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RESEARCH ARTICLE

Characterizing the memory of the GAL regulatory network in *Saccharomyces cerevisiae*

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Abstract Genetic regulatory networks respond dynamically to perturbations in the intracellular and extracellular environments of an organism. The GAL system in the yeast *Saccharomyces cerevisiae* has evolved to utilize galactose as an alternative carbon and energy source, in the absence of glucose in the environment. We present a dynamic model for GAL system in *Saccharomyces cerevisiae*, which includes a novel mechanism for Gal3p activation upon induction with galactose. The modification enables the model to simulate the experimental observation that in absence of galactose, oversynthesis of Gal3p can also induce the GAL system. We then characterize the memory of the GAL system as the domain of attraction of the steady states.

Keywords Cellular memory · Domain of attraction · ODE model · GAL system · *Saccharomyces cerevisiae*

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Introduction

Biological memory can be defined as a sustained cellular response to a transient stimulus (see Burrill and Silver 2010; Ajo-Franklin et al. 2007). One way that cells exhibit memory is through transcriptional states, which involve populations of molecules regulating gene expression. If the transcriptional response is bistable, a chemical state becomes defined as on or off and, given certain parameters, this state can be inherited through DNA replication and cell division. A cell can thus produce a lasting memory of a biological response (see Alon 1999; Monod and Jacob 1961). Such transcriptional responses are useful to synthetic biologists as well since much of the information processing in a cell is through transcription so that the transcription process enables a user with an useful set of characterized genetic units such as promoters, activators, and inhibitors that can be recombined to create new transcriptional circuits. The construction of synthetic memory circuits will improve our understanding of natural networks, further aiding the creation of useful, new biological tools. For example, a device capable of remembering a biological experience might be utilized in the long-term study of particular cells within a heterogeneous population following a defined event or applied in industry for the sustained production of desired proteins after induction by a brief stimulus (see Burrill and Silver 2010). In this paper, we show how the domain of attraction analysis results (see, for example, Materassi and Salapaka 2009) can be used to impart desirable memory to synthetic biological constructs built using the *galactose regulatory system* (the GAL system) of the yeast *Saccharomyces cerevisiae*, i.e., the Baker's yeast. The domain of attraction analysis technique itself is quite generic and may be applied to analyze the memory, and robustness thereof, of a variety of biological systems.

Our model of the GAL system is built on the model derived in Ruhela et al. (2004) by hypothesizing new interactions in the galactose sensing mechanism so as to capture the constitutive expression of the GAL system when Gal3p is overexpressed. In addition, so as to be consistent with the observed Gal2p mutant behaviour, it includes non-facilitated diffusion of galactose as a galactose sensing mechanism over and above the Gal2p mediated transport mechanism. Our model includes the growth and substrate consumption dynamics and, in addition, establishes a relationship between the dilution of the various protein complexes and the nutritional status of the medium. This model successfully predicts various mutant and experimental scenarios such as effect of preculturing on the memory of the cells (see Acar et al. 2005) and the effect of regulation of permease on the sensitivity of the switch (see Hawkins and Smolke 2006).

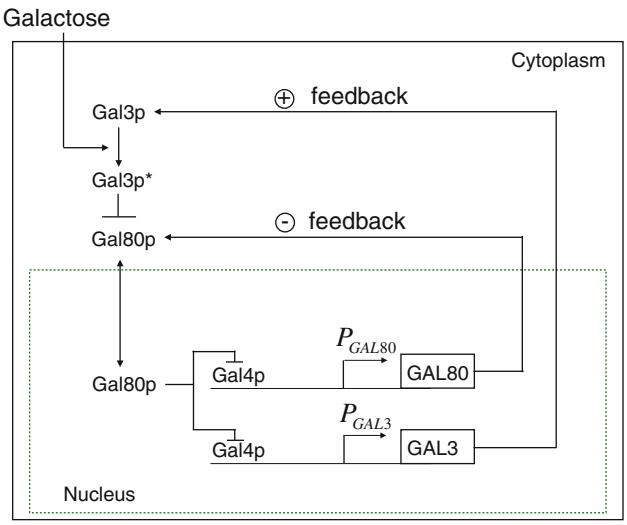

Fig. 1 The GAL regulatory system: The external galactose signal controls the transcriptional activity of the GAL genes. Galactose can shuttle between the cytoplasm and the nucleus. The galactose bound stage of the protein Gal3p is Gal3p*. The *pointed arrows* indicate activation whereas the *blunt arrows* indicate inhibition

The GAL system of *Saccharomyces cerevisiae*

Naturally occurring networks of genes and proteins, especially in eukaryotic organisms, feature multiple complex nested feedback loops. So, although gene expressions can be affected at many levels including protein-DNA interactions, protein-protein interactions, and protein-small molecule interactions, it is difficult to characterize, a priori, the systemic effect of these changes. An example of such networks is the galactose signalling pathway in the yeast *Saccharomyces cerevisiae*. Despite extensive data on its molecular interactions, an a priori prediction of its systemic behavior remains challenging (see Acar et al. 2005; Biggar and Crabtree 2001; Ideker et al. 2001). In the GAL regulatory network (see Fig. 1), the galactose signal propagates through a four-stage signalling cascade. At the uppermost stage is Gal2p, which imports extracellular galactose into the cell. Subsequently, intracellular galactose binds to and activates Gal3p (see Biggar and Crabtree 2001; Ideker et al. 2001). At the third stage of this cascade, the activated Gal3p binds to and sequesters Gal80p in the cytoplasm, depleting Gal80p from the nucleus. The transcriptional activator Gal4p, which is constitutively bound to promoters of the GAL genes, is then released from the inhibitory action of Gal80p and activates expression of genes at the output of the cascade, including GAL1, GAL2, GAL3 and GAL80. Because an increase in Gal2p and Gal3p concentration results in enhanced transcriptional activity, these two proteins each enforce a positive feedback loop whereas Gal80p enforces a negative feedback loop (see Acar et al. 2005).

Yeast metabolizes galactose using the enzymes of the well known Leloir pathway (Johnston 1987). When yeast is grown in the absence of galactose, the genes which encode the enzymes of the Leloir pathway are found to be transcriptionally inert (Platt et al. 2000). The enzymes in the Leloir pathway are regulated by a well-characterized genetic switch known as the GAL regulatory system. However, induction and transcription of GAL genes occur if galactose is the sole carbon source. On the other hand, GAL genes are repressed during growth in a medium containing glucose (see Johnston et al. 1994; Dong and Dickinson 1997). The GAL system is a complex genetic network with numerous interactions. The induction of GAL genes in *Saccharomyces cerevisiae* is controlled by the interplay between three regulatory proteins: a transcriptional activator Gal4p, a transcriptional repressor Gal80p, and an inducer Gal3p (Lohr et al. 1995). The activator Gal4p activates the GAL structural genes by recognizing and binding to the specific upstream activation sequence of the GAL structural genes (UAS_G) through its N-terminal DNA-binding domain. In the absence of galactose, Gal80p inhibits the function of Gal4p by binding to its C-terminal transcription activation domain (Giniger et al. 1985). The above interactions are shown schematically in Fig. 2.

The GAL gene family in *Saccharomyces cerevisiae* consists of three regulatory genes and five structural genes which enables it to use galactose as the carbon source (Lohr et al. 1995). The GAL network, shown in Fig. 2, has three genes MEL1, GAL3, and GAL80 with one binding site (referred to as D1 in Fig. 2) for activation by Gal4p and seven genes GAL2, GAL1, GAL7, GAL10, MTH1, PCL10, and FUR10 with two binding sites (referred to as D2 in Fig. 2) for the activator protein Gal4p. The activator Gal4p binds to the upstream activation sequences (UAS_G) of these genes as a homodimer and activates the transcription of the genes. The

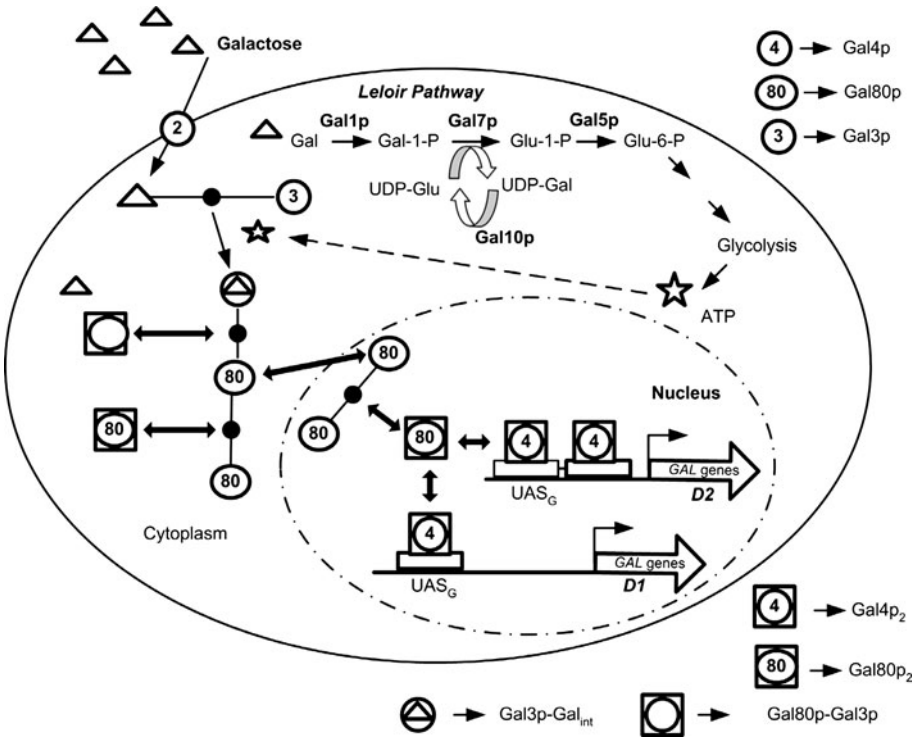

Fig. 2 Schematic representation of protein-protein and DNA-protein interactions in the cytoplasm and nucleus for the GAL genetic switch in presence of galactose. D1 and D2, represent genes with one and two binding sites, respectively. The numbers in circles namely 4, 80 and 3 represent Gal4p, Gal80p and Gal3p/activated Gal3p, respectively. The interactions in the nucleus include Gal4p dimerization, Gal80p dimerization, Gal4p dimer-DNA interaction with genes having one and two binding sites, Gal80p dimer-Gal4p dimer interaction and interaction of Gal80p dimer to Gal4p dimer bound to DNA. The interaction in the cytoplasm includes dimerization of Gal80p and binding of Gal80p to activated Gal3p. Gal80p shuttles between cytoplasm and nucleus to relieve its inhibition on Gal4p

repressor protein, Gal80p binds to the gene-Gal4p dimer complex and prevents it from recruiting RNA polymerase II mediator complex. Once galactose is inside the cell, the inducer protein Gal3p interacts with the repressor Gal80p in a galactose and ATP dependent manner in the cytoplasm to relieve the Gal80p inhibition (see Zenke et al. 1996; Platt and Reece 1998). The inducer Gal3p appears to require galactose and ATP so as to adopt a conformation that allows interaction with Gal80p to form a stable complex of Gal3p-Gal80p (Yano and Fukasawa 2008). The cytoplasmic interaction between Gal3p and Gal80p results in sequestration of Gal80p in the cytoplasm from the nucleus. The net result of this interaction is that inhibition of the switch by Gal80p is relieved and transcription of the GAL genes proceeds. These interactions are represented in form of a dynamic model for GAL regulatory network as discussed below.

System description

The performance of the GAL switch is observed as the fraction of the gene bound to the activator over the total available gene concentration. The fractional transcriptional levels for D1 and D2, viz., f_1 and f_2 are

$$f_1 = \frac{[D1 - \mathrm{Gal4}p_2]}{[D_{1t}]},$$
$$f_2 = \frac{[D2 - \mathrm{Gal4}p_2] + [D2 - \mathrm{Gal4}p_2 - \mathrm{Gal4}p_2]}{[D_{2t}]},$$

where [D1-Gal4p2] represents the interaction of dimer Gal4p with DNA for genes with one binding site, $[D2 - \mathrm{Gal4}p_2]$ represents the interaction of dimer Gal4p with DNA for genes with two binding sites, [D1t] is the total operator concentration of DNA with one binding site for Gal4p, and [D2t] is the total available operator concentration of genes with two binding sites for Gal4p. The fractional translation for both one and two binding sites is given by $f_{1p} = f_1^{0.5}$ and $f_{2p} = f_2^{0.5}$, where f_{1p} and f_{2p} represent the fractional translation of genes with one and two binding sites, respectively. The exponent 0.5 represents the co-response coefficient relating the fractional transcription to fractional translation and is determined by microarray data (Ideker et al. 2001). Presence of glucose turns off the GAL system by repressing the synthesis of Gal4p, which has been modelled by a Michaelis-Menten type relationship as follows,

$$R_{\mathrm{Gal4}p} = K_g[\mathrm{Gal4}p_{\max}]\frac{K_i}{K_i + Glu_{ext}},$$

Table 1 Physical meaning of state variables

State-variable	Meaning
x_1	[Gal4p]
x_2	[Gal4p_2]
x_3	[$D1$]
x_4	[$D2$]
x_5	[$D1$ − Gal4p_2]
x_6	[$D2$ − Gal4p_2]
x_7	[$D2$ − Gal4p_2 − Gal4p_2]
x_8	[Gal80pn]
x_9	[Gal80pn_2]
x_{10}	[Gal4p_2 − Gal80pn_2]
x_{11}	[$D1$ − Gal4p_2 − Gal80pn_2]
x_{12}	[$D2$ − Gal4p_2 − Gal80pn_2]
x_{13}	[$D2$ − Gal4p_2 − Gal80pn_2 − Gal4p_2]
x_{14}	[$D2$ − Gal4p_2 − Gal80pn_2 − Gal4p_2 − Gal80pn_2]
x_{15}	[Gal80pc]
x_{16}	[Gal80pc_2]
x_{17}	[Gal3p]
x_{18}	[Gal80pc − Gal3p]
x_{19}	[Gal3p −Gal$_{int}$]
x_{20}	[Gal2p]
x_{21}	[Gal$_{int}$]
x_{22}	[Mel_1]
x_{23}	[X]
x_{24}	[Gal$_{ext}$]
x_{25}	[Glu_{ext}]

where $R_{\text{Gal4}p}$, K_i and K_g represent the synthesis rates of Gal4p, half-saturation constant and kinetic rate constant, respectively. The synthesis of the other two regulatory proteins, Gal3p and Gal80p depends on the autoregulation of genes with one binding site. Therefore,

$$R_{\text{Gal3}p} = K_3 f_{1p} D1_t \text{ and } R_{\text{Gal80}p} = K_{80} f_{1p} D1_t,$$

where RGal3p, RGal80p and K_3, $K_8$0 represent the synthesis rate of Gal3p and Gal80p and the respective translational kinetic constants. In the previous model by Ruhela et al. (2004), it was assumed that the intracellular galactose (Galint) activates Gal3p, which then interacts with Gal80p in the cytoplasm to give a stable complex of Gal3p-Gal80p-Galint, thereby eliminating the possibility of induction of the GAL system in absence of galactose. In the dynamic model presented here, Gal3p interacts with internal galactose to form a complex Gal3p-Galint. The repressor Gal80p then interacts with Gal3p-Galint by a substitution mechanism wherein a new stable complex Gal3p-Gal80p is formed while freeing up Galint. The above interactions may be represented as follows,

Additionally, Gal3p can independently complex with Gal80p although with a significantly enhanced dissociation constant as follows (see Bhat and Hopper 1992):

$$\text{Gal3}_p + \text{Gal80}_p \leftrightarrow \text{Gal3}_p - \text{Gal80}_p. \quad (1)$$

Constants K_i represent the forward rate constants of the appropriate interaction while K_{-i} represents the corresponding backward rate constants. The above interaction is responsible for the induction of the GAL switch even in absence of galactose. The overall growth rate μ can be written as $\mu \doteq \mu_{\text{Gal}} + \mu_{Glu}$, where

$$\mu_{\text{Gal}} = \mu_{\text{Gal}_{\max}} \frac{\text{Gal}_{int}}{K_0 + \text{Gal}_{int}}, \quad \mu_{Glu} = \mu_{Glu_{\max}} \frac{Glu_{int}}{K_{Glu} + Glu_{int}}. \quad (2)$$

The galactose in the medium has been modelled as

$$\frac{d\text{Gal}_{ext}}{dt} = -K_p \text{Gal}_{2p} \frac{\text{Gal}_{ext}}{K_i + \text{Gal}_{ext}} X - K_a \text{Gal}_{2p} \frac{\text{Gal}_{ext}}{K_5 + \text{Gal}_{ext}} X, \quad (3)$$

where the first term represents the Gal2p regulated uptake of galactose while the second term quantifies the diffusion of galactose into the cell by a non-facilitated transport mechanism. The availability of internal galactose, Gal$_{int}$ is governed by the following equation,

The third and fourth terms above represent the independent weak interaction between Gal3p and Galint while the fifth and sixth terms represent the Galint assisted binding of Gal3p with Gal80p. The last term represents the growth related consumption of galactose. The uptake of glucose is modelled as follows,

$$\frac{dGlu}{dt} = \frac{-1}{Y} \mu_{Glu} X.$$

The growth is modeled as $\dot{X} = \mu X$.

The complete model consists of 25 differential equation and three algebraic equations, which are solved by MATLAB 6.5 of MathWorks Inc. All the model parameters are taken from Ruhela et al. (2004). The equilibrium dissociation constant K_4 corresponds to the interaction primarily responsible for Gal80p sequestration and has the same value as in Ruhela et al. (2004). The newly introduced dissociation equilibrium constants K_m and K_5 correspond to interactions between Gal3p and internal galactose and between the direct but weak interaction between Gal3p and Gal80p and their values are fixed based on a sensitivity study. Dynamic molar balance equations were written for interactions shown in Fig. 1 namely, protein-protein interactions, DNA-protein interactions and protein-substrate interactions. The interactions are adopted from Ruhela et al. (2004) while the proposed Gal3p-Gal80p interactions are discussed in the main text. The model parameter values are given in Ruhela et al. (2004). Few parameter values were modified to fit the model calculations. The complete dynamic model equations and notations used are presented below.

$$
\begin{aligned}
\dot{x}_1 &= k_1K_1x_2 - k_1x_1^2 + \frac{K_gK_i}{K_i + Glu_{ext}} - \mu x_1\\
\dot{x}_2 &= 0.5k_1x_1^2 - 0.5k_1K_1x_2 + k_dK_dx_5 - k_dx_2x_3\\
&\quad + k_dK_dx_6 - k_2x_2x_4 + k_dK_dx_7 - mk_dx_2x_6\\
&\quad + k_3K_3x_{10} - k_3x_2x_9 - \mu x_2\\
\dot{x}_3 &= k_dK_dx_5 - k_dx_2x_3\\
\dot{x}_4 &= k_dK_dx_6 - k_dx_2x_4\\
\dot{x}_5 &= k_dx_2x_3 - k_dK_dx_5 + k_3K_3x_{11} - k_3x_5x_9\\
\dot{x}_6 &= k_dx_2x_4 - k_dK_dx_6 + k_dK_dx_7 - mk_dx_2x_6\\
&\quad + k_3K_3x_{12} - k_3x_6x_9\\
\dot{x}_7 &= mk_dx_2x_6 - k_dK_dx_7 + k_3K_3x_{13} - k_3x_7x_9\\
\dot{x}_8 &= k_2K_2x_9 - k_2x_8^2 + \frac{k}{K}x_{15} - kx_8 - \mu x_8\\
\dot{x}_9 &= 0.5k_2x_8^2 - 0.5k_2K_2x_9 + k_3K_3x_{10} - k_3x_2x_9\\
&\quad + k_3K_3x_{11} - k_3x_5x_9 + k_3K_3x_{12} - k_3x_6x_9 + k_3K_3x_{13}\\
&\quad - k_3x_7x_9 + k_3K_3x_{14} - k_3x_9x_{13} - \mu x_9\\
\dot{x}_{10} &= k_3x_2x_9 - k_3K_3x_{10} - \mu x_{10}\\
\dot{x}_{11} &= k_3x_5x_9 - k_3K_3x_{11}\\
\dot{x}_{12} &= k_3x_6x_9 - k_3K_3x_{12}\\
\dot{x}_{13} &= k_3x_7x_9 - k_3K_3x_{13} + k_3K_3x_{14} - k_3x_9x_{13}\\
\dot{x}_{14} &= k_3x_9x_{13} - k_3K_3x_{14}\\
\dot{x}_{15} &= k_2K_2x_{16} - k_2x_{15}^2 + kx_8 - \frac{k}{K}x_{15} + k_4K_4x_{18}\\
&\quad - k_4x_{15}x_{19} + K_tD1_tf_{1p} - \mu x_{15}\\
\dot{x}_{16} &= 0.5k_2x_{15}^2 - 0.5k_2K_2x_{16} - \mu x_{16}\\
\dot{x}_{17} &= k_{m1}x_{19} - k_mx_{17} + K_tD1_tf_{1p} - \mu x_{17}\\
\dot{x}_{18} &= k_4x_{15}x_{19} - k_4K_4x_{18} - \mu x_{18}\\
\dot{x}_{19} &= k_4K_4x_{18} - k_4x_{15}x_{19} + k_mx_{17} - k_{m1}x_{19} - \mu x_{19}\\
\dot{x}_{20} &= K_pf_{2p}D2_t - \mu x_{20}\\
\dot{x}_{21} &= k_{m1}x_{19} - k_mx_{17}x_{21} + k_4x_{15}x_{19}\\
&\quad - k_4K_4x_{18}x_{21} + K_px_{20}\frac{x_{24}}{k_i + x_{24}}\\
&\quad + k_a\frac{x_{24}}{k_s + x_{24}} - \frac{\mu}{Y_{X/\mathrm{Gal}}} - \mu x_{21}\\
\dot{x}_{22} &= k_{me}f_{1p}D1_t - \mu x_{22}\\
\dot{x}_{23} &= \mu x_{23}\\
\dot{x}_{24} &= -K_px_{20}\frac{x_{24}}{k_i + x_{24}}x_{23} - k_a\frac{x_{24}}{k_s + x_{24}}x_{23}\\
\dot{x}_{25} &= -\frac{1}{Y_{X/\mathrm{Gal}}}\mu_{Glu}x_{23}\\
\mu &= \frac{\mathrm{Gal}_{int}}{K_0 + \mathrm{Gal}_{int}}\mu_{\mathrm{Gal}_{max}} + \frac{Glu_{ext}}{K_{Glu} + Glu_{ext}}\mu_{Glu_{ext}}\\
f_{1p} &= x_5^{0.5}\\
f_{2p} &= (x_6 + x_7)^{0.5}.
\end{aligned}
$$

Stability and multipliers

We now formally introduce the notation and the notion of stability; a detailed description of these notions is available in Willems (1971) and Desoer and Vidyasagar (1975). Let $(\mathbb{R}^+)\,\mathbb{R}$ denote the set of all (nonnegative) real numbers. Let $(\cdot)'$ (or $(\cdot)^T$) denote the transpose of a vector or a matrix $(\cdot)$. Let the inner-product $\langle x, y\rangle \doteq \int_{-\infty}^{\infty} y^T(t)x(t)dt$ and let the norm $\|x\| \doteq \sqrt{\langle x, x\rangle}$. The vector space $\mathcal{L}_2$ comprises all signals x for which $\|x\| < \infty$. The norm $\|z\|_1 \doteq \int_{-\infty}^{\infty} |z(t)|\, dt$. The Dirac delta function is denoted $\delta(\cdot)$. The time-truncation operator is denoted P_τ. In stability analysis, a given system $\mathcal{S}$ is often decomposed into two interconnected subsystems—a *linear time-invariant* (LTI) subsystem $\mathcal{S}_1$ in the feedforward path and an otherwise subsystem $\mathcal{S}_2$ in the feedback path. Stability of $\mathcal{S}$ is then deduced if there exists a quadratic functional that separates the graph of $\mathcal{S}_1$ from the inverse graph of $\mathcal{S}_2$ (see Safonov 1980). Certain classes of convolution operators, also called *stability multipliers* (see Safonov and Kulkarni 2000), specify such functionals. The larger the class of the stability multipliers, the lower the conservatism in the stability analysis (Megretski and Rantzer 1997). Stability multipliers for memoryless monotone nonlinearities are the Zames-Falb multipliers (1968) and their limiting cases include Popov multipliers (1962) and RL/RC multipliers (Cho and Narendra 1968). A key property of such a multiplier M is that it preserves the positivity of a memoryless monotone nonlinearity N in the sense that the positivity of N implies the positivity of MN. Well known examples of positivity preserving multipliers include the Popov multipliers and the Zames-Falb multipliers (see Safonov and Kulkarni 2000; Zames and Falb 1968; Willems 1971, Chapter 3).

Definition 1 A system $\mathcal{S}$ mapping $u \in \mathcal{L}_2$ into $y \in \mathcal{L}_2$ is said to be *finite gain stable* if there exists $\gamma \geq 0$ such that $\|\mathcal{S}(u)\| \leq \gamma\|u\|$ for all $u \in \mathcal{L}_2$.

Definition 2 The class $\mathcal{N}_M$ of *monotone nonlinearities* consists of all memoryless mappings $N : \mathbb{R}^n \mapsto \mathbb{R}^n$ such that: (i) N is the gradient of a convex real-valued function, and (ii) there exists $C \in \mathbb{R}^+$ s.t. $\|N(x)\| \leq C\|x\|\ \forall x \in \mathcal{L}_2$. The class $\mathcal{N} \doteq \{N \in \mathcal{N}_M | N(0) = 0\}$.

Definition 3 The class $\mathcal{M}_{ZF}$ of Zames-Falb multipliers denotes the class of convolution operators, either continuous-time or discrete-time, such that the impulse response of an $M \in \mathcal{M}_{ZF}$ is of the form

$$m(\cdot) = g\,\delta(\cdot) + h(\cdot) \quad \text{with} \quad \|h\|_1 < g,\ h(t) \leq 0\ \forall t,$$

where $g, h(\cdot) \in \mathbb{R}$.

Domain of attraction results to characterize the steady states

The ODE model derived in the previous section is of the form $\mathcal{S}_L : \dot{x} = Ax + \Phi(x) + Bu$, where A is a real-valued matrix of suitable size, Φ is a quadratic nonlinearity, and Bu is the forcing input. Note that a quadratic nonlinearity $\Phi(x)$ can be represented as $\Phi(x) = x^T N x$ where $N = [N_1 N_2 \ldots N_{25}]^T$, where N_i are real-valued matrices of suitable size. Literature on the stability analysis of such systems is sparse although sufficiency conditions have been established in Koditschek and Narendra (1983). In general, $\mathcal{L}_2$ stability cannot be expected of multistable models. We now first establish sufficiency conditions under which a polytope $\mathcal{P} \doteq \{\alpha_i x \leq 1 \mid i = 1, 2 \ldots, n+1\}$ belongs to the domain of attraction of the equilibrium point $x = 0$ given that the state feedback $u = Kx$ is used to control the galactose entering the cell. Let v_i denote the vertices of $\mathcal{P}$. The following result is well known (see Khalil 1992)).

Theorem 1 *Given a closed set $E \subset \mathbb{R}^n$ such that the equilibrium point x_o is contained in E, suppose the following conditions are satisfied: (i) E is an invariant set of the given system; and, (ii) a Lyapunov function $V(x)$ exists such that $V(x)$ is positive definite on E and, further, $\dot{V}(x)$ is negative definite along the trajectories of the given system in E. Then, E is an estimate of the domain of attraction of x_o.*

The above theorem can be specialized to our system as follows.

Theorem 2 *$\mathcal{P}$ is in the domain of attraction of an equilibrium point $x = 0$ of $\mathcal{S}_G$ if there exist scalars $\gamma \in (0, 1)$, $c > 0$, a symmetric positive definite matrix $P \in \mathbb{R}^{n \times n}$, and a matrix K such that*

$$\begin{bmatrix} 1 & \gamma \alpha_i^T P c \\ (\gamma \alpha_i^T P c)^T & Pc \end{bmatrix} \geq 0, \quad \text{and} \quad \begin{bmatrix} 1 & v_i^T \\ v_i & cP \end{bmatrix} \geq 0, \; i = 1, 2, \ldots, 25, \tag{4}$$

$$Herm\left(\gamma (A + BK)^T P + \begin{bmatrix} N_1^T v_i \\ N_2^T v_i \\ \vdots \\ N_5^T v_i \end{bmatrix} P \right) < 0, \; i = 1, 2, \ldots, 25, \tag{5}$$

where $Herm(\cdot)$ denotes the Hermitian of $(\cdot)$. The desired controller is given by $u = Kx$.

Proof Our proof uses the results derived in Amato et al. (2007, 2009), and can be sketched as follows. Let us consider the function $V(x) = x^T P^{-1} x$ as the candidate Lyapunov function. Since P is a symmetric positive definite matrix, $V(x)$ is positive definite. It needs to be shown that $\dot{V}(x)$ is negative definite along the system trajectories on $\mathcal{P}$. Observe that the inequality Eq. 5 holds not only for the vertices v_i but for all points x inside the scaled polytope $\widetilde{\mathcal{P}} \doteq 1/\gamma \mathcal{P}$ since the function on the left-hand side is an affine function of x. It can be observed that the left hand side of this inequality is $\dot{V}(x)$ along the trajectories of $\mathcal{S}_G$ so that $\dot{V}(x)$ is indeed a Lyapunov function for $\mathcal{S}_G$. We next show that the polytope $\widetilde{\mathcal{P}}$ contains a level curve of the chosen Lyapunov function. It is well known that the ellipsoid $\mathcal{E} \doteq \{x \in \mathbb{R}^{25} \mid x^T P^{-1} x \leq c\}$ contains the polytope $\mathcal{P}$ (see Boyd et al. 1994, pp. 69). Now, the polytope $\widetilde{\mathcal{P}}$ can be expressed as $\widetilde{\mathcal{P}} = \{x \in \mathbb{R}^{25} | \gamma a_i x \leq 1 \, i = 1, 2, \ldots, 25\}$. Now, using the Schur complement, the condition (4) can be re-written as $\gamma \, (a_i^T \, cP \, a_i) \, \gamma \leq 1 \; \forall \; i$. Hence, by Boyd et al. (1994, pp. 70), it follows that $\widetilde{\mathcal{P}}$ contains $\mathcal{E}$. Hence $V(x)$ is a Lyapunov function on $\mathcal{E}$. Further, the boundary of $\mathcal{E}$ is a level curve of $V(x)$ whence $\mathcal{E}$ is an invariant set. Hence, by Theorem 1, $\mathcal{E} \supset \mathcal{P}$ is an estimate of the domain of attraction. Hence the proof. □

Remark 1 Theorem 2 establishes a lower bound $\mathcal{P}$ on the domain of attraction of an equilibrium point and also yields a full-state feedback controller $u = Kx$ which asymptotically drives a state within $\mathcal{P}$ to the equilibrium point. The result applies only for the special case wherein the equilibrium point x_o is the origin, and can be extended to cover the case of other equilibrium points.

Remark 2 The domains of attraction of the equilibrium points have been experimentally reported as the regions of persistent and non-persistent memory in Acar et al. (2005). Theorem 2 characterizes the domain of attraction for the special case in which a linear time-invariant feedback from the expressed genes is used to control the input galactose.

If the objective is to control only GAL4 expression, as opposed to controlling *all* individual gene expression levels, the classical multiplier theory might provide a wide range of linear and nonlinear stabilizing controllers. We have experimentally observed that the GAL4 expression exhibits an aberration of monotone nonlinearity when the cell is excited with galactose; the expression is further inhibited in the presence of glucose. Some experimental set-ups require that the galactose be injected in a cell such that the GAL4 expression is regulated to a desired value. For these applications, a class of stabilizing controllers may be obtained as follows using the framework of Rantzer (2001). Let N denote this nonlinearity, and let Δ denote the dip in the nonlinearity curve. Let C be the controller to be designed. Then, feedback system Σ_R of interest is as follows: $y_1 = N(u_1)$, $u_1 = C(e_1)$, $e_1 = r - y_1$. Using Theorem 1 of Rantzer (2001), the following result is readily established.

Lemma 1 *Let $\mathcal{M}_R$ denote the class of convolution operators, either continuous-time or discrete-time, such that the impulse response of an $M \in \mathcal{M}_R$ is of the form*

$$m(\cdot) = (g + \Delta)\,\delta(\cdot) + h(\cdot) \quad \text{with} \quad \|h\|_1 < g,\ h(t) \leq 0\ \forall t,$$

where $g, h(\cdot) \in \mathbb{R}$. Then Σ_R is finite-gain stable if $C \in \mathcal{M}_R$.

Proof The proof follows as a ready consequence of [Rantzer 2001, Theorem 1]. This controller can be used to control the expression of GAL4. However, it cannot control the cellular memory since it cannot regulate the expression of other genes. □

Conclusions

We have derived an ODE model of the GAL regulatory network of *Saccharomyces cerevisiae*. We have shown that although the ODE model of Smidtas et al. (2006) gives an elegant explanation of the transient response of a subset of this network, it does not exhibit bistability, a key property of the GAL regulatory network. By including more chemical reactions in the approach of Smidtas et al. (2006), we have proposed a 25 state variable quadratic model of the GAL regulatory network. For this model, we have established sufficiency conditions for the domain of attraction of an equilibrium point for the special case of full-state feedback control. This result is useful in characterizing the persistence of cellular memory. Unlike the existing literature on GAL regulatory systems, our approach is not limited to 2 state-variables or 2 parameters; our LMI conditions scale well to address more state-variables and parameters, as is the case in the GAL regulatory system, and can be easily implemented in software.

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References

Acar M, Becksei A, van Oudenaarden A (2005) Enhancement of cellular memory by reducing stochastic transitions. Nature 435:228–232

Ajo-Franklin CM, Drubin DA, Eskin J, Gee E, Landgraf D, Philips I, Silver PA (2007) Rational design of memory in eukaryotic cells. Genes Dev 21:2271–2276

Alon U (1999) An introduction to systems biology: design principles of biological circuits. CRC Press, Chapman and Hall, Boca Raton, FL

Amato F, Cosentino C, Fiorilo A, Merola A (2009) Stabilization of bilinear systems via linear state-feedback control. IEEE Trans Circ Syst II Express Briefs 56(1):76–80

Amato F, Cosentino C, Merola A (2007) On the region of attraction for nonlinear quadratic systems. Automatica 43:2119–2123

Bhat P, Hopper J (1992) Overproduction of the GAL1 or GAL3 protein causes galactose-independent activation of the GAL4 protein: evidence for a new model of induction for the yeast GAL/MEL regulon. Mol Cell Biol 12(6):2701–2707

Biggar S, Crabtree G (2001) Cell signalling can detect either binary or graded transcriptional responses. EMBO J 20:3167–3176

Boyd S, Ghaoui LE, Feron E, Balakrishnan V (1994) Linear matrix inequalities in system and control theory. SIAM Press, New York

Burrill DR, Silver PA (2010) Making cellular memories. Cell 140(1):13–18

Cho Y, Narendra K (1968) An off-axis circle criterion for the stability of feedback systems with a monotonic nonlinearity. IEEE Trans Autom Control 13:413–416

Desoer C, Vidyasagar M (1975) Feedback systems: input-output properties. Academic Press, New York

Dong J, Dickinson R (1997) Glucose represses the lactos-galactose regulon in Kluyveromyces lactis through a SNF-1 and MIG1-dependent pathway that modulates galactokinase (GAL1) gene expression. Nucleic Acids Res 25(18):3657–3664

Giniger E, Varnum S, Ptashne M (1985) Specific DNA binding of GAL4, a positive regulatory protein of yeast. Cell 40(4):767–774

Hawkins KM, Smolke CD (2006) The regulatory roles of the galactose permease and kinase in the induction response of the GAL network in saccharomyces cerevisiea. J Biol Chem 281(19):13485–13492

Ideker T, Thorsson V, Ranish J, Christmas R, Buhler J, Eng J, Bumgarner R, Goodlett D, Aebersold R, Hood L (2001) Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science 292:929–934

Johnston M (1987) A model fungal gene regulatory mechanism: the GAL genes of Saccharomyces cerevisiae. Microbiol Rev 51(4):458–476

Johnston M, Flick J, Pexton T (1994) Multiple mechanisms provide rapid and stringent glucose repression of GAL gene expression in Saccharomyces cerevisiae. Mol Cell Biol 14(6):3834–3841

Khalil H (1992) Nonlinear systems. McMillan Press, New York

Koditschek D, Narendra K (1983) Stabilizability of second-order bilinear systems. IEEE Trans Autom Control 28(10):987–989

Lohr D, Venkov P, Zlatanova J (1995) Transcriptional regulation in the yeast GAL gene family: a complex genetic network. FASEB J 9:777–787

Materassi D, Salapaka MV (2009) Attraction domain estimates combining lyapunov functions. In: Proceedings of the American control conference. St. Louis, Missouri

Megretski A, Rantzer A (1997) System analysis via integral quadratic constraints. IEEE Trans Autom Control AC-42(6):819–830

Monod J, Jacob F (1961) Making cellular memories. Cold Spring Harbor Symposium on Quantitative Biology 26:389–401

Platt A, Reece R (1998) The yeast galactose genetic switch is mediated by the formation of a Gal4p-Gal80p-Gal3p complex. EMBO J 17(14):4086–4091

Platt A, Ross H, Hankin S, Reece R (2000) The insertion of two amino acids into a transcriptional inducer converts it into a galactokinase. PNAS 97(7):3154–3159

Popov V (1962) Absolute stability of nonlinear systems of automatic control. Autom Remote Control 3: 857–875 (Russian original published in 1961)

Rantzer A (2001) Friction analysis based on integral quadratic constraints. Int J Robust Nonlinear Control 11(10/11):645–652

Ruhela A, Verma M, Edwards JS, Bhat PJ, Bhatiya S, Venkatesh KV (2004) Autoregulation of regulatory proteins is key for dynamic operation of GAL switch in saccharomyces cerevisiea. FEBS Lett 576:119–126

Safonov M (1980) Stability and robustness of multivariable feedback systems. The MIT Press, Cambridge

Safonov M, Kulkarni V (2000) Zames-Falb multipliers for MIMO nonlinearities. Int J Robust Nonlinear Control 10(11/12): 1025–1038

Smidtas S, Schachter V, Kepes S (2006) The adaptive filter of the yeast galactose pathway. J Theor Biol 242:372–381

Willems J (1971) The analysis of feedback systems. The MIT Press, Cambridge

Yano K, Fukasawa T (2008) Galactose-dependent reversible interaction of Gal3p with Gal80p in the induction pathway of Gal4p-activated genes of Saccharomyces cerevisiae. PNAS 94(5): 1721–1726

Zames G, Falb P (1968) Stability conditions for systems with monotone and slope-restricted nonlinearities. SIAM J Control Optim 6(1):89–108

Zenke F, Engels R, Vollenbroich V, Meyer J, Hollenberg C, Breunig K (1996) Activation of Gal4p by galactose-dependent interaction of galactokinase and Gal80p. Science 272(5268):1662–1665