6 A Plea for More Theory in Molecular Biology

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Abstract. The integrationist principles of systems theory have proven hugely successful in the physical sciences and engineering. It is an underlying assumption made in the systems approach to biology that they can also be used to understand biological phenomena at the level of an entire organism or organ. Within this holistic vision, the vast majority of systems biology research projects investigate phenomena at the level of the cell, with the belief that unifying principles established at the most basic level can establish a framework within which we may understand phenomena at higher levels of organization. In this spirit, and to use a celestial analogy, if a disease – effecting an organ or entire body – is our universe of discourse, then the cell is the star we gaze at. In building an understanding of disease and the effect of drugs, systems biology makes an implicit assumption about direct causal entailment between cell function and physiology. A skeptic might argue that this is about the same as trying to predict the world economy from observations made at a local supermarket. However, assuming for the moment that the money and hope we are investing in molecular biology, genomics, and systems biology is justified, how should this amazing

intellectual achievement be possible? In this chapter we argue that an essential tool to progress is a systems theory that allows biological objects and their operational characteristics to be captured in a succinct yet general form. Armed with this conceptual framework, we construct mathematical representations of standard cellular and intercellular functions which can be integrated to describe more general processes of cell complexes, and potentially entire organ.

6.1 Crossing Levels

The cell is the basic building block for higher levels of structural organization, including the various tissues and organs within a body. It is for this reason that in trying to understand phenomena at the level of an organ or whole organism, the vast majority of systems biology research projects focuses on observations at the level of the cell. What is suggested here is that we are dealing with two kinds of organization: structural and functional organization. The cell's structural organization is given by the cell membrane, a nucleus in eucaryotic systems, and all those components that make up the cell as a biophysical entity. For the functional organization of a system, say an organ such as the liver, detoxification is achieved through hepatocyte cells realizing their role (function). At the level of the cell then, the concept of a pathway, understood as a network of biochemical reactions, is used to explain cell function, including cell differentiation, proliferation, and apoptosis.

To explain disease mechanisms, we need to understand the structural *and* functional organization of cells and organs. The internal structural organization of the cell, the molecular characterization of its components, has been the focus of modern molecular and cell biology, with the areas of genomics and bioinformatics taking center stage as a means of understanding the process. The recent emergence of systems biology on the other hand, signals a shift in focus, away from molecular characterization and cataloguing of components, toward an understanding of functional activity. This trend is illustrated in Fig. 1, where the diagram shows the continuity of development from the contribution of bioinformatic cataloging to the functional description using systems biology. Our proposition is that this shift of attention toward functional organiza-

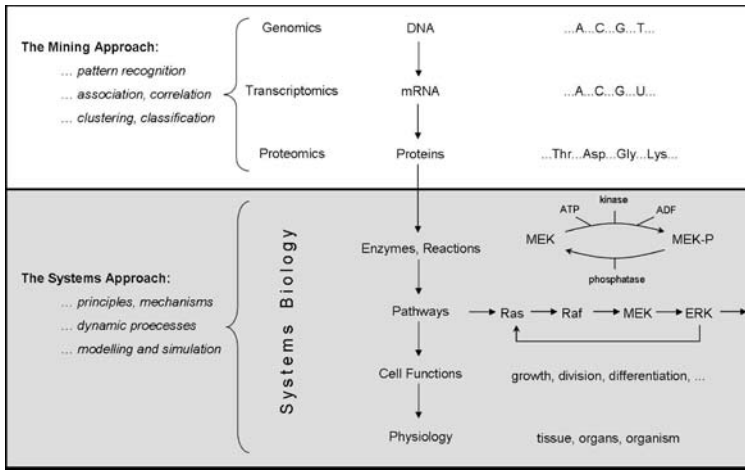


Fig. 1. The Emergence of Systems Biology as an Area of Research, Separate from Genomics and Bioinformatics, Signals a Shift of Focus, Away from Molecular Characterization and Toward an Understanding of Functional activity

tion is the crucial next step toward understanding the mechanisms of life. To use a culinary comparison, bioinformatics gave us the ingredients list in the recipe of life. But ingredients can combine in many different ways and with different outcomes. Therefore, to complete the recipe we must understand how to combine the ingredients in the correct way. We will argue that to do this will require new theoretical tools from systems theory.

While the structural units that make up the parts of a larger whole suggest themselves more easily, the identification of functional units (e.g., pathways) and the study of their interactions is a major challenge for systems biology research. To us the most important questions of systems biology are

> *How do the components within a cell interact, so as to bring about the cell's* structure *and realize its* functioning*?* (intra-cellular dynamics)
> *How do cells interact, so as to develop and maintain higher levels of* structural *and* functional organization*?* (inter-cellular dynamics)

With systems theory being the study of function and organization *per se*, systems biology is appropriately defined as a merger of systems theory with molecular biology [1, 2]. To demonstrate this in technical terms, the following section takes us from the general systems description of function and organization within an abstract setting, to a systems description of signal transduction pathways as functional units. By going from a general system to the model of a specific pathway, we show that once we look beyond single pathways there is a conceptual framework in which to study truly complex systems in the abstract, and which allows observations of biological function to be generalized. The reader with an allergic reaction to mathematical expressions may skip the next section, in order to read the discussion on why abstraction is useful and how theory can be practical.

6.2 Systems Thinking

Reviewing the book Systems Theory and Biology: Proceedings of the 3rd Systems Symposium (Cleveland, Ohio, October 1966, edited by M.D. Mesarović, [3]), Robert Rosen writes in 1968:

> "There is no doubt that system-theoretic ideas seem somewhat strange, and perhaps just a little frightening, to the present generation of structurally oriented biologists. It is not without irony that these system-theoretic ideas actually mark a return to the holistic, functionally oriented view of organisms entertained by biologists prior to the emergence of biochemistry and molecular biology, a view which was displaced by the rapid growth of these fields. (. . .) System theory is emerging as a force in modern biology because (i) extremely powerful new formal tools are now available for the study of functional activities (particularly regulation, control, and information processing) and (ii) the limitations of what we can really learn about basic biological problems in purely structural terms are rapidly becoming apparent." [4]

The early encounters of systems theory with biology unfortunately could not fulfill their promise. This was no fault of systems theory *per se*, but due to the lack of experimental data with which the systems theorist could test and develop methodologies. Optimists would of course argue

that the current situation is very different in that we have now vastly improved technologies to make observations and take measurements of the cell. Indeed, buoyed forward by the success of high-throughput measurement in the Human Genome Project, measurement technologies continue to improve and now extend beyond the measurement of static 'omic' properties to the crucial area for systems theoretic modeling – system dynamics. General systems theory [5, 6] not only provides us with techniques to model and tools to simulate systems, but also a methodology that is a way of thinking about complex hierarchical organizations. What we are therefore assuming is that the inside of the cell, as well as populations of cells, are not random collections of bio-molecules, but organized entities that we can describe in terms of interacting functional and structural units.

For complex systems, the well-known dictum is that "the whole is more than its parts, looked at in isolation." The correct conclusion from this is that the cell must not be looked at in isolation but must be considered in its social context. However, we cannot escape reductionism. Indeed, the very complexity of the systems under consideration and the difficulties in conducting experiments force us to look at parts or subsystems, but these must be considered in their context if we wish to understand how they realize their function in a larger whole.

The most basic definition of a system is that of a set of related objects. Formally, a general system is a relation on variables/indicators/items defined in set theoretic terms

$$\mathcal{S} \subset O_1 \times O_2 \times \cdots$$

The definition of a complex system follows naturally as a relation on systems/subsystems, i.e.,

$$\mathcal{S} \subset \prod_{j \in J} \mathcal{S}_j \,,$$

such that there is a distinct behavior of the complex system while the integrity of the subsystem is preserved. When (sub)systems interact, they do this through defined interfaces, which we may refer to as inputs and outputs. For example, in cell signaling, membrane receptors suggest themselves as inputs, receiving a stimulus in the form of ligands

binding to them. The expression level of a target gene may be defined as the response, respectively output of the pathway as a system. More formally, a signal transduction pathway may thus be described as the system

$$\mathcal{S} \subseteq \Omega \times \Gamma ,$$

where Ω and Γ are related to the stimulus and response, respectively. Most important for a philosophy of systems biology is that we understand a pathway not as a static graph, but as a network of biochemical reactions, that is to say, a dynamic system that establishes a causal connection between stimulus and response. This means that we define a pathway formally as the mapping

$$\begin{aligned} \sigma &: \Omega \to \Gamma \\ \omega &\mapsto \gamma \end{aligned}$$

where a stimulus ω (respectively, response γ) is defined as a temporal sequence of events

$$\Omega = \{\omega : I \to U\} , \qquad \Gamma = \{\gamma : I \to Y\} .$$

At any point in time $t \in I$, our system σ receives a stimulus $u(t)$ to which it responds at time t with $y(t)$. We assume that stimuli and responses take their values from constant sets U, respectively Y.

A key concept in systems biology is that of a signal $\omega:]t_0, t_1] \to U$ acting on the system between time t_0 and t_1, generating a response $\gamma:]t_0, t_1] \to Y$. In general, we denote the set of acceptable stimuli in terms of the mapping

$$\omega : I \to U ,$$

and for the response

$$\gamma : I \to Y ,$$

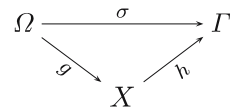
where for concentrations we usually assume positive real values $U \subseteq \mathbb{R}_+^m$ and $Y \subseteq \mathbb{R}_+^q$. For $m = 1$ and $q = 1$ the vector-valued notation reduces to a single signal or time series. If $I = \mathbb{Z}_+$ we have a discrete-time system model, which corresponds to the collection of experimental data, and

in which case we could consider Ω and Γ as finite-dimensional vector spaces, encoding sequences of events

$$\omega = \big(u(0), u(1), \dots\big) ,$$
$$\gamma = \big(y(1), y(2), \dots\big) .$$

In modeling it often makes sense to assume a signal that is continuous in value and time. For $I = \mathbb{R}_+$ a continuous-time system, with $\omega\colon]t_1, t_2] \to U$ in Ω and $\gamma\colon]t_2, t_3] \to Y$ in Γ. The entire sets of stimuli and responses that the cell can realize form the objects Ω and Γ of our definition of a stimulus–response system.

The description of a pathway σ as a mapping from Ω to Γ, is an external description, without consideration for the internal interactions that generate its input–output behavior. Extending therefore our abstract model with a state-space X, we have the following state-space representation:



Dynamic pathway modeling is the process by which we identify and characterize the mathematical objects Ω, Γ, X as well as the mappings that put these objects in relation to another. At any point in time, the behavior of the system is thus encapsulated by the state $x \in X$. The temporal evolution of the state, $x(t)$, implies the existence of a state-transition map

$$\varphi : I \times I \times X \times \Omega \to X$$

whose value is the state $x(t) = \varphi(t; t_0, x, \omega)$, that is, an element of the state-space X. In this setting, the state x at time t arises from an initial state $x_0 = x(t_0) \in X$ at some initial time $t_0 \in I$ under the action of stimulus $\omega \in \Omega$.

Depending on the nature of the biological process under consideration, but also motivated by personal preference and mathematical convenience, one can choose among a number formalisms by which to translate biological understanding into a mathematical model. To name but a few,

we can distinguish between mass-action, power-law, or S-systems, and Michaelis-Menten models [7, 8] that are based on differential equations. The computer scientist tends to find the setting of automata theory, machines and formal languages (π-calculus, Petri-nets, process algebras) more preferable. For any formalism mentioned, one question is always the role or relevance of randomness [9]. If the answer is that random fluctuations cannot be ignored, we are led to stochastic models, based on Markov-processes, Langevin-, or Chapman-Kolmogorov equations [10].

By far the most frequently employed approach is to represent temporal changes of protein concentrations as differential (rate) equations

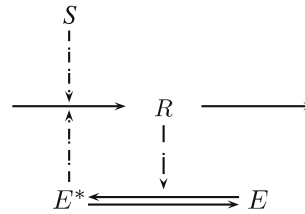
$$\dot{x} = V\big(x(t), u(t)\big)$$
$$y(t) = h\big(x(t)\big)$$

Here $\dot{x}$ describes the rate of change in state vector $x = (x_1, \ldots, x_n)$ at time t. Above V is again a map, which in the context of dynamic systems theory, is referred to as a vector field. The map h describes the observations we make on the states of the system. A special case of the above representation is a class of nonlinear systems models for which there exists some experience

$$\dot{x} = f\big(x(t)\big) + \sum_{i=1}^{m} g_i\big(x(t)\big)u_i(t), \;\; y_j = h_j\big(x(t)\big), \;\; 1 \le j \le q \; .$$

Variable u is considered a state-independent external control input to the system. Not surprisingly, most of the research around this formalism has emerged from the control engineering community (e.g. [11, 12, 13]). A computer simulation of the temporal changes, $x(t)$, of protein concentrations is referred to as the solution of the differential equations above. Toward this end we interpret the above introduced map φ as an evolution operator $\varphi^t : X \to X$, satisfying the semigroup properties $\varphi^0 = \mathrm{id}$ and $\varphi^{t+s} = \varphi^t \circ \varphi^s$. The action of φ^t on a set of initial conditions is then referred to as a flow. The graph of φ in $I \times X$, that is, the set $\{\varphi^t \circ x_0\}$ is called the orbit through x_0. The orbits $\{x(t)\}$ are also known as solution trajectories of the system.

Let us now look at an example and consider the following simple biochemical reaction network:



In this pathway map, S denotes the stimulus, R the response, and E is an intermediate component, and E^* denotes its activated form. The diagram immediately suggests the existence of some positive feedback loop: an increase in R causes an increase in the activation of E, which in turn should increase R. A rate equation that realizes this system is the following

$$\frac{dR}{dt} = k_0 E^*(R) + k_1 S - k_2 R$$

where $E^*(R)$ is short for the Goldbeter-Koshland function [14]. The analysis of the system proceeds as follows: The right-hand side of the rate equation above consists of two parts, positive contributions for the production of R, that is, $k_0 E^*(R) + k_1 S$, and its degradation, $k_2 R$. If we plot these two curves (Fig. 2), we at once see that this simple system can show some interesting behavior. The intersections of the two lines indicates steady states in which the rate of production and the rate of degradation are balanced. The number of such steady states and their nature are an important characteristic for the behavior of a dynamic system. We see that for different levels of the stimulus, the system may display rather different behaviors without the system itself having changed.

Figure 3 (left) illustrates the bistable behavior of this system. As a critical threshold of S is passed, the system switches to a high steady state level of R. The simulation of the system for different initial conditions $R(o)$ (shown on the right) reveals the sensitivity of the systems behavior on initial conditions.

What we have discussed so far provides us with an analysis of the qualitative behavior of the system. The stimulus–response curve predicts what kind of temporal behavior could display. This is very important since the observations we make strongly depend on the strength

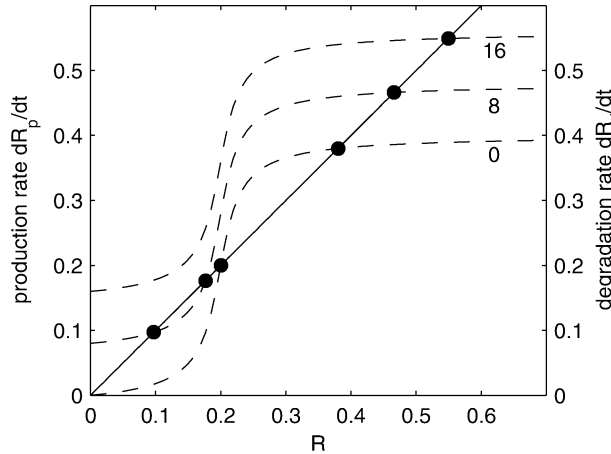


Fig. 2. Comparison of the rate of degradation (*solid line*) and the rate of production for different signal strengths (*dashed lines*)

and duration of the stimulus as well as initial conditions. As shown in Fig. 4, if in two separate experiments we choose a stimulus which is below/above the critical point or we choose a stimuli of different duration, we may observe rather different curves from the same system. It appears that without exhaustive perturbation studies, we are not able to unambiguously describe the system. The conclusions we can draw from this simple system are significant. If we accept the view that the cell realizes its function in space and time, then for even simple systems the design of experiments will require systematic perturbations, which are far more expensive and time- consuming than what is currently feasible or considered acceptable. To make matters worse, despite considerable advances of dynamic systems theory, there is a need for more research on methods that help us reconstruct or hypothesize rate equations from experimental data.

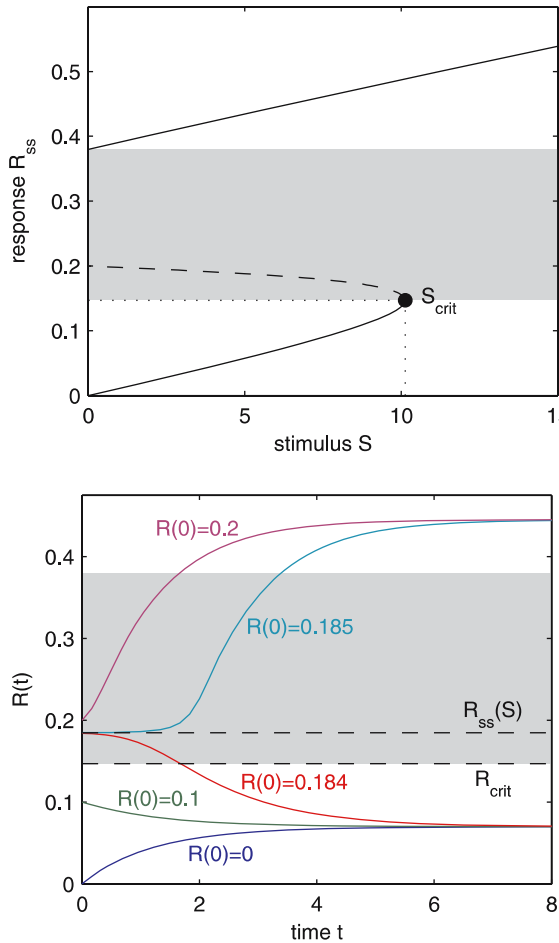


Fig. 3. First: Signal-response curve. The steady states (stable solutions) are shown as *solid lines*. The unstable solution is drawn as a *dashed line*. Because of the two stable branches, the system is called bistable. Second: Relaxation into the steady state for different initial response signals R_0. For $R_0 < R_{ss}(S)$ the system achieves a steady state on the lower branch. Outside this range the upper branch becomes the final state. For comparison, the unstable solution and the critical response signal R_{crit} are shown

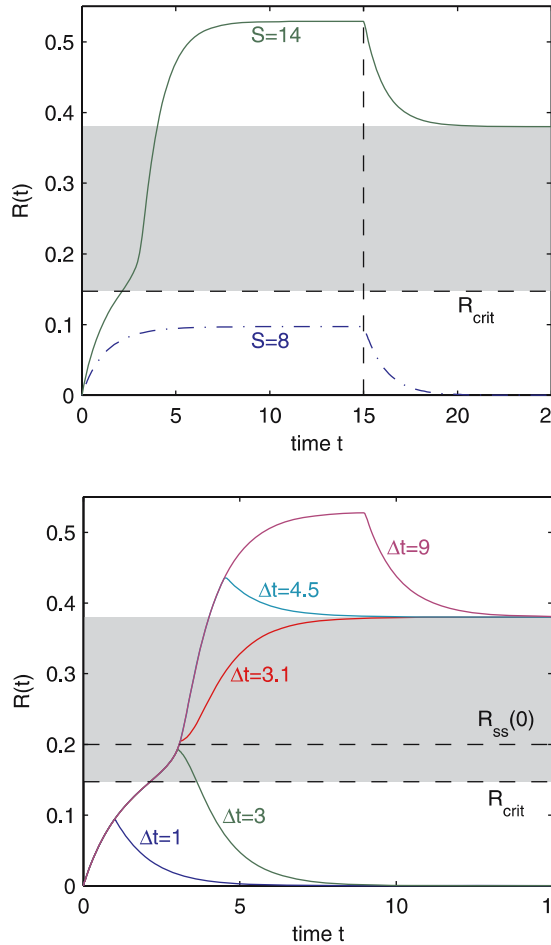


Fig. 4. First: The temporal evolution for two different stimuli, the first subcritical and the second supercritical. Once the response relaxes into the S-dependent steady state, the signal is switched off. The critical response signal R_{crit} is important for the change of the behavior of the system. At this point the activation/deactivation strongly increase from a lower to a high level. Second: temporal evolution for a supercritical signal $S = 14$ of different durations. The activated state is reached only and only if the separatrix $R_{\text{ss}}(0)$ is exceeded

6.3 The Role of Mathematical Modeling

Why do we need mathematical models in the life sciences? To answer this question, we need do no more than turn to the physical sciences, where mathematical modeling underpins everything we do, from fundamental research to the most practical of technologies. In the physical sciences, mathematical modeling is seen as a fundamental prerequisite to *understanding* observed phenomena as part of an integrated whole, rather than merely *explaining* them in isolation as did philosophers in antiquity [15]. As a tool for understanding, the act of developing a mathematical model adds to an integrated lexicon of knowledge. A mathematical model makes observed phenomena part of that universal language which all systems speak – namely mathematics. At a practical level, this understanding allows us to generalize and develop shorthand descriptions of complex dynamic phenomena in a way that clarifies their complexity.

In the physical sciences, these shorthand methods are embedded in standard modeling methods based upon dynamics as an energy- handling phenomenon [16]. The techniques developed from this approach are routinely used to explore physical system dynamics and design products *in-silico*. So why don't similar standard mathematical modeling techniques exist in the life sciences? The answer to this question lies in the complexity of cell-biological systems and their behavior. The simple energy- handling motifs used in the physical sciences are not adequate to describe the complexity of biomolecular dynamics. Complexity is of the essence here, and since it extends beyond the issues of complexity of energy handling into structure and plasticity of behavior, it merits some elucidation. Specifically, complexity in systems biology is the consequence of:

- The nonlinearity of relations between biological objects.
- The relatively large number of variables (proteins, genes).
- The large size and diversity of data sets (e.g., in whole genome studies).
- The heterogeneity of information.
- The fact that interactions among proteins are dynamic rather than static. Thus it is not the presence or absence of a protein that mat-

ters, but its spatiotemporal concentration profile, which in turn is an adaptive function of its context.
– The coordinated and multilevel (both in space and time) nature of biological processes within an organism.
– The difficulties in generating accurate and comprehensive quantitative measurements.

The objective of modeling as a means of dealing with complexity is to reduce it without losing predictability. Modeling is a process of abstraction, that is, a reduction of complexity by capturing the essential elements of complexity in a succinct manner. The energy-handling paradigm does this magnificently for the physical sciences. To do the same for biological systems, we must find models that reduce a complex biological process to its essence, capturing a general principle by which the cell functions (say feedback regulation of transcription) from its experimental context of a particular culture, cell line, or organism. The modeling methods of the physical sciences capture general principles in a set of primal energy-handling motifs that simplify apparently complex behavior and unify apparently diverse phenomena. It is natural to ask whether the same can be true of biological processes. In this connection, certain motifs – some of which are illustrated in Fig. 5 – can be identified in biology. This, however, is a work in progress since these motifs are not primal – rather they are macro-descriptors of frequently occurring objects. Given the increased complexity of biological function compared to the physical sciences, the job of finding general modeling tools will be a long one.

The search for motifs within a system is motivated by an aim to simplify *complexity of structure* within a model. A further, and equally challenging form of complexity is associated with *complexity of behavior*, whereby as parameters or operating conditions change the observed behavior changes radically. For some classes of systems, such behavioral complexity can be routinely clarified using systems theoretic methods, which transform temporal phenomena to simpler operator models. While all dynamic behavior shows complexity, the nonlinear spatiotemporal relationships found in biology in particular are incomprehensible without the support of formal modeling. For example, we have demonstrated in the foregoing that a simple three-component pathway can exhibit

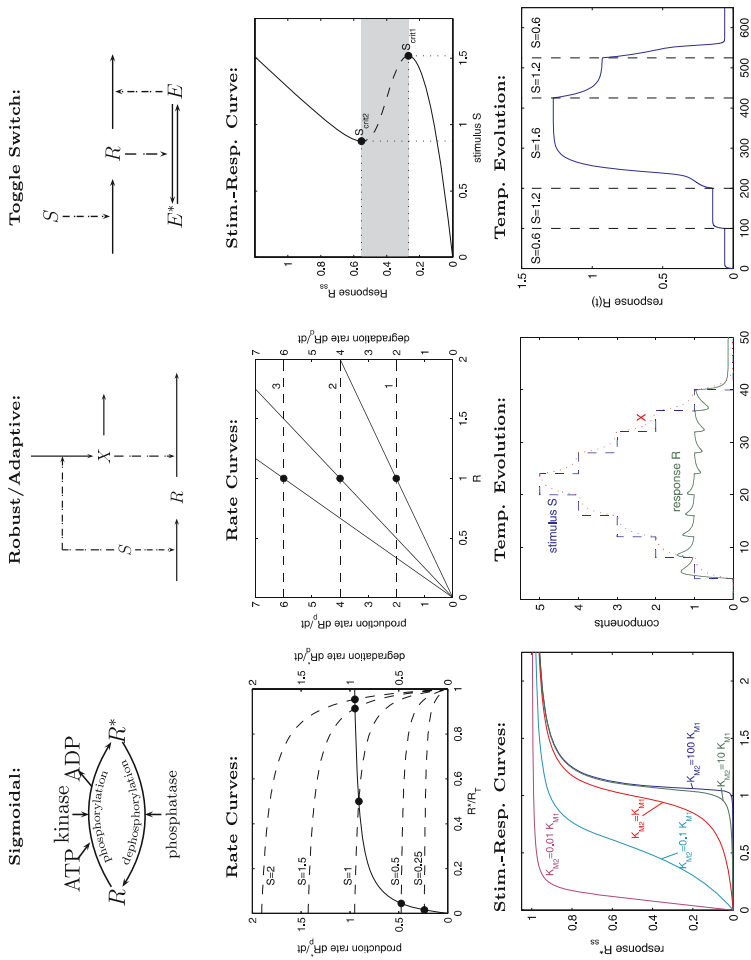


Fig. 5. A selection of dynamic motifs. For a more comprehensive survey see [17]

a strong dependence of the observed behavior on initial conditions, and both the strength and duration of the stimulus. If however, a systems theoretic representation, based on an operator model, is written for the

process, then the behavioral complexities are revealed as belonging to one system.

If we are to accept that cell functions (growth, differentiation, proliferation, apoptosis, and the cell cycle) are regulated/controlled nonlinear dynamic systems, then the use of modeling to demystify structural and behavioral complexity will be mandatory. This in turn will have radical consequences on the design of experiments – and in particular the operational conditions that pertain during an experiment. It is here that modeling and simulation can demonstrate that theory can be practical because it acts as a guide in the choice of experiment and procedure. In this spirit, mathematical modeling is an experiment-design and decision-making guide. It provides a formal framework within which the experimentalist can objectively:

– Decide which variables to perturb, and in what manner
– Determine what the experimental operating conditions should be
– Establish which variables should be measured and with what frequency

Experiment design is central to effectively and efficiently conducting practical experiments and measurements. Within this formal framework for experiment design, every feature of the experimental environment is important. For example, methods of test signal design [18] tell us that the perturbation of a system over time must be conducted in a systematic fashion if we are to efficiently and unambiguously identify a model. Structural issues, such as the presence of feedback, can also cause unanticipated ambiguities in poorly designed experiments [19].

The above are but two examples of experiment-design issues. There is, however, a general body of techniques available. In particular, the system-theoretic concepts of stability, identifiability, observability, and distinguishability [20] are of particular value. An interesting challenge for systems biology is to make these theoretical and somewhat difficult concepts applicable to pathway models and accessible to experimentalists. Specifically, these concepts have been highly developed in their current sphere of use – namely control and dynamical analysis of technological systems – but will require new systems theoretic advances to be generally applicable to the very different structures and functions found in biological processes. In this context, we firmly believe that

systems biology will only succeed if we can demonstrate that theory can be useful and practical. A mathematical model should not only confirm expectations but also challenge hypotheses. The modeling process itself can guide the experimentalist, helping him to save time and money for experiments. However, the aim of mathematical modeling is not simply to guide experiment or to produce exact *in silico* replicas of natural systems. Rather it is to provide a conceptual framework in which to analyze complex nonlinear dynamic systems.

The areas of genomics and bioinformatics have shown that molecular characterization of components and static associations will not suffice to understand disease mechanisms. High-throughput and whole-genome analysis are a vital step in drug target identification but this is not enough for the validation of potential drug targets. Most diseases will be related to cell function, i.e., cell growth, differentiation, proliferation and apoptosis. These cell functions are nonlinear dynamic processes. There is therefore no alternative but to apply systems theoretical analysis, develop corresponding mathematical models and numerical simulations if we wish to understand the mechanisms that generate the correlations and associations we observe across experiments.

6.4 The Role of Theory

Research projects in molecular and cell biology, genomics, bioinformatics, and systems biology aim at an improved understanding of how cells function. This knowledge in turn is the basis for drug development and combating diseases. In other words, biomedical research is trying to understand the physiology of an organ in terms of knowledge about the cell. How is this amazing intellectual achievement possible?

How can/do we unravel those chains of causal entailment that take us from the level of the cell to the level of an organ?

Clearly the development of general modeling tools, as advocated in the previous section, will address some aspects of these questions. However, an intellectual leap of a different dimension is required to answer the questions at their most profound level. Science must demonstrate its ability to cross levels not yet breached – levels that will take us:

– From cells to organs and organisms
– From cell function to physiological phenomena
– From model organisms to human diseases

A systems biology for medical applications has to study the cell not in isolation but in its social context, as part of a larger whole that is a tissue or organ. It may appear that advances in this area are incidental, individual research projects providing pieces for a large puzzle in which every so often a piece of the overall picture emerges and that from this the whole will eventually emerge. But such a piecemeal approach will not suffice. The complexity of the systems we deal with is such that the distance between pieces of the puzzle can be infinitesimally small, and still we will not be able to connect them by empirical means alone. An interdisciplinary approach is dictated in which systems theory plays an underpinning and unifying role.

The need for interdisciplinary research is widely accepted but unfortunately it is most commonly understood as an alliance between experimentalists and data analysts or software developers, not between experimentalists and theoreticians. We argue that if cell function is indeed a nonlinear dynamic system we need alliances between fields that could hardly be further apart: advanced theoretical research in systems theory and biomedical and clinical research. Data mining and computational simulations are fields in which computer scientists feel confident and it is fair to say that these fields are now well developed. This is, however, very different to the analysis of nonlinear dynamics, something that is in the remit of systems and control theory and that (as hinted earlier) is far from having suitable techniques available at present. Data mining and numerical simulations are a simpler route than formal analysis but no full alternative. They are only an approximation to the real thing; they cannot explain the mechanisms that generate the pattern in data.

If the aim is to develop predictive and personalized medicines, we first need to identify generic or universal principles and then adapt this information and knowledge to the biochemical make-up of an individual. Universal principles are therefore general rules that are valid *in vitro* as well as *in vivo* and *in situ*; which can be studied in model organisms and also translated to humans whose observations are independent of a chosen cell line or experimental set up. A predictive medicine relies on the

discovery of universal biomolecular principles. Personalized medicine, on the other hand, requires us to know the parameters that define the individuality of the particular patient. What conceptual framework is necessary for us to cross levels of structural and functional organization in cells and organs, to distinguish the general and the particular in disease, to integrate an understanding for a larger whole in terms of its parts?

What methodologies are required to predict a general disease mechanism from which to derive drugs that apply to the special case of an individual patient?

What is addressed here is what we would call the uncertainty principle of systems biology: *as the complexity of the experimental system increases, our ability to make general and yet relevant predictions diminishes*. To overcome this hurdle, new methodologies and scientific practices are required. More specifically, we need to integrate approaches:

- Experimental data and theoretical models.
- Combine data mining (correlations/associations) and systems theory (dynamic interactions).
- Move from molecular characterization to an understanding of functional activity.
- Extend single-model analysis to hierarchies of interacting systems; scaling up from pathway models to cells and beyond.
- In light of the many proteins one could consider, how do we identify subsystems or modules, suitable for experiments but without losing the context of the larger whole.
- Study single cells but in their social context.
- Integrate metabolic and signaling pathways.

The second section of the present text introduced a simple example of a pathway with only three components. Nonetheless, this simple example contains features that already challenge experimentalists and theoreticians alike. A key aspect of this challenge, as elsewhere in biological processes, is the presence of feedback. Indeed feedback mechanisms are the basis for all forms of regulation, control, and coordination at all levels within living organisms. They cannot be understood through conventional data analysis, data mining, or machine learning – instead they require dynamic systems theory. In contrast to the aforementioned

fields, there is a lack of theory to support the analysis of dynamic pathway models. Although undesirable for many, the simple conclusion is that we need to support theoretical research as part of systems biology. This will take time, a lot more time than most are prepared for. The only consolation is that it is inexpensive and that the reward of a true understanding is more satisfying than data engineering. As we tried to indicate in the second section, there are avenues to generalize pathway models and initial efforts are under way to develop categories of dynamical systems. As esoteric as these ideas may sound, we are convinced that they are going to be useful and of practical value in our endeavor to make sense of life itself.

On a final positive note, several Nobel Prizes for game-theoretic studies in economics are evidence that unlikely partnerships between abstract mathematics and the everyday world can exist and can be fruitful. What is more, such alliances frequently yield practical results that go far beyond any original intentions or vision of the theoretician. It is often thus with key mathematical theories, for – to paraphrase Hilbert – there is nothing more practical than good theory.

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