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Multiple Functionalities of Biochemical Reaction Networks

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Abstract

We consider a biological cell as a highly interconnected network of chemical reactions, which is constituted of a large number of semi-autonomous functional modules. Depending on the global state of the network, the separate functional modules may display qualitatively different behavior. As an example, we study a conceptual network of phosphorylation cycles, for which the steady-state concentration of an output compound depends on the concentrations of two input enzymes. We show that the input-output relation depends on the expression of the proteins in the network. Hence changes in protein expression, due to changes in the global regulatory network of the cell, can change the functionality of the module. In this specific example, changed expression of two proteins is sufficient to switch between the functionalities of various logical gates.

Introduction

The human body consists of over 200 distinct cell types, which display a large variety in both morphology and physiology. Often differences between cell types manifest themselves in the response to extracellular stimuli. The same type of molecule may even have a totally different function in different cell types. An example for this is the response of cells to the neurotransmitter acetylcholine (see Alberts et al. (2002)). The extracellular presence of this compound yields contraction in skeletal muscle cells but a decrease of contraction in heart muscle cells and even a multitude of different effects in many other cells.

On the other hand, as all cells in an organism descend from the same zygote, they share the same potential reaction network, i.e., they are capable of producing the same set of macromolecules, the interactions among which are based on the same rules (e.g., rate and diffusion constants). Therefore, the key to differences in behavior must lie in different configurations of the network. In a systems theory sense, these cells can be looked upon as similar systems that due to a different state show very diverse responses to identical stimuli. The state variables of these systems are the concentrations of chemical species, including species with an important regulatory function such as transcription factors.

During differentiation, a chain of extracellular stimuli and cell-cell interactions pushes the network for each cell in a certain region of its state space. Each of those regions is characterized by its pattern of present transcription factors (and other regulatory molecules) and consequently a corresponding pattern of protein expression. Not only through development, but also in adult individuals, subtle changes in configuration occur. Examples are synaptic plasticity in neurons and adaptations to a changed environment, but also many diseases coincide with a changed configuration of the reaction network of individual cells: in tumor cells the cell cycle control is disrupted (Hanahan and Weinberg (2000)) and in metabolic syndrome the cellular respiratory system is affected (Kitano et al. (2004)).

In order to deal with the vast number of interactions in an intracellular reaction network, it is common practice to observe semi-autonomous parts of the system as separate modules (Hartwell et al. (1999)). Such a separation is not necessarily artificial, as many biological systems have a tendency towards modularity. Interestingly, modularity has been reported by Variano et al. (2004) to emerge when evolving artificial networks described by linear differential equations with a fitness function that rewards network stability . Depending on the exact scale of observation and field of interest, many definitions for modules and modularity exist (see for instance Polani et al. (2005), Bongard (2002)). In this paper, we use the word *module* to refer to a group of proteins (and metabolites) that interact with each other on the level of protein-protein (and protein-metabolite) reactions, but not with proteins or metabolites outside the module. We do, however, allow the transcriptional and translational regulatory systems of the cell to alter the concentrations of proteins inside the module. Hence, as the tuning of transcription and translation differs between cell types, this may affect protein concentrations and, because of that, the dynamics of the module of interest. This applies to all intracellular reaction networks that depend on protein concentrations, among which metabolic pathways and signaling networks.

Here, we focus on the influence of protein expression on the functionality of a module. There are a number of ways

in which proteins expression can influence this functionality. Obviously, when none of the involved proteins are expressed, the module of interest would be switched off. As a result, many processes are present in only a few types of cells. For instance, only a small number of cells synthesize certain neurotransmitters, although all cells have the blueprints for the necessary enzymes in their DNA. Apart from that, the changes in protein expression can have many more effects on the input-output relations of functional modules. We can roughly distinguish two classes of effects. Firstly, the expression of enzymes involved in a module can have quantitative effects on the input-output relations of the module. For instance, muscle cells are set to take up larger amounts of glucose than less energy consuming cell types such as skin cells. Secondly, there is the possibility that the observed functionality itself is changed in a qualitative fashion. An example is the MAP kinase signaling network of Bhalla et al. (2002), in which the expression of MAP kinase phosphatase determines whether the network displays a gradual or a bistable response to extracellular stimuli.

In this paper we exemplify with a conceptual model, that the steady-state input-output relations of a signal transduction network consisting of phosphorylation cycles may change dramatically by changing only a few concentrations. Moreover, this model shows that even if we know the topology of a network and the sign (i.e., positive or negative influence) and strength of its interactions, the behavior depends heavily on the actual concentrations of proteins. We show that with the same topology and rate constants, this network, which consists of only 5 phosphorylation cycles, can have at least 8 different input-output relations, depending on the chosen protein concentrations. Note that, from a mathematical point of view, there is only one function that is calculated by the network. However, we consider some of the concentrations as parameters that are determined outside the model. The input-output relation for each parameter set is thus considered as a separate function.

The implementation of Boolean logic in biochemical reactions is not new. For instance, Strack et al. (2008) have recently build wet lab implementations of AND, OR, XOR and 'B AND NOT A' functions using enzymatic reactions. Boolean logic is also a subject of research in the field of genetic regulatory networks (see for instance Schilstra and Nehaniv (2008)). Here, we describe one single network that with suitable parameters can compute 8 different Boolean functions.

Model description

Phosphorylation cycles

Phosphorylation cycles are common building blocks of intracellular signaling networks (Cohen (2000)). The generic phosphorylation cycle involves a single type of protein which can be in two states: a phosphorylated and a dephosphorylated state. In a phosphorylation reaction, a phos-
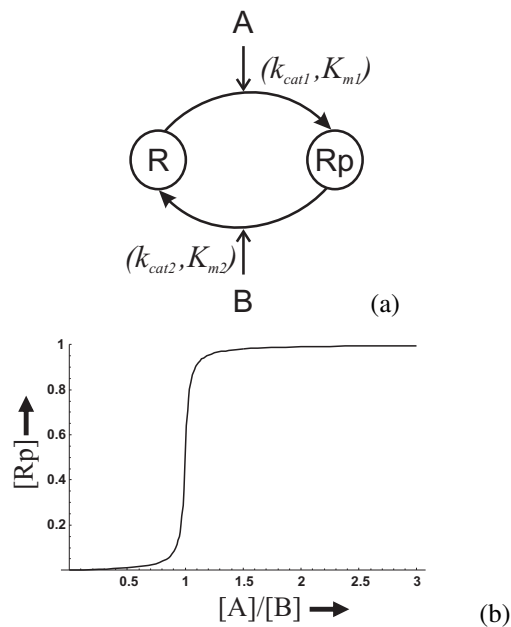
Figure 1: (a) Phosphorylation cycle with one kinase A and one phosphatase B, for which the dynamics can be described by two Michaelis-Menten reactions with constants k_{cat1} and K_{m1}, and k_{cat2} and K_{m2}, respectively. (b) The steady-state concentration of Rp as a function of the ratio of the concentrations of A and B, for the case $k_{cat1} = k_{cat2} = 1$ and $K_{m1} = K_{m2} = 0.01$.

phate group is transferred from a donor molecule (commonly ATP) to a specific site on the dephosphorylated protein. The enzymes that catalyze this 'forward' reaction are called kinases. The 'backward' reaction, i.e., the dephosphorylation reaction, is catalyzed by a phosphatase enzyme. A schematic representation of such a cycle is shown in Figure 1 a.

In this paper we restrict our description of the phosphorylation cycle to a highly idealized cycle consisting of two Michaelis-Menten type reactions (Fersht (1999)). Furthermore, we assume a fixed concentration of phosphate donors. In this way, we can describe the rates v_{phos} and v_{dephos} of the reactions

$$A + R \quad \rightarrow \quad A + Rp$$
$$B + Rp \quad \rightarrow \quad B + R,$$
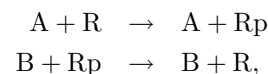
with Michaelis constants K_{m1} and K_{m2} and catalytic constants k_{cat1} and k_{cat2} by

$$v_{phos} = \frac{k_{cat1}[A][R]}{K_{m1} + [R]},$$

$$v_{dephos} \frac{k_{cat2}[B][Rp]}{K_{m2} + [Rp]}.$$

Note that square brackets indicate the concentration of the corresponding compound. The steady-state of such a cycle

is given by Goldbeter and Koshland (1981) and can be written as (Tyson et al. (2003)):

$$[\mathrm{Rp}] = \frac{2\, v_1 J_2 R_{\mathrm{tot}}}{v_2 - v_1 + v_2 J_1 + v_1 J_2 + D}\ ,$$

where

$$D = \sqrt{(v_2 - v_1 + v_2 J_1 + v_1 J_2)^2 - 4(v_2 - v_1)v_1 J_2}$$

and $R_{\mathrm{tot}} = [\mathrm{R}] + [\mathrm{Rp}]$, $v_1 = [\mathrm{A}]\, k_{\mathrm{cat1}}$, $v_2 = [\mathrm{B}]\, k_{\mathrm{cat2}}$, $J_1 = K_{m1}/R_{\mathrm{tot}}$, $J_2 = K_{m2}/R_{\mathrm{tot}}$.

Typically, the steady-state response of such a cycle is sigmoidal as function of $[\mathrm{A}]/[\mathrm{B}]$ (see Figure 1 b). As both the phosphorylated and dephosphorylated proteins can act as enzymes themselves, phosphorylation cycles can be coupled in a cascade, in which the substrate of one cycle is a kinase or phosphatase in another reaction. In the next section we introduce a network of such cycles, in which we assume that only the phosphorylated form is active as an enzyme and can have either kinase or phosphatase activity but not both. We do not take into account formation of protein-protein complexes other than the kinase-substrate and phosphatase-substrate complexes taken care of in the model of Goldbeter and Koshland.
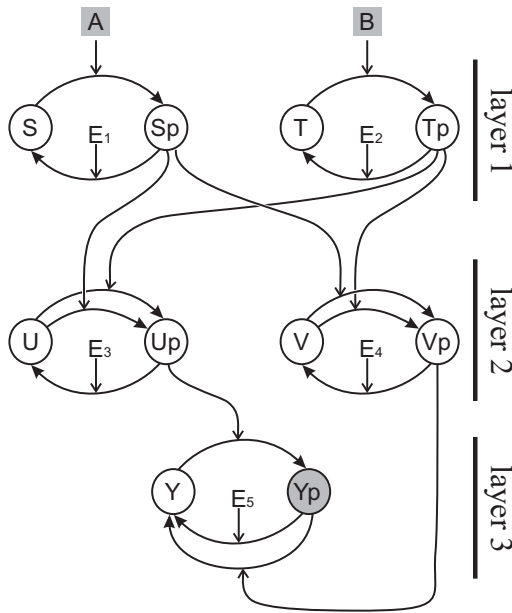

Figure 2: Reaction scheme of the network. System parameters $[\mathrm{E}_1]...[\mathrm{E}_5]$, $S_{\mathrm{tot}}, T_{\mathrm{tot}}, U_{\mathrm{tot}}, V_{\mathrm{tot}}$ determine the response of $[\mathrm{Yp}]$ to input concentrations $[\mathrm{A}]$ and $[\mathrm{B}]$.

Network topology

Consider the network of phosphorylation cycles as shown in Figure 2. Depending on the external concentrations $[\mathrm{A}]$ and $[\mathrm{B}]$ (which are further referred to as 'input concentrations'), the state of the network will change. We focus on

the steady-state concentration of Yp for given concentrations $[\mathrm{A}]$ and $[\mathrm{B}]$. Clearly, this concentration depends on the concentrations of uncoupled enzymes ($[\mathrm{E}_1]...[\mathrm{E}_5]$) and the total amount of mass in each phosphorylation cycle (i.e., $S_{\mathrm{tot}} = [\mathrm{S}] + [\mathrm{Sp}]$, $T_{\mathrm{tot}} = [\mathrm{T}] + [\mathrm{Tp}]$, $U_{\mathrm{tot}} = [\mathrm{U}] + [\mathrm{Up}]$, $V_{\mathrm{tot}} = [\mathrm{V}] + [\mathrm{Vp}]$ and $Y_{\mathrm{tot}} = [\mathrm{Y}] + [\mathrm{Yp}]$). The regulation of the amount of protein expression (i.e., transcription, translation and protein degradation) is part of the chemical network of the entire cell, but not of the module of interest. Therefore, the total concentrations of involved proteins are ways for the global system of the cell to tune the functionality of the described module. From here on, we refer to these concentrations as system parameters. Clearly, the rate constants of all the reactions are parameters of the module as well. However, it seems unlikely that the global network, apart from the expression of inhibitors or activators, can change the values of these parameters. Therefore, we keep the rate constants of all reactions at 1.

As there is no feedback, each phosphorylation cycle in our model has one single stable solution irrespective of its initial state. As there are only feed-forward connections, there is only one steady-state solution for the entire network as well for given system parameters and inputs ($[\mathrm{A}]$ and $[\mathrm{B}]$). When two or more enzymes catalyze the same reaction, we consider the sum of their concentrations as the concentration of the catalyst.

Parameter values

In the previous section we have defined system parameters as variables of the global cell network that determine the behavior of functional modules. The presented conceptual model of such a module has 10 system parameters ($[\mathrm{E}_1]...[\mathrm{E}_5]$, S_{tot}, T_{tot}, U_{tot}, V_{tot} and Y_{tot}), which can be used to configure the network. In order to obtain more insight in the possible behaviors of the network, we focus on its possibilities as a logic gate. For the input we define values below 0.5 as False and above 0.5 as True. We show that only two system parameters (S_{tot} and T_{tot}) have to be adjusted to obtain 8 different logical functions with 2 inputs. For all reactions we choose the Michaelis constant $K_{\mathrm{m}} = 0.01$ and the catalytic constant $k_{\mathrm{cat}} = 1$.

The first layer of the network functions as a thresholding device. That is, as we do not demand inputs $[\mathrm{A}]$ and $[\mathrm{B}]$ to be exactly 0 or 1, we want $[\mathrm{Sp}]$ be near 0 if $[\mathrm{A}] < [\mathrm{E}_1]$ and near S_{tot} if $[\mathrm{A}] > [\mathrm{E}_1]$. The same holds for the T-Tp cycle. We choose thresholds $[\mathrm{E}_1] = [\mathrm{E}_2] = 0.5$. As both phosphorylation cycles in the second layer receive input from both S_{p} and T_{p}, we consider the sum of their concentrations (i.e. $[\mathrm{Sp}] + [\mathrm{Tp}]$) as the output of the first layer. The input-output relation of layer 1 is given by Table 1. The output of the first layer $[\mathrm{Sp}] + [\mathrm{Tp}]$ is approximately one of the four numbers $0, T_{\mathrm{tot}}, S_{\mathrm{tot}}$ or $S_{\mathrm{tot}} + T_{\mathrm{tot}}$. Note that, as S_{tot} and T_{tot} are the only adjustable system parameters, these four numbers are not yet fixed.

Layers 2 and 3 take $[Sp] + [Tp]$ as input, and have $[Yp]$ as output. If we also take $U_{tot} > 0$, $[E_5] \approx 0.5\,U_{tot}$, $V_{tot} \gg U_{tot}$, and $Y_{tot} = 1$ and choose any combination of $[E_3]$ and $[E_4]$ for which $[E_3] < [E_4]$, we obtain the input-output relation for layers 2 and 3 that is shown in Figure 3. Now, the only parameters that are not yet fixed are S_{tot} and T_{tot}.

[A]	0	0	1	1
[B]	0	1	0	1
[Sp] + [Tp]	0	T_{tot}	S_{tot}	$S_{tot} +$ T_{tot}

Table 1: Input-output relation of layer 1
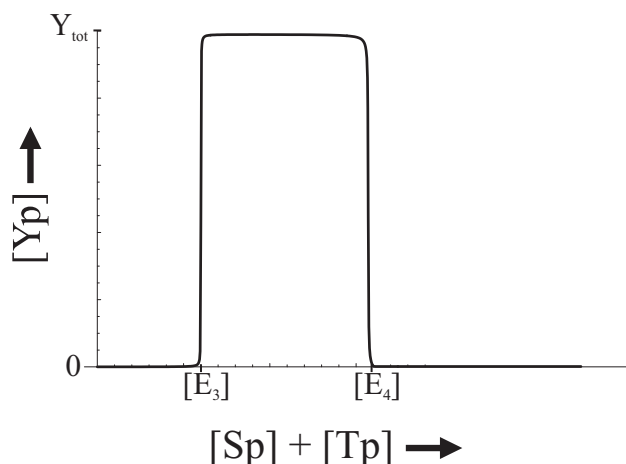

Figure 3: Response of layer 3, when layer 2 receives a total input $[Sp] + [Tp]$.

Network behavior

Different combinations of S_{tot} and T_{tot} can yield different computable functions, as shown in Figure 4. Because the response of the network is built up of sigmoidal functions, the borders between the shown regions do not indicate discrete changes in functionality but rather narrow continuous transitions.

For instance, to compute the XOR function, we choose S_{tot} and T_{tot} such that $[E_3] < S_{tot} < [E_4]$, $[E_3] < T_{tot} < [E_4]$ and $[E_4] < S_{tot} + T_{tot}$. Similarly, to compute the OR function we require $[E_3] < S_{tot} < [E_4]$, $[E_3] < T_{tot} < [E_4]$ and $[E_3] < S_{tot} + T_{tot} < [E_4]$. It is easily verified from Table 2 and Figure 3 that indeed the XOR respectively OR function are computed. Note that the conditions on S_{tot} and T_{tot} for the OR function require that $[E_3] < [E_4]/2$. As can be seen from Figure 4, all 8 Boolean functions that yield 0 for input $[A] = [B] = 0$ can be computed if $[E_3] < [E_4]/2$.

If we consider both concentrations $[Y]$ and $[Yp]$ as outputs, our network is able to calculate all 16 possible log-
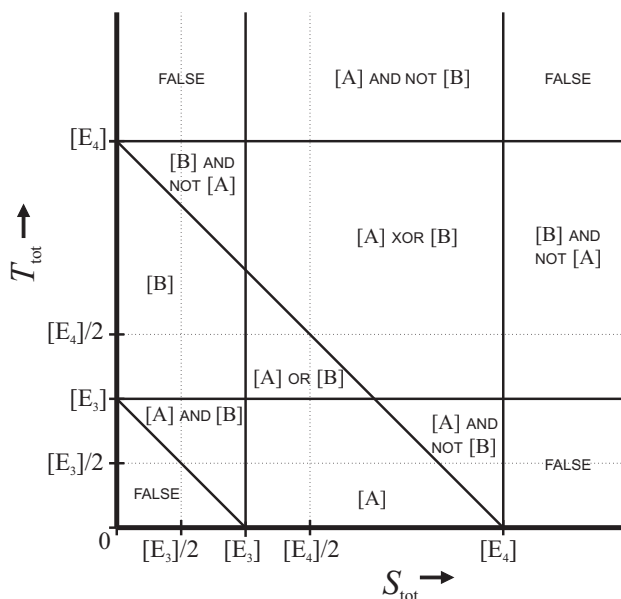

Figure 4: Network output for combinations of S_{tot} and T_{tot}. Only relative concentrations are shown, see Figure 5 for examples of actual parameter values.

XOR				
[A]	0	1	0	1
[B]	0	0	1	1
[Sp]+[Tp]	0	$> [E_3]$, $< [E_4]$	$> [E_3]$, $< [E_4]$	$> [E_4]$
[Yp]	0	1	1	0

OR				
[A]	0	1	0	1
[B]	0	0	1	1
[Sp]+[Tp]	0	$> [E_3]$, $< [E_4]$	$> [E_3]$, $< [E_4]$	$> [E_3]$, $< [E_4]$
[Yp]	0	1	1	1

Table 2: Computing the XOR or the OR function. The values of S_{tot} and T_{tot} (see Figure 4) determine the range of values of $[Sp] + [Tp]$ and by that the value of $[Yp]$ for the possible combinations of input values $[A]$ and $[B]$.

ical functions for two inputs. The reason is that, because $[Y] = 1 - [Yp]$, we can consider $[Y]$ as the negation of $[Yp]$.

The possible outputs of the network are shown in Figure 5. Also, without considering $[Y]$, other logical functions may be calculated, as well as functions with a more gradual response to different inputs. To this end, alternative choices for system parameters, other than S_{tot} and T_{tot} should be used. Even more possibilities may appear by adding extra enzymes (kinases or phosphatases) to some of the phospho-

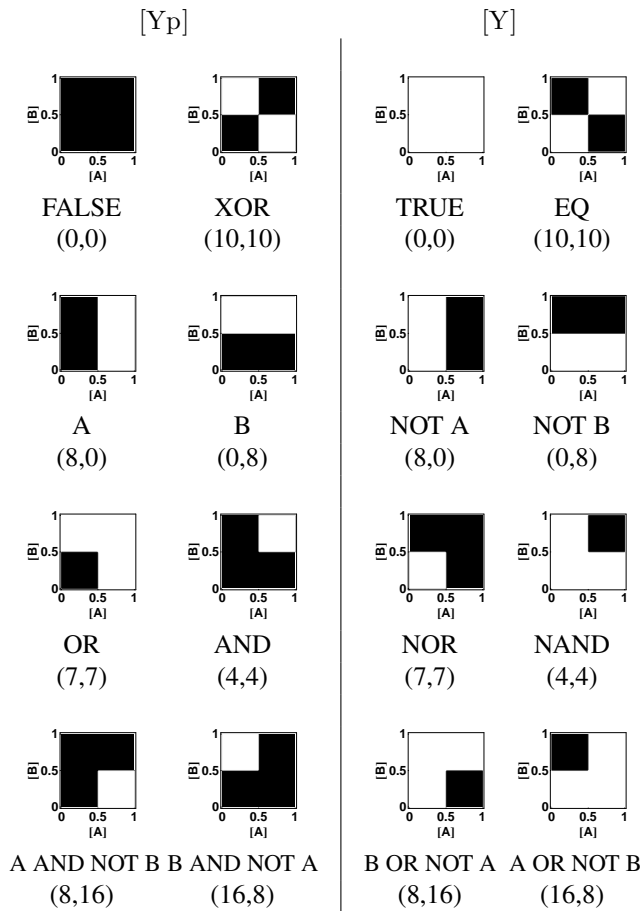rylation cycles. However, this is beyond the scope of this paper.

Figure 5: Depending on the network configuration, the steady-state concentrations of Yp and Y correspond to one of the 16 logical functions for 2 inputs [A] and [B]. (Gray levels indicate concentration: Black ≈ 0 , White ≈ 1). All sub-figures were plotted using $[E_3] = 6$, $[E_4] = 16$, $[E_5] = 0.5$, $U_{tot} = 1$, $V_{tot} = 10$ and the values for S_{tot} and T_{tot} that are given in brackets for each sub-figure.

We have shown how the values of only two parameters determine the response of the network. The importance of parameter values is even more strikingly exemplified if we use the same network as a unary logic operator. In order to do that, we consider [B] as a system parameter instead of an input, choose S_{tot} and T_{tot} within the 'XOR region' and leave the other parameter values unchanged. In that case changing the value of [B] is sufficient to switch the output [Yp] of the network from identity to negation of input [A].

Biological Plausibility

Although the topology and parameters of the model are optimized to display specific idealized behavior rather than to describe any known intracellular pathway, the model consists of building blocks that are common in intracellular signaling. It may therefore be possible to find a similar topology within existing biological networks. Indeed, in the Human Protein Reference Database (see Peri et al. (2003) and Mishra et al. (2006)), we have found a number of protein kinases and phosphatases that show similar patterns of interactions. More specifically, we have searched this database (Release 7, downloaded from http://www.hprd.org) for combinations of five proteins S,T,U,V and Y for which the proteins S and T are each known to phosphorylate at least one site on the proteins U and V. In addition, protein U has to phosphorylate protein Y and protein V has to dephosphorylate Y. Furthermore, we require that both S and T are targets for phosphorylation by other protein kinases. We did not discriminate between multiple phosphorylation sites on the same protein.

With these requirements, we have identified the combinations of S,T,U,V and Y, that are listed in Table 3. Note that proteins S and T are interchangeable. For each of the possible proteins for S and T, known kinases (i.e. potential network inputs A and B) are listed in Table 4. On protein PRKACA there are at least 5 different sites that are known targets for phosphorylation, but for which no upstream kinases are known. Finding these networks with similar topology makes it more plausible that this type of multifunctional modules can occur in nature. However, due to the current limited knowledge about parameters of signalling networks, it remains unclear whether such functionality is indeed used in biology.

S	T	U	V	Y
FYN	LCK	SHC1	ACP1	ZAP70
LCK	LYN	PRKCD	PTPN6	EGFR
MAPK1	PRKACA	RAF1	PTPN7	MAPK1
PRKCA	PRKACA	SRC	PTPN12	ABL1
PRKCA	PRKACA	SRC	PTPN12	PTK2

Table 3: Real networks with same topology. S and T are interchangeable.

Substrate (S or T)	Kinase (A or B)
FYN	FYN, CSK, PDGFRB
LCK	CSK, LCK, PRKCA, MAPK1, SYK, PRKACA, MAPK3
LYN	LYN, MATK, CSK
MAPK1	MAPK1, RET, MAP2K1, RAF1
PRKACA	Kinase unknown
PRKCA	PRKCZ, SYK

Table 4: Kinases working on possible substrates S and T.

Discussion

In order to get some grip on the overwhelming complexity of biochemical interactions within a cell, it is common practice in biology to analyze separate pathways or modules instead of the whole system. We have defined a module in such a way that it can be analyzed separately from the larger network to which it belongs. Changes outside the module are therefore considered as changes in parameters or boundary conditions, rather than changes of the state of the module itself. Because of this, the same module may have different parameters in two different cell types, and may therefore display different functionalities. Since a small module, such as the conceptual network presented in this paper, can already behave totally different depending on the values of two parameters, it is likely that also in real networks changes in protein expression result in changes in observed functionality. Note that, from a mathematical point of view, there is no such thing as a change in functionality, as the network of interactions is still the same. However, when dealing with small parts of the system, different behavior can be observed as different functionalities. The model presented in this paper gives an example how small changes in parameters of a phosphorylation network can already yield a different functionality. For example, the presence or absence of the compound B is sufficient to switch the input-output relation between [A] and [Yp] from identity to negation.

Although this model is not based on any known intracellular pathway, we have shown that similar topologies can be found in the Human Protein Interaction Database. Despite the idealized sigmoidal response of the phosphorylation cycles, this small network can already behave in at least 8 different ways, depending on how it is configured. This makes it plausible that modules of real biological networks display such multifunctional properties as well, which gives a clue how the same protein in different cells can be involved in different processes and can even show contradictory behavior among cell types. Moreover, this multifunctionality may also be exploited by evolution to use the same modules for different purposes in different cell types. Spatial isolation of proteins may even allow the exploitation of different functionalities in separate regions in the same cell. On the other hand, the same functionality may be needed in many different cell types. In that case it appears to be advantageous if the involved genes are co-regulated, as this would preserve the ratios between the protein concentrations and by that the functionality of the module.

As for many signaling networks quantitative data is lacking or unreliable, an often used technique is to use Boolean networks to model positive and negative interactions between nodes (Kauffman (1969), see de Jong (2002) for a review). Although this technique is useful to understand some interactions within a complicated network, a lot of qualitative effects are missing with this approach. We can illustrate this with our conceptual model. Cycles U-Up and V-Vp are both positively influenced by cycles S-Sp and T-Tp and indirectly by external concentrations [A] and [B]. As in a classical Boolean network interactions only have a sign but not a weight, the U-Up and V-Vp cycles are identical. The Y-Yp cycle receives positive influence from Up and negative influence from Vp. As the topology and interactions remain the same, a purely qualitative approach would be insufficient to describe the different 'modes' of the network. This also shows that for understanding the dynamics of these complex networks, it is necessary to perform multiple quantitative measurements at the same time.

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