

# Biological networks in metabolic P systems

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Received 30 March 2006; received in revised form 24 October 2006; accepted 29 November 2006

## Abstract

The metabolic P algorithm is a procedure which determines, in a biochemically realistic way, the evolution of P systems representing biological phenomena. A new formulation of this algorithm is given and a graphical formalism is introduced which seems to be very natural in expressing biological networks by means of a two level representation: a basic biochemical level and a second one which regulates the dynamical interaction among the reactions of the first level. After some basic examples, the mitotic oscillator in amphibian embryos is considered as an important case study. Three formulations of this biological network are developed. The first two are directly derived by Goldbeter's differential equations representation. The last one, entirely deduced by translating the biological description of the phenomenon in our diagrams, exhibits an analogous pattern, but it is conceptually simpler and avoids many details on the kinetic aspects of the reactions.

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**Keywords:** P systems; Membrane systems; Metabolism; Biological networks; Biological oscillators; Biological discrete models

## 1. Introduction

One of the most important problems mathematicians and computer scientists have to cope with, while modelling biological phenomena, is a deep understanding and a clear representation of the phenomena related to intercellular or intracellular information transfer. In fact, in the analysis of the interactions occurring in metabolic or signal pathways, very complex networks are expressed, in their specific mechanisms, by terms like *synthesis*, *production*, *catalysis*, *degradation*, *introduction*, *expulsion*, *consumption*, *influence*, *activation*, *inactivation*, *inhibition* and *promotion*. Many of these concepts have a simple translation in formal terms, but other are very often vague, ambiguous, or strongly depending on the specific contexts in which they are

embedded. The graphical notations which are used in the visualizations of these networks (Segel and Cohen, 2001; Voit, 2000) have only an intuitive basis and when one attempts to express them in mathematical terms, then many inconsistencies or confuse meanings are easily encountered; moreover, they do not provide information about the dynamics of the network they represent. Therefore, a natural question arises: is it possible to reduce the most important biological regulation mechanisms to some basic relationships which could permit rigorous mathematical translations? In this paper, we present some initial steps along this direction. In fact, a notation for representing some biological networks is here described which is related to the *metabolic algorithm* (Bianco et al., 2006a, b; Fontana et al., 2006; Manca et al., 2005), an effective method for “computing” biological dynamics we have developed in the framework of P systems (Păun, 2000, 2002). This notation provides not only the basic information of the biochemical reactions, but it also gives the reaction regulation functions which are the core of metabolic algorithm for computing

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the dynamics of a special class of biologically meaningful P systems. One of the main purposes of Systems Biology, is to understand the dynamic and molecular-level relationships among biological molecules in living systems. For this reason, tools which provide intuitive ways for representing and analyzing the dynamics of complex biological networks seem to be a necessary step in the assessment of a discipline that seeks to find the “hidden” structures underlying molecular data.

## 2. The P Metabolic Algorithm

P systems are a computational model based on the *compartmentalization of the workspace* and on *multi-set rewriting*. These concepts were introduced due to their strong biological motivation; in fact, they are intrinsically related to the basic role that membranes have in biological organisms and in the biochemical basis of any biological reality. In other words, the localization and the concentrations of any biochemical element at each instant determines all the relevant properties which underlie the form and the function a biological system exhibits at that time. We refer to Păun (2000, 2002) and the website at <http://psystems.disco.unimib.it>, for the definitions of P systems structure in all of the most important variants and for the most typical strategies which establish their evolution in time. However, from a biological viewpoint, the strategies of evolution considered in the standard P systems seem to be completely inadequate. In fact, the transition from a state to the next one is usually given by a maximally parallel application of rules which determines the way of transforming objects, of moving them from a membrane region to another one and, in the more general cases, of changing the topological setting of membranes. But, maximal parallelism or other strategies of this kind cannot express the dynamics of populations of chemicals governed by biochemistry laws. Therefore, we started with this main question: “How does a membrane system evolve in a biochemical realistic sense?” and, more specifically, “How is it possible to find a membrane system modelling some given biological phenomena, by reproducing its observed evolution in terms of concentration and localization of biochemicals?”. In order to address these questions we define an evolution strategy, called *Metabolic P Algorithm*, shortly MPA, which is a new formulation of algorithms given by Bianco et al. (2006a, b), expressing the essence of biochemical dynamics in a clearer and more intuitive way. An interesting feature of MPA is that it does not oblige to take a detailed account of the

exact biochemical kinetic of the system under investigation.

In the following, the main principles of this algorithm are formulated in a way that is directly related to the graphical formalism we present in the next section. For the sake of simplicity, we consider the simple case of only one membrane but, at the price of an heavier notational cost, the algorithm can be defined in the general context of multi-membrane systems as well. The leading principles of the MPA are the following:

- Reactants are distributed among all the rules step by step according to a “competition” strategy.
- If different rules need the same reactant, then each of these rules gets a portion of the available substance, in a percentage that is proportional to its reaction strength (*reactivity*) at that step.
- The reactivity of a rule at a given instant depends on the state of the system, defined as the concentration and localization of all substances.
- According to its stoichiometric “reading”, any rule determines its own reaction unit and therefore the amount of substances which it consumes and produces.

We summarize these principles with four statements:

- (1) Rules compete for object populations.
- (2) Objects are allocated to rules according to a *mass partition principle*.
- (3) Partition factors are determined by *reaction maps*.
- (4) A “Metabolic rule”  $r$  consumes/produces integer multiples of a *reaction unit*  $u_r$  which generalizes the notion of molar unit (Avogadro’s principle).

An example may be useful to clarify these principles. Let  $T = \{A, B, C, \dots\}$  be an alphabet of biological species (or types), we define  $q : T \rightarrow \mathbb{N}$  as the state of the system, that is, the concentration of each type at a certain observation instant. Assuming that, in a given instant, four rules, say  $r_2, r_3, r_5$  and  $r_7$ , need molecules of a certain type  $A$  for performing some biochemical reactions (see Fig. 1), then a partition strategy for species  $A$  is necessary.

The novelty of our approach is that of considering, for any state of the system, a real number as the strength of any rule, which is the value assumed by a *reactivity*, or *reaction map*, in the considered state. For example, with respect to Fig. 1, if  $a, b$ , and  $c$  are the concentrations of species  $A, B$ , and  $C$ , respectively, then the reactivities associated to the rules  $r_2, r_3, r_5$  and  $r_7$  in a state  $q$ , which

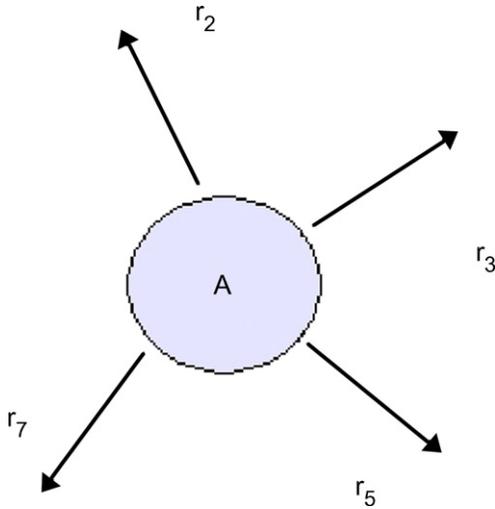


Fig. 1. Competition for object A.

ask for  $A$  molecules could be:

$$\begin{aligned} f_2(q) &= 200a, & f_3(q) &= 0.5a^{1.25}b^{-1}, \\ f_5(q) &= a^{1.25}(b+c)^{-1} & \text{and} & & f_7(q) &= 10. \end{aligned}$$

We define

$$K_{A,q} = \sum_{i=2,3,5,7} f_i(q)$$

as the *total pressure* on  $A$ . Then, for each of the competing rules  $r_j$  we consider the *partial pressure* (or *weight*) of  $r_j$  on type  $A$  as

$$w_{A,q}(r_j) = \frac{f_j(q)}{K_{A,q}}.$$

Getting back to the example discussed before, it should be easy to see that

$$w_{A,q}(r_2) = \frac{200a}{200a + 0.5a^{1.25}b^{-1} + a^{1.25}(b+c)^{-1} + 10}$$

while

$$w_{A,q}(r_3) = \frac{0.5a^{1.25}b^{-1}}{200a + 0.5a^{1.25}b^{-1} + a^{1.25}(b+c)^{-1} + 10}$$

and the other weights can be calculated analogously. These weights determine the partition factors of the amount of species  $A$ , available in the state  $q$ , among the rules which need objects  $A$  for performing their reactions.

At the end, let us assume that one of the rules competing for  $A$ , say rule  $r_2$ , has the following form:  $AAB \rightarrow AC$ , and let us suppose that, according to the point (3) expressed above,  $n$  objects of type  $A$  were allocated to

$r_2$  and  $m$  objects of type  $B$  were allocated to  $r_2$  too. The corresponding reaction unit turns out to be

$$u_{r_2} = \min\{n/2, m\}$$

and this means that  $2u_{r_2}$  objects of type  $A$  and  $u_{r_2}$  objects of type  $B$  are consumed, while  $u_{r_2}$  objects of type  $A$  and  $C$  are produced.

This globally states that  $u_{r_2}$  objects of type  $A$  and  $u_{r_2}$  objects of type  $B$  are replaced by  $u_{r_2}$  objects of type  $C$ . But, the important thing to point out here is that rule  $r_2$  is absolutely different from a rule  $r'$  having the form  $AB \rightarrow C$ , and this is due to the fact that the two rules imply different competition factors, and consequently, different mass partitions. In fact, in the second case the reaction unit would have been  $u_{r'} = \min\{n, m\}$ .

In general, if  $S_r$  is the set of substances which are reactants of the rule  $r$ , if we set:

$$R(Y) = \{r | Y \in S_r\}$$

and

$$K_{Y,q} = \sum_{r \in R(Y)} f_r(q)$$

then the reaction unit  $u_r(q)$ , of a rule  $r$  in the state  $q$ , is given by

$$u_r(q) = \min \left\{ \frac{q(Y)f_j(q)}{K_{Y,q}} \mid Y \in S_r \right\}.$$

The strategy outlined here suggests a natural representation of rules as graphs with two levels. The first level describes the reaction itself (the *stoichiometry*, that is the network of physical connections between species), the second level expresses the layout of *regulation* which tune the relative strengths of rules. The formal representation of biological networks is the subject of the following section.

### 3. Metabolic P Graphs

A *Metabolic P system of level 0* (with only one membrane), shortly a *MP system*, is given by a structure  $M = (T, Q, R, F, q_0)$  where  $T$  is an alphabet of types of  $M$ ;  $Q$  are the *states* of  $M$ , functions from  $T$  to the set  $\mathbb{N}$  of natural numbers;  $R$  is the set of rules of  $M$  which are denoted by  $\alpha \rightarrow \beta$  with  $\alpha, \beta$  strings over  $T$ ;  $F = \{f_r | r \in R\}$  is the set of *reaction maps* of  $M$ , with  $f_r : Q \rightarrow \mathbb{R}$  taking values in the set  $\mathbb{R}$  of real numbers; and  $q_0 \in Q$  is the *initial state* of  $M$ .

The evolution of  $M$  in time is given by a *dynamical function*  $\varphi : \mathbb{N} \rightarrow Q$  such that  $\varphi(0) = q_0$  and, for any  $n \in \mathbb{N}$ ,  $\varphi(n+1)$  is calculated by  $\varphi(n)$  by means of the *MPA*.

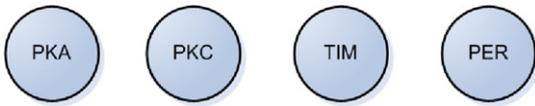


Fig. 2. Some examples of type nodes.



Fig. 3. Some examples of metabolic reactions nodes.



Fig. 4. Some examples of reactivity nodes.

We propose to represent the “dynamical structure” of metabolic P systems, in a way directly readable in terms of MPA algorithm, by graphs which extend the *Stoichiometric Network Analysis* formalism, SNA for short, (Clarke, 1980; Segel and Cohen, 2001) developed in the context of complex reaction networks. Namely, a *MP graph* is a structure  $G = (T, R, F, E, C)$ , where

- $T$  is the set of nodes representing types (we can think of each  $t \in T$  as a container holding a certain amount of a peculiar kind of substance). We represent such kind of nodes as big circles labelled with the type of objects contained in it, that for the sake of simplicity is depicted inside the circle (see Fig. 2).
- $R$  is the set of nodes representing biochemical reactions between types. We represent each of the nodes in  $R$  as a full bullet and we label it with the name of the reaction represented by that node (see Fig. 3).
- $F$  is the set of nodes labelled by reaction maps. We represent such kind of nodes (see Fig. 4) with rectangles. These nodes are connected with a, possibly empty, set of circles (types) but they are also connected with exactly one bullet node (simple lines are put from circles to squares while arrows are drawn from squares to bullets).
- $E$  is a set nodes presenting input or output gates. It contains two different kind of nodes: *input gates* and *output gates*. Both of them have the triangular shape depicted in Fig. 5, where input gates have an arc exit-

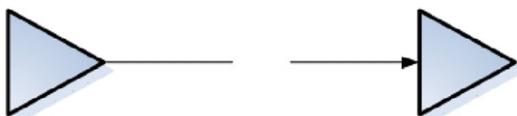


Fig. 5. An input gate (left) and an output gate (right).

ing from a triangle vertex, and output gates have an arc entering in the middle of a triangle edge.

- $C$  is a set of edges (arcs and connections) between nodes. Edges are of two different kinds: plain edges or dashed edges.
  - Plain edges* connect types to biochemical reactions, in particular they specify *reactants* and *products* of the reaction. Arcs connecting reactants to reactions are depicted as lines while arcs connecting reactions to products appear as arrows (oriented arcs).
  - Dashed edges* which connect types with square nodes (reactivity nodes) are depicted as dashed lines, while dashed edges which connect square nodes with bullets are depicted as dashed arrows (see Fig. 6). When specifying the reactivity labels, we will denote amounts of objects by small letters (or words) corresponding to the capital ones denoting species. For example, at each instant, the reactivity  $Fr$  in Fig. 6 is equal to a certain constant  $k$  multiplied by the square of the amount of elements of type  $Z$  present into the system at that instant.

Two components are easily distinguishable in MP graphs: a *stoichiometric* component and a *regulation* component. The stoichiometric component is the subgraph obtained after removing from a MP graph  $G = (T, R, F, E, C)$  the nodes  $F$  and the dashed arcs which connect them to the other nodes. This removed part is the *reaction regulation layout* of  $G$ .

Fig. 6 represents a first very basic example of network described by our formalism. Species  $X$ ,  $Y$  and  $Z$  are connected to a reaction node  $R$  by means of plain edges.

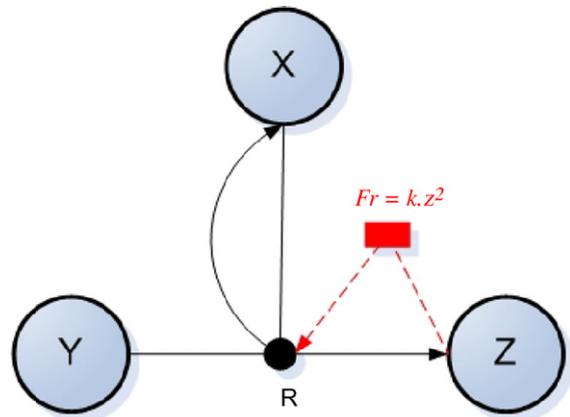


Fig. 6. A very simple MP graph with three biochemical elements  $X$ ,  $Y$ , and  $Z$ , a reaction node  $R$  and a reactivity node  $Fr$  whose reactivity is equal to  $kz^2$ , that is the square of amount of elements of type  $Z$  multiplied by a constant factor  $k$ .

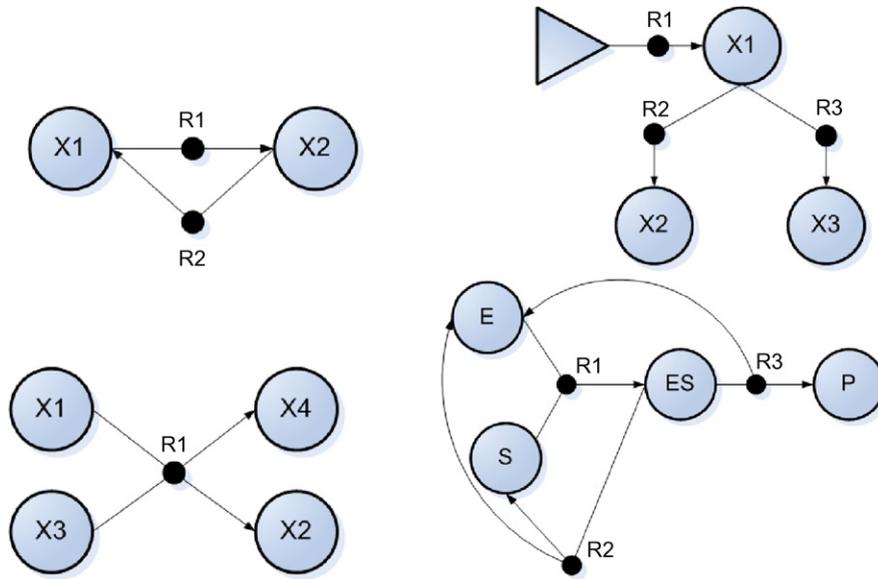


Fig. 7. Examples of stoichiometric components of P metabolic graphs.

As usual in P systems, we can represent the biochemical reaction  $R$  as the following rewriting rule:



in fact, species  $X$  and  $Y$  are connected to the reaction  $R$  by means of a line and according to the formalism they are reactants, while  $X$  and  $Z$  are connected to  $R$  by an arrow and this means that they are products of the reaction. The reaction  $R$  specified in this way is a transformation of species  $Y$  into species  $Z$  that is catalyzed by the presence of the species  $X$ . Moreover, Fig. 6 contains the specification of a reactivity relation between  $Z$  and  $R$  and the corresponding reactivity map is a quadratic polynomial on type  $Z$ . This states that, according to the model

depicted, the production of species  $Z$  has a promoting effect.

In Fig. 7 and in Fig. 8 some examples of stoichiometric components of MP graphs are shown.

The first example (Fig. 7, top left) represents the case of a reversible reaction in which species  $X 1$  is transformed into  $X 2$  by means of the reaction  $R 1$  and the converse way is performed by the reaction  $R 2$  transforming species  $X 2$  into  $X 1$ .

The second example in Fig. 7 (top right) involves the creation of a species  $X 1$  by means of a reaction  $R 1$  that has an input gate as a reactant. Then element  $X 1$  is subject to two different reactions, labelled  $R 2$  and  $R 3$ , that compete for the usage

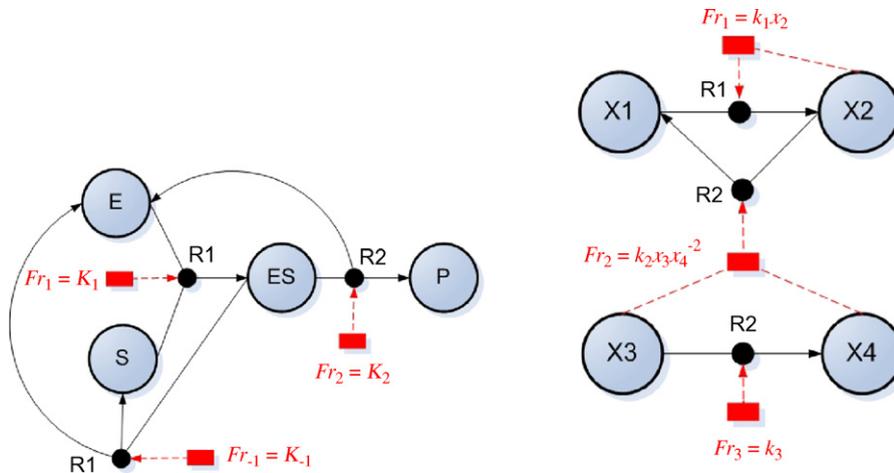


Fig. 8. Examples of metabolic P graphs.

of  $X_1$ , and which produce elements  $X_2$  and  $X_3$ , respectively.

The third example in Fig. 7(bottom left) depicts the simple case of a transformation of species  $X_1$  and  $X_3$  into another two  $X_2$  and  $X_4$ .

The last example considered in Fig. 7(bottom right) represents a well known example of an enzymatic reaction. According to the Michaelis and Menten (1913) model of enzymatic reactions (Briggs and Haldane, 1925), an enzyme  $E$  and a substrate  $S$  form a complex  $ES$  by means of a rapid and reversible reaction  $R_1$ , then the complex  $ES$  can be subject to two different behaviours: it can either split back into its components ( $E$  and  $S$ ) by means of reaction  $R_2$  (stating the reversibility of the complex formation) otherwise the translation of the substrate into the product  $P$  can take place by means of the reaction  $R_3$  that also releases the enzyme  $E$  that gets ready to start again its work.

All the examples of Fig. 7 deal with the first level of a regulated stoichiometric network. In fact, all of them lack a description of reactivities necessary to regulate the dynamics of a reaction network. On the contrary, we have the description of both levels of specification in the two examples depicted in Fig. 8. The first example (Fig. 8, left side) completes the description of an enzymatic reaction (depicted in Fig. 7) by adding reaction maps. In this simple case, according to the Michaelis–Menten formulation already discussed, each of the three rules has a constant kinetic rate, respectively  $K_1$ ,  $K_{-1}$  and  $K_2$ . Note that, to be consistent with literature and to highlight the reversibility of reaction  $R_1$  we have renamed  $R - 1$  the reaction labelled  $R_2$  in the model depicted in Fig. 7.

The second example (Fig. 8, right side) depicts the case of a reversible reaction  $R_1$  that transforms type  $X_1$  into  $X_2$ . The production of type  $X_2$  is promoted by  $X_2$  itself, as stated by the reactivity of rule  $R_1$  equal to  $k_1 x_2$ . The other way round is performed by rule  $R_2$  with a reaction map which is a function of two types not directly involved in the reaction ( $X_3$  and  $X_4$ ), and in particular, reaction  $R_2$  is promoted by the element  $X_3$  while it is inhibited, with a square power, by  $X_4$ . Finally, a transformation of  $X_3$  into  $X_4$  proceeds at a constant rate  $k_3$ .

In the next section, we are going to use all the elements discussed in the previous sections with the purpose to simulate the dynamics of a model of the mitotic oscillations as they occur in amphibian embryos.

#### 4. A Case Study: The Mitotic Oscillator in Amphibian Embryos

In this section, we apply our modelling framework to a case study of mitotic oscillator (Goldbeter, 1991, 2004).

Mitotic oscillations are a mechanism exploited by nature to regulate the onset of mitosis, that is the process of cell division aimed at producing two identical daughter cells from a single parent cell. More precisely, mitotic oscillations concern the fluctuation in the activation state of a protein produced by *cdc2* gene in fission yeasts or by homologs genes in other eukaryotes. The model here considered focuses on the simplest form of this mechanism, as it is found in early amphibian embryos. Here, the progressive accumulation of the *cyclin* protein leads to the activation of *cdc2 kinase*. This activation is achieved by a bind between cyclin and *cdc2 kinase* forming a complex known as M-phase promoting factor (or *MPF*). The complex triggers mitosis and degrades cyclin as well, the degradation of cyclin leads to the inactivation of the *cdc2 kinase* that brings the cell back to the initial conditions in which a new division cycle can take place. In yeasts and in somatic cells, the cell cycle is subject to the control of many checkpoints, but the relaying mechanism based on the activation–inactivation of *cdc2 kinase* remains the same (Alberts et al., 1997).

Goldbeter proposed a minimal structure for the mitotic oscillator in early amphibian embryos in which the two main entities are cyclin and *cdc2 kinase*. According to this model, depicted in Fig. 9, the signalling protein cyclin is produced at a constant rate  $v_i$  and it triggers the activation (by means of a dephosphorylation) of *cdc2 kinase* that passes from the inactive form labelled  $M^+$  to the active one,  $M$ . This modification is reversible and the other way round is performed by the action of another kinase (not taken into account in the model) that brings back  $M$  in its inactive form  $M^+$ . Moreover, active *cdc2 kinase* ( $M$ ) elicits the activation of a protease  $X^+$ , that when in the active (phosphorylated) form ( $X$ ) is able to

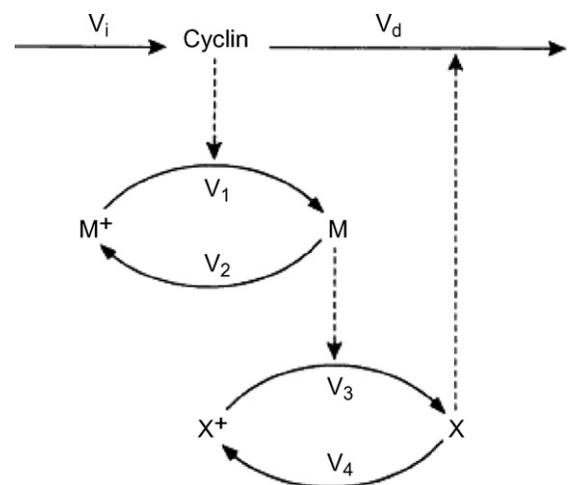


Fig. 9. The model provided by Goldbeter (1991).

degrade the cyclin. This activation, as the previous one, is reversible as stated by the arrow connecting  $X$  to  $X^+$ . The set of differential equations that are used to model the dynamics of such a system are the following:

$$\begin{aligned} \frac{dc}{dt} &= v_i - v_d \cdot x \frac{c}{K_d + c} - K_d c \\ \frac{dm}{dt} &= V_1 \frac{1 - m}{K_1 + (1 - m)} - V_2 \frac{m}{K_2 + m} \\ \frac{dx}{dt} &= V_3 \frac{1 - x}{K_3 + (1 - x)} - V_4 \frac{x}{K_4 + x} \end{aligned} \quad (1)$$

where  $V_1 = (c/K_c + c)V_{M_1}$  and  $V_2 = mV_{M_3}$ . All the details about the elements involved in this set of differential equations can be found in Goldbeter’s paper, but we recall their meaning here for the ease of reading. Symbol  $c$  denotes concentration of cyclin,  $m$  and  $x$  the fraction of active cdc2 kinase and cyclin protease respectively, thus  $(1 - m)$  and  $(1 - x)$  represent respectively the fraction of inactive cdc2 kinase and of inactive cyclin protease. Parameters  $v_i$  and  $v_d$  are the constant rate of cyclin synthesis and the maximum rate of cyclin degradation by protease  $x$  reached for  $x = 1$ ,  $K_d$  and  $K_c$  are the Michaelis constants for cyclin degradation and for the activation of the phosphatase acting on  $M$ , while  $k_d$  is the first-order rate constant of degradation of cyclin. Moreover,  $V_i$  and  $K_i$ ,  $1 \leq i \leq 4$ , represent the normalized parameters characterizing the kinetics of the four enzymes involved in the reactions dealing with cdc2 kinase and cyclin phosphatase. More precisely, for each of the four enzymes (not explicitly represented as entities of the model)  $V_i$  and  $K_i$  are the effective maximum rate and Michaelis constant divided by the total amount of relevant protein target. Finally,  $V_{M_1}$  denotes the maximum rate of the enzyme associated to the dephosphorylation of  $M^+$ , while  $V_{M_3}$  is the maximum rate of the kinase  $M$  reached for  $m = 1$ . The dynamics obtained by a numerical solution of differential Eq. (1), in Fig. 10, shows an

oscillatory behaviour in the activation of the three elements  $M$ ,  $C$ ,  $X$  that repeatedly go through a state where cell enters in a mitotic cycle.

In what follows, we show three models implementing the system described by Eq. (1). This highlights the fact that, in general, there is no unique way to translate a differential equation system into a set of rules suitable for the P metabolic algorithm. In particular, in the first two models we make two different translations of the differential Eq. (1), while in the third one we explicitly introduce four enzymes catalyzing the reactions  $M \rightarrow M^+$ ,  $M^+ \rightarrow M$ ,  $X \rightarrow X^+$  and  $X^+ \rightarrow X$ .

The first model described here is depicted in Fig. 11. It contains three cooperative rules, the first one is  $r_2$  that models the degradation of the cyclin  $C$  by means of the active phosphatase  $X$ , the second one is rule  $r_4$  and it models the activation of the cdc2 kinase ( $M^+$ ) elicited by cyclin  $C$ , while the last one is rule  $r_6$  representing the activation of the phosphatase  $X^+$  performed by means of the active cdc2 kinase  $M$ . From this representation we can easily derive the following set of rewriting rules and reaction maps:

$$\begin{aligned} r_1 : \lambda \rightarrow C & , \text{Fr}_1 = k_1 \\ r_2 : XC \rightarrow X & , \text{Fr}_2 = \frac{k_2}{k_3 + c} \\ r_3 : C \rightarrow \lambda & , \text{Fr}_3 = k_4 \\ r_4 : M^+C \rightarrow MC & , \text{Fr}_4 = \frac{k_5}{(k_6 + c)(k_7 + m^+)} \\ r_5 : M \rightarrow M^+ & , \text{Fr}_5 = \frac{k_8}{k_9 + m} \\ r_6 : X^+M \rightarrow XM & , \text{Fr}_6 = \frac{k_{10}}{k_{11} + x^+} \\ r_7 : X \rightarrow X^+ & , \text{Fr}_7 = \frac{k_{12}}{k_{13} + x} \end{aligned} \quad (2)$$

in which all the parameters are taken directly from the model proposed by Goldbeter<sup>1</sup>.

Another important aspect to highlight here is that we specify an *inertia* for each of the species considered in the model. The inertia is a resistance opposed by each species against the pressure forces pushing them to react according to the corresponding rules. This aspect is modelled in our system by introducing a rule  $Z \rightarrow Z$  for each one of the species  $Z \in \{C, X, X^+, M, M^+\}$ . We call this kind of rules *inertial rules*. These rules (an example of

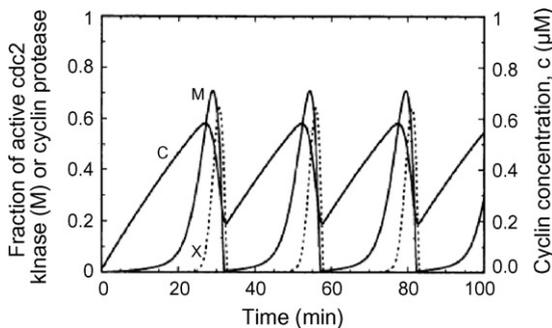


Fig. 10. A numerical solution of the set of differential Eq. (1) implementing the model provided by Goldbeter (1991).

<sup>1</sup> Here, for a easier reading, the notation is slightly different from that of Goldbeter. The correspondence with Goldbeter’s model is the following:  $k_1 = v_i$ ,  $k_2 = v_d$ ,  $k_3 = K_d$ ,  $k_4 = k_d$ ,  $k_5 = V_{M_1}$ ,  $k_6 = K_c$ ,  $k_7 = K_1$ ,  $k_8 = V_2$ ,  $k_9 = K_2$ ,  $k_{10} = V_{M_3}$ ,  $k_{11} = K_3$ ,  $k_{12} = V_4$  and  $k_{13} = K_4$ .

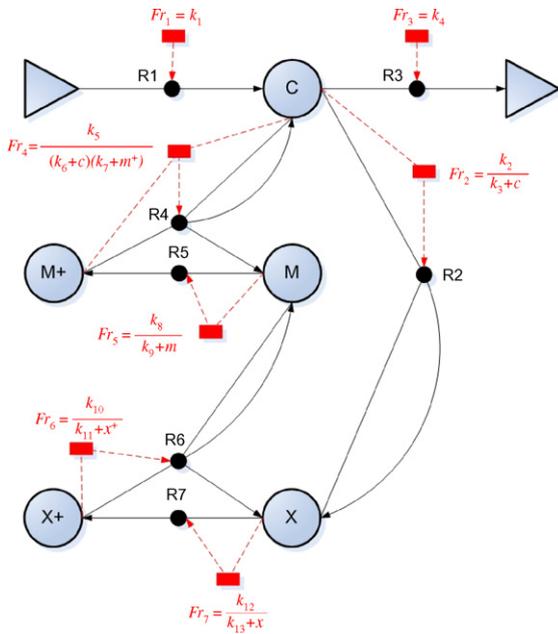


Fig. 11. The first model of the mitotic oscillator represented by an MP graph.

which is depicted in Fig. 12) correspond, in MP graphs, to loop edges.

The dynamics obtained by the simulation of this first model of the mitotic oscillator by using MPA is depicted in Fig. 13. Hereafter, quantities are expressed in terms of unspecified population units (conventional moles) and reactivities provide, for any state, pure numbers specifying the relative strength of rules. What is important is only the relationship between rule strengths. For this reason, we could normalize the reactivities, with respect to a chosen constant  $k$ , by multiplying them by a factor  $1/k$ .

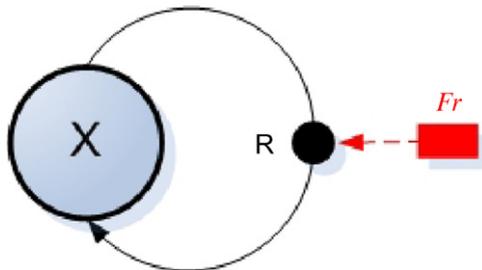


Fig. 12. An example of inertial rule associated to species X, the reactivity of the rule is a constant value, but this is not mandatory. In all examples discussed in this paper reactivities of inertial rules will be constant values.

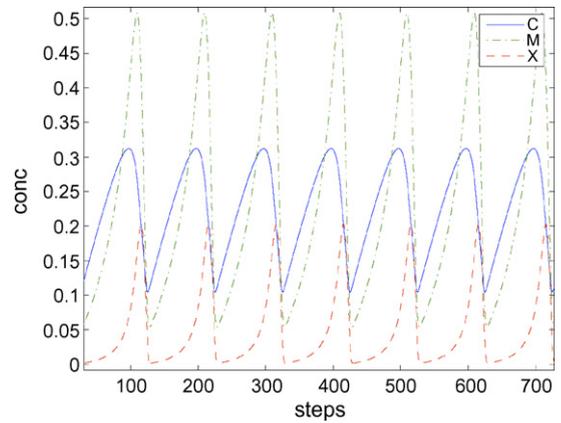


Fig. 13. MPA evolution of the model described by Fig. 8. All the parameters and initial concentrations are derived from Goldbeter (1991) and are the following:  $k_1 = 0.025/6.4$ ,  $k_2 = 0.25$ ,  $k_3 = 0.02$ ,  $k_4 = 0.01$ ,  $k_5 = 3$ ,  $k_6 = 0.5$ ,  $k_7 = 0.005$ ,  $k_8 = 1.5$ ,  $k_9 = 0.005$ ,  $k_{10} = 1$ ,  $k_{11} = 0.005$ ,  $k_{12} = 0.5$  and  $k_{13} = 0.005$ . Initial quantities are 0.01 for species C, M and X, while 0.99 for species  $M^+$  and  $X^+$ . Moreover, reactivities of inertial rules are all constant and fixed to 6.4.

If we “read” in a different way the set differential Eq. (1) we obtain the second model of the mitotic oscillator as represented in Fig. 14. In this model, there is no rule involving more than a single species, in fact, C in R 4 and M in R 6 were removed as “catalyst” in the rules and were introduced as arguments in the corresponding reaction maps. The following is the complete

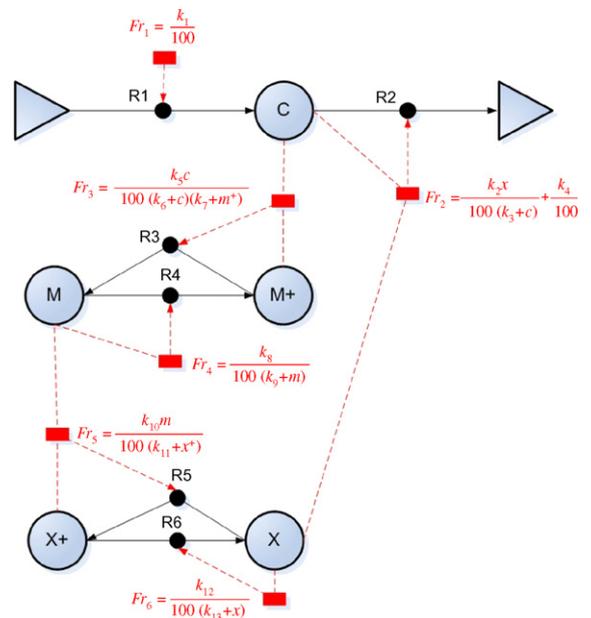


Fig. 14. The second model of the mitotic oscillator represented by an MP graph.

list of rules and reaction maps. The graphical representation leads directly to the following set of rules and reaction maps:

$$\begin{aligned}
 r_1 : \lambda &\rightarrow C & , \quad Fr_1 &= \frac{k_1}{100} \\
 r_2 : C &\rightarrow \lambda & , \quad Fr_2 &= \frac{k_2 x}{100(k_3 + c)} + \frac{k_4}{100} \\
 r_3 : M^+ &\rightarrow M & , \quad Fr_3 &= \frac{k_5 c}{100(k_6 + c)(k_7 + m^+)} \\
 r_4 : M &\rightarrow M^+ & , \quad Fr_4 &= \frac{k_8}{100(k_9 + m)} \\
 r_5 : X^+ &\rightarrow X & , \quad Fr_5 &= \frac{k_{10} m}{100(k_{11} + x^+)} \\
 r_6 : X &\rightarrow X^+ & , \quad Fr_6 &= \frac{k_{12}}{100(k_{13} + x)}
 \end{aligned}
 \tag{3}$$

that can be used to simulate the second model of the mitotic oscillator, producing the dynamics depicted in Fig. 15.

The last model of the mitotic oscillator here proposed has a different shape. Now we take into consideration the set of enzymes  $E_1, E_2, E_3,$  and  $E_4$  that catalyze the reactions involving species  $M, M^+, X,$  and  $X^+$ . All of these four reactions are cooperative and the constant rates used in the calculation of the system's dynamics are the effective maximum rates of the four enzymes divided by the total amount of protein target.

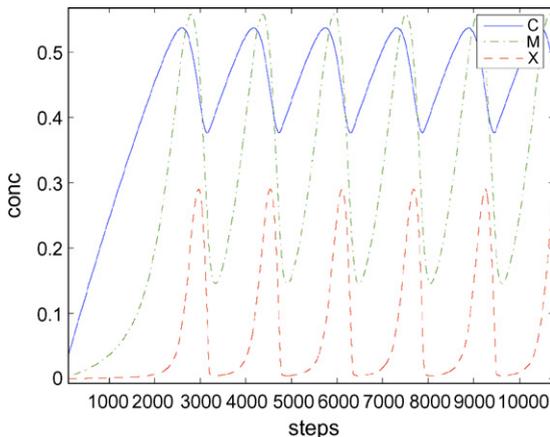


Fig. 15. MPA evolution of the second model, described by Fig. 14. All the parameters and initial concentrations are taken directly from Goldbeter (1991) and are here the following:  $k_1 = 0.025, k_2 = 0.25, k_3 = 0.02, k_4 = 0.01, k_5 = 3, k_6 = 0.5, k_7 = 0.005, k_8 = 1.5, k_9 = 0.005, k_{10} = 1, k_{11} = 0.005, k_{12} = 0.5$  and  $k_{13} = 0.005$ , initial quantities are 0.01 for species  $C, M$  and  $X$ , while 0.99 for species  $M^+$  and  $X^+$ . Moreover, reactivities of inertial rules are all constant and fixed to 1.

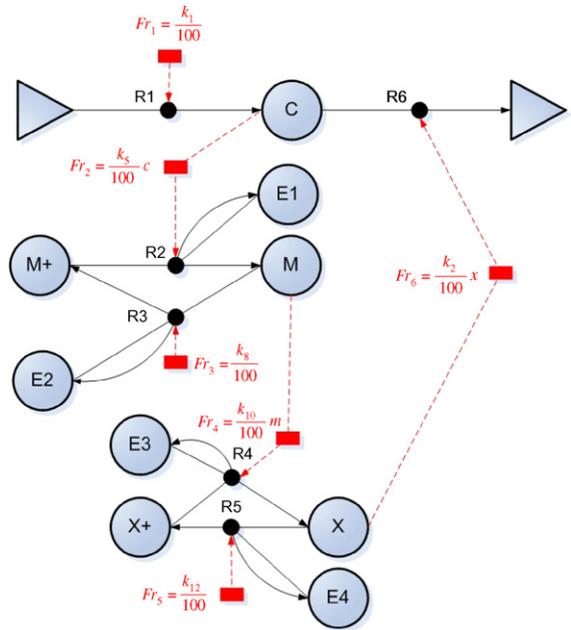


Fig. 16. The third model of the mitotic oscillator represented by an MP graph.

The graphical representation of the model is depicted in Fig. 16 while the set of rules and reaction maps are the following:

$$\begin{aligned}
 r_1 : \lambda &\rightarrow C & , \quad Fr_1 &= \frac{k_1}{100} \\
 r_2 : E_1 M^+ &\rightarrow E_1 M & , \quad Fr_2 &= \frac{k_5 c}{100} \\
 r_3 : E_2 M &\rightarrow E_2 M^+ & , \quad Fr_3 &= \frac{k_8}{100} \\
 r_4 : E_3 X^+ &\rightarrow E_3 X & , \quad Fr_4 &= \frac{k_{10} m}{100} \\
 r_5 : E_4 X &\rightarrow E_4 X^+ & , \quad Fr_5 &= \frac{k_{12}}{100} \\
 r_6 : C &\rightarrow \lambda & , \quad Fr_6 &= \frac{k_2 x}{100}
 \end{aligned}
 \tag{4}$$

The dynamics of this last model oscillates as well as in the case of the two previous models and it is represented in Fig. 17. The important thing to consider here is that this last model is very simple and it is directly obtainable by the description of the phenomenon, but the oscillatory behaviour is reproduced quite well too. This final model highlights the capabilities of our formalism to represent all the information needed by the metabolic algorithm for calculating the dynamics of a biological network.

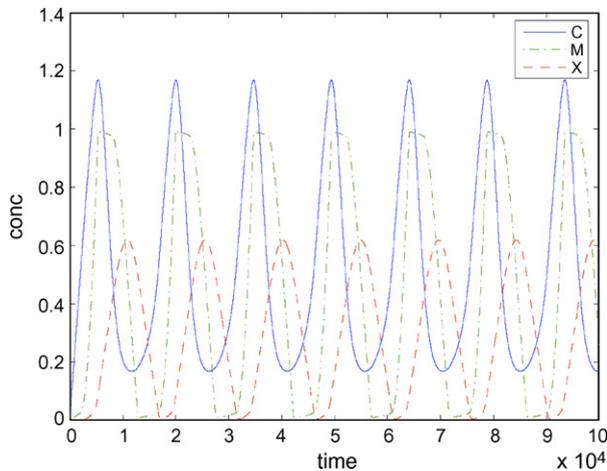


Fig. 17. MPA evolution of the third model, described by Fig. 16. All the parameters and initial concentrations are taken directly from Goldbeter (1991) and are here the following:  $k_1 = 0.025$ ,  $k_2 = 0.25$ ,  $k_5 = 3$ ,  $k_8 = 1.5$ ,  $k_{10} = 1$  and  $k_{12} = 0.5$ , initial quantities are 0.1 for species C, M and X, while 0.99 for species  $M^+$  and  $X^+$  and 0.5 for  $E_1$ ,  $E_2$ ,  $E_3$  and  $E_4$ . Reactivities of inertial rules of enzymes  $E_1, \dots, E_4$  are fixed to 20 while they are chosen equal to 1 for all other species.

## 5. Conclusions

In this paper, we continue our investigation regarding the application of P systems to biological phenomena. A new version of Metabolic P Algorithm (MPA) is presented which is strictly related to the graphical formalism of MP graphs, here introduced for providing natural descriptions of biochemical systems. Interestingly enough, MP graphs can also be seen as particular *neuron-like* membrane systems, according to Păun (2002) and Ciobanu et al. (2006) terminology. The direct application of MPA to the mitotic oscillator in amphibian embryos reveals its adequacy in the biological modelling. In fact, three formulations of this biological network have been developed here. The first two are directly obtained from Goldbeter's differential equations representation. The last one is entirely deduced by translating the biological description of the phenomenon in our diagrams. It is worthwhile observing that in this third case an analogous oscillatory pattern is obtained, but its derivation is conceptually simpler because directly driven by our formalism and avoiding many details on the kinetic aspects of the reactions.

This work suggested some important questions which have been addressed in subsequent papers: how is it possible to compare and evaluate the translations of the same differential model into different MP graphs? What is the

role of inertial rules and their interpretation in differential terms? (see Fontana and Manca, 2007). And finally, another fundamental issue, for a wider application of MP graphs in the modelling of biological dynamics, is the definition of general methods for evaluating the values of parameters occurring in reaction maps (see Manca, in press).

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