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Modeling the Dynamics of the *XlnR* Regulon Network in *Aspergillus niger*

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Abstract In this paper the dynamics of the transcription-translation system for *XlnR* regulon in *Aspergillus niger* is modeled. The simulations are based on Hill regulation functions and ordinary differential equations. The response to a single trigger of D-xylose to the system is considered, stability analysis is performed and the effects of activating and repressive feedback are also considered. Simulation and systems analysis showed significant influence on metabolite expressions, the effect of the combined activating and repressing feedback was significant on influencing the expression outputs. The responses for genes and proteins can be understood through modeling system dynamics like we have shown.

Keywords Perturbation; *Aspergillus niger*; *XlnR* regulon; dynamic response; stability; feedback

1 Introduction

The filamentous fungus *A. niger* is a main organism in the production of enzymes and precursors for the food and chemical industries. Citric acid is one of the most well known products. The xylanolytic activator gene *xlnR* is a main controlling gene in the *XlnR* regulon of *A. niger* and is also one of the most studied parts of this organism. The *XlnR* regulon is activated by D-xylose as a culturing media [1]. The current description of this system is, however, based on static interpretation of the system. As the activity of the organism shows dynamic properties a quantitative model for the behavior of the *XlnR* regulon is hereby proposed.

The challenge with genetic network modeling is with determining a specific equation formalism to represent the network structure. One of the suggested strategies of modeling using differential equations is to fix the form of the equation [2]. Prior knowledge on the network structure is essential to develop a quantitative model [3]. The descriptive information on the *XlnR* regulon [1] enables us to hypothesize models for the interaction in the different network components.

Modeling and simulation of the *XlnR* regulon is explored by using nonlinear differential equations and Hill functions for the transcription and linear reaction kinetics for

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the translation process. To ensure that detailed aspects of the system are captured, some assumptions are incorporated in the modeling. Perturbation experiments are performed by triggering the genetic network at steady state. A stability analysis is performed and the effect of feedback in the system is explored.

2 Methodology

2.1 Regulation mechanism for the *XlnR* regulon

In *Aspergillus niger* transcription of genes encoding xylanolytic and cellulolytic enzymes take place [1]. Activation enables the degradation of the cellulose and hemicellulose from the plant cell walls. *XlnR* is a zinc binuclear cluster protein consisting of about 875 amino acids, it is suspected that *XlnR* binds as a monomer. The *XlnR* gene is induced in the presence of D-xylose as a culturing media and repressed by the presence of *CreA*.

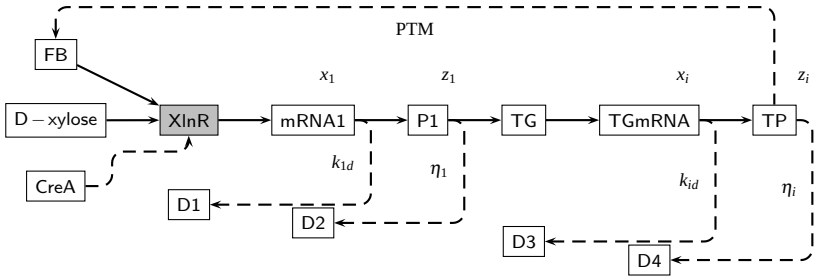

Figure 1: The *XlnR* regulon induced by D-xylose in the presence or absence of *CreA*. P1 and TP are the corresponding proteins from the *XlnR* gene and target genes respectively. mRNA1 and TGmRNA are the transcription products from the *XlnR* gene and target genes respectively. FB is the feedback protein.

Gene regulation can take place at different stages of the central dogma of molecular biology (DNA$\rightarrow$ RNA $\rightarrow$ Protein). These stages include among others transcription, translation and PTMs of the associated protein. In Figure 1 a scheme of the activities in the *XlnR* regulon is given. The *XlnR* gene is induced by D-xylose. At induction the *XlnR* gene produces mRNA which is translated in proteins. These proteins then activate the target genes (TG). For the *XlnR* regulon, the number of target genes are estimated to be in the order of 20 to 40. In Figure 1 all target genes are represented by TG. After transcription and translation of the target genes, proteins are obtained (TP). Protein from post-translational modifications (PTM) can be involved in the regulation of the *XlnR* gene trough a feedback loop. At each step in transcription and translation mRNA and proteins can be degraded and/or used for other processes (D1-D4).

2.2 Transcription model

Commonly, hyperbolic functions and the sigmoid class of functions are used to represent the kinetics of gene regulation [4]. Such functions mimic the nonlinearity in gene regulation, by assuming that a critical amount of protein build up have to be reached before a gene can be considered regulated or repressed. The most common forms of functions

used for modeling gene transcription are the Hill functions [5][6]. Let $\mathbf{z} = [z_1, \ldots, z_n]^T$ represent the concentrations of the translated proteins corresponding to the genes $1, \ldots, n$; where n the number of involved genes, then the activating and repressing functions are given by

$$\Psi(z_i, \theta_i) = \begin{cases} \psi^+(z_i, \theta_i) = \dfrac{z_i^h}{\theta_i^h + z_i^h} & \text{Activator.} \\ \psi^-(z_i, \theta_i) = \dfrac{\theta_i^h}{\theta_i^h + z_i^h} & \text{Repressor.} \end{cases} \tag{1}$$

where $\psi^-(z_i, \theta_i) = 1 - \psi^+(z_i, \theta_i)$, with the gene specific half-saturation parameter θ_i and the positive number h. The regulation mechanism for each target gene i is captured by the function $\Psi(z_i, \theta_i)$ in (1). According to Hasper et al., [7] there is evidence that although most zinc binuclear cluster proteins bind as a dimer, it seems that *XlnR* binds as a monomer - therefore, a Hill coefficient with $h = 1$ is used. Given the availability of structural prior knowledge and that the master regulator activates the target genes, the nonlinear system is given by

$$\Sigma_{\text{nls}} = \begin{cases} \dot{x}_1 = \rho_1 - k_{1d}x_1 + b_1u_1 \\ \dot{x}_2 = \rho_2 + k_{2s}\dfrac{k_{21}z_1}{1 + k_{21}z_1} - k_{2d}x_2 \\ \quad \vdots \qquad\qquad\qquad\qquad \mathbf{x}(0) = \mathbf{x}_0 \\ \dot{x}_n = \rho_n + k_{ns}\dfrac{k_{n1}z_1}{1 + k_{n1}z_1} - k_{nd}x_n \end{cases} \tag{2}$$

where ρ_i - Basal (*leaky*) transcription rate for gene i, k_{i1} - effective affinity constant for gene 1 activating gene i ($i = 2, \ldots, n$), k_{is} - synthesis parameter for gene i, k_{id} - first order degradation rate (or consumption rate) for gene i, θ_{i1} - gene 1 activated transcription rate constant for gene i, $\mathbf{x}_0$ - vector of initial mRNA concentration, z_i - concentration of translated protein from gene i, $\mathbf{b} = [b_1, \ldots, b_n]^T$ - input matrix and $\mathbf{u} = [u_1, \ldots, u_n]^T$ - input vector (gene triggering compounds).

2.3 Translation model

Next a system of linear differential equations (3) to model the protein abundance (translation process) is considered.

$$\dot{z}_1 = r_1x_1 - \eta_1z_1; \;\; \dot{z}_2 = r_2x_2 - \eta_2z_2; \;\; \ldots; \;\; \dot{z}_n = r_nx_n - \eta_nz_n; \;\; z_i(0) = z_{i0} \tag{3}$$

where r_i - translation rate for gene i, η_i - degradation rate for protein i and x_i - mRNA concentration for gene i. The z_i's are represented by the TP's in scheme 1. At steady state the difference between the response rate and degradation rate balances out, i.e. $\dot{x}_1 \approx \dot{x}_2 \approx \ldots \approx \dot{x}_n \approx 0$. By setting $\dot{x}_i = 0$ for all i in (2), we have

$$\tilde{x}_1 = \frac{1}{k_{1d}}(\rho_1 + b_1u_1); \;\; \tilde{x}_i = \frac{1}{k_{id}}\left(\rho_i + k_{is}\frac{k_{i1}z_1}{1 + k_{i1}z_1}\right) \;\; i \geq 2 \tag{4}$$

2.4 System stability

The interesting case to analyze is the systems behavior in the absence of the inhibitor, *CreA*. Let us denote the equilibrium concentrations of mRNA and protein quantities by $\tilde{\mathbf{x}} = [\tilde{x}_1, \ldots, \tilde{x}_n]^T$ and $\tilde{\mathbf{z}} = [\tilde{z}_1, \ldots, \tilde{z}_n]^T$ respectively. Using (3) the steady states lead to the relationships $\tilde{x}_i = \eta_i \tilde{z}_i / r_i$ for all i. The stability of each steady state (from (2) and (3)) can be analyzed using Hopf Bifurcation. Let $F : \mathbb{R}^{2n} \to \mathbb{R}^{2n}$ be a set of functions (with $F = (F_1, \ldots, F_{2n})$) that capture the system dynamics. In this case we have $F_1 = \dot{x}_1, \ldots, F_n = \dot{x}_n$, and $F_{n+1} = \dot{z}_1, \ldots, F_{2n} = \dot{z}_n$ in (2) and (3) respectively. The Jacobian matrix is given by

$$\mathbb{J}_n(\cdot) \quad := \quad \begin{pmatrix} \partial F_1/\partial x_1 & \ldots & \partial F_1/\partial z_n \\ \vdots & \ddots & \vdots \\ \partial F_{2n}/\partial x_1 & \ldots & \partial F_{2n}/\partial z_n \end{pmatrix}_{\big|_{[\tilde{\mathbf{x}} \;\; \tilde{\mathbf{z}}]}} \tag{5}$$

This Jacobian matrix is then used to assess the regulon stability and to identify which parameters dictate the transcript abundance. Let us first consider a case of three genes, $n = 3$. The Jacobian is given by $\mathbb{J}_3(\cdot) = \partial F / \partial [\mathbf{x} \;\; \mathbf{z}]_{\big|_{[\tilde{\mathbf{x}} \;\; \tilde{\mathbf{z}}]}}$ where $\mathbf{x} = [x_1, x_2, x_3]^T$ and $\mathbf{z} = [z_1, z_2, z_3]^T$. Using expressions (2) and (3) in (5) we obtain

$$\mathbb{J}_3(\cdot) \quad = \quad \begin{pmatrix} -k_{1d} & 0 & 0 & 0 & 0 & 0 \\ 0 & -k_{2d} & 0 & \varphi_{24} & 0 & 0 \\ 0 & 0 & -k_{3d} & \varphi_{34} & 0 & 0 \\ r_1 & 0 & 0 & -\eta_1 & 0 & 0 \\ 0 & r_2 & 0 & 0 & -\eta_2 & 0 \\ 0 & 0 & r_3 & 0 & 0 & -\eta_3 \end{pmatrix} \tag{6}$$

where

$$\varphi_{i4} \quad = \quad \frac{k_{is} k_{i1}}{1 + k_{i1} \tilde{z}_1} \Big(1 - \frac{1}{1 + k_{i1} \tilde{z}_1} \Big); \qquad \text{for} \qquad i = 2, 3 \tag{7}$$

A similar generalized expression for $\varphi_{i,n+1}$ can be obtained given a regulon with n transcripts. The characteristic polynomial obtained from $\mathbb{J}_3(\cdot)$ is given by

$$\lambda(\mathbb{J}_3(\cdot)) \quad = \quad \prod_{i=1}^{3} (\lambda + k_{id})(\lambda + \eta_i) \tag{8}$$

where $\lambda(\mathbb{J}_3(\cdot)) = |\mathbb{J}_3(\cdot)|$. Basing on (8), conditions can be established on the parameters to ensure global stability. The formulations of the Jacobian matrix and the eigenvalue spectra can be extended to an n-dimensional system. The generalization for the eigenvalue spectra can be shown to be

$$\lambda(\mathbb{J}_n(\cdot)) \quad = \quad \prod_{i=1}^{n} (\lambda + k_{id})(\lambda + \eta_i) \tag{9}$$

for $n \in \mathbb{Z}^+$. Note that $\lambda(\mathbb{J}_n(\cdot))$ is independent of the translation rate parameters r_i, and the gene synthesis coefficient k_{is} and the terms in the expression (7). Clearly, from (9) the

system is globally stable ($Tr(\mathbb{J}_n(\cdot)) < 0$ and $|\mathbb{J}_n(\cdot)| > 0$ for all $\mathbf{x}, \mathbf{z}$). The system stability behavior is dictated by how fast the translation and transcription processes proceed (i.e. magnitudes of k_{id} and η_i). In metabolic terms this equates to demand and supply of essential components in and out of the cell.

According to Aro et al., [8]; de Vries et al., [9]; Hasper et al., [10] the *A. niger* gene: *eglA, eglB, eglC, cbhA, cbhB, xlnB, xlnC* and *xlnD* contain binding sequences (GGC-TAAA) to *XlnR* protein as well as binding sequences to *CreA*, a repressor protein acting in the presence of monomeric sugars (i.e., glucose) as a self-regulating mechanism. This property ensures that most target genes have similar expression dynamics in time. An example is considered to investigate the time evolution of gene activity and protein abundance in the *XlnR* regulon.

> **Example.** Consider a regulon network of three genes given a perturbation $u(t) = u(0)(1/(\beta + e^{Kt}))$, where $u(t) \equiv$ [D-xylose] and $\beta > 0$, to trigger the system; with $K = 0.3$ and $u(0) = 50$ mM as the initial D-xylose concentration. The parameters used for the simulation are: $b_1 = 1$, $\rho_1 = 2e-3$, $\rho_2 = 2.5e-3$, $\rho_3 = 1e-3$, $k_{1d} = 0.5$, $k_{2d} = 0.4$, $k_{3d} = 0.3$, $k_{2s} = 5$, $k_{3s} = 6$, $k_{21} = 0.1$, $k_{31} = 0.1$, $r_1 = r_2 = r_3 = 0.5$, $\eta_1 = 1$, $\eta_2 = 1$ and $\eta_3 = 1$. The phase plots for the mRNA and protein availability are shown in Figure 2.

In Figure 2 both the gene expressions in plot (A) and protein abundance plot (B) show similar behavioral dynamics. Moreover, with the chosen input pattern of D-xylose the target genes show phase plots similar in patterns but with variations that are dictated by individual gene or protein kinetic parameters. A relaxation time of $\tau_{R1} = 1/k_{1d} \approx 2$ hours is noticed for the master regulator and for the target genes, $\tau_{R1} < \tau_{R2}, \tau_{R3}$. The relaxation time is an approximation for the time required for the system to relax into steady state. This represents the time it takes a system to react to an external disturbance (D-xylose).

2.5 Feedback in the network

So far we have only considered a case of no feedback in the network. Next, we consider a feedback into the network and study the system dynamics when some target gene products are involved in the regulation of the master regulator, *XlnR*. Assume that the PTMs have some kind of time delay (τ) associated with each of them. Competitive feedback effects were modeled at the promoter sites of the *XlnR* regulator gene. In the modeling and simulation a fixed number of promoter sites for gene regulation was assumed. Next the effect of having an activating and repressing feedback are analyzed.

2.5.1 Activating and repressing feedback

We hypothesize that the target proteins (TP's) and PTMs in the feedback loop in scheme 1 only act on the regulator gene *XlnR*. Therefore, only the equation for x_1 has to be modified accordingly. The adapted equation is given by

$$\begin{aligned} \dot{x}_1(t) &= \rho_1 - k_{1d}x_1(t) + \Big[b_1 u_1(t) \\ &+ k_{ls}\Big(\frac{1}{1 + k_{jL}\sum_{j\in \mathbf{S}_1} z_j(t-\tau)} \frac{k_{lL}\sum_{l\in \mathbf{S}_2} z_l(t-\tau)}{1 + k_{lL}\sum_{l\in \mathbf{S}_2} z_l(t-\tau)}\Big)\Big] H \end{aligned} \tag{10}$$

where $H = 1/(1+k_A C_A(t))$ is the repressor Hill function and C_A - quantitative activity state for *CreA*, k_A - inverse of the Hill constant of *CreA*. The sets $\mathbf{S}_1 = \{j \mid j = 1,\ldots,m\}$ and $\mathbf{S}_2 = \{l \mid l = m+1,\ldots,n-1\}$ where $\mathbf{S}_1 \bigcup \mathbf{S}_2 = \{1,\ldots,n-1\}$ i.e. collection of all the target proteins in the regulon. All the supposed repressing and activating proteins are lumped in the sets $\mathbf{S}_1$ and $\mathbf{S}_2$, respectively. The effect of the D-xylose and the feedback loop is modeled as additive. Equation (10) also specifies the build up of proteins and repression or activation of the *XlnR* gene through the feedback loop. Through the sequence of PTMs the protein availability in the feedback loop is delayed. All the other components representative of the target genes in the network models (2) and (3) remain unchanged.

Since the presence of *CreA* is a strong repressor that inhibits the *XlnR* gene activity by blocking the promoter binding site, we chose to model this influence by considering a switch function with $H \in \{0,1\}$. Here $H = 0$ and $H = 1$ means *CreA* is present and absent respectively. In absence of *CreA* the protein products from the target genes are involved in regulating the activity of the master regulator. These protein products may either inhibit or activate the *XlnR* gene.

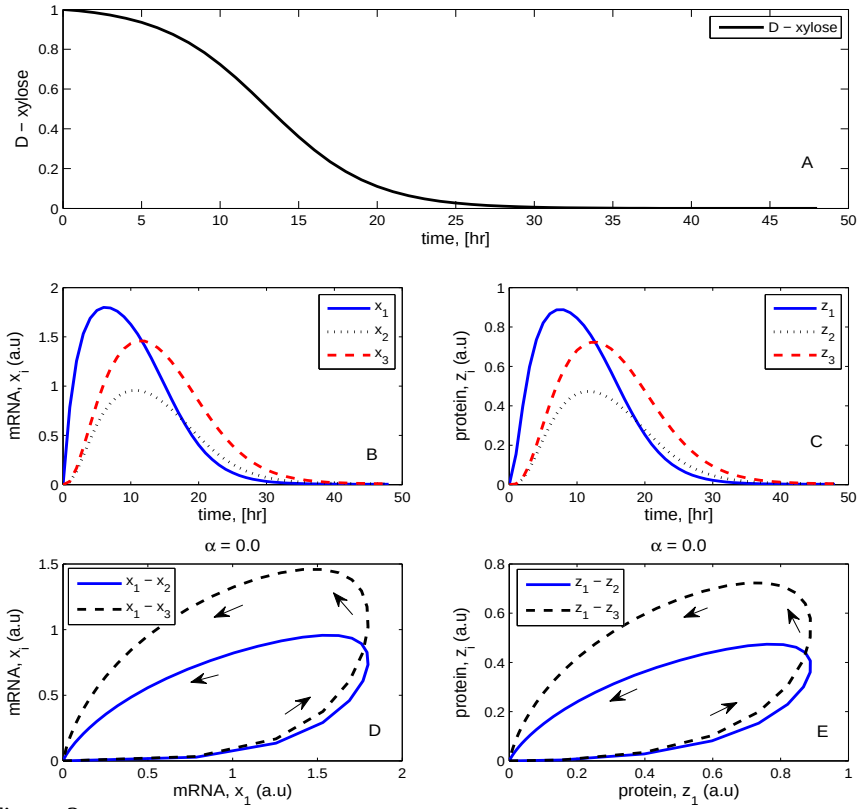

Figure 2: (A): The simulated trajectory for D-xylose consumption. (B): Expressions profiles for genes, (C): Proteins abundance plots. (D): Phase plot for gene expression showing variation of mRNA concentrations of the *XlnR* gene and the other target genes (x_i). (E): Corresponding protein abundance phase plot.

In practice, quantifying the feedback effect of each individual gene in a network is far from trivial. In fact, it is interesting to assess the joint effect of the feedback of the target genes on the single master regulator. In genetic networks the effects of PTMs can be either activating, repressing or non at all. This range of regulation possibilities is

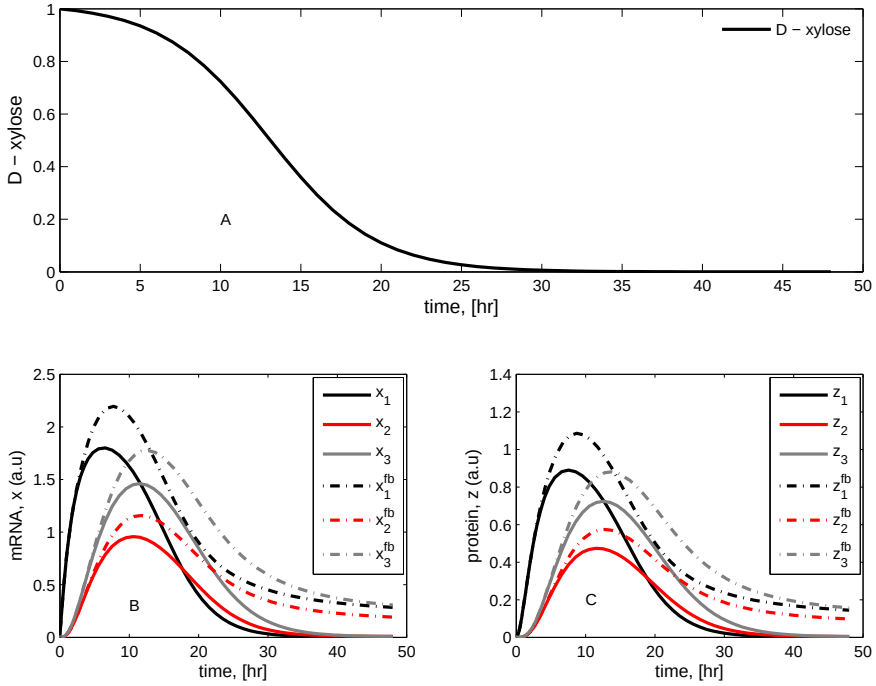

Figure 3: (A) The simulated trajectory for D-xylose. (B) Gene expression profiles with solid lines (−) showing the expression profiles for the genes in the absence of *CreA*. The corresponding dotted lines (···) show the simulated effect of competitive feedback. (C) Protein abundance profiles (solid lines).

considered and modeled in (10). Considering feedback loops enable better understanding of any fluctuations in the protein availability. For both activating and repressing feedback loops with time delay, we definitively specified $x_i(t) = 0$ and $z_i(t) = 0$ for $t < 0$ and for all $i = 1,\ldots,n$ and $\tau = 1$ hour.

A comparison of the metabolite expression dynamics for the network with and without feedback loops is shown in Figure 3. The same parameter values in the **Example** above were used for the simulation with the extra parameters from (10) being $k_{jL} = 1$ and $k_{lL} = 1$ and the lumped synthesis parameter from (10) chosen as $k_{ls} = 1$. Figure 3 indicates the enhanced metabolite expression as a result of incorporating a feedback loop in the model. Using the adapted model (10), the computed entry in the $(1, n+i)$-th cell ($i = j, l$ for all values of j and l) of the Jacobian matrix (5) is given by (11) and/or (12) depending on

which proteins are involved in the feedback loop regulation.

$$\frac{\partial F_1}{\partial z_j}\Big|_{j\in \mathbf{S}_1} = -\frac{k_{jL}k_{lL}k_{ls}\sum_{l\in \mathbf{S}_2} z_l(t-\tau)}{\left(k_{jL}\sum_{j\in \mathbf{S}_1} z_j(t-\tau)+1\right)^2\left(k_{lL}\sum_{l\in \mathbf{S}_2} z_l(t-\tau)+1\right)} < 0 \qquad (11)$$

$$\frac{\partial F_1}{\partial z_l}\Big|_{l\in \mathbf{S}_2} = \frac{k_{lL}k_{ls}}{\left(k_{jL}\sum_{j\in \mathbf{S}_1} z_j(t-\tau)+1\right)\left(k_{lL}\sum_{l\in \mathbf{S}_2} z_l(t-\tau)+1\right)^2} > 0 \qquad (12)$$

The expressions (11) and (12) have the potential to yield oscillatory behavior in the metabolite response profiles. The oscillatory behavior (when it exists) is purely governed by the values of the system mechanistic parameters.

2.5.2 *XlnR* gene promoter activity

The competitive effect of the activators and repressors for the promoter binding sites were also simulated. The effect of which transcription factor, TF (activator or repressor) wins occupancy of a promoter binding site depends partly on the strength of the synthesis parameter k_{ls} (see Figure 4). The promoter is most active (activity around $50-80\%$) when

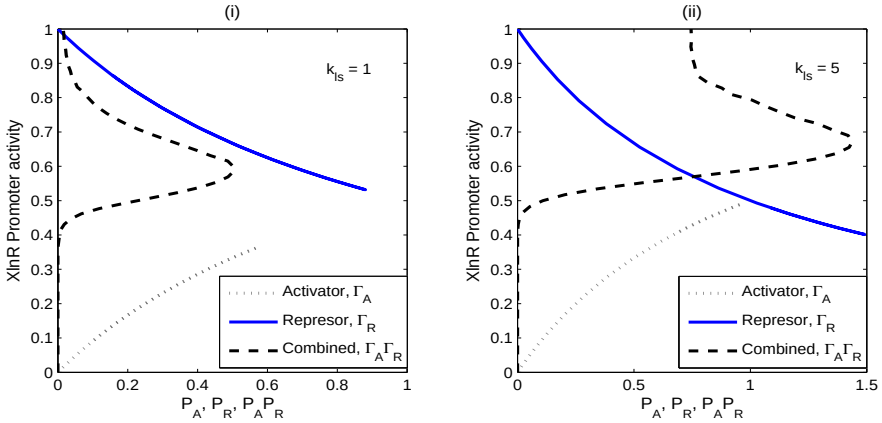

Figure 4: Plot of the *XlnR* promoter region activity, Γ_{A}, Γ_{R}. The term $\Gamma_{\mathrm{A}}\Gamma_{\mathrm{R}}$ - is the combined affect of competitive binding to promoter region by activators and repressors. Plots (i) and (ii) show the influence of weak ($k_{ls}=1$) and strong ($k_{ls}=5$) synthesis parameters respectively.

the regulon is fully active. This corresponds to the time window at which the network is fully responsive to the external perturbation. Let us define the promoter activities by (13) and extracts of the denominator functions by (14)

$$\Gamma_{\mathrm{A}} = \frac{k_{lL}\sum_{l\in \mathbf{S}_2} z_l(t-\tau)}{1+k_{lL}\sum_{l\in \mathbf{S}_2} z_l(t-\tau)}; \quad \Gamma_{\mathrm{R}} = \frac{1}{1+k_{jL}\sum_{j\in \mathbf{S}_1} z_j(t-\tau)} \qquad (13)$$

$$\mathrm{P}_{\mathrm{A}} = k_{lL}\sum_{l\in \mathbf{S}_2} z_l(t-\tau); \quad \mathrm{P}_{\mathrm{R}} = k_{jL}\sum_{j\in \mathbf{S}_1} z_j(t-\tau) \qquad (14)$$

For the sake of illustrations, two target genes were considered (i.e. values of $j=1$ and $l=2$) in the simulation with one as an activator and the other as a repressor (we used

$k_{jL} = k_{lL} = 1$). Extending the model with lumped feedback regulatory effects further complicates the analysis. We consider the sets $\mathbf{S}_1$ and $\mathbf{S}_2$ of unit elements. We observe that the activator has a tendency of occupying most of the promoter sites at any given time (see Figure 4).

To assess the effect of time delays in the transcription and translation processes, we simulated some cases (Figure 5). The simulations were performed for specific cases of $\tau = 1$ hour and $\tau = 5$ hours and the outputs compared.

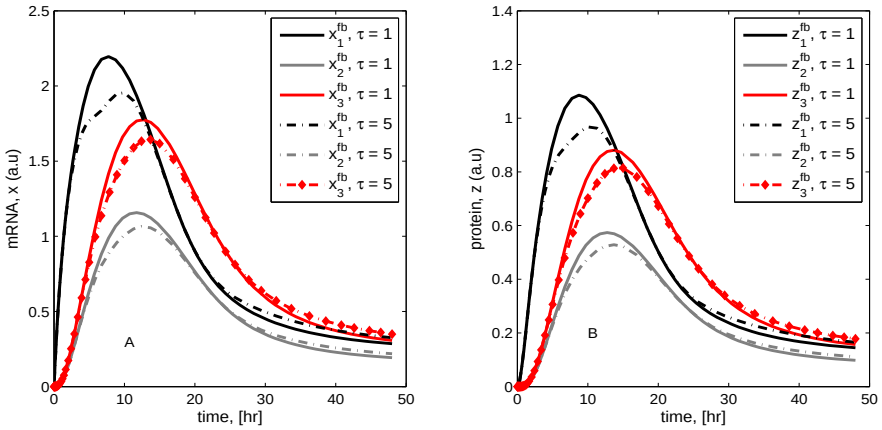

Figure 5: (A)-(B) Plots showing the effect of variation in time delay in the feedback loops corresponding to the transcription and translation processes, respectively. The observed effect on the responses is small except for the slight deviation at the peak of the expression profiles.

3 Discussion

Additional simulations showed that the dynamics of the D-xylose input function considered in the examples has an important effect on the profiles of the individual metabolite concentrations. This is particularly dictated by the value of the parameters in the input function $u(t)$. The larger the value of the K, the faster the consumption of D-xylose. This depends on the chemical reactions taking place in any given cell, or the saturation levels of the individual compounds in a cell.

Feedback affects the response of the output profiles for the metabolites seriously (Figure 3). Further simulations showed that variations of the time delay in the feedback loop ($\tau = 1, 2, \ldots, 5$ hours) have a small effect on the response (Figure 5). The investigations in subsection 2.4 show that the network system dynamics exhibits no oscillatory behavior. Nevertheless, many biological systems exhibit some delays in transcription-translation processes. These delays can be attributed to (i) material transportation mechanisms in and out of the nucleus, (ii) binding of TFs, (iii) interaction of TFs and gene promoter sites. Even after a perturbation, the system trajectories gradually settle to their respective steady states. For a system with feedback, the steady states are slightly changed. In both cases the observed dynamics is as a result of the change in eigenvalues from the Jacobian matrix (5) which is determined by the individual parameter values.

The modeling approach used in this paper provides good information for understanding network behavioral dynamics particularly for small-sized networks. This is illustrated with the *XlnR* regulon network in which even the simplest of structures can yield interestingly complex dynamics. The primary reason for limiting our goals to the modeling and systems dynamics investigations is that experimental work is needed to obtain the basal and other parameters. Having such information in advance would enhance the results. Nevertheless, with parameter guesses, simulation studies provide good information into the systems behavior.

According to Balsa-canto et al., [12], having powerful mathematical analytic tools highlight the value for successful study of many biological systems. However, such success can mainly be attributed to the unrelenting endeavors for an in-depth understanding of both computational methods and the biological problems of interest. For the case of the *A. niger* regulon, work provides a basis for understanding the behavioral dynamics of genes and proteins after network perturbation. This will form a basis for future wet-lab experiments, particularly with the genes from the *XlnR* regulon.

4 Conclusions and outlook

The study shows that the *XlnR* regulon should be considered as a dynamic system instead of a static system. The work provided insight into the dynamic properties of the *XlnR* regulon. By studying this system, it has also become clearer that the parameters that dictate most of the dynamics in the regulation properties of the network are the transcription and translation degradation rates.

The dynamics in the regulation properties of the network are dictated mainly by the transcription and translation degradation rate parameters, and the D-xylose consumption profile. This is an observation based on the modeling approach we considered. The analysis of the network dynamics has provided useful information for future *in vitro* experimental work. Particularly the potential for hypothesis testing basing on this work and design of related perturbation experiments to generate time course data. Thereafter, techniques for the network structural identification and parameter estimation for the *XlnR* regulon can be investigated.

The role played by feedback in the network dynamics was found to influence the expression dynamics of genes and proteins. This means that the effect of the feedback should be considered in the model if there is sufficient supportive biological need. Just like for most biological systems, this is no doubt an important piece of information for the accurate modeling of biological network.

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