Is the regulation of galactose 1-phosphate tuned against gene expression noise?

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The average number of mRNA molecules per active gene in yeast can be remarkably low. Consequently, the relative number of copies of each transcript per cell can vary greatly from moment to moment. When these transcripts are encoding metabolic enzymes, how do the resulting variations in enzyme concentrations affect the regulation of metabolic intermediates? Using a kinetic model of galactose utilization in yeast, we analysed the transmission of noise from transcription and translation on metabolic intermediate

regulation. In particular, the effect of the kinetic properties of the galactose-1-phosphate uridylyltransferase reaction on the transmission of noise was analysed.

Key words: galactose-1-phosphate uridylyltransferase, galactose uptake, gene expression, metabolic pathway, noise transmission, *Saccharomyces cerevisiae*.

INTRODUCTION

The cellular translation machinery allows the synthesis of large quantities of proteins [1] from a small number of transcripts [2]. The obvious consequence of such a small number of transcripts is that stochastic variations in this number can potentially translate into large oscillations in protein concentrations. Is this compatible with cellular homeostasis? It has been suggested that noise in gene expression is a biologically important variable, is generally detrimental to organismal fitness, and is selected against [3]. Galactose uptake in the yeast *Saccharomyces cerevisiae* has been described extensively at the metabolic and genetic levels [4–8], and provides an attractive opportunity to analyse how these two levels integrate. It also provides an example of a metabolic intermediate that needs to be maintained at low levels (see below). Evolutionary forces may have optimized network structures [9–11]. We recently analysed how the structure of the genetic network for galactose uptake is affecting the transmission of intrinsic noise in yeast [12]. In the present paper, we describe how the kinetic properties of enzymes can also provide robustness against intrinsic noise in yeast.

Genetic regulatory interactions control switches that allow yeast cells to adapt to changing environments. Galactose uptake begins with the entry of galactose into the cell through a galactose-inducible transport process that is dependent on the protein Gal2p encoded by the gene *GAL2*, followed by the conversion of galactose into glucose 1-phosphate through the Leloir pathway (reviewed in [4,5]). This requires the action of three galactose-inducible enzymes, GK (galactokinase; EC 2.7.1.6), GT (galactose-1-phosphate uridylyltransferase; EC 2.7.7.12) and EP (UDP-galactose 4-epimerase; EC 5.1.3.2). These correspond to the proteins Gal1p, Gal7p and Gal10p encoded by the genes *GAL1*, *GAL7* and *GAL10* respectively. The system can be seen in Scheme 1.

The galactose transporter and the three enzymes that metabolize galactose are encoded as part of the *GAL* regulon. The *GAL* regulon contains the genes that encode the transporter and the three enzymes, together with other genes that include *MEL1*, for

external α -galactosidase activity, and *GAL3* and *GAL80*, which encode two proteins, Gal3p and Gal80p, that are involved in the regulation of the complete regulon.

The induction of the *GAL* genes is subject to a dual regulation, where synthesis is induced by galactose, but is repressed by glucose (reviewed in [6–8]). Induction is co-ordinately mediated by the action of one common activator, Gal4p, a common repressor, Gal80p, and two common releasers of the repressor, Gal3p and Gal1p, which are activated in the presence of galactose. Glucose repression includes specific proteolysis of Gal2p [13] and transcriptional repression of the *GAL* genes.

The co-ordinated expression of *GAL* genes allows the entry of carbon, but it can have a secondary consequence in the form of accumulation of metabolic intermediates. These levels are a consequence of the combined effect of the enzyme concentrations (dependent on the gene induction) and also of the kinetic properties of the enzymes, their affinities for substrates and catalytic constants. GA_{1P} (galactose 1-phosphate) is the product of GK and the substrate of GT, and an example of an intermediate that needs to be maintained at low levels. An accumulation of GA_{1P} (e.g. deficiency in GT) causes an inability to grow in galactose conditions [14]. In humans, this deficiency produces the most common form of galactosaemia [15]. The mechanism of GA_{1P} toxicity remains unclear; however, through experiments, in part in yeast, it has been suggested that accumulated GA_{1P} inhibits the normal function of key enzymes, such as UDP-glucose pyrophosphorylase, inositol monophosphatase or phosphoglucosyltransferase (discussed in [14,16–19]). Lai et al. [19] have found in human fibroblasts that intracellular concentrations of GA_{1P} , as found in classic galactosaemia, inhibit competitively the UDP-glucose pyrophosphorylase, and reduce the intracellular concentrations of U_{GL} (UDP-glucose) and U_{GA} (UDP-galactose), potentially affecting membrane-bound glycoproteins and glycolipids.

The level of mRNA per cell for GK has been measured as 33 mRNA per cell by Iyer and Struhl [20] at saturation levels of GA_E (external galactose). For lower levels of galactose, lower mRNA levels can be expected, as Biggar and Crabtree [21] have shown graded changes in *GAL1* promoter activity.

Abbreviations used: EP, UDP-galactose 4-epimerase; GA_E , external galactose; GA_I , internal galactose; GA_{1P} , galactose 1-phosphate; GK, galactokinase; GL_{1P} , glucose 1-phosphate; GT, galactose-1-phosphate uridylyltransferase; MCA, metabolic control analysis; ODE, ordinary differential equation; U_{GA} , UDP-galactose; U_{GL} , UDP-glucose.

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Table 2 Parameter values

Using i as an index, τ_{50,R_i} is the half life of mRNA of gene *GALI*. For conversion from molecules per cell (m/c) into mM, we assume 15×10^{-12} g of cell dry weight per yeast haploid cell [50] and 2.38 ml of cell volume per g of cell dry weight [18].

Parameter	Value	Units	Note
V_{TR}	$K_{m(TR,GA)}$	1	mM
	α	1	Value for the inducible high-affinity transport from [29]
	k	4350	min ⁻¹
V_{GK}	$k_{cat}(GK)$	3350	min ⁻¹
	$K_{m(GK,GA)}$	0.6	mM
	K_{IJ}	19.1	mM
	K_{IC}	160	mM
V_{GT}	$k_{cat}(GT)$	59200	min ⁻¹
	$K_{m(GT,GA_{1P})}$	4.0	mM
	$K_{m(GT,U_{GL})}$	0.26	mM
V_{EP}	$k_{cat}(EP)$	40×3890	min ⁻¹
	$K_{m(EP,U_{GA})}$	0.22	mM
	K_{BQ}	3.5	—
	$K_{m(EP,U_{GL})}$	0.25	mM
F	Variable	—	In <i>Vicia faba</i> [48]
k_{S,R_2}	0.52	m/c · min ⁻¹	Fractional saturation
$k_{S,R_{1,7,10}}$	1.09	m/c · min ⁻¹	$k_{S,R_2} = (k_{D,R_2} + k_{dil}) \cdot R_2/F$
k_{D,R_2}	0.014	min ⁻¹	$k_{S,R_{1,7,10}} = (k_{D,R_{1,7,10}} + k_{dil}) \cdot R_{1,7,10}/F$
$k_{D,R_{1,7,10}}$	0.032	min ⁻¹	$k_{D,R_2} = (\ln 2)/\tau_{50,R_2}$
k_{S,G_2}	6.94	min ⁻¹	$k_{D,R_{1,7,10}} = (\ln 2)/\tau_{50,R_{1,7,10}}$
$k_{S,G_{1,7,10}}$	9.92	min ⁻¹	$k_{S,G_2} = (k_{D,G_2} + k_{dil}) \cdot G_2/R_2$
$k_{D,G_{2,1,7,10}}$	0.022	h ⁻¹	$k_{S,G_{1,7,10}} = (k_{D,G_{1,7,10}} + k_{dil}) \cdot G_{1,7,10}/R_{1,7,10}$
k_{dil}	0.097	h ⁻¹	Average for 50 proteins [39]
Values used only for the deduction of values above:			
τ_{50,R_2}	49	min	Poly(A) half-life estimates are from supplementary material of [49] (the value for R_1 , R_7 and R_{10} is the average of values reported)
$\tau_{50,R_{1,7,10}}$	22	min	[20]
R_1	33 ($F = 1$)	m/c	[25]
$R_{2,7,10}$	R_1	m/c	
G_2/R_2	3500	—	Approximate average protein/mRNA molecule for "Transport facilitation" and "Metabolism" functional categories (see supplementary material of [1])
$G_{1,7,10}/R_{1,7,10}$	5000	—	

Table 3 Steady-state values at 10% and 100% fractional saturation levels at 0.5 mM GA_E

For conversion from μ mol or mmol/g of dry weight into mM, we assume 2.38 ml of cell volume per g of dry weight as used by Ostergaard et al. [18] (*), and 2.7 ml of cell volume per g of dry weight as suggested by Daran et al. [28] (†). It should be noted that our model gives a value of 0.26 mM for GA_{1P} at $F = 1$ and 2% GA_E , in agreement with the 0.3 mM from Daran et al. [28] (‡), and the flux of galactose uptake (J) reported by Ostergaard et al. [18] probably reflects the fact that cells were previously pre-incubated in conditions of full induction, and they were then allowed to deplete the GA_E concentration to 0.47 mM, resulting in a higher flux (this situation would correspond to $F = 1$) (§).

	F		Reference	
	0.1	1	Converted	Original
GA_{1P} (mM)	0.09	0.09	0.09*	0.21 μ mol/g of dry weight [18]
			0.3†‡	0.81 μ mol/g of dry weight (2% GA_E) [28]
U_{GL} (mM)	0.82	0.82	0.82†	2.21 μ mol/g of dry weight (2% GA_E) [28]
U_{GA} (mM)	0.24	0.24	0.24†	0.65 μ mol/g of dry weight (2% GA_E) [28]
J (mM/min)	0.5	5	7.4*§	1.06 mmol/(g of dry weight · h) [18]
U_{GL}/U_{GA}	3.4	3.4	3.4	(2% GA_E) [28]
			3.9	(0.1% GA_E in human fibroblast) [19]

a maximum attained at an initial GA_E concentration of between 1 and 3 g/l. The value of 33 transcripts of *GALI* per cell reported by Iyer and Struhl [20] was under full induction, and this level of induction should be similar for *GAL2*, 7 and 10, as observed when comparing results from microarray experiments of steady-state induction (galactose) with those of repression (glucose)

[25]. Taking all these results together, in the present paper, full induction is assumed to result in 33 *GAL2*, 1, 7 or 10 mRNA molecules per cell, and low (10%) induction is assumed to produce 3.3 molecules per cell. Our simulations compute the mRNA and protein concentrations within a single cell over several generations of asexual cell division at low induction.

MCA (metabolic control analysis) was applied to the analysis of steady-state sensitivities (see [26,27] for a review). These are divided into system and local sensitivities. The latter denote the sensitivity of an isolated reaction step (an enzyme or a block of reactions) and are called elasticities:

$$\varepsilon_x^{v_i} = (\partial \log v_i / \partial \log x)_{ss} \quad (1)$$

where v_i refers to the rate of the reaction i , x refers to a variable that affects it, and ss refers to the steady state. System sensitivities are divided into control coefficients and response coefficients. Control coefficients can be defined as the sensitivity of a dependent variable Y (concentration, flux) with respect to the concentration of one enzyme, or more generally, with respect to the activity of a reaction step i :

$$C_i^Y = (d \log Y / d \log v_i)_{ss} \quad (2)$$

A response coefficient refers to a system sensitivity with respect to a perturbation of any parameter. When the parameter p acts only on the reaction step i , the response coefficient can be expressed

Figure 1 Model sensitivity with respect to GA_{1P}

The curves show the steady-state value of GA_{1P} for ± 10 -fold changes of all transporter and enzyme activities, and the only parameters of the metabolic pathway model [$K_m(GT,GA_{1P})$ and GA_E] that can produce a change in GA_{1P} greater than 0.2 mM. The steady state corresponds to $GA_E = 0.5$ mM.

as:

$$R_p^Y = C_i^Y \cdot \varepsilon_i^{v_i} \quad (3)$$

System characteristics that the model reproduces

(1) The kinetic parameters of this model were adjusted to result in a steady state closely matching the values provided by Ostergaard et al. [18] for GA_{1P} , and by Daran et al. [28] for U_{GL} and U_{GA} and the ratio U_{GL}/U_{GA} .

(2) Ostergaard et al. [18] analysed the steady state in a chemostat continuous culture at 0.47 mM GA_E . The value for GA_{1P} was adjusted by changing the value of the parameter k for the transporter. The resulting value for the parameter k provides a V_{max} close to that published by Reifengerger et al. [29] for inducible high-affinity transport.

(3) $U_{GL} + U_{GA}$ is a conserved quantity in the model, and can be fixed to that published by Daran et al. [28]. The equilibrium constant for the EP reaction was measured from purified samples by Fukasawa et al. [30], $K_{eq} = U_{GL}/U_{GA} = 3.5$, which agrees closely with the ratio $U_{GL}/U_{GA} = 3.4$, as measured by Daran et al. [28] from cell samples. We adjusted the value of $k_{cat(EP)}$ to reproduce the reported U_{GL}/U_{GA} ratio.

(4) Schell and Wilson [31], and Timson and Reece [32] have reported product inhibition for GK at high concentrations of GA_{1P} . This product inhibition has been included in the model from the data provided by Timson and Reece [32], who analysed the GK reaction mechanism, and showed that GA_{1P} is a mixed inhibitor in the GK reaction (see Tables 1 and 2 for details).

(5) To simulate low induction (10%), we set the fractional saturation regulating transcription (parameter F in Tables 1 and 2) to 0.1, and GA_E to 0.5 mM ($F = 1$ for full induction). The value of 0.5 mM GA_E for low induction was selected because Li et al. [24] have reported gene expression rates of 10% at around 0.1 g/l GA_E .

(6) The model was validated by analysing the dependence of GA_{1P} on all parameters of the transporter and enzymic parts of the model. Each parameter was analysed in a range from 0.1- to 10-fold change, and the most significant are plotted in Figure 1. The activities can be changed by adjusting the concentration of transporter or enzymes, or through changes of catalytic constants. The simple nature of the metabolic pathway results in a significant

dependence on the transporter activity and the enzymic activities for GK and GT (discussed below) and on the K_m of GT for GA_{1P} [$K_m(GT,GA_{1P})$]. This last point is not surprising considering that GA_{1P} is the substrate of the enzyme encoded by *GAL7*.

System characteristics not included in the model

(1) Galactose transport can be divided into three components [29,33]: two galactose-inducible transport systems, a high-affinity process and a low-affinity process, both dependent on Gal2p; and one residual constitutive low-affinity transport system, independent of Gal2p. The mechanism of this inducible two-component transport mechanism is unknown, and we are assuming only the high-affinity component.

(2) GK is described by an irreversible reaction dependent only on GA_i (internal galactose). The only available information refers to the forward reaction. However, a scenario of reversibility is implausible because it would imply that the Leloir pathway could reverse-metabolize GL_{1P} (glucose 1-phosphate) into GA_i , which would induce the GAL regulon in the absence of GA_E .

(3) GK and GT catalyse bi-substrate reactions, but the K_m used in the model corresponds to an apparent K_m obtained by fixing the concentration of the other substrate [31,34]. The effect of variations in these K_m s is negligible with respect to GA_{1P} accumulation, with the exception of the K_m of GT for GA_{1P} (Figure 1).

(4) EP is a bifunctional enzyme with aldolase 1-epimerase (mutarotase) activity [35], not modelled here.

(5) U_{GL} has a strategic position in yeast metabolism, and, as for GA_{1P} , its regulation appears to be important. It is fundamental for the synthesis of the cell wall, the synthesis of glycogen and trehalose, N-glycosidation of proteins, and other roles (see [28]). Among the enzyme activities that affect U_{GL} is that of UDP-glucose pyrophosphorylase, where GL_{1P} with UTP is transformed into U_{GL} and PP_i by the action of UDP-glucose pyrophosphorylase. Also, UDP-glucose pyrophosphorylase has been shown to be a bifunctional enzyme [17,19], which catalyses the conversion of GA_{1P} into U_{GA} , although its activity is not relevant under physiological conditions [19]. All these transformations affecting U_{GL} are not included in the model. The reaction catalysed by EP appears to be enough to reproduce the ratio U_{GL}/U_{GA} measured by Daran et al. [28] (discussed above).

(6) We assumed that induction in continuous chemostat culture results in a steady-state response, as assumed by Ostergaard et al. [18]. However, Braun and Brenner [36] have reported recently that, over several generations, the system converges to a single robust steady state, independent of the carbon source. The steady state we refer to should therefore be considered meta-stable on long time scales.

(7) Yeast growth involves relatively fast asexual cell division by budding [37]. The way that the induced state is transmitted to the daughter cells could impose fluctuations in the steady state in a continuous culture, and is not addressed by our model.

(8) Based on the experimental evidence of Gal7p subcellular localization, which requires co-expression of Gal1p and Gal10p, Christacos et al. [38] have suggested that substrate/product channelling or other interactions could alter the kinetics of the enzymes.

(9) mRNA and protein turnover are modelled with first-order degradation. Both decays depend on two rate constants, the cell volume dilution rate constant (k_{dil}) and the intrinsic decay constant ($k_{D,R}$ or $k_{D,G}$). The growing machinery of yeast, a mixture of cell growing and cell division by budding [37], is responsible for cell volume dilution. We adopted, as an estimate of the cell volume dilution rate, the dilution rate of 0.097 h^{-1} reported for the chemostat steady-state culture of Ostergaard et al. [18].

Figure 2 Transmission of noise to GA_{1P}

(a) Protein time courses. (b) GA_{1P} time course. The 2000 min time course shown corresponds to the period with the highest transmission in noise to GA_{1P} in a total time course simulation of 30 000 min. The values in parentheses correspond to the coefficients of variation (S.D./mean) estimated from the total time course. These are in broad agreement with recent results reported in [22].

(10) Protein degradation and the ratio of protein/mRNA are based on general measures in *S. cerevisiae*, not specific for *GAL* products [1,39].

(11) We did not consider differences between mRNA and protein expression [40] owing to a lack of pertinent data.

RESULTS AND DISCUSSION

Transmission of transcriptional noise

Figure 2 illustrates transmission of noise from mRNA and protein concentrations to the level of GA_{1P} under low induction. However, this transmission does not produce significant variations in the level of GA_{1P} , and we can conclude that the system is robust. The transmission of noise depends on the relative time scales affecting the components of the network. The turnover of mRNA, proteins and metabolic intermediaries represent three different time scales. Inspection of rate constants in Table 2 provides an idea of the difference between mRNA and proteins. The time scale for proteins to return to steady state after perturbations is clearly larger than that of the other species. A longer time scale for turnover implies a longer time to steady state after a perturbation. The consequence of this is that the effect of noise in mRNA concentrations, with apparent oscillations with short periods, is smoothed into small oscillations of longer period. In Figure 2(b), the steady-state concentration of GA_{1P} can be estimated at each time point using the corresponding transporter and enzyme concentrations at this point. Interestingly, this steady-state prediction closely matches the dynamic time course plotted for GA_{1P} . This shows that the large periods of the apparent

Figure 3 Supply and demand analysis

Reaction rates of the supply block (v_{supply}) against GA_{1P} , and the demand block (v_{demand}) against GA_{1P} . Broken lines correspond to increases or decreases in activities for supply or demand. The intersections of the two sets of curves correspond to steady-state flux and GA_{1P} levels, and are represented with numbered points (see text for details). (a) Default model. (b) Modified model with a 20-fold increase of the ratio $GA_{1P}/K_{m(GT,GA_{1P})}$.

oscillations that affect the levels of proteins are larger than the time that GA_{1P} needs to change its steady state.

GA_{1P} regulation

The fact that the apparent oscillations in the protein levels are much slower than the time that GA_{1P} needs to change its steady state simplifies the analysis of noise transmission to the analysis of the steady-state regulation of GA_{1P} . In order to analyse the steady-state regulation of a particular intermediate, a multi-step system can be simplified to a simple system with a supply block, the intermediate, and a demand block [41]. In Scheme 1(b) (galactose uptake), we show the supply and demand system around the GA_{1P} intermediate as a simplification of the entire pathway in Scheme 1(a). In Figure 3, we apply a visual and quantitative analysis [41] to describe how the properties of supply and demand blocks control the steady-state flux, and contribute to the regulation of the intermediate, here GA_{1P} . The six possible changes that we can introduce by changing the activities of supply and/or demand blocks are presented, where the numbered point 1 corresponds to the original steady state, and the others refer to the possible changes. The points in Figure 3 refer to the following situations: (1) reference steady state at 0.5 mM GA_E and 10% induction ($F = 0.1$), (2) proportional increase in the activities of both supply and demand blocks, (3) proportional decrease in the activities of both supply and demand, (4) increase in supply activity alone, (5) decrease in supply activity alone, (6) increase in demand activity alone, and (7) decrease in demand activity alone. Scenarios (2) and (3) correspond to co-ordinate regulation (constant ratio of supply and demand), while scenarios (4)–(7) correspond to changing the supply/demand ratio. In scenario (2), the co-ordinate expression of structural genes during galactose induction is in keeping with the observation that a co-ordinate

Table 4 Numerical evaluation of the components of eqns 6 and 7

	Whole system	Demand block alone		Elasticities	
	C_{GT}^J	$C_{GT}^{J_{demand}}$	$R_{GA_{1P}}^{U_{GL}}$	$\varepsilon_{GA_{1P}}^{v_{GT}}$	$\varepsilon_{U_{GL}}^{v_{GT}}$
Default model	0.00	1.00	-0.01	0.97	0.01
Model with 20-fold increased $GA_{1P}/K_{m(GT,GA_{1P})}$	0.00	1.00	-0.01	0.63	0.09

increase in the activity of all the enzymes of the pathway allows an increase of flux without affecting the level of intermediates [42,43]. In scenario (5), the stronger repression of supply by glucose, through the proteolysis of Gal2p helps to ensure against transient increases in GA_{1P} . The simultaneous change in absolute values in scenario (2) affects the flux, but not GA_{1P} levels, while the relative change in scenario (5) affects both flux and GA_{1P} . These observations are a consequence of the summation theorems of MCA (see [26,27] for a review):

$$C_{supply}^J + C_{demand}^J = 1 \quad \text{and} \quad C_{supply}^{GA_{1P}} + C_{demand}^{GA_{1P}} = 0 \quad (4)$$

Thus the degree of transmission of noise depends on the relative activity of supply and demand blocks, and not on their absolute value.

How do the kinetic parameters of GT affect noise transmission?

A formalization of the relationships in Figure 3 for infinitesimal changes can be easily derived in terms of elasticities and control coefficients [41]:

$$C_{supply}^{GA_{1P}} = -C_{demand}^{GA_{1P}} = 1/(\varepsilon_{GA_{1P}}^{v_{demand}} - \varepsilon_{GA_{1P}}^{v_{supply}}) \quad (5)$$

The slope of the curve at each (numbered) steady state in Figure 3 corresponds to the elasticity at that point. The slope of the demand curve is positive, i.e. $\varepsilon_{GA_{1P}}^{v_{demand}} > 0$. For the supply block, the horizontal line for supply in Figure 3 corresponds to an elasticity $\varepsilon_{GA_{1P}}^{v_{supply}} = 0$. This is a consequence of both the assumption for GK of irreversibility and the fact that the effect of the inclusion of product inhibition is negligible.

Figure 1 shows that the concentration of GA_{1P} depends mainly on the kinetic properties of GT. We can explore this fact algebraically in terms of MCA. If we analyse the demand block as a separate system, the block elasticity $\varepsilon_{GA_{1P}}^{v_{demand}}$ of the whole system corresponds to the response coefficient of the flux J through the demand block with respect to GA_{1P} ($R_{GA_{1P}}^{J_{demand}}$). This response coefficient can be described by either of two expressions (see the Appendix for derivation):

$$R_{GA_{1P}}^{J_{demand}} = C_{GT}^{J_{demand}} \cdot \varepsilon_{GA_{1P}}^{v_{GT}} \quad (6)$$

$$R_{GA_{1P}}^{J_{demand}} = \varepsilon_{GA_{1P}}^{v_{GT}} + \varepsilon_{U_{GL}}^{v_{GT}} \cdot R_{GA_{1P}}^{U_{GL}} \quad (7)$$

Numerical analysis of the values of the components of eqns 6 and 7 (Table 4) reveals that the only effective contributor to the value of $R_{GA_{1P}}^{J_{demand}}$ is the elasticity $\varepsilon_{GA_{1P}}^{v_{GT}}$. Thus all flux control of the demand block is in GT (eqn 6), and the contribution of U_{GL} variability is negligible (eqn 7). The elasticity $\varepsilon_{GA_{1P}}^{v_{GT}}$ is described by the following expression:

$$\varepsilon_{GA_{1P}}^{v_{GT}} = \frac{1}{1 + \frac{GA_{1P}}{K_{m(GT,GA_{1P})}} \left(1 + \frac{K_{m(GT,U_{GL})}}{U_{GL}}\right)} \quad (8)$$

From this expression, we can see that the elasticity increases as the ratios $GA_{1P}/K_{m(GT,GA_{1P})}$ or $K_{m(GT,U_{GL})}/U_{GL}$ decrease, and vice versa, with a theoretical maximum value of one. Interestingly, the

situation predicted by the model is close to the maximum value of 1 (Table 4). The resulting control coefficients ($C_{supply}^{GA_{1P}}$ and $C_{demand}^{GA_{1P}}$) are both close to the minimum absolute value of 1, according to eqn 5.

We can analyse how the transmission of noise to GA_{1P} concentration is affected by the kinetic parameters of GT. However, it is not possible to change the value for a parameter without changing the steady state. At the steady state, an equality involving the flux (J) on one side, and the equation describing v_{GT} (see Table 1) on the other, constrains the values of V_{maxGT} , $K_{m(GT,GA_{1P})}$ and $K_{m(GT,U_{GL})}$. This constraint can be written by grouping the ratios involving substrates and K_m as:

$$J = V_{maxGT} \cdot \frac{1}{\left(1 + \frac{GA_{1P}}{K_{m(GT,GA_{1P})}}\right) + \frac{K_{m(GT,U_{GL})}}{U_{GL}} + 1} \quad (9)$$

If we want to maintain the reference steady state deduced from the bibliography, a decrease or increase in one parameter needs to be compensated for by a change in other parameters.

If we want to change the value for the ratio $GA_{1P}/K_{m(GT,GA_{1P})}$ without changing the steady state for GA_{1P} , it is necessary to impose a change on one of the other parameters in eqn 9. For example, a 20-fold increase of the ratio $GA_{1P}/K_{m(GT,GA_{1P})}$ can be achieved by decreasing the $K_{m(GT,GA_{1P})}$ to a value of 0.2 mM and the $k_{cat(GT)}$ to 4600 min⁻¹. All steady-state values for flux and intermediates are maintained, but at the cost of changes in the elasticities and control coefficients (see eqns 5–8). For the preceding example, $\varepsilon_{GA_{1P}}^{v_{demand}} \approx 0.6$ and $C_{supply}^{GA_{1P}} = -C_{demand}^{GA_{1P}} \approx 1.6$. (Table 4). The consequence is higher transmission of noise to GA_{1P} , as shown graphically in Figure 2. Figure 3(b) shows why the sensitivity to the transmission of noise to GA_{1P} is increased.

More complex scenarios

The scenario analysed is the most simple, with the first block being described by an irreversible process with negligible product inhibition, and the second block being described by an equation showing a hyperbolic curve with respect to GA_{1P} . In this scenario, $\varepsilon_{GA_{1P}}^{v_{supply}} = 0$ and $\varepsilon_{GA_{1P}}^{v_{demand}} \approx 1$ (Table 4). Optimal homeostasis is provided by maximizing the value of the denominator of eqn 5 [44]. Thus more complex scenarios can be modelled with more optimal reductions of noise transmission as follows.

Supply block

The consequence of the assumption of irreversibility and negligible product inhibition for GK is that $\varepsilon_{GA_{1P}}^{v_{supply}} = 0$ and that all control in the flux is in the supply block, as is evident from Figure 3. As noted by Hofmeyr and Cornish-Bowden [41], when flux is controlled by one block, the other block determines the degree to which concentration of the linking metabolite is homeostatically maintained. In other words, all the sensitivity of GA_{1P} depends on $\varepsilon_{GA_{1P}}^{v_{demand}}$ only. $\varepsilon_{GA_{1P}}^{v_{supply}}$ would be lower than 0 if either the reverse reaction for GK or product inhibition for GK were not negligible. It is interesting to note that, under such conditions, noise transmission would be reduced further, as the absolute value for $C_{supply}^{GA_{1P}}$ and $C_{demand}^{GA_{1P}}$ would be lower.

Demand block

Eqns 6 and 7 show the contribution of the elasticity $\varepsilon_{GA_{1P}}^{v_{demand}}$ to the block elasticity $\varepsilon_{GA_{1P}}^{v_{GT}}$. For $\varepsilon_{GA_{1P}}^{v_{GT}}$, more optimal situations could be reached: for example, a demand block reaction rate following co-operative kinetics with respect to its substrate. In this case

$\varepsilon_{GA_{1P}}^{demand}$ could be higher than 1. The other elements contributing to the value of $\varepsilon_{GA_{1P}}^{demand}$ in eqns 6 and 7 can change according to U_{GL} regulation. Our model does not include the UDP-glucose pyrophosphorylase and other enzyme activities that affect U_{GL} . The activities of these reactions could produce a change in the value of eqns 6 and 7, potentially with worse noise transmission.

Is a very low level of induction possible?

Our simulations are based on a model that only tries to reproduce qualitatively the regulation of GA_{1P} , and we cannot establish the exact level of GA_{1P} that is toxic. However, there are measurements that allow estimation of the approximate GA_{1P} concentration values that could be toxic. Lai et al. [19] analysed galactose toxicity in human fibroblasts, and reported that a GA_{1P} concentration of 2.5 mM increases the apparent K_m of purified UDP-glucose pyrophosphorylase for GL_{1P} from 19.7 μ M to 169 μ M. Also, they reported a concentration of GA_{1P} of 5.2 mM for GT-deficient cells. From our analysis, we can speculate that incrementally reducing the level of induction below 10%, one reaches a threshold beyond which intrinsic mRNA noise would translate to large GA_{1P} oscillations. However, this situation is probably never reached. Multiple binding sites for the common activator Gal4p in all the genes for transporter and the enzymes could result in strong repression by Gal80p under non-induction that could also be effective at very low induction.

APPENDIX

Derivation of eqn 6

GA_{1P} acts only through GT and no other enzymes in the system, thus eqn 6 is a particular case of eqn 3. Note that the flux control coefficient for the demand block alone ($C_{GT}^{J_{demand}}$) and the flux control coefficient for the whole system (C_{GT}^J) are different (see Table 4).

Derivation of eqn 7

Provided that the reaction rate through the demand block (v_{demand}) corresponds to the reaction rate through GT (v_{GT} , which depends on GA_{1P} and U_{GL}), and that U_{GL} depends on GA_{1P} through the action of EP, then an expression for v_{demand} can be written as:

$$v_{demand} = v_{GT}(GA_{1P}, U_{GL}[GA_{1P}]) \quad (A1)$$

In the context of a separate block demand, GA_{1P} is an independent variable, and U_{GL} is a dependent variable. By application of the chain rule, $R_{GA_{1P}}^{J_{demand}}$ can be derived:

$$\begin{aligned} R_{GA_{1P}}^{J_{demand}} &= \left(\frac{d \log v_{demand}}{d \log GA_{1P}} \right)_{ss} = \left(\frac{dv_{demand}}{dGA_{1P}} \right)_{ss} \cdot \left(\frac{GA_{1P}}{v_{demand}} \right)_{ss} \\ &= \left(\frac{\partial v_{GT}}{\partial GA_{1P}} + \frac{\partial v_{GT}}{\partial U_{GL}} \cdot \frac{dU_{GL}}{dGA_{1P}} \right)_{ss} \cdot \left(\frac{GA_{1P}}{v_{GT}} \right)_{ss} \\ &= \left(\frac{\partial \log v_{GT}}{\partial \log GA_{1P}} \right)_{ss} + \left(\frac{\partial \log v_{GT}}{\partial \log U_{GL}} \right)_{ss} \cdot \left(\frac{d \log U_{GL}}{d \log GA_{1P}} \right)_{ss} \end{aligned} \quad (A2)$$

Now, considering the definitions for elasticity (eqn 1) and response coefficient (eqn 3), $R_{GA_{1P}}^{J_{demand}}$ can be described by the following expression:

$$R_{GA_{1P}}^{J_{demand}} = \varepsilon_{GA_{1P}}^{v_{GT}} + \varepsilon_{U_{GL}}^{v_{GT}} \cdot R_{GA_{1P}}^{U_{GL}} \quad (A3)$$

The repression of *GAL* genes is in part based on the common repressor Gal80p that interacts with the activator Gal4p. Under non-induced and non-repressed carbon conditions (for example in glycerol) a basal expression level is found for *GAL3* and *GAL80* (each with a single Gal4p-binding site), which contrasts with the non-expression of genes that encode the enzymes for galactose uptake [45]. Melcher and Xu [45] suggested a mechanism to explain the basal levels of the *GAL* genes with single Gal4p-binding site and the complete repression of *GAL* genes with multiple Gal4p-binding sites. They suggested that a Gal80p–Gal80p interaction on adjacent Gal4p-binding sites could stabilize the repression of Gal80p in genes with multiple Gal4p-binding sites. In addition, the subcellular localization and formation of a complex involving Gal7p, Gal1p and Gal10p [38] is another potential way to reduce noise.

Conclusions

We have shown that for biologically plausible concentrations of GA_{1P} and mRNA in the yeast galactose-utilization pathway, the level of the toxic intermediate GA_{1P} is well regulated at 10% induction. We traced this robustness to be primarily due to the kinetic parameters of one of the Leloir pathway enzymes, GT.

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