Analysis and modelling of signal transduction pathways in systems biology

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Abstract
There is general agreement that a systems approach is needed for a better understanding of causal and functional relationships that generate the dynamics of biological networks and pathways. These observations have been the basis for efforts to get the engineering and physical sciences involved in life sciences. The emergence of systems biology as a new area of research is evidence for these developments. Dynamic modelling and simulation of signal transduction pathways is an important theme in systems biology and is getting growing attention from researchers with an interest in the analysis of dynamic systems. This paper introduces systems biology in terms of the analysis and modelling of signal transduction pathways. Focusing on mathematical representations of cellular dynamics, a number of emerging challenges and perspectives are discussed.

Introduction
A principal challenge for the life sciences is to understand the ‘organization’ and ‘dynamics’ of those components that make up a living system, i.e. to investigate the spatio-temporal relationships between (macro-)molecules, cells and tissues, that give rise to cause and effect in living systems. A major problem is that networks of cellular processes are regulated through complex interactions among a large number of genes, proteins and other molecules. From these considerations, the fundamental goal of systems biology [1–5] is to understand the nature of this regulation in order to gain greater insight into the mechanisms that determine the functions of cells, and ultimately their consequences at the physiological or phenotypic levels. In systems biology, this is achieved not through cataloguing and characterizing physical components, but through the integration of this information in mathematical models. Hence, the emergence of systems biology signals a shift of focus away from molecular characterization of the components in the cell to an understanding of functional activity through the interactions in molecular dynamics.

The dynamic relationship between components, their organization and regulation in complex cellular networks is still largely an open question. The fact that the cell is a dynamic system (or a large set of interacting dynamic processes) requires us in many cases to rethink the way in which we conduct experiments. The apparent success of mining genomic data has led to large collections of experiments, in which we are looking for differences or ‘patterns’. System theoreticians would argue that the ‘mining approach’ can, at most, identify associations between elements or variables, rendering inferences about causal entailment a scientific art-form that is based on the experience and creativity of the scientist. If it is accepted that system dynamics give rise to biological function, a different approach is necessary to identify causal entailment directly from experimental data. The systems approach is however more demanding on the experimental design in that only a systematic manipulation (through defined perturbations) of the system dynamics will allow us to identify these from experimental data directly. This also implies the need for quantitative, reliable and sufficiently rich data sets.

While our definition of systems biology may be an exciting proposal, the existing experimental limitations to generating data that are suitable for a systems approach, as well as the difficulties for systems theory to integrate space and time into a reasonably usable mathematical framework, provide enormous challenges. Systems biology is consequently not providing answers to all questions in genomics, but it does take genomics towards its natural conclusion: the understanding of cellular dynamics. Since any particular technology (e.g. proteomic gel techniques, transcriptomic microarrays) provides only part of the picture of gene expression, there is a need to fuse data obtained by using different technologies, and to combine information from experiments conducted by various research groups around the world. Dynamic mathematical modelling of large-scale networks meets difficulties because the necessary parameters for such models are rarely accessible. For predictive mathematical models and simulation of inter- and intracellular dynamics to fulfill their promise, it is thus crucial to investigate any particular system or problem with the full range of technologies and methodologies available. For this we have to combine data and models in a meaningful way: standards and
operating procedures are a problem for ‘wet-lab’ experiments as much as they are for ‘dry-lab’ modelling and simulation. The complexity of genetic pathways, the costs of experiments and the effort going into them, forces us to concentrate on models that are part of a larger whole. Pathways (i.e. regulated networks of biochemical reactions) are the conceptual framework of molecular and cell biology to describe inter- and intracellular dynamics. Research focuses on individual pathways and usually only a subset of proteins for any particular experimental set-up. In the following section we discuss the challenges and opportunities in systems biology using a specific signal transduction pathway as an example. While acknowledging the enormous complexity of such systems, the lack of reliable, accurate and sufficiently rich data sets, added to the inadequacies of our formal methodologies, we nevertheless find that even simple simulations, and the modelling process itself, can provide useful information, guiding experimental design and generating new hypotheses.

**Systems biology**

The most important recent development in the life sciences is that many biological problems are no longer just experimental but are increasingly conceptual. This is largely due to novel technologies that allow us to study many variables simultaneously, from different perspectives and with increasing accuracy. As a consequence, the analysis of data, generated by post-genomic technologies and mathematical modelling, based on these data, is becoming increasingly important [4,5]. Systems biology aims at a system level and signal-oriented understanding of pathways by investigating ‘inter-relationships’ (organization or structure) and ‘interactions’ (dynamics or behaviour) of RNA transcripts, proteins and metabolites. Biological function, an observed change, phenotype or physiological effect, does not reside in material objects (stretches of DNA, identified as genes), but arises from the spatio-temporal interactions of molecules that are the product of the information in the genome. Although a signal- and systems-oriented approach is the way forward in the understanding of gene expression and regulation, the limitations of a signal-oriented approach are obvious. The spatial organization of the cell is vitally important for its functioning but systems theory has had its difficulties in extending mathematical models and analyses to account for the position of dynamically interacting objects. Another problem for a systems-theoretic approach is that the direction of an information flow, i.e. the definition of inputs and outputs, is not always obvious in cellular processes. One common use of the term ‘network’ is evidence for this, suggesting a web-like structure rather than a ‘black-box’ system, which is associated with defined inputs and outputs. While in many cases the identification of what the inputs (independent variables) and outputs (dependent variables) are is in fact the objective of the analysis, in signalling we can often assume the hormone stimulation of receptors as our ‘input’ and the protein affecting transcription being the ‘output’. To identify causal relationships, one has to be able to manipulate the independent variables (‘inputs’) in a systematic fashion. In other words, a particular system is perturbed in different ways. A common strategy for grouping related genes from microarray data is to combine a series of quite unrelated experiments and if any two genes have similar expression profiles across experiments, this is used as evidence that they belong to a related functional class. In system identification we focus on one system and manipulate the input to the system with defined input profiles, allowing the quantification of dynamic interactions between system variables.

The ability to generate data for a large number of experiments, using high-throughput technologies, combined with the ‘heuristic’ or ‘mining’ approach to data analysis, has led to the creation of large databases. The idea is then to search (‘mine’) these datasets for pattern and associations between variables. The field of data mining has been successful with this strategy in the analysis of economic, financial and consumer data. It appears therefore natural to apply these ideas to what is often described as “vast amounts of biological data, waiting to be explored”. The mining approach is supported by newspaper headlines that suggest ‘the’ gene for disease ‘x’ has been discovered, with the small print suggesting that this discovery will lead us to a better ‘understanding’ of the disease. It is true that by identifying a key contributing factor, the likelihood for the discovery of a drug target has increased, but by no means is this a certain outcome, nor does it necessarily help the understanding of a disease. In fact, the discovery of a drug target may often be a chance result rather than the consequence of a better understanding of the complex mechanisms that underlie a disease. A mining approach is usually limited to associations (as one variable varies, it can describe the co-variation of another, its direction and intensity of others). The systems approach captures the causal entailment between chosen system variables and may thus contribute more to an understanding of the mechanisms that are responsible for biological function, or physiological effects.

To conclude this section, we would like to point out the relevance of signals in the transmission of information. The similarity of genome sequences between organisms is often commented on. For example, *Mycobacterium tuberculosis* (in humans) and *Mycobacterium bovis* (in cattle) are over 99.5% identical at the DNA sequence level and yet, at the metabolic, and phenotypic level they are rather different. The difference is less surprising by considering the following experiment. For a universe of discourse with eight possible spatial locations, there are 2 to the power of 8, that is 256, possible different patterns to be discerned in a comparative study. If we however allow the objects to be ordered as a sequence of temporal events, for only three time points, we have 256 to the power of 3, that is more than 16 million, different trajectories or carriers of information. The number of possible trajectories carrying information increases exponentially over time and with a more refined state space. If we accept the notion that it is system dynamics that gives rise to biological function, the variety of metabolic and physiological differences, despite
near-identical genome sequences, does not come as a surprise (see also Figure 1).

**Dynamic pathway modelling**

Cells are not running a program, but rather continually sensing their environment through receptors. To determine how cells act and interact we need to understand how information is transferred between and within cells. Cell signalling or ‘signal transduction’ is the mechanism by which this transfer of biological information comes about. In the previous section we argued that it is dynamic interactions of gene products and other molecules, not the genome itself, that gives rise to biological ‘function’ [6]. For experimental design this means that instead of trying to identify genes as causal agents for some change, function or phenotype, we should relate these observations to sequences of events. In other words, time-course experiments that investigate signal transfer and transient behaviour form the basis for dynamic pathway modelling. The aim is then to identify the direction and strength of relationships between variables in a pathway. The fact that most of these relationships are non-linear, and there exist feedback connections, makes this a non-trivial problem. Non-linearity implies a breakdown of the superposition principle (i.e. the whole is more than the sum of its parts). As a consequence, observations often do not match an intuitive or expected response. Feedback loops provide a different challenge in that their existence is often a key aspect of the biological investigation but mathematical formalisms to detect and quantify them are in short supply. Since in signal transduction we are primarily interested in dynamic interactions and transient behaviour, tools for steady-state analysis, developed for metabolic pathways, are only of limited use. Recent developments in the area of dynamic pathway modelling are as follows. Huang and Ferrell [7] developed a mathematical model of MAPK (mitogen-activated protein kinase) cascade and showed that the simulated response curve of MAPK with regard to MAPK kinase follows the experimental characteristic of p42 MAPK/ERK2 (extracellular-signal-regulated kinase 2) from Xenopus oocyte. Bhalla and Iyengar [8] proposed a signalling network model composed of multiple signalling pathways to identify network-level synergetic characteristics such as persistent activation, developmental and immunological memories, and strong information. Fussenegger et al. [9] developed a mathematical model describing the receptor-mediated and stress-induced caspase activation mechanism. Asthagiri and Lauffenburger [10] studied a negative-feedback mechanism of MAPK pathway through mathematical modelling and identified conditions for ultra-desensitization. Schoeberl et al. [11] investigated various responses of TNF (tumour necrosis factor) pathway and the related apoptosis mechanism in the HeLa cell. Heinrich et al. [12] developed a mathematical model of the receptor-stimulated kinase/phosphatase signalling cascade and studied various general properties of signal transduction pathways. Their analysis showed that phosphatases have a more pronounced effect than kinases on the rate and duration of signalling, whereas signal amplitude is controlled primarily by kinases. The models also revealed a surprising effect on the length of the signalling pathway. On the one hand, longer pathways tend to increase signalling time and duration. However, longer pathways also permit amplification to be distributed over more steps, which allows the same signal output to be achieved with faster phosphatase reactions. Bhalla et al. [13] proposed a computational model of MAPK1,2/protein kinase C signalling network and analysed its stability for growth factor stimulation. Hoffmann et al. [14] developed a mathematical model to study the dynamic properties of the IκB (inhibitory κB)/NF-κB (nuclear factor κB) signalling module. Schoeberl et al. [15] proposed a topological representation and a computational model of epidermal growth factor receptor signal transduction pathways. Cho et al. [16] developed a mathematical model to investigate the influence of RKIP (Raf kinase-inhibitor protein) on the ERK pathway.

Most of the mathematical models that have been developed are based on reaction kinetics and employ non-linear ordinary differential equations. The model parameters are usually estimated from in vitro experiments and/or complemented by information from the literature. In order to obtain a more comprehensive picture of cellular dynamics, there are still numerous hurdles to take: concerning means to identify suitable model topologies, parameter estimation techniques that can handle sparse data sets, and the generation of quantitative and sufficiently rich data sets. The next section provides an illustrative example of dynamic pathway modelling and the associated challenges.

**Example**

In this section, we consider a TNFα-mediated NF-κB signal transduction pathway in Figure 2 to illustrate the idea of analysis and modelling of signal transduction pathways, employing systems theoretic concepts.
Figure 2 | Biological cartoon of the TNFα-mediated NF-κB signal transduction pathway
For definitions see text.

TNFα is a potent pro-inflammatory cytokine that plays an important role in immunity and inflammation, and in the control of cell proliferation, differentiation and apoptosis [17,18]. TNFα exerts its effects through two distinct TNF receptors, TNFR1 and TNFR2. Binding of the inherently trimeric TNFα to TNFR1 and TNFR2 induces receptor trimerization and recruitment of several signalling proteins to the cytoplasmic domains of the receptor [19,20]. The first protein recruited to TNFR1 is TRADD (TNFR1-associated death domain protein), which serves as a platform to recruit at least three additional mediators, FADD (Fas-associated death domain), RIP (receptor-interacting protein) and TRAF2 (TNFR-associated factor 2). TRAF2 is required for recruitment of the IKK (IκB kinase) complex, which also contains the IKKa and IKKB catalytic subunits, to the activated TNFR1. FADD binds to TRADD and induces the caspase cascade via caspase-8 binding and proteolytic activation. Thus, caspase-8 is the initiating caspase where caspase-3 and caspase-7 represent major executional caspases (i.e. effector caspases) that cleave protein targets for apoptosis. NF-κB is regulated primarily by phosphorylation of inhibitory proteins, the IκB, which retain it in the cytoplasm of non-stimulated cell. In response to TNFα and other antagonists, the IκB is phosphorylated by the IKK complex, resulting in its ubiquitination and degradation, followed by the nuclear translocation of the freed NF-κB. The anti-apoptotic activity of NF-κB depends on gene induction. In fact, NF-κB induces the expression of a number of genes whose products can inhibit apoptosis; c-IAPs, Flice, A1, TRAF1 and TRAF2. The best studied protein of them is the c-IAP (cellular inhibitor of apoptosis protein), which directly binds and inhibits effector caspses such as caspase-3 and caspase-7. Figure 3 describes the so-called ‘circuit diagram’ of the biokinetic reactions for which a mathematical model is used to simulate the influence of ligand variation on the pathway.

The mathematical model corresponding to Figure 3 comprises a cell proliferation module, an apoptosis module and an anti-apoptosis module. The cell proliferation branches off from the TNFα/TNFR1/TRADD complex (m5) and transfers through TRAF2 (m6) and IKK (m8). The signal further propagates such that NF-κB (m14) translocates into the nucleus and transcribes the target gene(s). On the other hand, the apoptosis signal diverges from the TNFα/TNFR1/TRADD complex (m5) and transfers through caspase-8 (m17) and the effector (m20). The activated effector (m22) translocates into the nucleus and induces the fragmentation of the target DNA. The anti-apoptosis module triggers NF-κB (m14) to express the anti-apoptosis factor c-IAP (m23) and this c-IAP inhibits the activation of the effector by binding it in the apoptosis module.

Based on reaction kinetics [21] and the topology of the given signal transduction pathway, the whole pathway can be modelled in general by a set of non-linear differential equations and a set of algebraic conditions (depending on the loop conditions) as follows:

\[
\frac{dm(t)}{dt} = h[m(t), k(t), \xi(t)]
\]

subject to \(g[dm(t)/dt, m(t)]\) where \(m(t) = [m_1(t), m_2(t), \ldots, m_n(t)], k(t) = [k_1(t), k_2(t), \ldots, k_q(t)], \xi(t)\) denotes uncertainty, such as noise effects in \textit{in vivo} environments, \(m_i(t)\) represents \(\text{cellular inhibitor of apoptosis protein}\), which directly binds and inhibits effector caspses such as caspase-3 and caspase-7. Figure 3 describes the so-called ‘circuit diagram’ of the biokinetic reactions for which a mathematical model is used to simulate the influence of ligand variation on the pathway.

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Figure 3  |  Graphical model (‘circuit diagram’) of the TNFα-mediated NF-κB signal transduction pathway

A circle represents the state variable relating to the concentration of a signalling protein, a rectangle represents a rate of reaction, and directed arcs (arrows) connect the circles and the rectangles.

The accurate numerical estimation of parameter values for these reduced nonlinear differential equations remains a limiting step in biomathematical modelling [22,23]. If a reasonable model is constructed from parameter estimates, this can then be used in a variety of ways to validate and generate hypotheses, or to help experimental design [16,24,25]. Based on the mathematical model illustrated in Figure 3, and the estimated parameter values as for example obtained using a discretization of the non-linear ordinary differential equations, we can perform simulation studies to validate the signal transduction mechanism (via simulation of fixed initial conditions) as illustrated in Figure 4 and also to analyse the signal transduction system with respect to the sensitivity for the ligand (via simulation of variable initial conditions) as illustrated in Figure 5.

Summary and conclusions

The post-genomic challenge is to understand both the ‘organization’ and the ‘dynamics’ of genetic pathways. More specifically, we can summarize the challenges for dynamic pathway modelling as follows. (i) Numerical issues introduced by time delays affect simulation and parameter estimation. (ii) The identification and quantification of feedback

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Figure 4 | Simulation results of the mathematical model for fixed initial conditions
The upper left plot shows the dynamics for TNFα/TNFR1, TRADD and their complex TNFα/TNFR1, the upper right plot shows the activity of IKK, IKK* and IκB/NF-κB complex, the lower left shows the activity of the IκB/NF-κB/IKK* complex, the freed NF-κB and the TNFα/TNFR1/TRADD/FADD complex, and the lower right shows the activity of the activated caspase-8, the effector caspase and c-IAP which binds with the effector and thereby inhibits apoptosis.

Figure 5 | Simulation results according to the variation of the concentration of TNFα
The upper left plot shows the change of concentration of TNFα, the upper right shows TRADD, the lower left shows the activated IKK and the lower right shows IκB/NF-κB/IKK* complex.
relations is at the core of dynamic pathway modelling. However, feedback loops cannot be easily identified from a system which is under control when observed. (iii) For most systems, we deal with a relatively large number of variables but get relatively few measurements. (iv) Mathematical modelling and simulation should be employed to identify key components and subsystems in a large network, so as to help experimental design. This requires techniques to reduce the complex, large-scale systems, without a loss of predictive power. (v) Numerical solutions and simulations are a suitable approach but rely on knowledge about the initial conditions and parameter values. Analytical solutions are usually not feasible with existing tools. Tools for bifurcation or phase-space analysis should be developed to support the analysis of signal transduction pathways. (vi) Regulation and control takes place in cells over a wide range of time scales. This not only poses challenges for numerical simulations, but also suggests hybrid models that combine stochastic formalism (e.g. dealing with few molecules or modulating at transcriptional level) with deterministic representations (e.g. dealing with the flow of material). Qualitative or rule-based formalisms may provide sufficiently predictive models at ‘higher levels’, describing the emergent behaviour, while agent-based simulations may be better suited to model the interactions of large numbers of small components. (vii) Dealing with spatio-temporal dynamics, a direct translation of proteins into mathematical variables is often not feasible; the cell is not a homogeneous soup of molecules floating freely about. For example, longer diffusion times would suggest the use of partial differential equations rather than ordinary differential equations. Well-defined regions or compartments are less of a problem as we can deal with them by simply adding more variables to the model, accounting for different compartments.

In the context of post-genome technologies, the key challenges in systems biology can be summarized as dealing with ‘complexity’ (defined by the usually large number of variables) and ‘uncertainty’ (caused by the difficulties in observing cellular dynamics). Modelling the interaction of phenomena that happen on a wide range of scales in space, and time, organized complexity, is a primary post-genomic challenge. The data we currently have available on the other hand, do not permit the use of well-established engineering tools for parametric system identification. A conclusion from this is that mathematical modelling and simulation of cellular systems may have the same fate as weather forecasting: regardless of the computer power and time available, the predictions remain uncertain. However, even if we will never be able to build accurate predictive models of cellular or genetic systems, systems thinking and the modelling process itself will prove valuable to the biologist, helping him to identify which variables to measure and why. In fact, a common engineering experience is that we learn most from those models that fail. The quest for precision is analogous to the quest for certainty and both precision and absolute certainty are impossible to attain in understanding cellular dynamics, at present if not in general. Experience shows that even dramatically simplified models, based on many assumptions, can still capture essential phenomena and thereby support hypothesis testing and experimental design.

Systems biology attracts new people, new disciplines, and new ideas to the life sciences. In particular, new ideas are necessary if we are to make sense of the data and information that are being accumulated. It is not their existing toolbox for which, say, mathematicians and engineers are wanted but for the development of new mathematics, novel methodologies, and computational tools to realize those. Systems biology is connecting people and ideas. Only open-minded interdisciplinary research integrating the ‘wet-lab’ with ‘dry-lab’ can pave the way to a better understanding of cellular dynamics.

This work was supported by a grant from the Korean Ministry of Science and Technology (Korean Systems Biology Research Grant, M10309000006-03B5009-00211).

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Received 24 July 2003

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