approaches did not connect to the more rapidly expanding experimental molecular biosciences, largely because of the lack of sufficient quantitative molecular data. Worthwhile exceptions were developments in metabolic control analysis [7], biochemical systems theory [8], kinetic modeling (of metabolism and genetic circuitry) [9–11] and mechanistic non-equilibrium thermodynamics [12], with genomics, bioinformatics and metabolic engineering [6] all considered among the roots of systems biology [13].

The molecular biosciences deliver lots of data that require systems approaches to understand their implications for cell function. The more systems-oriented sciences avail of several systems approaches that have worked well for inanimate systems. Not all molecular bioscientists are familiar with current mathematical methods, nor are all mathematicians aware of acceptable biological mechanisms. Of course, implementation of mathematics to large datasets will deliver results that might look remarkable enough to be published. However, unless the systems biology is carried out correctly, it might not deliver an understanding of cell function that is based on molecular interactions. Therefore, in this review, we outline the challenges faced by systems biology and discuss two approaches that are helping to address such challenges: top-down and bottom-up systems biology. We outline the limitations of these approaches but also describe how they have already led to discoveries concerning the mechanisms and principles that underlie cell function.

Challenges faced by systems biology

The complete sequencing of the genome of an organism or the determination of the crystal structures of all of its proteins might constitute the biology of that system, and

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the processing of the data can require lots of mathematics. However, this does not contribute much to understanding how the interactions of the individual components lead to function and, thus, it does not constitute systems biology. A complete systems biological approach requires: (i) a (complete) characterization of an organism in terms of what its molecular constituents are, with which molecules they interact, and how these interactions lead to cell function; (ii) a spatio–temporal molecular characterization of a cell (e.g. component dynamics, compartmentalization, vesicle transport); and (iii) a thorough systems analysis of the 'molecular response' of a cell to external and internal perturbations. In addition, information from (i) and (ii) must be integrated into mathematical models to enable knowledge-testing by formulating predictions (hypotheses), the discovery of new biological mechanisms, calculation of the system behavior obtained under (iii), and finally, development of rational strategies for control and manipulation of cells. To accomplish all of these challenges, systems biology must integrate methods and approaches developed in other disciplines, which did not previously interface much with each other.

Microbiology: the haven of systems biology

The sheer number of unknown components of organisms made the falsification or verification of hypotheses *in vivo* virtually impossible. Functional genomics now enables the experimental analysis of complete sets of molecules at the mRNA level. In multicellular organisms, however, average expression levels are determined over various cell types. Moreover, the number of proteins in most multicellular organisms is so large that their identification through proteomics is still incomplete. Accordingly, notwithstanding the pressure towards human systems, systems biology should now be ready to prove itself in microbiology or with well-defined cultures of mammalian cells.

Top-down systems biology

With the introduction of the 'omics', the top-down approach emerged as a new and dominant method. It starts from a bird's eye view of the behavior of the system – from the top or the whole – by measuring genome-wide experimental data, and aims to discover and characterize biological mechanisms closer to the bottom – that is, the parts and their interactions (Figure 1). In this 'top-down' systems biology, the main objective is to discover new molecular mechanisms using an iterative cycle that starts with experimental data, followed by data analysis and data integration to determine correlations between concentrations of molecules, and ends with the formulation of hypotheses concerning co- and inter-regulation of groups of those molecules. These hypotheses then predict new correlations, which can be tested in new rounds of experiments or by further biochemical analyses. The major strengths of top-down systems biology are that it is potentially complete (i.e. genome-wide) and that it addresses the metabolome, fluxome, transcriptome and/or proteome [13] (Figure 1).

Any top-down systems biology study is faced with large experimental datasets (typically thousands of data points [14]) for a single organism subjected to a few perturbations.

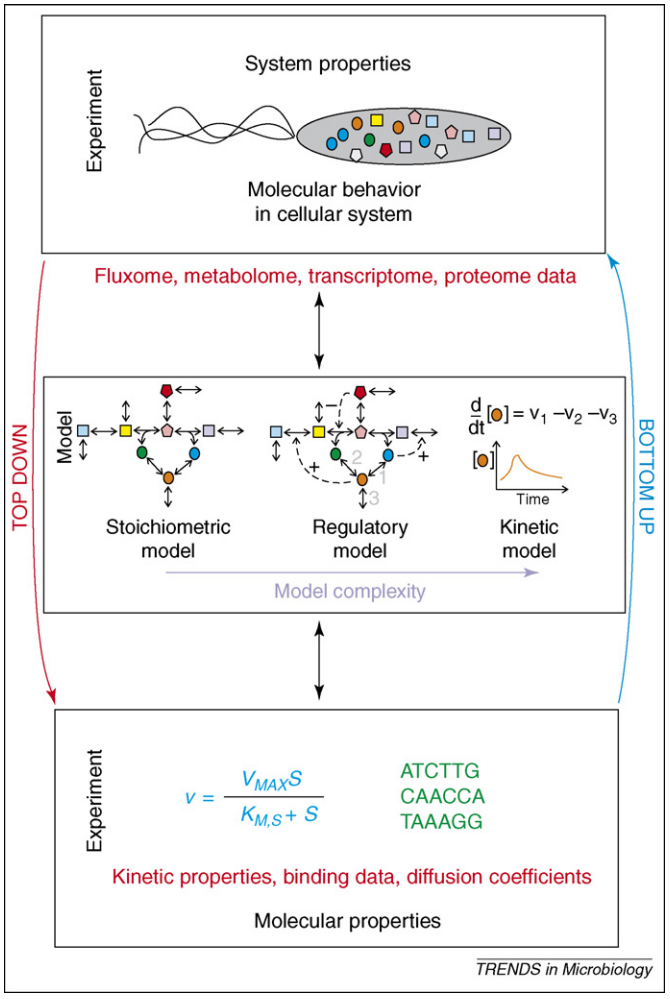

Figure 1. Diagram portraying the top-down and bottom-up approach to systems biology. The molecular properties, deriving from experiments carried out in the molecular biosciences and from bioinformatics, lie at the basis of the construction of various network descriptions (models). Three types of models that are often applied in systems biology are shown. Bottom-up systems biology starts with the molecular properties to construct models to predict systemic properties followed by experimental validation and model refinement. By contrast, top-down systems biology is systemic-data driven. It starts with experimental data to discover or refine pre-existing models that describe the measured data successfully. In this way, previously unidentified interactions, mechanisms and molecules can be identified. Contemporary bottom-up systems biology often considers kinetic models whereas top-down systems biology predominantly typically focuses on regulatory models to analyze data. Molecular species such as enzymes, transcription factors or metabolites are shown as colored shapes. Reactions are displayed as full arrows. Dashed arrows depict regulatory influences (e.g. inhibitory allosteric feedback interactions).

These perturbations can be genetic (e.g. resulting from one or more mutations or protein overexpression), environmental (e.g. changes in nutrients, growth factors or stress levels) or induced by RNA interference, by intrinsic dynamics such as the cell cycle, or by the administration of drugs. These studies aim to obtain a large enough view of system behavior to enable the discovery of behavioral patterns that are sufficiently generic to have predictive power about biological mechanisms present in the system [15–17], and to discover functionally related processes (modules) that might be under the control of a common set of transcription factors [18–20]. However, no matter how large the dataset, it remains difficult to bring about the large number of truly independent perturbations that are needed to disentangle the many mechanisms that

operate in living cells; the claims of the published studies can seem impressive but they cannot reveal more than the tip of the iceberg.

Most top-down systems biology uses different models at different phases of the characterization of the system. In the early stages, the experiments aim to discover the identity of the components, the model being that components correspond to DNA sequences. In the phase in which occurrences or concentrations of the identified components are correlated, mathematical models of sets of linearly dependent and independent variables can motivate principal component analysis, or Bayesian models can produce regulatory trees. The results suggest dependencies that guide new experiments, which leads to the discovery of new proposed interactions and mechanisms in a further iteration (for an example, see Ref. [21]). This iterative process can even be accomplished computationally [22].

The models used in top-down systems biology are phenomenological – that is, they are not based on mechanisms and, mostly, they do not even employ knowledge about relationships between molecular components. It should be noted that correlations are mapped onto virtual mechanisms, which might well describe the correlations but, nonetheless, can still be far from reality. This type of systems biology is mostly undertaken with cellular (sub-) systems that have not yet been characterized to a high level of mechanistic detail and in which much remains to be discovered (e.g. elucidation of protein–protein interaction networks or genetic networks). The approach is useful but only if its pitfalls are appreciated. One example is the use of Bayesian networks (which assume the absence of feedback) for those biological regulatory networks that are known to abound in feedback.

A second example is the frequent description of cellular regulation only in terms of gene networks, although it is clear that proteins, signal transduction and metabolism are involved in this regulation in addition to mRNAs [23]. The 'integrative' studies of '-omics' datasets that simultaneously measure transcriptomic, proteomic and fluxomic data address this issue properly but offer major challenges to data analysis [21,24–26]. One obtains 'vertical genomics' in which the change in the rate of a process can be traced back quantitatively to the various underlying causes (i.e. changes in the levels of its substrates, products, effectors, enzyme and mRNA) [26–30]. Such developments offer a new approach to cell biology by studying the extent to which various regulatory routes inside the networks of organisms contribute simultaneously to a particular behavior. In the study of adaptive responses of cells, considerable potential is seen for the application of methods derived in control theory in combination with detailed kinetic models of the associated networks [31–35].

Top-down systems biology concerns the identification of the structure of the molecular network that underlies system behavior, that is, 'reverse engineering' from system data alone. This can concern the 'real' mechanistic structure, a phenomenological structure such as a gene network (see earlier) [23,36,37] or merely the determination of the values of its parameters [38,39]. Proposed interaction structures of, for example, protein–protein interactions or gene networks can be tested by direct experimental measurement provided the proposal is explicit enough to mention proteins as the modes in the network [40–42]. The issue of how to choose the most informative experiments impinges on experimental design, standardization and system identification [17,43].

Bottom-up systems biology

In this article, systems biology is described as it has developed, in the perspective of what is needed to understand the functioning of living organisms in terms of molecular interactions. Whereas top-down systems biology gives insights through induction, bottom-up systems biology deduces the functional properties that could emerge from a subsystem that has been characterized to a high level of mechanistic detail using molecular methods. Bottom-up systems biology starts from the bottom (the constitutive parts) by formulating the interactive behavior (rate equation) of each component process (e.g. enzymatic process) of a manageable part of the system. It then integrates these formulations to predict system behavior. The ultimate aim of this approach is to combine the pathway models into a model for the entire system level – the top or the whole (Figure 1). As research and understanding progresses, such models are typically enlarged by the incorporation of more processes at high mechanistic detail. Examples of this approach include the series of combined modeling and experimental papers by Kholodenko and coworkers concerning the signaling network downstream of the epidermal growth factor receptor [44–47], and the modeling of central carbon metabolism in *Escherichia coli* [48–50] and *Trypanosome brucei* [51–53] (Box 1).

Bottom-up systems biology studies rely on: (i) experimental studies that determine the kinetic and physicochemical properties of the components (e.g. enzyme kinetics, diffusion properties) either by studying enzymes in isolation or by using parameter estimation strategies; (ii) data concerning responses of the subsystem to perturbations while it is in the context of the cell; (iii) the construction of detailed models to calculate the data from (ii) (for model validation and improvement) and to improve experimental design; and (iv) the development of tools for model analysis and representation. These models are mechanism-based rather than phenomenological.

The determination of kinetic parameters using parameter estimation strategies [38,39] suffers from an inherent risk. Because the kinetic models are often simplified, a parameter estimation strategy would not yield the 'true' kinetic parameters. As soon as the model is enlarged and the same data are again used for parameter estimation, the parameters will assume different values. In addition, gaps in our knowledge can be missed by falsely fitting them to a model ('fitted away'). However, in some cases (e.g. for eukaryotic signaling and eukaryotic gene expression), it is extremely difficult – if not impossible – to measure kinetic parameters *in vitro*, which leaves parameter estimation as the only option. One possibility to overcome this problem would be the development of technology to measure enzyme kinetics *in vivo*. Such an approach is now more feasible since the development of high-resolution imaging techniques.

Box 1. Models in systems biology

Various models are used in systems biology. Bottom-up systems biology is mostly concerned with kinetic models whereas top-down systems biology often considers structural or regulatory models. These three types of models are distinguished on the basis of the resources required to construct them and are compared in Table I.

Table I. Three different types of model used in systems biology[a]

Models	Structural		Kinetic
	Stoichiometric	Regulatory	
Model input			
Reaction stoichiometry[b]	+	+	+
Effectors of reactions[c]	–	+	+
Kinetic mechanisms[d]	–	–	+
Model output			
Steady-state fluxes	+[e]	+[f]	+[g]
Steady-state concentrations	–	–	+
Modules, motifs, small world	+	+	+
Control coefficients (MCA)	–	+[h]	+[i]
Dynamic rates of reactions	–	–	+
Dynamic concentrations	–	–	+

[a]Abbreviations: MCA, metabolic control analysis; +, incorporated in model; –, not incorporated in model.
[b]Reaction stoichiometry (e.g. hexokinase: glucose + ATP ↔ glucose-6P + ADP).
[c]Effectors of reactions (e.g. AMP is an activator of phosphofructokinase).
[d]Reaction mechanism. For example, for hexokinase:

$$V_{HK} = \frac{V_{MAX,HK}\frac{[GLC][ATP]}{K_{M,GLC}K_{M,ATP}}\left(1-\frac{[G6P][ADP]}{[GLC][ATP]K_{EQ,HK}}\right)}{\left(1+\frac{[GLC]}{K_{M,GLC}}+\frac{[G6P]}{K_{M,G6P}}\right)\left(1+\frac{[ATP]}{K_{M,ATP}}+\frac{[ADP]}{K_{M,ADP}}\right)}$$

[e]With flux balance analysis, an optimal flux distribution can be calculated provided that an optimality criterion is supplied [67].
[f]By application of flux balance analysis (the effector interactions are now redundant information).
[g]The numerical values of the fluxes can be calculated without use of optimality criteria.
[h]Qualitative distribution of control can be determined for particular systems [71].
[i]Quantitative distribution of control can be calculated.

One approach to bottom-up systems biology is the silicon cell program (http://www.siliconcell.net) [54]. In this program, the explicit aim is to make computer replicas of actual pathways, including the experimentally determined properties of all the molecular components [55]. Systems behavior is not fitted but calculated. If the calculated behavior does not correspond to experimentally observed system behavior, then this is seen as a falsification of the proposed silicon cell model and is not 'fitted away'. The silicon cell program is the combined effort of a global consortium of groups that construct precise biological models. After reviewing by associated journals, the models are made available through the web-based model JWS online, which enables online *in silico* experimentation such as the overexpression of an enzyme in the pathway to see whether that should increase productivity. JWS online is not about software development: its aim is to provide real precise models that are inclusive of the experimentally determined parameter values and that have been refereed by collaborating scientific journals. JWS is unique in that online experiments can be carried out without any knowledge of computer programming. Several other model databases now exist but they require downloading and the integration of models and modeling software.

Although all bottom-up systems biology studies have a common aim to obtain mechanism-based descriptions of subsystems of organisms, they often differ in the resources they use for model building (Box 1). Typically, the model structure is taken to reflect the 'real' stoichiometric structure and the structure of allosteric regulation accurately. However, whereas some modeling studies put experimental effort into precisely determining the kinetic parameters and enzyme mechanisms for the organism they are studying, others might take that information from the literature or fit kinetic parameters to simplified catalytic mechanisms. Thus, some bottom-up studies can be more accurate than others.

General principles of system behavior

Many efforts in biology are inspired by the observation that different species have many systemic properties and molecular mechanisms in common. This might be the result of ancestral relationships, convergent evolution, comparable evolutionary constraints (implicit in the organization of organisms and their niches) or the general character of evolutionary dynamics. Such interspecies commonalities lead to general principles that offer predictive power and a fundamental understanding of living systems that transcends single species. This has already resulted in exciting developments in the analysis of genomes and protein structures by bioinformatics.

One success story of systems biology is the general principles that have already been discovered; metabolic and hierarchical control analysis [7,12,56–58] and biochemical systems theory [8] were among the systems approaches that led to the discovery of such principles. These include the fact that the control of a function need not reside in a single 'rate-limiting' step but can be distributed over network components, provided that the sum of control equals 1 for the control of flux, zero for the control of transient concentrations, and minus 1 for duration, area under the curve and frequency [33,59,60]. Likewise, metabolic and gene-expression regulation must sum to 1 [61]. Distribution of control over gene expression and metabolic levels has been documented quantitatively for DNA supercoiling in *E. coli* [58]. Control is the system inverse of the interaction properties of the molecular components. All these principles lead to invariant relationships between network structure and control properties that transcend single species and, in this respect, they resemble the laws of physics. Many biological networks share structural characteristics with each other but also with networks of a non-biological origin: for example, a small-world and modular organization [1] and bow-tie organization [62]. Similar principles underlie the robustness of networks to intrinsic fluctuations and external perturbations [12,63,64]. Analyses of the fluctuations are especially exciting because they combine single-cell experimentation with theory and modeling [65,66]. The analysis of flux distributions through metabolic networks within the framework of 'constrained-based modeling' is an example of a popular (predominantly) theoretical framework for the analysis of certain general properties of molecular networks [67]. Other studies focus on the modular organization of networks [68] or the presence of network motifs [69]. It seems that this aspect of systems biology could lead to the development of appreciable fundamental insights into the principles that underlie biology.

Concluding remarks and future perspectives: challenges for systems biology

Systems biological studies on ill-characterized cellular (sub-) systems frequently take the form of top-down systems biology to identify correlations between the various variables of the systems. These are then formulated in terms of empirical relations between properties. This rarely (if at all) leads to the formulation of relations between properties in terms of molecular mechanisms. Although the emphasis formally lies on inductive discovery science, such discoveries rarely lead to molecular knowledge. We feel that these studies must either transform into or associate with the more mechanism-based systems biology associated with bottom-up systems biology. Given the scale of its daunting tasks, systems biology is still in its infancy, and it is important that it is done correctly to ensure meaningful results and to avoid ending up with another type of 'stamp collecting'.

Likewise, bottom-up systems biology should be kept to its proper mission. The aim should not merely be to see whether certain molecular interactions might cause a functional phenomenon – it is crucial that a phenomenon is actually demonstrated to occur. Therefore, much effort needs to go into the experimental determination of the actual interaction parameters and in the corresponding precise modeling. It should then be realized that bottom-up systems biology cannot tolerate possible unknown factors and, hence, will need to integrate with top-down, genome-wide systems biology to ensure completeness.

Furthermore, one should not forget that systems biology is a science and, hence, should discover generic principles. Consequently, silicon cells should not be seen as an aim but as a tool that can help to discover such general principles. In addition, systems biology should not be limited to finding principles of metabolic networks without taking gene-expression regulation into account, nor should it project the entire cell biology onto 'gene networks'. Principles that relate to all the dimensions of cellular organization should be discovered.

It seems that systems biology is here to stay just as much as molecular biology. The combination of synergistic application and further development of quantitative experimentation, modeling and theory is a promising approach that will bring biology to the next systems level. Further innovations include synthetic biology, which focuses on the experimental synthesis of pathways predicted by systems biology to exhibit new, exciting and useful properties (see Ref. [70] for an example).

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