Modelling of the TH-dependent regulation of tadpole tail resorption

S. Troncale,[1,*] R. Thuret,[2] A.-C. Fierro,[2] C. Ben,[2] N. Pollet,[2] J.-P. Comet[1, 3] and G. Bernot[3]

[1]*IBISC, FRE 2873, Université d'Evry Val-d'Essonne, Génopole, France*
[2]*Laboratoire de Développement et Evolution, UMR 8080, Université de Paris Sud, France*
[3]*Programme d'Epigénomique, Génopole et Université d'Evry, France*

Tail resorption observed at the time of amphibian metamorphosis is controlled by the thyroid hormone (TH). The inherent regulation network is complex and involves an important number of different factors. Consequently, global understanding of this biological process needs elaborate experiments. However, these experiments may be difficult to realize because of the need to manipulate in space and in time gene expression and hormonal treatments. Hence, we first modelled and simulated the biological process using Hybrid Functional Petri Nets. This powerful formalism offers a number of useful features and flexibility. Curves obtained *in silico* by simulations are in agreement with those observed *in vivo* and *in vitro*. Our modelling approach led us to ask pertinent biological questions, from which new hypotheses for experimental testing have emerged.

Keywords: Hybrid Functional Petri Nets, metamorphosis, modelling, tail resorption, thyroid hormone

1. INTRODUCTION

Most amphibians undergo numerous morphological changes at the tadpole stage, a biological process called metamorphosis. Amphibian metamorphosis can be divided into three periods. During premetamorphosis the feeding tadpole grows. During prometamorphosis hind limbs grow and differentiate. Finally, tail resorption characterizes metamorphic climax. All these modifications are under the control of the thyroid hormone [1].

Among all the changes related to metamorphosis, we were particularly interested in the regulatory mechanisms responsible for tail resorption [2]. Indeed, apoptotic mechanisms are triggered [1], and a better understanding of the regulation inducing apoptosis could have important therapeutic consequences. However, experimental analysis of the actually occurring regulations is difficult. Indeed, the relevant molecules (hormones, proteins) are difficult to observe and manipulate in a spatio-temporal manner in the context of metamorphosis.

Therefore, the experimental study of this process is hindered. As a first step, modelling is much more flexible. *In silico* tests of biological hypotheses are then made much easier. In this paper, we construct a model representing the kinetics of different factors that interfere in tail resorption of the tadpole *Xenopus tropicalis*. A formalism offering a maximum of flexibility is required to study this complex biological process, which implicates a large number of interacting factors. Accordingly, we chose Hybrid Functional Petri Nets (HFPN) [3, 4], a formalism allowing discrete and continuous processes, and consumption and production of resources, and relations between factors.

Elaboration of the model to be considered was enabled thanks to close collaboration between biologists and modelling specialists. The interaction productively encouraged the modellers, the asking of pertinent questions from a biological system point of view, and the formulation of hypotheses on the rôle of some cells as a function of their location with respect to plasmatic flow. The kinetics obtained from the simulations are in agreement with such experimental data as is available in the literature.

This paper is organized as follows: the biological context is first presented in section 2, and the HFPN formalism is described in section 3. Construction of our model is detailed in section 4 and our simulation results are exposed in section 5. Section 6 presents an *in silico* test of enzymatic overexpression. Finally, we discuss these results as well as the contribution of inter-disciplinarity in the study of biological complex systems.

2. BIOLOGICAL CONTEXT

The whole development of the *Xenopus* frog, starting from the zygote (fertilized egg) and ending at the adult form, has been described as a succession of discrete stages classified using anatomical traits (Nieuwkoop and Faber [5]; Figure 1 extracted from [6]). Metamorphosis encompasses the period covering the stages 54 to 66 (the last stage of development). During metamorphosis, the *Xenopus tropicalis* tadpole undergoes a series of morphological changes. All metamorphosis-associated morphological changes are under the control of thyroid

*Corresponding author. E-mail: sylvie.troncale@ibisc.fr

Journal of Biological Physics and Chemistry **7** (2007) 45–50
Received 4 December 2006; *accepted* 28 May 2007

hormones, denoted TH. It is relevant to distinguish two molecular forms of TH [2]. Thyroxine (tetraiodothyronine, T4) corresponds to the major form secreted by the thyroid gland; it is an "inactive" form of TH and is considered a prohormone. Triïodothyronine (T3) is the biologically active form but it is secreted in a smaller quantity [7]. Complex regulations in which signals emitted by many organs, such as pituitary gland or liver, intervene regulate the synthesis of thyroid hormones. Newly produced TH is secreted in the plasma and supplies organs. From this regulation a strong increase of plasmatic TH concentration during prometamorphosis results, up to a peak at the climax of metamorphosis (stages 62–63) [6] (Figure 1).

The TH-dependant regulation at the origin of tail resorption requires a high intracellular TH concentration [1]. This regulation is triggered at the climax of metamorphosis when plasmatic TH concentration is maximal. Intracellular TH binds its nuclear receptors existing in two forms: TR-α and TR-β [8, 1, 9]. The TH/TR complex bound to DNA recruits a co-activator complex that activates expression of downstream genes, ultimately resulting in programmed cell death. On the one hand, TR-α is observed ubiquitously in the tail (Figure 1), it binds its co-factor RXR-α and will not be considered in our model because of its ubiquitous character. On the other hand, TR-β is a direct-response gene of TH [1]: its expression increases with endogenous TH concentration (Figure 1).

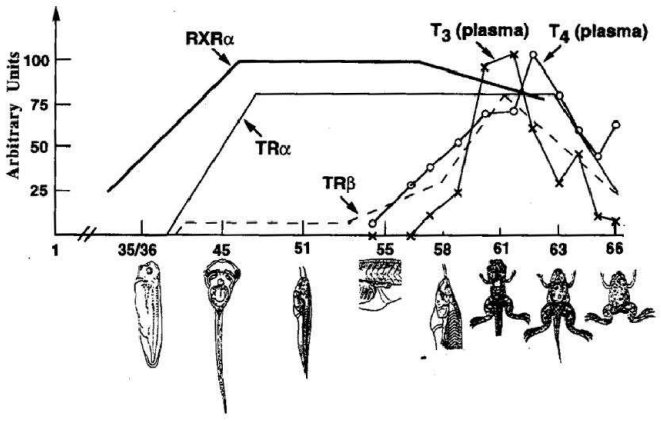

Figure 1. Correlations between levels of endogenous TH and mRNA, of TR-α, TR-β and RXR-α genes during the different development stages. The abscissa is marked with the stage number. Figure extracted from [6].

The intracellular TH concentration depends on the plasmatic contribution, but it is also regulated by two enzymes, type 2 deiodinase, D2 [10] and type 3 deiodinase D3 [11]. Type 2 deiodinase synthesizes the active form of the hormone (T3) from its inactive form (T4): D2 + T4 → T3. On the contrary, type 3 deiodinase inactivates the two hormone types T3 and T4: D3 + T4 → *r*T3 and D3 + T3 → T2, where *r*T3 and T2 are inactive. The gene expressing D3 is a direct-response gene of TH [11]. D3 concentration increases with that of T3, and D3 concentration is then down-regulated at the climax of metamorphosis (stages 60–61) [10]. Expression of the gene encoding D2 is also regulated by the thyroid hormone, but in an indirect manner. D2 concentration then increases with that of T3, but response time is much longer (a few days) [10]. The concentration of this enzyme reaches a peak at the climax of metamorphosis (stages 62–63) [12], and decreases when the tail degenerates.

3. HFPN FORMALISM

Modelling of tail resorption in the *X. tropicalis* tadpole must integrate notions of consumption and production of resources in a way commensurate with the quantitative observation of the evolution of intracellular TH concentration. Moreover, two kinds of biological processes can be distinguished: continuous processes, such as enzymatic activity defined by the Michaelis constant (K_M) and by the maximal rate, and discrete processes matching here essentially biological processes activated after a time delay. We then considered hybrid modelling allowing both discrete and continuous processes. Hybrid functional Petri nets (HFPN) [3, 4] represent an adequate formalism for hybrid models. The functional aspect of these nets is related to the possibility to define consumed or produced quantities as well as reaction rates using functions depending on other factors in the net. For example, if we consider the rate of a continuous transition, this can be expressed as a function of marking of one or some places. The incidence condition between the transition and a place is not necessary to express the rate dependence as a function of marking of a place. Then HFPN enable a large freedom in the interaction description, conserving the usual representation of Petri nets [13]. They are composed of places, transitions and arcs, and are either discrete or continuous (Figure 2):

• Discrete places represent entities that can be numbered (thanks to tokens).

• Continuous places represent entities that cannot be numbered. A real number (representing a concentration, for example) is associated with each continuous place.

• Discrete transitions are fired after a delay of time dt.

• Continuous transitions are continuously fired at a rate $v(t)$.

• Normal arcs activate transitions by consuming resources.

• Inhibitory arcs activate transitions only when a resource is absent.

• Test arcs activate a transition without consumption of resources.

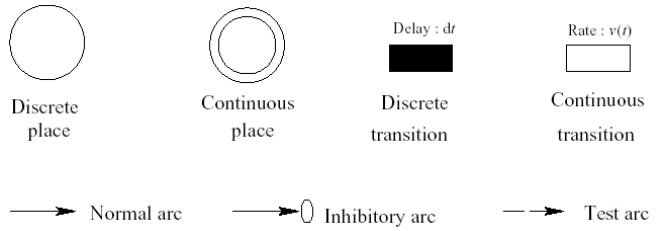

Figure 2. Representation of the different HFPN components.

4. MODEL CONSTRUCTION

Since our goal is to study causalities leading to tail resorption from plasmatic TH concentration, plasmatic TH concentration curves are then considered as result of regulations upstream of the studied process.

The model presented below generates T3p and T4p concentration curves in agreement with those observed in Figure 1 (see subsection 4.1, where the function describing hormone evolution is constructed to obtain such curves). These kinetics stand for input data in our model. The model also integrates regulations related to intracellular TH, D2, D3, TR-β,.... Its elaboration is addressed through the construction of submodules. We begin with the rôle of plasmatic TH.

4.1 TH plasmatic modelling

The first step in our model construction was to calculate plasmatic TH concentration related to those observed on Figure 1. These curves are used as the model base. Plasmatic T4 and T3 concentrations are modelled thanks to two continuous places, called T4p and T3p in the final model (Figure 3). The continuous transition t_1 represents continuous synthesis of thyroid hormone by the thyroid gland. When T3p and T4p respectively reach their peak, the transitions t_2 and t_3 provide a continuous token to places $p1$ and $p2$. This signal models metamorphic climax, and plasmatic TH concentration can then decrease.

The resulting sub-model only uses one place and one transition for each hormone. For example, the modelling of plasmatic T4 (T4p) only needs t_2 and $p1$. This modelling was made easier thanks to the use of functions on arcs and transitions. For example, the arc from the place T4p to the transition t_2 possesses a function explained by: *if the climax of metamorphosis is still not reached, then plasmatic T4 concentration linearly increases, or else this concentration linearly declines until a basal value.* The use of functions allows complex reactions with few places and transitions to be modelled.

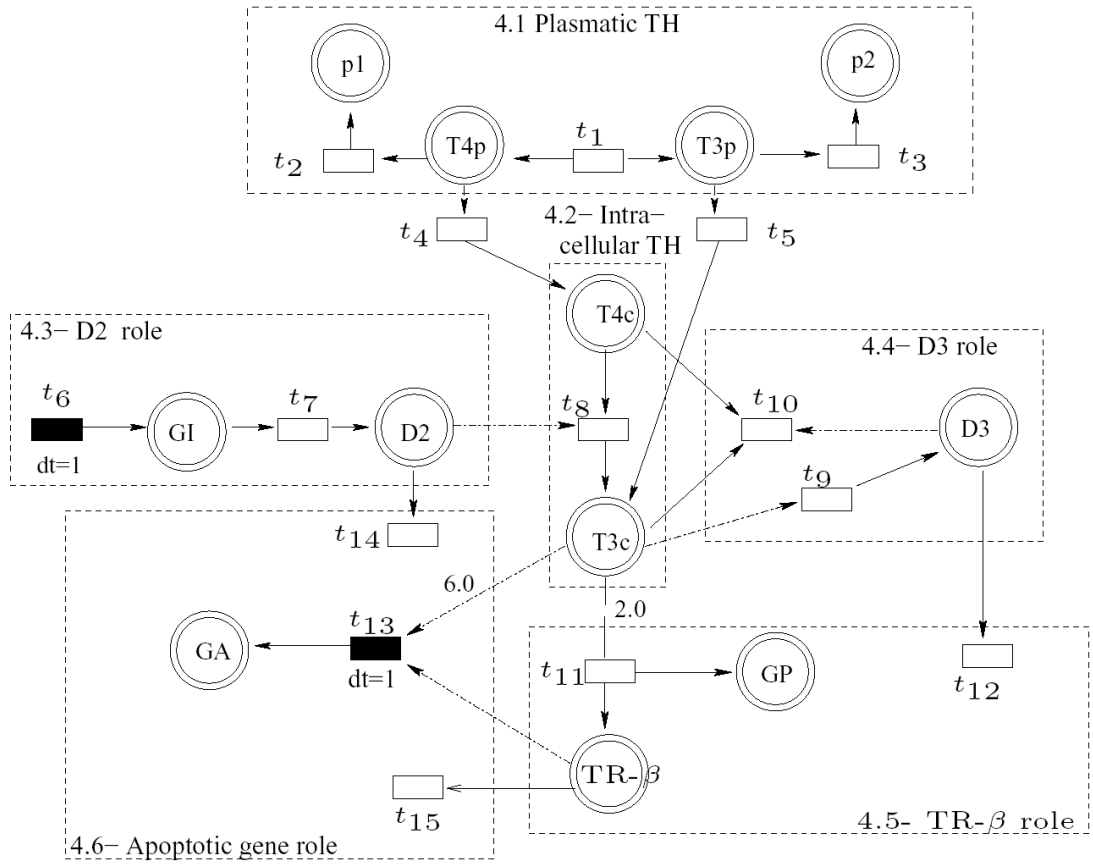

Figure 3. Final model representing interactions between plasmatic TH (T4p and T3p), intra-cellular TH (T4c and T3c), deiodinases of type 2 and type 3 (D2 and D3) and the TH receptor TR-β. Intermediate genes (GI), early genes (GP) and apoptotic late genes (GA) are also represented.

4.2 Intracellular TH modelling

A proportion of plasmatic TH passes into tail cells and contributes to the increase of intracellular TH concentration. The plasmatic T4 crossing is modelled by the continuous transition t_4 (Figure 3) which supplies the cell with intracellular T4 (continuous place T4c). The same process is used for intracellular T3 (continuous transition t_5 and continuous place T3c).

4.3 D2 rôle modelling

When the intracellular T3 concentration reaches a threshold, T3 activates intermediate genes [12], which then induce D2 expression, initially absent in the tadpole tail [10]. Consequently, a time delay exists between the threshold reached by T3c and the observation of D2 in the tail. This time delay is modelled by the discrete transition t_6 (Figure 3). Once the time is elapsed, intermediate genes (continuous place GI) cause D2 concentration increase (continuous place D2). The active form of thyroid hormone (T3c) is synthesized by D2 from the inactive form (T4c) (continuous transition t_8). Since D2 is not consumed at the moment of reaction, a test arc is used.

4.4 D3 rôle modelling

Contrarily to D2, D3 is initially present in the tadpole tail (continuous place D3) [11]. D3 concentration is directly regulated by the intracellular T3 (T3c). This activation is modelled by a continuous transition t_9 (Figure 3). Since T3c concentration does not decrease at the moment of this regulation, a test arc is used. Type 3 deiodinase inactivates the two kinds of hormone, T3 and T4. This inhibition is modelled by the continuous transition t_{10}, which diminishes T3c and T4c concentration when it is fired. Functions were also integrated so that the enzymatic reaction is proportional to the resource quantity (D3 and TH).

4.5 TR-β rôle modelling

One of the thyroid hormone receptors, TR-α, is ubiquitously present in tail cells, while TR-β is a direct-response gene of TH [1, 8]. One hypothesis considers TR-α as a transcription factor of early genes and TR-β as a transcription factor of late genes leading to apoptosis [1]. Due to the ubiquitous character of TR-α, this is not explicitly represented in our model. When T3c reaches a threshold, it activates early gene transcription, among which we distinguish TR-β. This continuous process is modelled by the transition t_{11} (Figure 3), which fills continuous places GP and TR-β, respectively associated with early genes and TR-β. This transition is only fired when the threshold indicated on the arc is reached. Finally, it is strongly probable that one of the early genes is responsible for D3 inhibition [11]. When early genes are activated (place GP is filled), the continuous transition t_{12} is activated in turn, decreasing D3 concentration.

4.6 Apoptosis activation modelling

Once TR-β is activated, it forms a complex with T3c. The transcription factor formed enables the induction of late apoptotic genes (place GA on Figure 3). This reaction can only occur when a high T3c concentration is present in the cell during a certain delay [1]. This delay is modelled by the discrete transition t_{13}. The threshold enabling activation of this transition is more important than the threshold inducing early genes previously cited.

Finally, among apoptotic genes there are proteases that damage still-present proteins, that is to say D2 and TR-β. This process is modelled by transitions t_{14} and t_{15}.

5. RESULTS

The *Cell Illustrator* software [3, 4], available at the web site http://www.GenomicObject.Net/, implements HFPN formalism and allows us to test our model through a run of simulations. We observed concentration evolution of the following elements as a function of time: plasmatic T3 and T4 (T3p and T4p), intracellular T3 and T4 (T3c and T4c), D2, D3, TR-β and the expression of the apoptotic gene (GA). Results are presented in Figure 4.

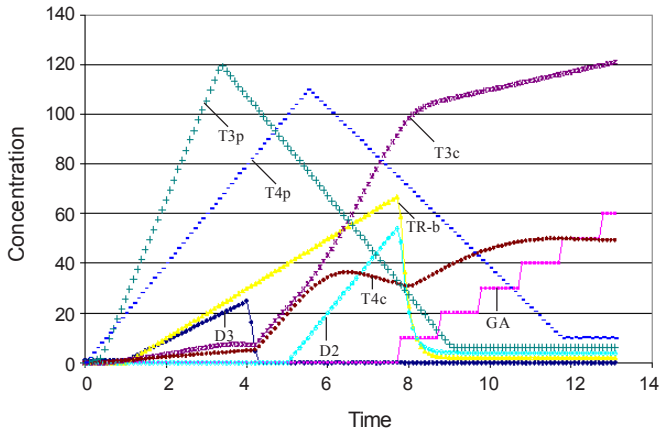

Figure 4. Concentration evolution of the different factors (simulations made by *Cell Illustrator*).

From a qualitative viewpoint, curves observed *in silico* are in agreement with the kinetics (expressed in arbitrary units) observed *in vivo* and *in vitro* [6, 1, 10, 11, 12]. As a matter of course, T3p and T4p curves (piecewise linear) abstract the behaviour resulting from upstream regulations (see subsection 4.1). While D3 is present in the cell, intracellular TH concentration stays

low. It then increases when type 3 deiodinase is down-regulated. *In vivo*, D3 inhibition marks D2 activation. This activation is observed *in silico* at time 5.0 of our simulation. D2's presence enables a concentration of intracellular T3, higher than the intracellular T4 concentration, to be obtained very quickly.

The induction of genes expressing TR-β and D3 is similar. Nevertheless, contrarily to D3, TR-β is only down-regulated when the tail degenerates (time 8.0 on Figure 4). Finally, when the intracellular T3 concentration reaches a threshold, the transcription factor T3/TR-β enables induction of apoptotic gene expression. Apoptosis triggering is observed with an increase of the apoptotic gene concentration. It should be noted that the steps in curve GA are not biologically significant: the rôle of this curve is similar to that of a toggle switch, it indicates whether apoptosis is triggered.

6. D3 OVEREXPRESSION

So as to validate our model informally, a test already performed *in vivo* was tested *in silico*. A tadpole overexpressing deiodinase of type 3 was simulated. *In vivo* these tadpoles were obtained by the usual protocols of transgenesis [11]. A consequence of such an experiment is the ubiquitous expression of D3 in the tail cells, D3 concentration is then high during the entire metamorphosis. *In silico*, this process was modelled by removing transitions responsible for D3 regulation. Then transitions t_9 and t_{12} were removed from the final model (Figure 3) and the initial value of D3 marking was also increased in order to model overexpression. Simulations led to the results presented in Figure 5.

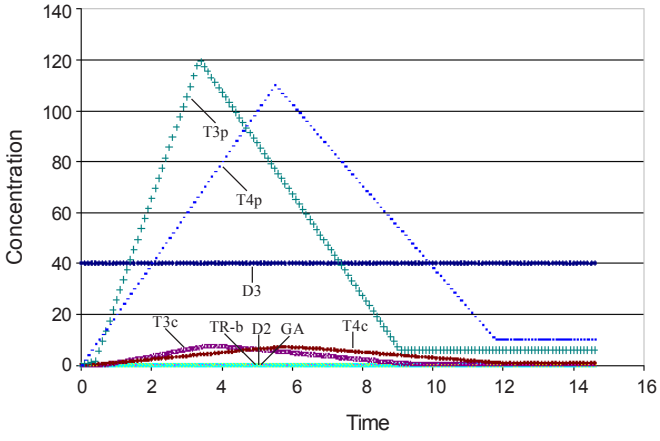

Figure 5. Concentration evolution of the different factors when D3 is overexpressed (simulations made by *Cell Illustrator*).

T3p and T4p curves are unchanged compared with those shown previously. T3c and T4c concentrations stay low all along the metamorphosis. Curves show that T3c and T4c do not reach threshold leading to expression of D2 or TR-β. Consequently, apoptotic genes are never induced. The tail death program is then not set in motion. We can conclude that the tadpole modelled *in silico* keeps its tail despite metamorphosis.

Such *in vivo* experiments are hard to perform. Indeed, most of the animals do not survive. A single tadpole reached the adult age and this animal kept its tadpole tail [11].

7. DISCUSSION

In this paper, a model simulating the TH-dependant regulation responsible for tail resorption in the *Xenopus tropicalis* tadpole has been developed. The model obtained provides results in agreement with those reported in the literature. Modelling of this biological process required the use of a flexible formalism. We concentrated on the HFPN formalism, which enables modelling of discrete and continuous processes as well as the modelling of consumption and production of resources. This formalism has the distinctiveness to facilitate modelling of complex regulations thanks to the use of functions on arcs and transitions. Despite the expressiveness of the HFPN formalism, it would be interesting to compare and precisely evaluate contributions and limits of the HFPN formalism with respect to the other formalisms used in biology.

The main goal of modelling is to lead to better understanding of the biological system. In our case, the model was constructed with the goal of elucidating the details of our knowledge about the specific functionalities of the thyroid hormone in the tail. The initial question refers to the nature of TH: can we talk about a morphogen for the thyroid hormone? A morphogen is a secreted molecule, which has the property of inducing different cellular types with different concentrations. Once the model was constructed, *in silico* tests led us towards an in-depth study of the rôle and activities of type 2 and 3 deiodinases. The kinetics data (K_M and V_{max}) given by Germain et al. [14] show that D2 is 10 times less active than D3. Moreover, since plasma supplies single-handedly a high T3 concentration, we question the matter of the D2 rôle. Intermediate models were then developed to simulate competition between D2 and D3. We first suggested that our regulation network presented in this paper was mutual to each tail cell. Nevertheless, simulation results contradicted this hypothesis and new questions were then asked: which cells express D2 and D3 in the tadpole tail? do some cells express D2 and do the others express D3? A bibliographical study concluded that deiodinases are exclusively expressed in a minority group of tail cells [10, 9]. Cells expressing these enzymes are surprisingly those that are the most vascularized, and

consequently those receiving a maximum of plasmatic TH. At the opposite extreme, cells that are the least vascularized do not express D2.

We can then ask how these cells undergo apoptosis. Two hypotheses are conceivable:

• Cells expressing D2 could be used as a spatial relay towards cells that do not express the enzyme. The most vascularized cells would accumulate a high T3 concentration, which would then diffuse towards cells lacking this hormone. This spatial relay would also be temporal because it would allow the maintainance of a high T3 concentration in cells expressing D2, in spite of the decline observed in the plasma, which does not maintain the regular T3 supply.

• The second hypothesis considers two types of death in the tail. Cells expressing D2 would die by apoptosis, while the death of the other cells would be the consequence of apoptosis in the first cell class. Some papers already identified several resorption programs [15, 9], but none correlates it with D2 expression.

These two hypotheses are not in contradiction and could coexist in the same regulation system.

Currently, we work on elaboration of experimental validation methods enabling the confirmation or the invalidation of these hypotheses.

This model fits only the first step in the understanding of the biological system studied. This first step was performed using numerical values of the different parameters that are consistent with published knowledge. In the future we envisage working on *in silico* parameter estimation (range of consistent parameters, stability, robustness, etc.). We also envisage adding new factors, such as corticosteroids, which enable D3 inhibition and D2 activation. This model enrichment will further enhance the possibilities of connecting our specific model to relevant biological processes.

REFERENCES

1. Z. Wang and D. Brown. Thyroid hormone-induced gene expression program for amphibian tail resorption. *J. Biol. Chem.* **268** (1993) 16270–16278.
2. C. Rose. Integrating ecology and developmental biology to explain the timing of frog metamorphosis. *Trends Ecol. Evolution* **20** (2005) 129–135.
3. H. Matsuno, Y. Tanaka, H. Aoshima, A. Doi, M. Matsui and S. Miyano. Biopathways representation and simulation on hybrid functional Petri nets. *In Silico Biol.* **3** (2003) 389–404.
4. A. Doi, S. Fujita, H. Matsuno, M. Nagasaki and S. Miyano. Constructing biological pathway models with hybrid functional Petri nets. *In Silico Biol.* **4** (2004) 271–291.
5. P.D. Nieuwkoop and J. Faber, *Normal Table of Xenopus laevis (Daudin)*. Amsterdam: North-Holland (1956).
6. J. Leloup and M. Buscaglia. Triiodothyronine, hormone of amphibian metamorphosis. *C. R. Acad. Sci.* **284** (1977) 2261–2263.
7. J.D. Furlow and E.S. Neff. A developmental switch induced by thyroid hormone: *Xenopus laevis* metamorphosis. *Trends Endocrinol. Metabolism* **17** (2006) 40–47.
8. A. Kawahara, B. Baker and J.-R. Tata. Developmental and regional expression of thyroid hormone receptor genes during *Xenopus* metamorphosis. *Development* **112** (1991) 933–943 .
9. D. Berry, R. Schwartzman and D. Brown. The expression pattern of thyroid hormone response genes in the tadpole tail identifies multiple resorption programs. *Developmental Biol.* **203** (1998) 12–23.
10. L. Cai and D. Brown. Expression of type 2 iodothyronine deiodinase marks the time that a tissue responds to thyroid hormone-induced metamorphosis in *Xenopus laevis*. *Developmental Biol.* **266** (2003) 87–95.
11. H. Huang, N. Marsh-Armstrong and D. Brown. Metamorphosis is inhibited in transgenic *Xenopus laevis* tadpoles that overexpress type 3 deiodinase. *Proc. Natl Acad. Sci. USA* **96** (1999) 962–967.
12. H. Huang, L. Cai, B. Remo and D. Brown. Timing of metamorphosis and the onset of the negative feedback loop between the thyroid gland and the pituitary is controlled by type 2 iodothyronine deiodinase in *Xenopus laevis*. *Proc. Natl Acad. Sci. USA* **98** (2001) 7348–7353.
13. R. David and H. Alla. *Discrete, Continuous and Hybrid Petri Nets*. Heidelberg: Springer (2005).
14. D. Germain, R. Schwartzman, W. Croteau, A. Kanamori, Z. Wang, D. Brown and V. Galton. A thyroid hormone-regulated gene in *Xenopus laevis* encodes a type III iodothyronine 5-deiodinase. *Proc. Natl Acad. Sci. USA* **91** (1994) 7767–7771 .
15. R. Elinson, B. Remo and D. Brown. Novel structural elements identified during tail resorption in *Xenopus laevis* metamorphosis: Lessons from tailed frogs. *Developmental Biol.* **215** (1999) 243–252.