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Review

Understanding complex signaling networks through models and metaphors

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Abstract

Signaling networks are complex both in terms of the chemical and biophysical events that underlie them, and in the sheer number of interactions. Computer models are powerful tools to deal with both aspects of complexity, but their utility goes beyond simply replicating signaling events in silicon. Their great advantage is as a tool to understanding. The completeness of the description demanded by computer models highlights gaps in knowledge. The quantitative description in models facilitates a mapping between different kinds of analysis methods for complex systems. Systems analysis methods can highlight stable states of signaling networks and describe the transitions between them. Modeling also reveals functional similarities between signaling network properties and other well-understood systems such as electronic devices and neural networks. These suggest various metaphors as a tool to understanding. Based on such descriptions, it is possible to regard signaling networks as systems that decode complex inputs in time, space and chemistry into combinatorial output patterns of signaling activity. This would provide a natural interface to the combinatorial input patterns required by genetic circuits. Thus, a combination of computer modeling methods to capture the complexity and details, and useful abstractions revealed by these models, is necessary to achieve both rigorous description as well as human understanding.

Keywords: Biochemical pathways; Computer simulation; Logical networks; Neural networks; Databases; Bioinformatics

Contents

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1. Introduction

Biology, it is said in jest, is nothing but applied chemistry. While the application of chemical understanding to biology has had spectacular success in studying metabolism and the foundations of molecular biology, it has been harder to apply it to signaling pathways. In part this is due to experimental and conceptual limitations in describing cellular processes in terms of chemistry, and in part due to the essential complexity of biology.

Recent experimental and theoretical work has begun to fill in the gaps in our description of biological detail. In a small but growing number of systems (Bayer et al., 2001; Shimizu et al., 2000) it is possible to examine signaling events down to the level of individual molecular interactions. The theoretical and computational counterparts to such experimental findings are also now emerging (Arkin et al., 1998; Lamb and Pugh, 1992). These tools enable us to apply basic chemical principles to predicting and analyzing the functioning of molecular circuits at the single-molecule level. Thus, in principle at least, it is possible to describe biology at a level not too far from the reductionist chemical viewpoint (Endy and Brent, 2001).

The complexity of biology is also something that is beginning to look more finite, even as biological databases burgeon. In some ways, this is because of technology. The idea that the human genome can be stored in one or two CDs worth of data puts things into a somewhat surprising perspective. If the blueprint of man is approximately equivalent to Beethoven's 9th in information content, it is clearly not all that large from a modern technical viewpoint. The very act of amassing data in a database suggests that someday it may *all* be in there. The problem of actually understanding all this data is more fundamental. Technology continues to scale up exponentially, but the human brain does not. Given even current levels of simulation detail, and database sizes, the cell looks like a system not really suited for human intuition. Is it time to turn it all over to another level of bioinformatics, using vast databases and huge computer simulations, and just marvel at the results?

This review suggests that there is hope for human understanding despite this avalanche of detail. Despite the popular image of a computer model being a complex representation of a complex biological system, such models do provide deep insights into signaling function that may make it easier to think about them. The process of specifying biological complexity in a reductionist manner leads to parallels with other, better understood systems. Such parallels and metaphors provide several possible paths for thinking about signaling networks. Thinking in metaphors may be a hazardous way of drawing scientific conclusions, but combined with numerical simulations and experimental anchors, it may well be the best way the human mind can usefully grapple with biological complexity.

This review first considers the two kinds of complexity that characterize cellular signaling: the chemical and physical details of individual signaling pathways, and the numbers of interactions. It then considers various abstractions of these interactions. Finally it examines approaches to integrating this data and abstractions into a form that is useful for human understanding.

2. A reductionist approach to defining signaling systems

2.1. Putting in the details

Biological systems often resemble a fractal—the closer you look, the more detail that emerges. This is a familiar complaint of all modelers. There is always more to put in. Suppose, for example we were to try to model a ligand (L) interacting with a G-protein coupled receptor (R). A good starting point would be to consider the signaling events in terms of simple chemistry (Fig. 1a).

Already some readers would point out that this reaction scheme is too simple: the GDP.G$\alpha\beta\gamma$ may bind to the receptor before the ligand, and the separation of GTP.Gα from the receptor–ligand–G-protein complex is a multistep process. In principle, there may be hundreds of additional reaction details and side reactions with various cellular molecules that might have a diminishing effect on the signaling pathway. In that sense, a chemical description of this kind is only an approximation to the signaling pathway. Thus signaling models are always understood to be successively better approximations to biology. For practical purposes, we rather quickly reach the point where experimental uncertainties dwarf the additional precision coming from greater reaction-level detail (Bhalla, 2000).

The next issue that comes up with this chemical approximation is compartmentalization. We should really consider the fact that the ligand occupies extracellular space, the receptor and bound G-protein are on the membrane, and GDP and GTP exchange with the cytosol (Fig. 1b). Now the volumes of the respective cellular compartments become important. Additional steps may be necessary to describe the movement of molecules between compartments. This level of detail, including compartmentalization, is where most current signaling models operate (Fig. 1b) (Bhalla and Iyengar, 1999).

Beyond simple compartmentalization, there are two obstacles to further detail in modeling. The first is that the numerical methods become sharply more difficult. The second is that good kinetic parameters are not easy to come by at this level of detail. The bulk of available data comes from test-tube experiments, but the cell is not a test tube. There are spatial details and issues of individual molecular function that require finer experimental techniques.

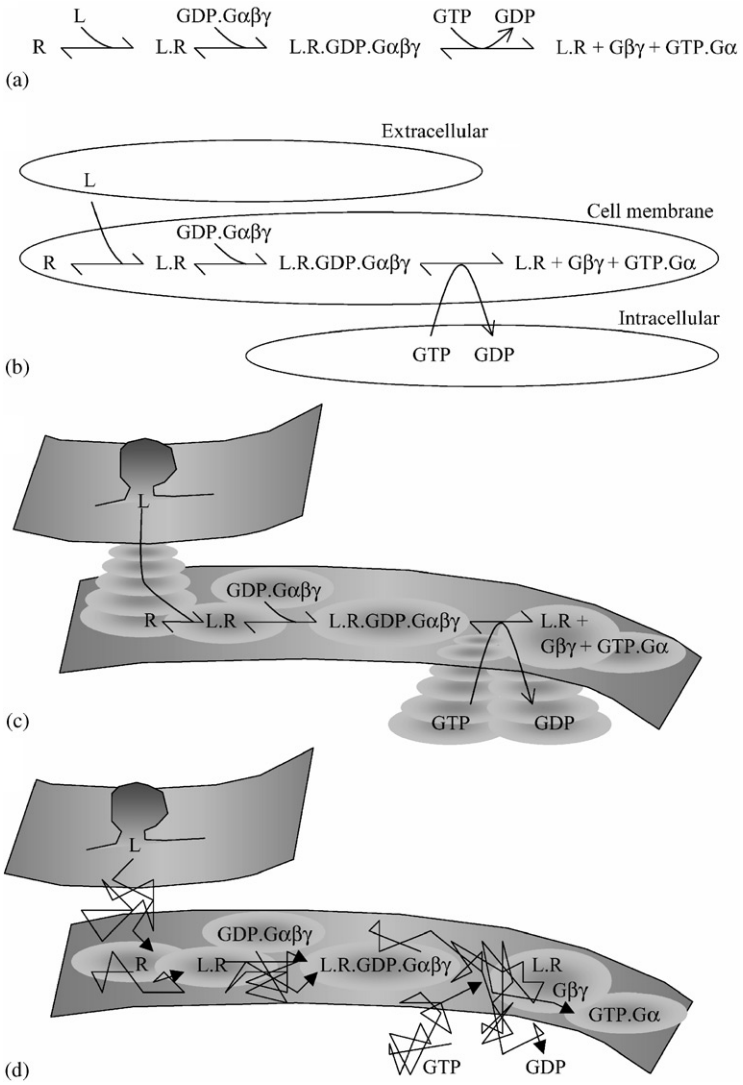

Fig. 1. Levels of signaling representation. In all cases the model represents ligand interaction with a G-protein coupled receptor. Abbreviations: L: ligand, R: receptor, GDP: guanosine diphosphate, GTP: guanosine triphosphate, G$\alpha\beta\gamma$: trimeric form of G-protein, Gα: alpha subunit of G-protein, G$\beta\gamma$: G-protein beta–gamma dimer. (a) Mass-action reaction level, in terms of simple chemical steps. (b) Mass-action plus compartmentalization. Three compartments are shown: extracellular, membrane and intracellular. Interactions between compartments may involve an extra translocation step. (c) Reaction-diffusion level: The ligand must diffuse across the extracellular space, each of the signaling molecules can diffuse within the membrane, and the GTP and GDP must diffuse to and from the membrane to interact with the G-protein. (d) 3-D stochastic level: Each molecule undergoes Brownian motion in space. The motion is three-dimensional for the ligand L and for GTP and GDP, and two-dimensional for the membrane-bound components. When the molecules are in close proximity, they may react with a probability dependent on their chemical properties.

The first step up in detail is to recognize that all these reactions really occur in three-dimensional space, or two-dimensional for membrane-bound reactions. A reaction–diffusion system of equations is therefore a more appropriate description (Fig. 1c). This is not too difficult to compute if we restrict ourselves to simple geometries such as spheres, cylinders, or cubes. Unfortunately, real cells are not simple. It turns out to be extraordinarily difficult both to obtain experimental data for, and to solve reaction–diffusion equations in arbitrary three-dimensional geometry. It becomes necessary to partition the complex geometry into fine grids, necessitating the application of finite difference or finite element methods. The computation time scales up rapidly as the geometry becomes more involved (Press et al., 1988).

A further elaboration would be to point out that we should really consider the reactions in terms of events happening at individual molecules. After all, it does not take very many activated receptors to cause a large cellular effect. This means that we must abandon mass-action kinetics, and consider instead the probabilities of random collisions giving rise to reaction events. Also, of course we should continue to do these calculations in three-dimensional space; hence we have to consider random directions of movement of the molecules too, constrained by compartment boundaries, the cytoskeleton and so on (Fig. 1d) (Stiles and Bartol, 2000).

At this point we are near the limits of modern simulation technology (Le Novere and Shimizu, 2001; Stiles and Bartol, 2000). Interestingly, for small numbers of molecules in complex geometries, it is actually more efficient to compute things in terms of probabilistic reactions and Brownian motion than it is to do so using reaction–diffusion systems. Unfortunately, except for a few special cases, we are already well past the limits of experimental technology (e.g., Shimizu et al., 2000). Even at this detail, we are just starting to touch the realm where signaling intersects with cell biology. Our reactions are very likely occurring on cytoskeletal complexes or scaffolds. It is difficult to see how mass-action, test-tube data would relate to molecular events at this scale. But there is more detail still to consider.

Proteins are not just static chemical machines. Many proteins undergo mechanical changes during their normal functioning, and proteins are frequently transported to different parts of the cell (Bray, 2001). Signaling events can be the cause and also the outcome of mechanical changes at this level, and a good representation of these signaling events may have to take movement into account. Signaling-coupled movement may occur in the formation and alteration of macromolecular complexes (Bayer et al., 2001; Lanzetti et al., 2000), at the level of molecular motor control (Hammer and Wu, 2002), or at the level of cytoskeletal matrices changing the structure of the cell itself (Davie and Spencer, 2001; Hata and Takai, 1999).

We have not separately considered the nucleus, where we have another entire edifice of regulatory control, three-dimensional structure, and monstrous molecular machines to account for. Nor have we considered the synthesis and turnover processes of the signaling molecules themselves. Fortunately, we now appear to be repeating ourselves, at least as far as the categories of molecular detail are concerned. For example, stochastic chemical signaling descriptions have been applied both to cellular signaling (Holmes, 2000) and to genetic circuits (Arkin et al., 1998). Purists may point out that this is just where the intractable protein structure problem comes in, but (we hope) the fractal of chemical detail has finally reached a reasonable end point here at least as far as signaling is concerned. To summarize, the molecular details that current knowledge regards as critical from a signaling viewpoint include the action and movement of individual

molecules in the three-dimensional context of the cell and its organelles. This level of detail is, in principle at least, manageable using modeling methods.

2.2. Putting in the complexity

If the molecular details are a measure of the depth of the problem, then the sheer number of signaling interactions is a measure of its breadth (Weng et al., 1999). It is relatively easy for a modeler to draw a line and say, "thus far, no further" with regard to molecular detail. However, when modeling signaling networks, it is difficult indeed to decide when a new regulator or isoform might be crucial to the overall function of a signaling network.

A glimpse of the scale of the problem is given by some numbers from genome analysis and studies of protein–protein interactions. There are perhaps 10,000 signaling proteins in the human genome, each of which can exist in at least two, and perhaps thousands of states (Endy and Brent, 2001). An approximate number of potential pair-wise interactions for each molecule is 5, based on several studies including yeast 2-hybrid screens, binding assays, and analyses of databases (Bhalla, 2002a; Jeong et al., 2001). A typical cascade from receptors at the cell surface to the nucleus involves some 4 or more steps, so a branching of 5 per step would lead to over 500 potential signaling effects. An example of the chemical complexity of even a small signaling network with this order of branching is shown in Fig. 2.

Many databases have sprung up in recent years to manage such data. Their emphasis ranges from qualitative descriptions of signaling interactions and networks (TRANSPATH: Schacherer et al. (2001), GeneNet: Kolpakov et al. (1998), BIND: Bader et al. (2001)) to pair-wise protein interaction databases (KEGG: Kanehisa et al. (2002), DIP: Xenarios et al. (2002), SPiD: Hoebeke et al. (2001)) to enzyme kinetic databases (BRENDA: Schomburg et al. (2002), EMP: Selkov et al. (1996)) and databases of signaling models (DOQCS: http://doqcs.ncbs.res.in) and even to ambitious projects to describe and simulate entire cells (AFCS: http://afcs.swmed.edu/afcs, E-Cell: Tomita et al., (1999)). There are also a number of efforts to come up with a better formulation of complex signaling systems than a mass of chemical interactions. There are a number of object-oriented simulators (E-Cell: Tomita et al. (1999), V-Cell: Loew and Schaff (2001)) that use organizing principles from computer science to structure the data representation. A recent analysis of signaling interactions suggests that the object-oriented view may be taken one step further, so that reactions can cleanly be encapsulated within pathway 'blocks' (Bhalla, 2002a). From this analysis, the interactions between pathways occur in a small number of stereotyped ways. Thus the object-oriented representation may be feasible at the level of entire signaling pathways, rather than individual reactions. Other developments to manage signaling complexity include development of cell description languages based on the standard Extensible Markup Language, XML (Hedley et al., 2001; Hucka et al., 2000).

Beyond these first-order issues of numbers of interacting molecules, cellular compartmentalization and spatial segregation details introduce further complexity. On the one hand, compartmentalization frequently acts to isolate signaling cascades from each other and reduce the number of potential interactions. On the other hand, the communication between compartments themselves is now a major contributor to signaling complexity (Weng et al., 1999). In addition to the technical issues mentioned above in simulating spatially organized structures, there are enormous data-gathering and handling issues in describing the structures themselves (Bassingthwaighte, 2000).

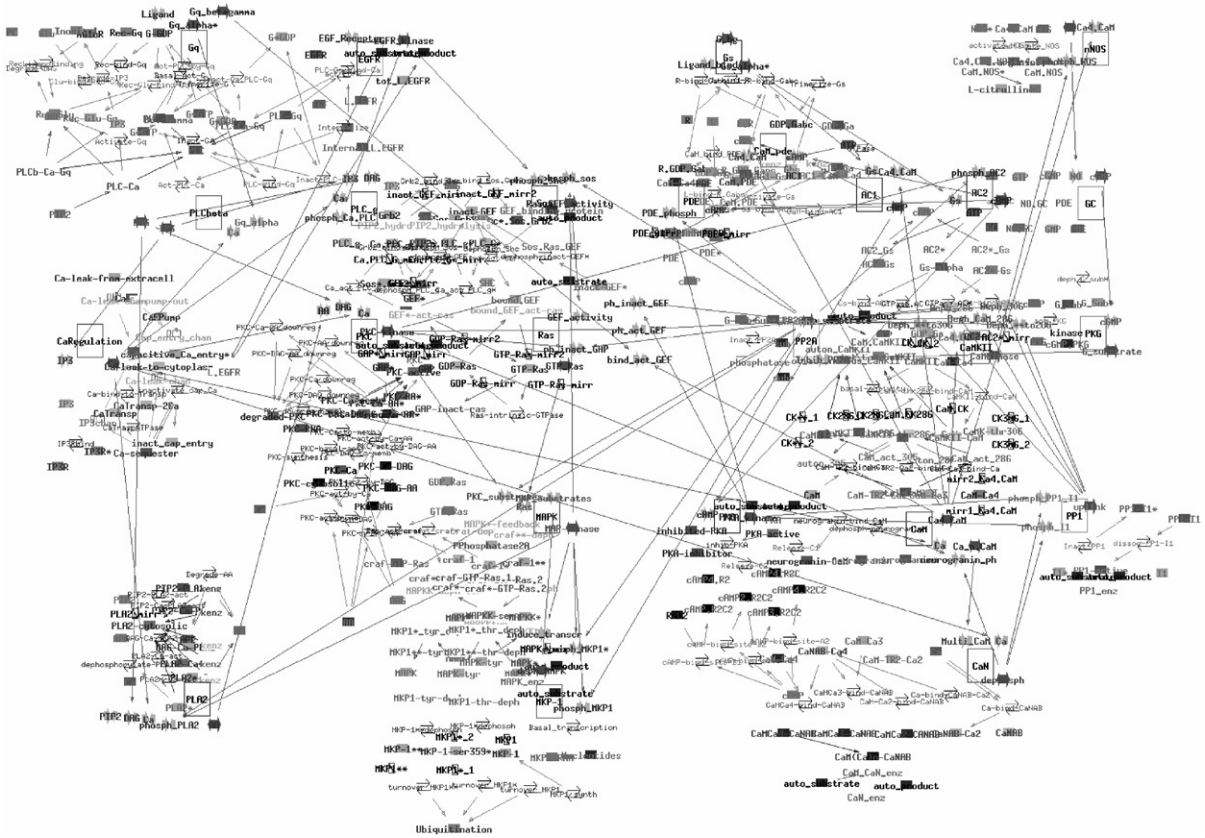

Fig. 2. Complexity in signaling. A computer screen-dump of a moderate-sized model at the mass-action level including individual reactions, enzymes, and molecules. A more comprehensible block-level diagram of the network is shown in Fig. 9. This model involves only 16 major signaling pathways, with very few isoforms.

Overall, despite the immense complexity of biological signaling, many of the tools for handling it are taking shape. The current limitations are primarily to do with good experimental methods to produce large-scale datasets at the resolution required for molecular-level modeling. An impressive array of high-throughput techniques is emerging that may soon reach this level of detail.

2.3. Limits to reductionism

From the above, it is clear that the reductionist approach to understanding signaling is difficult and perhaps somewhat beyond current computing or experimental capacities. The purpose of the above enumeration is not to show that it is complex—this is something that all biologists know. The point is to show that biology has finally reached a stage where it is conceptually possible to describe, define, and analyze cellular signaling at a molecular level. Further, the above enumeration shows that in many respects we are quite close to a level of description that would be satisfying from the reductionist viewpoint.

This is all very well, but the objective of modeling is not to replace a complex cell with a marvelously complex model. Having reduced things to the molecular level, what does this tell us in terms useful to mere humans, about how cells work? It turns out that the tools of simulations give us handles to work with the complexity, and these tools reveal similarities to familiar metaphors that may help us understand.

3. Abstractions for complex signaling

3.1. Drawing the boundaries: systems analysis

One of the classical tools for analyzing complex systems is systems analysis. A key concept here is that such systems tend to have a restricted number of stable states that the system will gravitate toward. The differential equations obtained from the preceding reductionist analysis can be used to find these stable states (Ermentrout, 2002).

Systems analysis has shown that there are certain repeating patterns of connectivity that possess special properties. One of the most interesting motifs is that of feedback loops. Feedback loops abound in signaling. Strictly speaking, every reaction has a back reaction and thus affects its substrates. The concept of end-product inhibition is an old one, and this is an example of a negative feedback loop. The properties of negative feedback loops include stabilization of system responses, and suppression of any amplification effects. In some cases, negative feedback with delays can introduce oscillations (Baier and Sahle, 1998; Tyson et al., 2001).

Positive feedback loops have particularly been the subject of analysis, because they can give rise to a multiplicity of stable states (Thomas and Thieffry, 1994; Tyson et al., 2001). In a typical bistable system there is a resting stable state of low activity, an intermediate unsteady stable state that can be regarded as a threshold, and a second stable state of high activity. There is a somewhat restrictive set of chemical properties of signaling pathways that can give rise to bistability, but nevertheless, such properties are biologically plausible in a subset of cases. These conditions include high cooperativity and a large dynamic range of responses of at least one of the pathways. An example of a putative bistable feedback loop involving signaling pathways that are commonly found in eukaryotic cells is shown in Fig. 3. This set of pathways, especially the mitogen activated protein kinase (MAPK) is involved in many cellular events such as the decision to remain quiescent or proliferate (Chang and Karin, 2001). It seems plausible that sustained activity of MAPK due to positive feedback may play a role in such decisions.

It is unlikely that there could be more than two stable states within a single feedback loop, given the known properties of signaling chemistry. However, nested stable states have been proposed to occur in many systems (Thomas and Thieffry, 1994). In such situations, more than one feedback loop may operate in a loosely coupled manner, and each may either be in an inactive or active state. Thus, nested feedback loops may give rise to many levels of stability.

The presence of a putative feedback circuit in signaling does not necessarily imply bistable or oscillatory behavior. The actual properties of the system are strongly dependent on the exact parameters of the chemical kinetics (Fig. 3d) (Bhalla et al., 2002). Thus systems analysis can suggest a range of likely system properties, but experiments and simulation analysis are required to further narrow down the system behavior.

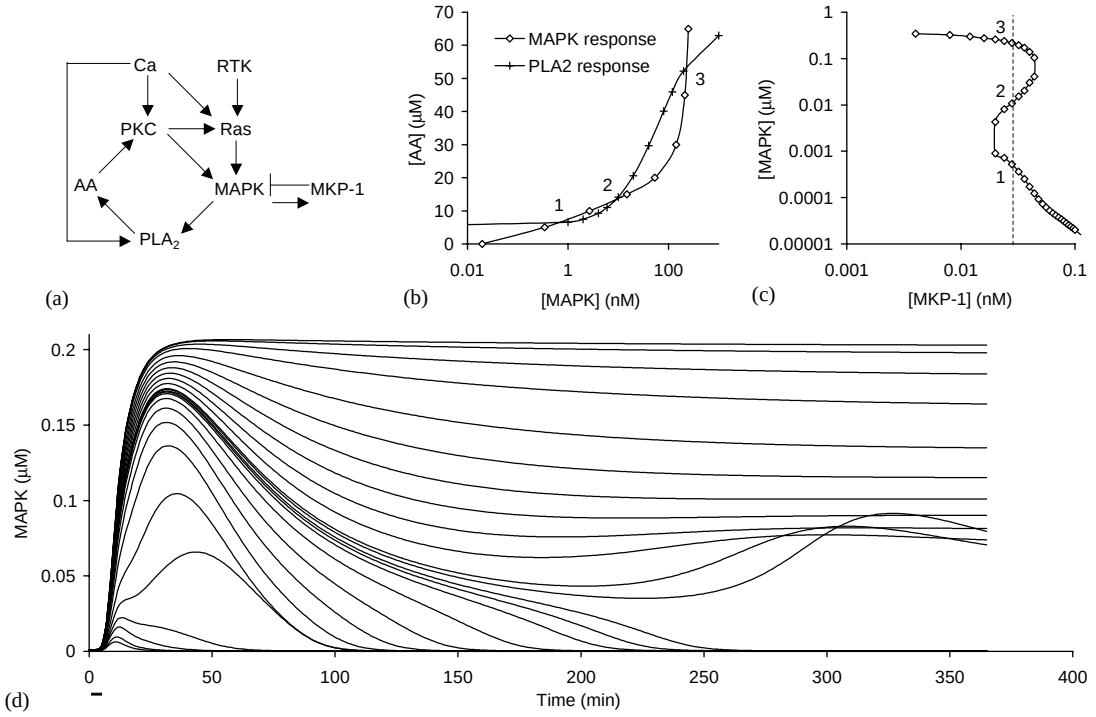

Fig. 3. States of a feedback loop. (a) Signaling pathways in loop: This set of signaling pathways is commonly found in eukaryotic cells and is involved in many cellular events such as the decision to remain quiescent or proliferate. Each name in the figure represents a complete signaling pathway, which in turn involves many individual reactions and molecules. Abbreviations—Ca: calcium, RTK: receptor tyrosine kinase, PKC: protein kinase C, AA: arachidonic acid, MAPK: mitogen associated protein kinase, MKP-1: MAPK phosphatase type 1, PLA_2: phospholipase A type 2. The positive feedback loop involves PKC, MAPK, PLA_2 and AA. There is also a negative feedback loop involving MAPK and MKP-1. Ca and RTK provide inputs to the system. All arrows indicate stimulatory interactions, except for the T-shaped arrow from MKP-1 to MAPK, which is inhibitory. (b) Finding stable points of the feedback loop. Dose response curves are calculated first by holding AA at a series of steady levels and measuring the MAPK response, and then by holding MAPK at a series of steady levels and measuring PLA2 response in terms of AA concentrations. The intersection points are the stable points of the system. States 1 and 3 are stable, and state 2 is metastable and acts like a threshold point for switching between states 1 and 3. (c) States of the loop as a function of a regulatory molecule, MKP-1. The activity of MAPK is used as a measure of the activity of the feedback loop. At low MKP-1 levels, there is little inhibition of the feedback loop and it has only one stable state, at high activity. At intermediate MKP-1 the system is bistable. Under these conditions, state 1 is stable at low activity, state 3 is stable at high activity and state 2 is metastable. When MKP-1 is present at high levels, it inhibits the feedback loop to an extent where the loop has only one stable state, at low activity. (d) Multiple states and modes of response of feedback loop to a 5 min stimulus (black bar). The range of states is obtained by varying only one parameter, the rate of synthesis of MKP-1. In this series, the loop can behave as an amplifier, thresholding device, timer, tuner, bistable system or constitutively active system. Panels (b) and (d) are reproduced with permission from Bhalla et al. (2002) Science 297:1018, Copyright 2002 American Association for the Advancement of Science.

The cell cycle is an example of a process that has been analyzed in this manner, combining experiments with theory (Aguda, 1999; Kohn, 1999; Tyson et al., 2001). There are multiple control modules in the cycle, two of which are bistable states balancing the G1 phase with the S phase and the S/G2 phase with the M phase, respectively. At large cell size, the M phase itself

ceases to be stable and the cell divides, restoring the system to a bistable regime. This analysis has been further supported by the identification of mutations in the pathways controlling these cycling processes. Such mutations lead to disruptions in normal cycle, in a manner which is consistent with the systems analysis.

Thus systems analysis is useful in setting boundaries for the range of behaviors of a signaling network, and to identify characteristic states and decision points in the system.

3.2. Logical metaphors for signaling

Many of the concepts from systems analysis are also reflected in various metaphors for signaling. Electronic circuits are a particularly rich source of such metaphors. Commonly used descriptions, in increasing order of detail, are logical networks, neural networks, steady-state analog circuits, and circuits with temporal dynamics.

The logical network is a suitable tool for cases where there is abundant qualitative experimental information about signaling and genetic networks (Glass and Kauffman, 1973; Tucker et al., 2001). The most basic approach only requires three pieces of information: the connectivity, the sign of interactions and the nature of the summation. The connectivity is required to specify the inputs and outputs of each signal. The sign is required to show whether the interaction is excitatory or inhibitory. The summation defines how signals combine. For example, if both inputs must be present in order to generate an output, it is an AND configuration. This level of information in fact forms the bulk of what is currently available in the field. Data sources include information about which proteins interact with which, which combination of repressors and promoters act on a gene and which signal is upstream of which (Sanchez and Thieffry, 2001). Because the qualitative information required is a common denominator of all of these kinds of network, the method is applicable equally to signaling and genetic networks. The conventional signaling block diagram maps rather closely onto the logical representation (Fig. 4).

A further elaboration of the logical networks introduces an element of time, again in a semi-quantitative manner. The analysis reveals that the outcome of a set of signals may be

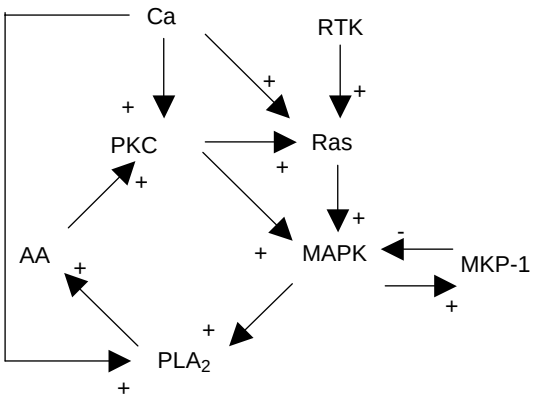

Fig. 4. Logical network representation of the same feedback loop as in Fig. 3. Note the close correspondence with the conventional qualitative block diagram. The + and − symbols on the arrows indicate whether the interaction is excitatory or inhibitory.

indeterminate. For example, one branch of a signaling event may turn on an output, whereas another branch may turn it off. However, this indeterminacy can often be resolved if the sequence of events can be ordered (Thomas and Thieffry, 1994).

An important conceptual result of this kind of study is that the basic building block of signaling and genetic networks should be considered in terms of feedback loops rather than individual molecules. As expected from systems analysis, negative feedback gives rise to homeostasis or oscillations. Positive feedback loops can give rise to multistability, and this defines the possible states of the system. Feedback loops can be nested to give rise to a multitude of possible states. The process of development, for example, involves many sequential choices between alternative states, each maintained through its own feedback process. The end result is a highly specific final state where a certain set of genes is 'on' and others 'off', and maintained in this state through feedback mechanisms.

Logical networks, therefore, are useful tools for the all-too-common situation where there is insufficient quantitative data about reaction kinetics, but the network topology is well known. Like systems analysis, these techniques highlight the importance of feedback in defining possible stable states of the signaling system.

3.3. Neural network metaphors for signaling

Since signaling pathways are obviously networks, it is interesting to examine how signaling operations might be considered in terms of neural networks (Bray, 1990). Unlike logical circuits, many neural networks are analog in operation and thus intermediate values do convey information (Rumelhart and McClelland, 1986). Neural networks are closer to signaling pathways in this respect. The fundamental building block of a neural network is a 'neuron' or 'unit' that is only a very distant cousin of its biological counterpart. These neurons have the capacity to receive multiple inputs, each with a specific weight. The inputs are all summed up according to their weights, and the output of the neuron is computed by running this summed value through an output function, usually a sigmoid (Fig. 5a).

A typical neural network consists of multiple layers of such units, each with a high degree of interconnectivity (Fig. 5b). In feed-forward networks, information flows only in one direction, but in feed-back networks, the output of the network can come back as an input. Classical neural networks do not include an explicit time dimension. The topological similarity of neural networks to highly interconnected signaling networks is evident (Figs. 5 and 7). What kinds of properties might be expected to arise from this analogy?

First, information storage and processing in a neural network is distributed (Rumelhart and McClelland, 1986). This means that the information is not concentrated in any one node or weight. This gives rise to the important property of robustness. A neural network will typically function well even if it loses a few neurons. This is often the case with biological signaling networks as well, where redundancy both in terms of isoforms and in terms of parallel pathways is well known. Many knockout experiments of single signaling genes have little effect due to this redundancy (Doetschman, 1999). Fault tolerance is clearly a useful attribute for biological networks.

Second, neural networks are very good at recognizing patterns that involve complicated combinations of inputs. This ability persists even when the inputs are somewhat degraded. This is

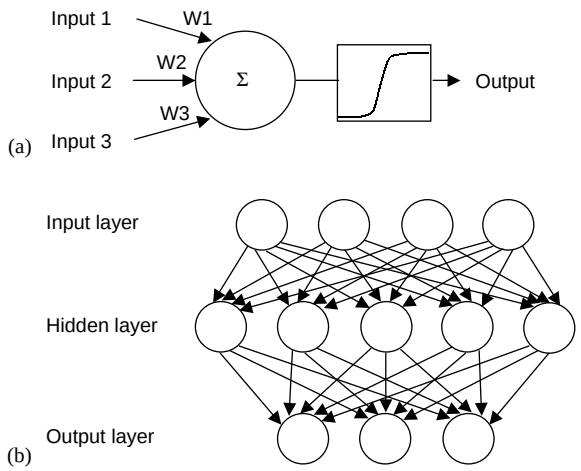

Fig. 5. Organization of an artificial neural network. (a) Schematic of a 'neuron' or 'unit' in an artificial neural network. The three inputs contribute to the activity of the neuron in proportion to their respective weights, W1, W2 and W3. The neuron performs a weighted sum of these inputs and then generates an output using a transform, typically a sigmoid as shown. (b) Circuit for a feed-forward neural network. Some combination of activities is presented at the neurons of the input layer. These go through one or more hidden layers and the output is monitored at the output layer. Each neuron performs a weighted sum of inputs followed by an output transform, as described in (a) above. Note the extensive connectivity in the network. Information is thus stored and processed not at individual connections or neurons but in a distributed manner.

important for biological signaling, where inputs are complicated, and subject to many forms of variation.

Neural networks are typically trained to recognize patterns through rather complex procedures such as back-propagation (Rumelhart and McClelland, 1986). Signaling networks can also be trained through evolutionary events to recognize relevant signaling inputs (Bray, 1990; James et al., 1999). As we discuss later, the incorporation of an explicit time dimension in the analogy with neural networks may improve their correspondence to biological signaling networks.

3.4. Analog electronic metaphors

The operation of analog electronic devices maps very closely to the flow of information in chemical reactions (McAdams and Shapiro, 1995). The buildup of charge, for example, is analogous to the accumulation of a particular molecule. Amplifiers, like enzymes, permit a small charge (or molecular concentration) to have a large effect on another. The identity of signals in an electronic circuit is maintained by distinct, insulated wires. In signaling circuits this identity is maintained by the fact that distinct molecules convey different signals. Even at a mathematical level, the equations describing equilibration of charge and amplifier function can faithfully mimic the equations describing chemical reactions. Thus, nearly all the concepts that one needs to analyze signaling circuits can be drawn from electronic circuit theory (Fig. 6). Indeed, the logical and neural network analogies considered above are special cases of analog circuits. For example, logical circuits arise when thresholding is applied to analog operations. Neural network 'neurons' can be directly implemented in silicon.

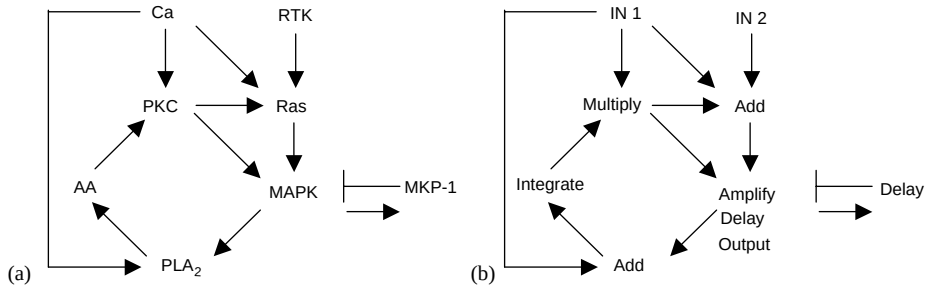

Fig. 6. Electronic equivalents of a signaling network. Possible functions of each of the nodes of the signaling network are illustrated.

Considering our example of the MAPK pathway, we can identify at least four direct mappings to electrical analogies: summation, amplification, delays and integration (Fig. 6b). G-protein cascades are routinely regarded as amplifiers, cooperative interactions as thresholding operations, and feedback inhibition is a classical analog configuration to introduce stability and linearity in a circuit response (Horowitz and Hill, 1989). Many of these concepts have been reviewed by Bray (1995).

A limitation of the neural network analogies is the lack of representation of time. In contrast, electrical circuit theory lays great stress on the time dimension. Signaling pathways, even when considered at the simplest level of mass-action kinetics, have an abundance of time courses. They range from millisecond action of calcium buffers and fast kinases, to many minutes for complex cascades such as the MAPK cascade, to many hours for genetically coupled loops like the cell cycle and diurnal rhythm. The most familiar form of signaling timing is the oscillator (Tang et al., 1996). As discussed above, feedback loops can oscillate when parameter conditions are suitable. Bacterial chemotaxis illustrates several interesting signaling computations in the temporal domain. In electronic terms, these can be regarded as timers, integrators and differentiators (Bray, 1995). The neuronal synapse exhibits many aspects of temporal selectivity (Bliss and Collingridge, 1993). It has been shown that signaling pathways can give rise to temporal tuning and filtering properties, with close analogy to their electronic counterparts (Bhalla, 2002c; Bray, 1995). Thus the temporal domain of signaling input can also be grist to the mill of the signaling network.

The electronic metaphor is therefore a particularly powerful and apt analogy for signaling. Familiar electronic concepts such as amplification and feedback are routinely used when describing the functions of signaling networks.

3.5. The combinatorial decoder

Many signaling functions are relatively easy to map onto one or more of the above signaling themes. There is a large class of cellular decisions, however, which involve much of the signaling machinery in a manner that is difficult to tease apart. Such complex cellular decisions often occur in processes such as development, differentiation, apoptosis, and synaptic plasticity. A characteristic of these signaling computations is that they are combinatorial, both at the input and output level. For example, synaptic plasticity requires that a number of cellular regulators should be at permissive levels and the specific type of plasticity depends on rather complex

temporal sequences of synaptic input (Bliss and Collingridge, 1993). In differentiation, cell fate may depend on a combination of history of the cell, signals from its neighbors, and extrinsic signals such as long-range gradients (Gurdon and Bourillot, 2001). The output in both situations involves expression of a selected set of genes, as well as coordinated activation of signaling and cell biological processes (Fig. 7).

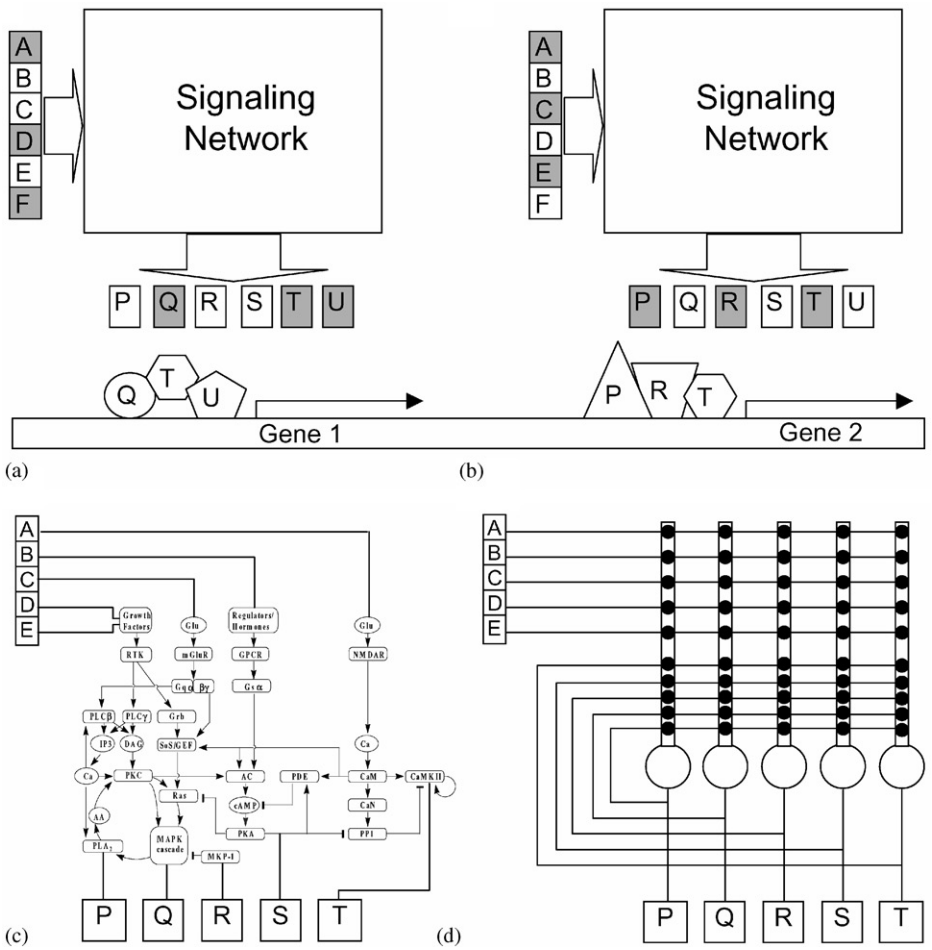

Fig. 7. Combinatorial decoding by signaling networks. (a, b) Transformation of different combinations of signaling input patterns (A–F) into combinations of activity at the output (P–U) of the network. The selective activation of different gene products as a result of this combinatorial decoding is shown. (c) Possible mapping of inputs and outputs onto a signaling network, reproduced with permission from Bhalla (2002b). (d) Associative memory metaphor for signaling network as a combinatorial decoder. The open circles represent artificial neural network 'neurons' as in Fig. 5a. The lines emerging from these 'neurons' are output 'axons' which go to the respective outputs (P–T) and also loop back to connect back to the inputs of the neurons. The black dots are inputs to these 'neurons', each with its own weight. Thus this network has recurrent (feedback) connections from the output back onto the neurons in the network. A combinatorial pattern of inputs may give rise to a specific combination of outputs when the input pattern is close to a memory or 'attractor' determined by the weights in the network.

For such systems, a higher level of abstraction is desirable. At a functional level, these situations involve a transformation from temporal and signaling inputs, to a combinatorial pattern of signaling activity. A few biologically inspired simulations of signaling networks do exhibit such properties (Bhalla, 2002b). These studies utilize simulation techniques on complex signaling networks and examine network responses to a range of inputs including patterns in time. The results suggest that complex signaling network can transform input patterns in time and in chemistry into combinatorial output patterns of signaling activity. There is an obvious compatibility of such outputs with the combinatorial patterns of repressor and promoter activation involved in gene regulation.

This transformation has some similarities with the behavior of a class of neural networks called associative memories (Fig. 7d) (James et al., 1999; Rumelhart and McClelland, 1986). These

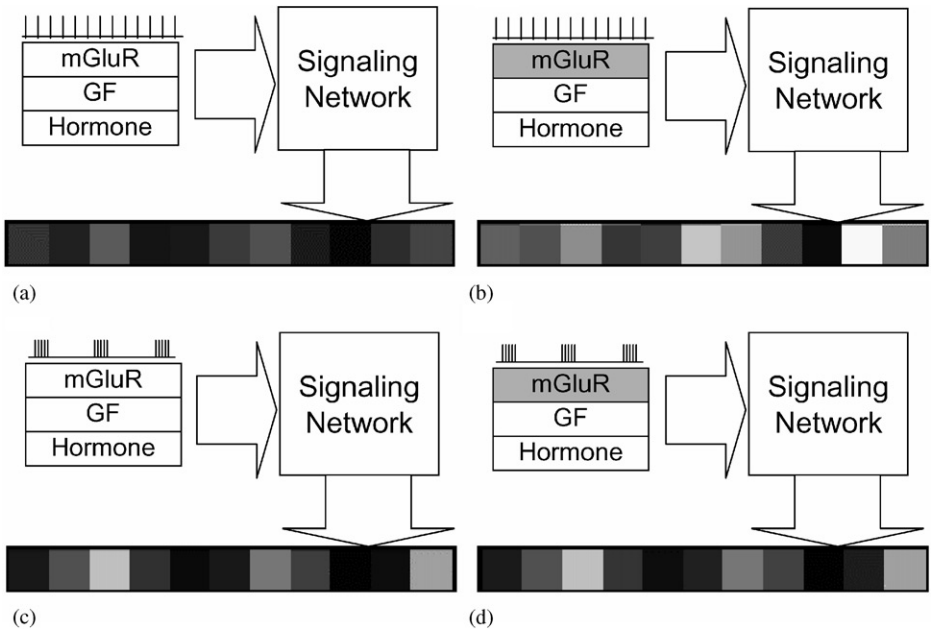

Fig. 8. Combinatorial decoding in the time domain. Inputs to the network can include temporally patterned inputs (represented as a series of action potential timings drawn as vertical lines on a horizontal time axis). In addition, various combinations of signaling input (metabotropic glutamate receptor input, mGluR; growth factor, GF; or hormone) may also affect network response. The signaling network is the same as shown in Fig. 7c. The output of the network is a combination of activities of 11 signaling molecules, using a gray scale where black is inactive and white is strongly active. The output signaling molecules include calcium and the four major kinases MAPK, PKA, PKC, and CaMKII (reproduced with permission from Bhalla, 2002b). Four combinations of temporal pattern (indicated by time series of stimuli to network) and signaling input (indicated by shaded mGluR input) are delivered to the signaling network. (a) Steady 1 Hz stimulus, no mGluR. (b) Steady 1 Hz stimulus, mGluR active. (c) 3 bursts of pulses separated by 10 min each, no mGluR. (d) 3 bursts of pulses separated by 10 min each, mGluR active. The network transforms these stimuli into different patterns of activation of signaling molecules. Temporal patterns as well as chemical signals determine which combination of outputs will be activated. The presence of the mGluR input makes a large difference to the network responses to the steady pulse sequence in (a) and (b), suggesting that they are on different 'attractors' in the signaling network. However, mGluR does not cause much difference in the outputs of the pulsed stimulus in (c) and (d), indicating that these patterns share an 'attractor'.

networks have extensive feedback and thus map onto the architecture of an arbitrary signaling network. The capabilities of such networks, as expected, include robustness and the ability to generalize. The associative memory has the ability to store a number of input/output transformations. When an input is close to one of the stored 'memories', the associative memory generates the appropriate output. Each memory is described as an *attractor* since many similar inputs are 'attracted' to this memory and all generate the same output.

A limitation of conventional associative memories is that they do not represent time directly. We have earlier seen that signaling pathways can in fact perform quite interesting temporal operations, so this should be an added capability of our temporal-pattern associative network. The nodes on the network could be temporal devices such as filters, tuners, integrators and so on. How might such a network function? First, the input and output of the network are both a complex combination of temporal sequences and chemical signals. This satisfies our functional criterion. Second, one would expect to see many of the familiar properties of neural networks: robustness, generalization and so on. This is obviously desirable for any biological system. Third, the network should be able to 'recognize' and respond in a distinct manner to inputs that include the temporal domain, since the units in the network are themselves temporally selective. In neural network terms, the network would have a set of attractors, or preferred input combinations, and it would respond with a given output combination to any input reasonably close to the preferred input. Fourth, the response preferences of the network should be tunable by various regulatory inputs to change the current set of attractors.

These match well with the properties reported for a simulation of synaptic signaling networks (Fig. 8) (Bhalla, 2002b). This temporal-pattern associative network therefore seems like a plausible analogy for complex signaling networks. Evolutionary pressures may act to store certain 'memories' or attractors in the signaling network, such that useful cellular outcomes are seen (Bray, 1990; James et al., 1999). Such networks would form a very versatile, evolutionarily plausible system for cellular control through combinatorial decoding.

4. The modular and functional viewpoints

The metaphors listed above are rich and, to this reviewer at least, suggest some intuitively useful abstractions for the signaling tapestry. Given the range of powerful tools and analogies from systems analysis, logical and neural network viewpoints, and electrical analogies, it may appear that we have a good set of functional modules to form a basis for thinking about signaling even in very complex networks. Indeed, such a functional module viewpoint has been proposed by several groups (Hartwell et al., 1999; Lauffenburger, 2000).

In many cases these analogies are both appropriate and reasonably complete. For example, the details of phototransduction are intricate and remarkable in providing a near optimal combination of speed and sensitivity over many orders of magnitude illumination. This is where the detailed quantitative description comes in (Lamb and Pugh, 1992). Nevertheless, one has a reasonable intuitive description of phototransduction in terms of a very simple analogy to an amplifier with gain control. It takes careful experimentation and simulations to work out the details, but for the purposes of human understanding, the concept of an amplifier is fairly accurate.

As the target of regulation becomes more complex, this simple approach of applying suitable analogies starts to become murky. Cell cycle control, for example, involves multiple regulatory modules and begins to take on a fair degree of complexity even when using concepts such as feedback loops (Kohn, 1999). Nevertheless, modularizing the problem makes it far simpler than the underlying system of chemical reactions.

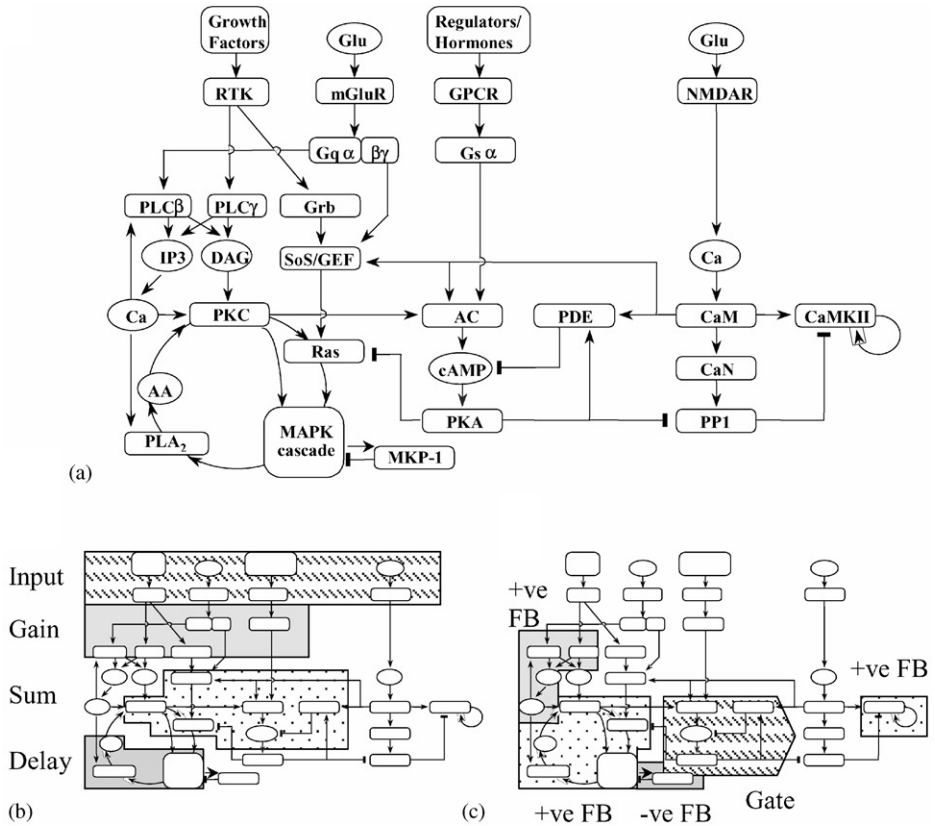

Fig. 9. Overlapping modules in a signaling network. (a) Network block diagram (reproduced with permission from Bhalla, 2002b). Pathway abbreviations are as in Fig. 3, with the following additions: PLCβ: phospholipase C beta, PLCγ: phospholipase C gamma, IP3: inositol trisphosphate, DAG: diacylglycerol, SoS/GEF: son of sevenless/guanine nuclotide exchange factor, Gq: G-protein type q, GPCR: G-protein coupled receptor, Gs: G-protein type s, AC: adenylyl cyclase, cAMP: cyclic adenosine monophosphate, PKA: protein kinase A, PDE: phosphodiesterase, Glu: glutamate, NMDAR: *N*-methyl *D*-aspartate receptor, CaM: calmodulin, CaN: calcineurin, PP1: protein phosphatase 1, CaMKII: calcium calmodulin kinase type II. (b) Possible decomposition of blocks into functional modules. Blocks performing common categories of functions are enclosed in shaded regions. The input modules transduce signals. The gain modules carry out amplification. Many modules perform summation of multiple inputs. MAPK and PLA_2/AA act as signaling delays. (c) Another possible decomposition into functional modules. Although the molecules involved overlap heavily with those in (b), they play quite different roles in this interpretation. Here a number of feedback loops have been identified, which could lead to properties such as bistability, thresholding, or oscillation. There is also a set of pathways that collectively act as a gate whereby the MAPK feedback loop may control the activity of the CaMKII loop.

Once we get to really complex signaling networks such as those involved in cell fate decisions, the modular approach becomes difficult to use. First, it may be difficult to find a clean mapping from signaling molecules to functional modules. Protein Kinase C (PKC), for example, might act as a summation point, a timer, part of a feedback loop, and a thresholding device, all at the same time (Fig. 9). It is not yet clear whether these functions can be separated or even identified from the circuit elements themselves. If one were presented with a complex signaling network diagram, one could readily point out *possible* functional modules emerging from the network. For example, feedback loops, as already discussed, are important circuit motifs that immediately suggest certain possible functions. The problem is that the exact function of the system is highly dependent on parameters. The same feedback loop might act as an amplifier, thresholder, timer, oscillator, or memory (Bhalla et al., 2002) (Fig. 3d). Thus modules may be more useful in making sense of a network post-facto, after we already understand a great deal of how it works and what it does.

Second, and more seriously, functional modules themselves are not very 'high-level' when thinking about seriously complex signaling situations. Indeed, the process of using functional modules to think about signaling circuits is not too different from the original ideas of qualitative modeling. For example, a moderately complex network could perhaps be decomposed into half-a-dozen or so modules (Bhalla and Iyengar, 2001). What next? Since we already had to use a simulation to figure out what the appropriate modules were, it is not much use to now make a qualitative model of the modules themselves to find out what they would do together. The simulation already does that. Given the scale of known signaling networks, we will shortly have to think in terms of tens or even hundreds of these functional modules.

In such situations, a more functional view may be appropriate. Here the network is first considered as an integral functional unit, rather than many interacting modules. The combinatorial decoder is one example of an abstraction based on a functional description of what the network does as a whole (or appears to do, to the best of our current knowledge). It is only after the decoder concept is applied that one could tentatively decompose it to simpler abstractions such as associative memories and temporal filters. Relatively few large networks have been simulated in sufficient detail to apply this abstraction, so is not yet clear whether the functional view will be generally useful, or just one of the many approaches to understanding signaling in a specific domain.

5. Summary

Biology is now at a level of understanding where it is in principle possible to describe many aspects of cellular signaling at the level of chemistry. Many of the technical advances that have made this possible have also unleashed a flood of raw biological information from genome, proteome, and related projects. The biologist is therefore confronted with a double dose of too much of a good thing: a depth of detail, from the cellular to the molecular level; and a breadth of biological data, including thousands of genes, proteins, and their interactions. Computers are the immediate tools for organizing all this data, both to define the details and to predict complex behavior. Computer models and databases are notoriously complex. However, such models also provide critical tools to help mere humans to understand cellular complexity. Often, understanding comes when one can map complex situations onto familiar concepts. There are

several such mappings in the case of signaling networks. Systems analysis provides tools for recognizing functionally important states of signaling networks. Many analogies from neural networks and electronics provide higher-order concepts to help understand signaling. Finally, in a few cases one can recognize an overall network process of transformation of temporal and chemical inputs into combinations of chemical signals that are compatible with genetic control through promoter and repressor combinations.

While there is a long way to go in filling out the biological wiring diagram, computers, simulations and metaphors have helped the human mind keep pace so far. It would be enormously valuable if these conceptual tools for understanding signaling continue to evolve in parallel with the growth of biological knowledge.

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