Computational systems biology

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To understand complex biological systems requires the integration of experimental and computational research — in other words a systems biology approach. Computational biology, through pragmatic modelling and theoretical exploration, provides a powerful foundation from which to address critical scientific questions head-on. The reviews in this Insight cover many different aspects of this energetic field, although all, in one way or another, illuminate the functioning of modular circuits, including their robustness, design and manipulation. Computational systems biology addresses questions fundamental to our understanding of life, yet progress here will lead to practical innovations in medicine, drug discovery and engineering.

It is often said that biological systems, such as cells, are 'complex systems'. A popular notion of complex systems is of very large numbers of simple and identical elements interacting to produce 'complex' behaviours. The reality of biological systems is somewhat different. Here large numbers of functionally diverse, and frequently multifunctional, sets of elements interact selectively and nonlinearly to produce coherent rather than complex behaviours.

Unlike complex systems of simple elements, in which functions emerge from the properties of the networks they form rather than from any specific element, functions in biological systems rely on a combination of the network and the specific elements involved. For example, p53 (a 393-amino-acid protein sometimes called 'the guardian of genome') acts as tumour suppressor because of its position within a network of transcription factors. However, p53 is activated, inhibited and degraded by modifications such as phosphorylation, dephosphorylation and proteolytic degradation, while its targets are selected by the different modification patterns that exist; these are properties that reflect the complexity of the element itself. Neither p53 nor the network functions as a tumour suppressor in isolation. In this way, biological systems might be better characterized as symbiotic systems.

Molecular biology has uncovered a multitude of biological facts, such as genome sequences and protein properties, but this alone is not sufficient for interpreting biological systems. Cells, tissues, organs, organisms and ecological webs are systems of components whose specific interactions have been defined by evolution; thus a system-level understanding should be the prime goal of biology. Although advances in accurate, quantitative experimental approaches will doubtless continue, insights into the functioning of biological systems will not result from purely intuitive assaults. This is because of the intrinsic complexity of biological systems. A combination of experimental and computational approaches is expected to resolve this problem.

A two-pronged attack

Computational biology has two distinct branches: knowledge discovery, or data-mining, which extracts the hidden patterns from huge quantities of experimental data, forming hypotheses as a result; and simulation-based analysis, which tests hypotheses with *in silico* experiments, providing predictions to be tested by *in vitro* and *in vivo* studies.

Knowledge discovery is used extensively within bioinformatics for such tasks as the prediction of exon–intron and protein structure from sequence[1], and the inference of gene regulatory networks from expression profile[2–4]. These methods typically use predictions based on heuristics, on statistical discriminators that often involve sophisticated approaches (such as hidden Markov models) and on other linguistic-based algorithms (see review in this issue by Searls, pages 211–217).

In contrast, simulation attempts to predict the dynamics of systems so that the validity of the underlying assumptions can be tested. Detailed behaviours of computer-executable models are first compared with experimental observation. Inconsistency at this stage means that the assumptions that represent our knowledge on the system under consideration are at best incomplete. Models that survive initial validation can then be used to make predictions to be tested by experiments, as well as to explore questions that are not amenable to experimental inquiry.

Although traditional bioinformatics has been used widely for genome analysis, simulation-based approaches have received little mainstream attention. This is now changing. Current experimental molecular biology is now producing the high-throughput quantitative data needed to support simulation-based research. Combined with rapid progress of genome and proteome projects, this is convincing increasing numbers of researchers of the importance of a system-level approach[5]. At the same time, substantial advances in software and computational power have enabled the creation and analysis of reasonably realistic yet intricate biological models.

There are still issues to be resolved, but computational modelling and analysis are now able to provide useful biological insights and predictions for well understood targets such as bifurcation analysis of the cell cycle[6,7], metabolic analysis[8,9] or comparative studies of robustness of biological oscillation circuits[10].

It is crucial that individual research groups are able to exchange their models and create commonly accepted repositories and software environments that are available to all. Systems Biology Markup Language (SBML; http://www.sbml.org/), CellML (http://www.cellml.org/) and the Systems Biology Workbench are examples of efforts that aim to form a *de facto* standard and open software platform for modelling and analysis[11,12]. These significantly increase the value of the new generation of databases concerned with biological pathways, such as the Kyoto

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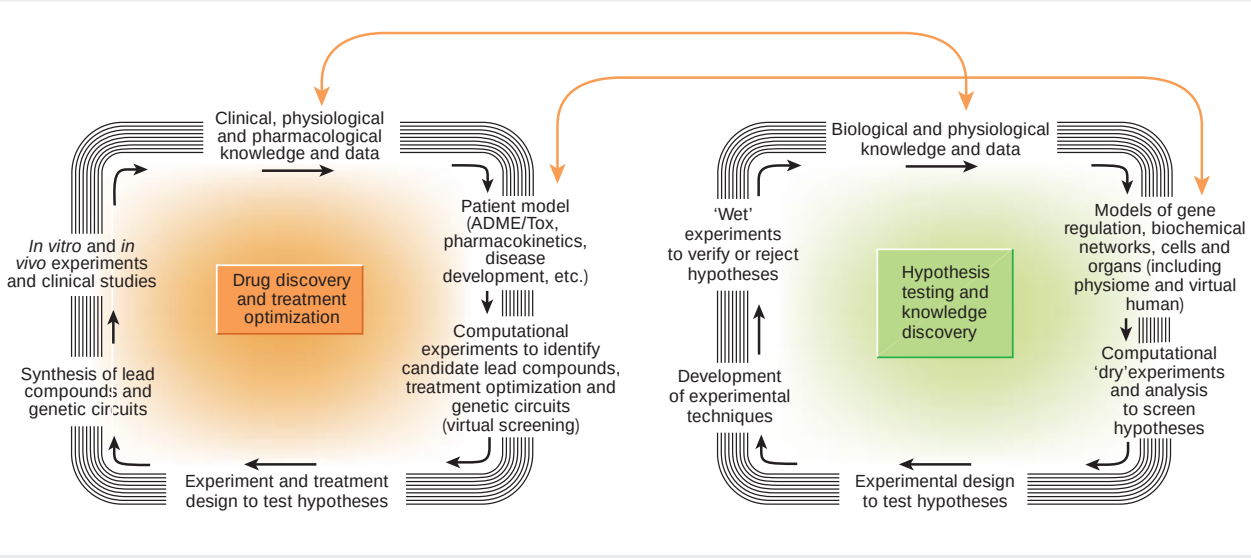

Figure 1 Linkage of a basic systems-biology research cycle with drug discovery and treatment cycles. Systems biology is an integrated process of computational modelling, system analysis, technology development for experiments, and quantitative experiments[18]. With sufficient progress in basic systems biology, this cycle can be applied to drug discovery and the development of new treatments. In the future, *in silico* experiments and screening of lead candidates and multiple drug systems, as well as introduced genetic circuits, will have a key role in the 'upstream' processes of the pharmaceutical industry, significantly reducing costs and increasing the success of product and service development.

Encyclopedia of Genes and Genomes (KEGG)[13], Alliance for Cellular Signaling (AfCS)[14] and Signal Transduction Knowledge Environment (STKE)[15], by enabling them to develop machine-executable models, rather than mere human-readable forms.

Such changes are fuelling a renewed interest in a system-level approach to biology, but we should not forget that this is an area with a long history[16,17], rooted as much as anywhere in classical physiology (see review in this issue by Buchman, pages 246–251). However, the close linkage between system-level understanding and molecular-level knowledge was made possible only by the recent progress in genomics and proteomics. The approach attempts to understand biological systems as systems, specifically targeting the identification of their structures and dynamics, and the establishment of methods to control cellular behaviours by external stimuli and to design genetic circuits with desired properties. These aims will be achieved only by combining computation, system analysis, new technologies for comprehensive and quantitative measurements, and high-throughput quantitative experimental data[18,19].

Multiple faces of robustness

Among various scientific questions, one issue receiving considerable attention is how robustness is achieved and how it evolves within various aspects of biological systems. Robust systems maintain their state and functions against external and internal perturbations, and robustness is an essential feature of biological systems, having been studied since the earliest attempts at a system-oriented view (for example, Cannon's homeostasis and Weiner's cybernetics[16]). Biological systems have been found to be robust at a variety of levels from genetic switches to physiological reactions (see review in this issue by Buchman, pages 246–251).

Robust systems are both relatively insensitive to alterations of their internal parameters and able to adapt to changes in their environment. In highly robust systems, even damage to their very structure produces only minor alterations in their behaviour. Such properties are achieved through feedback, modularity, redundancy and structural stability.

A variety of feedback and feed-forward control is observed throughout biology. For example, integral feedback is central to bacteria chemotaxis[20–22]. And p53-based cell-cycle arrest displays what is known in the engineering field as 'bang-bang control', a subtype of feedback control. Damage to DNA is sensed by proteins such as ATM (for ataxia telangiectasia mutated, named after a disease in which this enzyme is mutated) and DNA-dependent protein kinase, which activate the p53 protein. Active p53 then transactivates p21, which results in G1 arrest; this state is released when DNA damage is repaired, thus forming a feedback loop.

Cells themselves provide the most obvious form of biological modularity by physically partitioning off biochemical reactions. However, biochemical networks within cells also form modular compartments isolated by spatial localization[23], anchoring of proteins to plasma membranes and by dynamics.

Cells also provide redundancy, with many autonomous units carrying out identical roles. But redundancy also appears at other levels by having multiple genes that encode similar proteins, or multiple networks with complementary functions. For example, *Per1*, *Per2* and *Per3* genes encode proteins in the circadian oscillator, but knock-out of one or two of these produces no visible phenotype. The *Cln* gene family form redundant pairs for the cell cycle[24]. The stringent response of *Escherichia coli* activates alternative metabolic dynamics depending upon the availability of lactose and glucose[25].

Structurally stable network configurations increase insensitivity to parameter changes, noise and minor mutations. For example, elegant experiments on the archetypal genetic switch — the lambda phage decision circuit — have shown it to be robust against changes in binding affinity of promoters and repressors; its stable switching action arises from the structure of its network, rather than the specific affinities of its binding site[26]. Additionally, a number of networks for biological oscillations and transcriptional regulations have been shown to be tolerant against noise (ref. 27; and see review in this issue by Rao and colleagues, pages 231–237). But only computer simulation could have shown the degree to which the gene regulatory networks for segmentation during *Drosophila* embryogenesis remain robust over a large range of kinetic parameters[28,29].

The robustness of a system is not always to an organism's advantage. Cancer cells are extremely robust for their own growth and survival against various perturbations. They continue to proliferate, driven by the engine of the cell cycle, eliminating

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communication with their external environment, thus making it insensitive against external perturbations. In addition, many anti-cancer drugs are rendered ineffective by the normal functioning of a patient's body, including defence systems such as the metabolism of xenobiotics (most notably by cytochrome P450), the brain–blood barrier, and the dynamics of gene regulatory circuits, which can adjust the concentration of drug targets through feedback mechanisms and redundancy. To establish treatments that move patients from a stable but diseased state to a healthy one will require an in-depth, system-level understanding of biological robustness.

Although the general principles of robust systems are well established, there remain a number of unresolved issues concerning their evolution and execution in specific biological systems, and how they can be manipulated or designed. Control theory has been used to provide a theoretical underpinning of some robust systems, such as adaptation through negative feedback[21]. However, this approach has limitations. For example, current control theory assumes that target values or statuses are provided initially for the systems designer, whereas in biology such targets are created and revised continuously by the system itself. Such self-determined evolution is beyond the scope of current control theory.

No free lunch

Although robustness is critical in assuring the survival of a biological system, it does not come without cost. Carlson and Doyle emphasize the "robust, yet fragile" nature of complex systems exhibiting highly optimized tolerance[30,31]. Systems designed or evolved to be robust against common or known perturbations can often be fragile to new perturbations.

Another view on the vulnerability of complex network comes from a statistical perspective[32–34]. Comparative studies on robustness of large-scale networks show that scale-free networks (also known as 'small world' or Erdös–Rényi networks) are more robust than randomly connected networks against random failure of their components[34]. However, scale-free networks are more vulnerable against malfunction of the few highly connected nodes that function as hubs.

Scale-free networks can form by growth such that new nodes are connected preferentially to nodes that are already highly connected. Barabasi and colleagues claim that protein–protein interaction networks, which constitute the protein universe (see review in this issue by Koonin and colleagues, pages 218–223), are scale-free[32,35] and that mutations in highly connected proteins are more likely to be lethal than are mutations in less-connected nodes[33]. Although they estimated connectivity from yeast two-hybrid data, which are notoriously noisy, this hypothesis is intuitively attractive. For example, the p53 protein is one of the most connected hubs in the protein universe, and its mutations cause serious damage to cellular functions, particularly in repair of DNA damage and tumour suppression[36].

Nevertheless, some of the claims for scale-free networks are still controversial[37], and evidence for mechanisms leading to preferential attachment in biological systems remains equivocal. Furthermore, yeast two-hybrid assays produce many false-positive outcomes, and the current hand-crafted pathway maps may be heavily biased towards connection to functionally important genes simply because these have been popular targets for research.

Even when these shortcomings are surpassed, such statistics-based theories — despite providing insights on macroscopic properties of the network — will still have difficulty making predictions about specific interactions. It is analogous to telling a stock-market investor that "one in 50 companies will go bankrupt", advice that is of little help if you are unable to identify which one. The challenge for statistical theories is to identify how they can be linked to specific behaviours and so make useful predictions.

Design patterns of functional modules

Just as the principles behind robust networks can be classified into several types, so too can the various functional circuits or modules from which they are assembled, such as genetic switches, flip-flops, logic gates, amplifiers and oscillators. Good examples come from the mechanisms of biochemical oscillations (see review in this issue by Goldbeter, pages 238–245), which have been the focus of numerous groups[38–41]. These studies have facilitated their classification into several schemes, such as substrate-depletion oscillators, positive feedback loops, the Goodwin oscillator and time-delayed negative feedback oscillators[41]. Similar attempts have also been made for other functional networks. Jordan and colleagues have identified various examples of multitasking in signal transduction[42]; Bhalla and Iyengar reported several circuits that may function as temporal information stores (that is, memory devices)[43]; and Rao and colleagues have uncovered several circuits that mitigate the effect of noise and exploit it for specific functions (see review in this issue, pages 231–237).

Although these functional networks have analogues in electronic and process engineering, they have been formed by evolution, which makes it unlikely that any kind of 'first principle' underlies their design. However, a set of principles can be envisaged and identified through studying the structure and function of biological circuits, and their origin at the system level[44–46]. What are their basic functional building blocks? What are their dynamical properties and operating principles? How has each module evolved? And how can they be adapted or designed for alternative applications?

Recently, a systematic, high-throughput computational study was carried out by Shen-Orr and colleagues, which identified common motifs in the gene regulatory networks of *E. coli* using the RegulonDB database[47]. They found that feed-forward loops, single-input modules and dense overlapping regulons appeared frequently. While this study only used a gene regulation database, this type of approach can be augmented to include protein–protein and protein–DNA interactions to systematically identify network design patterns from large-scale data.

Such data, combined with function-driven identification of circuit patterns, will allow the creation of a large repository of functional biological networks, so enabling the systematic analysis of design patterns and their evolution. We already know of cases where the same circuit patterns and homologous genes produce similar system behaviours, but with unrelated physiological outcomes. We also know of cases where the same circuit patterns use different sets of genes to attain similar system behaviours, and where identical functions are achieved with degenerate paths involving different circuit patterns and different genes[46]. More systematic surveys will be needed to determine how many evolutionary conserved circuits exist, in what functions and how they relate to the evolution of genes. It may be that functional circuits should be considered the units of evolution.

Systems drug and treatment discovery

The systems biology approach, with its combination of computational, experimental and observational enquiry, is highly relevant to drug discovery and the optimization of medical treatment regimes for individual patients. Although the analysis of individual single nucleotide polymorphisms is expected to reveal individual genetic susceptibilities to all forms of pathological condition, it may be impossible to identify such relationships when complex interactions are involved.

Consider a hypothetical example where variations of gene A induce a certain disease. Susceptibility relationships may not be apparent if circuits exist to compensate for the effects of the variability. Polymorphisms in gene A will be linked to disease susceptibility only if these compensatory circuits break down for some reason. A more mechanistic, systems-based analysis will be necessary to elucidate more complex relationships involving multiple genes that may create new opportunities for drug discovery and treatment optimization.

Computer simulation and analysis, along with traditional bioinformatics approaches, have frequently been proposed to significantly increase the efficiency of drug discovery[48–50]. At present, empirical ADME/Tox (absorption distribution metabolism excretion/toxicity) and pharmacokinetic predictions have been used with some success.

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For example, a human intestinal absorption model based on correlations between the passive permeation measurement of over 300 compounds and known structural features, such as hydrogen-bond donors, hydrogen-bond acceptors and molecular weight, has been used to predict the absorption of novel compounds by the human intestine[51]. However, such models are not easily converted for use in other situations and they often require extensive data sets in order to address specific questions. What is needed are reliable, mechanism-based ADME/Tox and pharmacokinetic models[52–56], built on molecular-level models of cells, that are more easily transferable and accountable than are traditional, empirical, quantitative structure–activity relations.

Scaling up

So far, most systems biology simulations have tended to target relatively small sub-networks within cells, such as the feedback circuit for bacteria chemotaxis[20,21], the circadian rhythm[57,58], parts of signal-transduction pathways[43,59], simplified models of the cell cycle[7,60,61] and red blood cells[62–64]. Notable larger simulations have attempted to model bacterial metabolic networks for analysis of metabolic control[62,63] and flux balance[8,65], but these deal with steady-state rather than dynamic behaviour. Recently, research has begun on larger-scale simulations. At the level of the biochemical network, simulation of the epidermal growth factor (EGF) signal-transduction cascade has been carried out. The simulation involves over 100 equations and kinetic parameters and will be used to predict complex behaviours of the pathway, as well as to identify roles of external and internal EGF receptors[59]. The physiome project is an ambitious attempt to create virtual organs that represent essential features of organs *in silico*[66,67]. Simulation of the heart was one of the early attempts in this direction, integrating multiple scales of models from genetics to physiology[68]. Even whole-patient models for specific disease, such as obesity and diabetes, are being developed for prediction of disease development and drug discovery.

Building a full-scale patient model, or even a whole-cell or organ model, is a challenging enterprise. Multiple aspects of biological processes have to be integrated and the model predictions must be verified by biological and clinical data, which are at best sparse for this purpose. Integrating heterogeneous simulation models is a non-trivial research topic by itself, requiring integration of data of multiple scales, resolutions and modalities.

Simulation often requires integration of multiple hierarchies of models that are orders of magnitude different in terms of scale and qualitative properties (for example, gene regulations, biochemical networks, intercellular communications, tissue, organ and patient). Although some processes can be modelled by either stochastic computation or differential equations alone, many require a combination of both methods. But some biochemical processes take place within a millisecond whereas others can take hours or days. Additionally, biological processes often involve the interaction of different types of process, such as biochemical networks coupled to protein transport, chromosome dynamics, cell migration or morphological changes in tissues. Although biochemical networks may be reasonably modelled using differential equations and stochastic simulation, many cell biological phenomena require calculation of structural dynamics, deformation of elastic bodies, spring-mass models and other physical processes.

Nevertheless, development of precision models and their applications to ADME/Tox models are expected to revolutionize the process of drug discovery by providing a capability for multiple-target identification and high-throughput virtual screening of compounds. Furthermore, target identification using cellular models may provide desirable structures for candidate compounds by applying multiple constraints to parallel virtual screening[54], rationalizing drug discovery into a more systematic process (Fig. 1).

Systems therapy

Surpassing its scope for efficient improvements in the current paradigm of drug discovery and treatment, the introduction of a system-oriented view may drastically change the way treatments are conducted. Two somewhat speculative scenarios illustrate these opportunities.

Consider a feedback compensation circuit involving a drug target protein. Changes in the concentration of the protein resulting from drug administration may be neutralized by feedback control. High dosages of drugs will need to be administered to overcome this compensation mechanism, but this could produce serious side effects. Alternatively, small dosages of drugs could mitigate the feedback mechanism, so that the effect on the target protein will not be neutralized. Considering the p53 system, if there is abnormal overexpression of MDM2 (a protein that regulates p53), simply increasing p53 transcription may not restore the system to normal, as the excessive MDM2 protein will quickly ubiquitinate p53, targeting it for destruction. Additionally, p53 itself transactivates MDM2. MDM2 activity must be suspended or reduced to a normal level, at least temporarily, to make p53 stimulation effective in inducing cell-cycle arrest or apoptosis. The highly effective administration of multiple drug regimes can be accomplished only with a system-level analysis of the dynamics of gene regulatory circuits.

A far more futuristic approach proposes the introduction of functional genetic circuits to control cellular dynamics *in vivo* (see review in this issue by Hasty and colleagues, pages 224–230). Already, a set of basic functional circuits, such as oscillators and toggle switches, has been constructed and its viability confirmed in *E. coli* (refs 69–71; and see review by Hasty and colleagues). Computer simulation and comprehensive analysis will be needed to ensure that such circuits function as intended and do not result in significant side-effects. In the future, perhaps a genetic circuit can be devised to sense the level of p53 protein when DNA is damaged and switch on circuits to further increase transcription of p53.

The application of systems biology to medical practice is the future of medicine. Its realization will see drug discovery and the design of multiple drug therapies and therapeutic gene circuits being pursued just as occurs now with modern, complex engineering products — through iterative cycles of hypothesis and simulation-driven processes (Fig. 1). Although the road ahead is long and winding, it leads to a future where biology and medicine are transformed into precision engineering. □

doi:10.1038/nature01254

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Acknowledgements

I thank S. Imai, J. Doyle, J. Tyson, T.-M. Yi, N. Hiroi and M. Morohashi for their useful comments on the manuscript. This research is, in part, supported by: the Rice Genome and Simulation Project (Ministry of Agriculture), International Standard Development area of International Joint Research Grant (New Energy and Industrial Technology Development Organization (NEDO)/Japanese Ministry of Economy, Trade and Industry (METI)), Exploratory Research for Advanced Technology (ERATO) and Institute for Bioinformatics Research and Development (BIRD) program (Japan Science and Technology Corporation), and through the special coordination funds for promoting science and technology from the Japanese government's Ministry of Education, Culture, Sports, Science, and Technology.

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