Quantitative Analysis of GAL Genetic Switch of Saccharomyces cerevisiae Reveals That Nucleocytoplasmic Shuttling of Gal80p Results in a Highly Sensitive Response to Galactose*

Received for publication, April 4, 2003, and in revised form, September 25, 2003
Published, JBC Papers in Press, September 25, 2003, DOI 10.1074/jbc.M303526200

Malkhey Verma‡, Paike Jayadeva Bhat§, and K. V. Venkatesh‡‡§§

From the ‡Department of Chemical Engineering and §School of Biosciences and Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai-400076, India

The nucleocytoplasmic shuttling of the repressor Gal80p is known to play a pivotal role in the signal transduction process of GAL genetic switch of Saccharomyces cerevisiae (Peng, G., and Hopper, J. E. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 8548–8553). We have developed a comprehensive model of this GAL switch to quantify the expression from the GAL promoter containing one or two Gal4p-binding sites and to understand the biological significance of the shuttling process. Our experiments show that the expression of proteins from the GAL promoter containing one and two binding sites for Gal4p is ultrasensitive (a steep response to a given input). Furthermore, the model revealed that the shuttling of Gal80p is the key step in imparting ultrasensitive response to the inducer. During induction, free Gal80p concentration is altered by sequestration, without any change in the distribution coefficient across the nuclear membrane. Furthermore, the estimated concentrations of Gal80p and Gal3p allow basal expression of α-galactosidase, but not β-galactosidase, from the GAL promoter containing one and two binding sites for Gal4p, respectively. Conversely, the expression from genes with two binding sites is more sensitive to inducer concentration as compared with one binding site. We show that autoregulation of Gal80p is coincidental to the autoregulation of Gal3p, and it does not impart ultrasensitivity. We conclude from our analysis that the ultrasensitivity of the GAL genetic switch is solely because of the shuttling phenomena of the repressor Gal80p across the nuclear membrane.

Regulation of gene expression occurs mainly at the level of transcription by recruiting specific factors in response to a given stimulus. In general, the genetic switch responds to a given stimulus in an ultrasensitive manner. Ultrasensitive systems have a built in stimulus threshold value below which there is very little response, but as the stimulus is increased, the system output responds steeply (1, 2). In prokaryotes and bacteriophages, the ultrasensitivity in the genetic switch is due to cooperative binding of specific protein to DNA. However, in eukaryotes the compartmentalization of transcription machinery from the rest of the cellular components due to the presence of nuclear membrane poses a unique problem in transmitting the signal to the transcriptional switch. GAL switch in Saccharomyces cerevisiae is a prototypical eukaryotic genetic switch and is well characterized at biochemical and genetic levels. In this switch, galactose (an inducer) elicits a sigmoidal switch-like response (3). Recently, it has been shown that the shuttling of Gal80p (a repressor of GAL switch) between the nucleus and cytoplasm is responsible for turning on the switch (4). This genetic switch thus offers an opportunity to study the effect of shuttling on the signal response.

When yeast cells are growing in a non-inducible and non-repressing medium such as glycerol, Gal4p, a DNA binding transcriptional activator, remains bound to its cognate DNA-binding element in the nucleus. However, the ability of bound Gal4p to activate transcription is inhibited by Gal80p, the repressor, by directly binding to Gal4p (reviewed in Ref. 5). Recently, it has been shown that Gal80p dimerizes with high affinity and appears to stabilize Gal4p and Gal80p as well as Gal4p and DNA interaction (6). The dimer-dimer interaction of Gal80p has also been implicated as important for completely shutting off the expression of GAL genes from GAL promoters containing two but not one Gal4p-binding site. The lack of stabilization of dimer-dimer Gal80p on promoters containing the single Gal4p-binding site has been speculated to be the reason for basal expression of α-galactosidase (MEL1), which is expressed from the GAL promoter containing one binding site (6).

Experiments carried out in the past decade suggested that Gal80p remains bound to Gal4p even upon induction (7). This implied that Gal3p translocates into the nucleus and binds to Gal80p to alter the Gal80p-Gal4p interaction (8). Recently, however, it has been shown that Gal80p is a nucleocytoplasmic shuttle protein, and Gal3p is exclusively a cytoplasmic protein (9). Furthermore, it has been shown that the activation occurs due to the sequestration of Gal80p in the cytoplasm (4). This implies that there is a redistribution of Gal80p concentration such that the effective concentration of Gal80p in the nucleus to inhibit Gal4p-mediated transcription is altered. Therefore, shuttling of Gal80p appears to be the key feature of this nucleocytoplasmic signal transduction as suggested by Peng and Hopper (4).

It has been shown that regulation of nucleocytoplasmic transport of regulatory factors is intricately regulated in many other systems. The two most commonly found regulatory mechanisms, phosphorylation (10–12) or dimerization/tetramerization (13), regulate the transport across the nuclear membrane by altering the rate of entry or exit (14). These mechanisms will henceforth be referred to as “modification.” However, in case of the GAL switch, shuttling of Gal80p is...
cytoplasm and nucleus with a distribution coefficient of binding of Gal80p to activated Gal3p. Gal80p shuttles between the cytoplasm and nucleus includes dimerization of Gal80p and when the first site is already bound, for genes with two binding sites. This indicates that the Gal4p-DNA interaction does not contribute to the ultrasensitive response of the switch to galactose. This phenomenon of regulatory proteins, whose expression is regulated by the same switch in which they participate, is termed as autoregulation (28). Thus, the total concentrations of these proteins are related to status of the genes with one binding site.

The GAL system was evaluated by considering all the interactions to be at equilibrium and by applying mass balances on the species \( [D1], [D2], [G4], [80]_G, [80]_C, \) (see Supplemental Material for details). \( [D1] \) and \( [D2] \) were fixed by assuming three genes with one binding site (\( ME1, GA80, \) and \( GA3 \)) and seven genes with two binding sites (\( GA1, GA2, GA7, ME10, MTH1, PCL10, \) and \( ME15 \)). Gal4p, an activator, dimerizes and interacts with the DNA binding domain in the nucleus (16–19). The model also includes the cooperative binding of the Gal4p dimer to the second binding site in the case of genes with two binding sites (20). We thus define fractional transcriptional expression for one binding site (\( f_1 \)) and two binding sites (\( f_2 \)) as shown in Equations 1 and 2,

\[
f_1 = \frac{[D1] - [G4]}{[D1]_0}
\]

\[
f_2 = \frac{[D2] - [G4]}{[D2]_0} + \frac{2[D2] - [G4] - [G4]}{[D2]_0}
\]

This means that \( f_1 \) and \( f_2 \) are the ratio of mRNA that is transcribed to a given input stimulus to the maximum mRNA that can be transcribed by the system for the gene with one and two binding sites, respectively. It should be noted that the binding sites present in different GAL promoters have intrinsic differences in affinities for Gal4p (21). In our model, we have not considered different binding affinities of Gal4p for promoters of \( ME1 \) (gene with one binding site) and \( GA1 \) (gene with two binding sites).

Furthermore, to quantify the translation, we assume that a fixed logarithm fold change in mRNA would yield a net logarithm fold change in protein concentration as shown in Equation 3.

\[
\log_{10}(\text{fold change protein expression}) = x \log_{10}(\text{fold change mRNA expression})
\]

where \( x \) is the co-response coefficient (22) of protein expression and mRNA. It has been reported that the value of \( x \) is about 0.3, when all the mRNA is translated to the protein in \( S. \) cerevisiae (23). We have recalculated the value of \( x \) as 0.5 for GAL genes from the data of Ideker et al. (24). In terms of fractional translation, the fractional protein expression can be related to the fractional transcription as shown in Equation 4.

\[
f_p = f_1^{0.5} \quad \text{and} \quad f_p = f_2^{0.5}
\]

where \( f_p \) and \( f_t \) are fractional protein expressed for one and two binding sites, respectively.

For Gal80p, the shuttling protein dimerizes (6) and interacts with the Gal4p dimer bound to DNA to switch off transcription. The shuttling of Gal80p between cytoplasm and nucleus is assumed to be a reversible process with a distribution coefficient of \( K \). The value of \( K \) is equal to the ratio of free monomeric Gal80p in the cytoplasm to that in the nucleus. The definition of \( K \) based on the ratio of dimer concentration in cytoplasm to nucleus does not alter the final response as compared with that obtained based on the ratio of monomer. Gal3p, the cytoplasmic protein (9), in the presence of galactose, gets activated to bind to Gal80p monomer in the cytoplasm (4, 25, 26). Gal1p also has been shown to have regulatory function of Gal3p. However, it was shown by Broach (27) that the induction kinetics in a strain lacking Gal1p is not different from those for a wild type strain, suggesting that Gal1p has no role in induction in a wild type strain. Gal3p and Gal80p are regulatory proteins that are expressed from the GAL promoter containing one binding site. They have a basal expression as well as expression induced in response to galactose. This phenomenon of regulatory proteins, whose expression is regulated by the same switch in which they participate, is termed as autoregulation (28). Thus, the total concentrations of these proteins are related to status of the genes with one binding site.

The model accounts for genes with one binding site (D1), such as \( ME1 \) (encodes \( \alpha \)-galactosidase), \( GA3 \), and \( GA80 \), and for genes with two binding sites (D2), such as \( GA1, GA2, GA7, GA10, MTH1, PCL10, \) and \( FUR4 \) (15). Gal4p, an activator, dimerizes and interacts with the DNA binding domain in the nucleus (16–19). The model also includes the cooperative binding of the Gal4p dimer to the second binding site in the case of genes with two binding sites (20). We thus define fractional transcriptional expression for one binding site (\( f_1 \)) and two binding sites (\( f_2 \)) as shown in Equations 1 and 2.

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**RESULTS**

Comparison of Transcriptional Response to Nucleocytoplasmic Transport by Modification and Shuttling—We begin the analysis by comparing the regulated translocation of proteins across the nuclear membrane due to either protein modification or through shuttling of protein across the membrane. Fig. 2a shows the schematic of the first mechanism due to modification. A cytoplasmic protein “Ac” is transported across the nuclear membrane through modification. Once in the nucleus, the protein (An) activates transcription. Fig. 2b shows the transcriptional response based on equilibrium interactions and mass balance on the translocating protein (A) and DNA-binding site (D) (refer to derivations in the Supplemental Material). The response is a typical Michaelis-Menten type with a Hill coefficient of unity regardless of the distribution coefficient $K$ of the protein across the nuclear membrane. For a fixed normalized protein concentration, 10 for example, the transcription is shut off if the value of the distribution coefficient $K$ is 1000. In response to a signal, the protein gets modified followed by a decrease in the value of distribution coefficient $K$ resulting in translocation of the modified protein. In this case, the switch mainly operates due to change in the distribution coefficient by altering the rate of entry into the nucleus because of modification (14). This means that due to modification of the protein the response shifts from point X to point Y as shown by a line with an arrow in Fig. 2b.

A similar analysis applied to the mechanism of shuttling, such as in GAL system, demonstrates different stimulus response curves. Fig. 2c shows the schematic of the shuttling protein $B$, a repressor, whose concentration distribution across the nuclear membrane is dependent on the inducer concentration (C). In this case, the distribution coefficient $K$ is fixed, and protein $B$ shuttles between the nucleus and the cytoplasm to maintain the equilibrium. Fig. 2d shows various response curves at different values of distribution constant $K$. When $K$ is low, that is more of $B$ is present in the nucleus, the response has a Hill coefficient of unity, and the response becomes ultrasensitive with an increase in the value of $K$. The switch-like response is due to shuttling of repressor $B$ brought about by the sequestering of the repressor molecules in the cytoplasm by the inducer. The degree of sensitivity as quantified by the Hill coefficient is directly dependent on the value of the distribution coefficient $K$.

Steady State Response of GAL Genes to Galactose—Fig. 3a shows the simulation results for fractional transcriptional expression at different steady state galactose concentrations. The fractional transcriptional expressions of 42 and 70% were observed from the GAL promoter containing one and two binding sites, respectively. Fig. 3b shows the simulation results for fractional protein expression at different steady state galactose concentrations (see Supplemental Material for details). It is clear from the profiles of protein expression that the genes with one binding site are expressed at about 5% of the maximum level even in the absence of galactose. This basal transcription is not obvious in Fig. 3c, which is due to high maximum mRNA concentrations in the cell. In the absence of galactose, genes with two binding sites are completely shut off. It has been experimentally observed that the basal expression of $MEL1$ is reduced by overexpression of Gal80p (6). This indicates that the basal expression of $MEL1$ in a wild type strain is due to the limitation of Gal80p. Our analysis indicated that more than 100 mm of total Gal80p concentration (2000-fold more than that present in the wild type, see below for details) is required to completely shut off genes with one Gal4p-binding site. This result is consistent with the experimental observation that overexpression of Gal80p from a multicopy plasmid did not
result in a complete shut off of the basal expression of α-galactosidase (6). The maximal expression in a wild type organism in the presence of saturating galactose concentration was estimated to be 63 and 80% for one and two binding sites, respectively. The predicted results of percentage expression are consistent with experimental observations (29, 30, 36). The activity of α-galactosidase and β-galactosidase expressed from one and two binding sites, respectively, were
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experimentally determined for different steady state galactose concentrations. The theoretical predictions matched the experimental data very well for Gal80p distribution coefficient of 0.4 and $K_p = 1.0 \text{ mM galactose}$. The expression in response to galactose concentration was switch-like with a Hill coefficient of 1.5 and 1.9 for one and two binding sites, respectively (Fig. 3, c and d). This validates that the ratio of free Gal80p in the cytoplasm to nucleus is 0.4. In the absence of galactose, 96% of the total Gal80p concentration of 0.05 $\mu M$ resides in the nucleus. Our simulations indicate that at saturating galactose concentration, 99.9% of Gal80p is bound with activated Gal3p in the cytoplasm (total Gal80p concentration under these conditions changes from 0.05 to 0.6 $\mu M$ due to autoregulation). At the galactose concentration more than 10 mM, about 0.6 $\mu M$ of Gal80p, is sequestered by 3.0 $\mu M$ of activated Gal3p in the cytoplasm. This indicates that only about 0.06% of 0.6 $\mu M$ of Gal80p present in the nucleus is sufficient to repress 37 and 20% of the maximum for one and two binding sites, respectively (that is to express up to 63 and 80%, respectively). In a wild type strain in the presence of saturating galactose concentrations, about 20 $\mu M$ of Gal3p (that is 30-fold of total Gal80p), is required (data not shown) for complete expression. It should be noted that the sensitivity of the expression to varying galactose concentrations is due to shuttling of Gal80p, whereas the presence of Gal4p in the nucleus determines the maximum expression levels.

Fig. 3, c and d, shows the effect of distribution coefficient $K$ on protein expression in the case of genes with one and two Gal4p-binding sites, respectively. It is clear that an increase in the value of $K$ (relative to $K = 0.4$) results in a high basal expression in the absence of galactose and an overexpression at saturating concentration. This also results in a response that is highly ultrasensitive. In case of a decrease in the value of $K$, the response does not reach the expected expression values and is subsensitive. At a lower value of $K$, because more Gal4p is present in the nucleus, more Gal3p is required to sequester. At a higher value of $K$, a higher amount of Gal80p is required to shut off the switch in the absence of galactose. Thus, the value of distribution coefficient of 0.4 was found to be optimal for the switch to perform with a minimum Gal3p requirement.

Fig. 4a shows the variation of total Gal80p, Gal3p, and active Gal3p* at different galactose concentrations. The [Gal80p] varies from 0.05 to 0.6 $\mu M$, whereas [Gal3p] varies from 0.25 to 3.2 $\mu M$. This change in the concentration of Gal80p and Gal3p is due to autoregulation. The concentration of Gal80p that is maintained in the absence of galactose (due to the basal expression of Gal80p from the GAL promoter with one Gal4p-binding site) is sufficient to keep the switch in the off state. Gal3p (encoded by GAL3 contains one Gal4p-binding site) also has a basal expression in the absence of galactose, which is inactive but poised to activate the system in the presence of the inducer. Why does Gal80p concentration increase with an increase in the inducer (galactose) concentration? To study this phenomenon, simulation was carried out with both Gal80p and Gal3p being expressed independent of the GAL switch. The results show that (curves i and ii in Fig. 4b) for total Gal80p, and [Gal3p], of 0.05 and 0.25 $\mu M$ (present in absence of galactose), respectively, the switch operates as efficiently as with autoregulation. This indicates that autoregulation of Gal80p and Gal3p does not contribute to the ultrasensitivity. Then why are Gal80p and Gal3p autoregulated? Simulation demonstrated that the switch does not operate (curve iii in Fig. 4b) when Gal3p is autoregulated but not Gal80p. In this case, the fold change in Gal3p required to switch on the GAL switch is about 60-fold instead of the experimentally observed 5-fold. This demonstrates that if Gal3p is under autoregulation, Gal80p should also be under autoregulation for the operation of the switch. Thus, our simulation shows that the existence of autoregulation of Gal80p is merely coincidental to autoregulation of Gal3p.

**DISCUSSION**

Recently, many regulatory systems of biological significance have been demonstrated to exhibit robust behavior (37, 38). Robustness ensures efficient system performance regardless of the changes in environmental conditions. However, our analysis indicates that the GAL switch is highly sensitive to system parameters like the value of distribution coefficient $K$, binding constants, and relative concentrations of Gal4p, Gal80p, and Gal3p. The plausible reason for the above is that because galactose, the inducer, is also a substrate for the catabolic pathway (source of carbon and energy), the induction of GAL enzymes has to be regulated commensurate to galactose concentration. Moreover, under fully induced conditions, GAL enzymes constitute ~5% of the total cellular proteins (26, 39). Therefore, it is conceivable that the cell has evolved GAL switch to be highly vulnerable to system parameters so as to synthesize the requisite amount of enzymes required for the optimal metabolism of galactose. Although the GAL switch is not so robust with respect to system parameters, it still provides ultrasensitive response to galactose. Cooperativity, multistep binding, cascades, and zero order effects have been shown to be responsible for ultrasensitive response in signaling, genetic switches, and metabolic regula-
It is becoming increasingly clear that the nucleocytoplasmic transport of regulatory proteins is a key determinant of the transcriptional regulation in eukaryotes. For example, tumor suppressor p53 has been shown to be a nucleocytoplasmic shuttle protein, whose distribution varies throughout the cell cycle (13). Although the biological consequence of the switching of p53 in terms of turning on or off the transcription is known, the quantitative basis of the fine control brought about by the shuttling of p53 is still not clear. The quantitative analysis of Gal80p shuttling provided in this article illustrates the significant contribution of this nucleocytoplasmic transport process per se to the fine control of the GAL switch. An important issue that still needs to be addressed with regard to the GAL system is how induction starts in response to galactose addition. The insights provided by the steady state analysis, however, will be crucial for the development of a dynamic model to address the above issue in the future.

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J. Biol. Chem. 2003, 278:48764-48769. doi: 10.1074/jbc.M303526200 originally published online September 25, 2003

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