Online Supplementary Information

Model Development for one-step repression and two-step repression (through transcriptional activator)

We analyze the three mechanisms of transcriptional repression by Mig1p as illustrated in Fig. 1**b.** In the mechanism (***i***) repressor (*R*) binds to the upstream repression sequence (URS) of a gene (*D*1) with dissociation constant K_{d1},

$$D1 + R = D1R \tag{A-1}$$

$$K_{d1} = \frac{[D1][R]}{[D1R]} \tag{A-2}$$

The molar balances for DNA and repressor protein are as follows:

$$[D1]_t = [D1] + [D1R] \tag{A-3}$$

$$[R]_t = [R] + [D1R] \tag{A-4}$$

The probability of transcription (f_1) can be defined as the ratio of free DNA to total DNA. Thus,

$$f_1 = \frac{[D1]}{[D1]_t} \tag{A-5}$$

In the mechanism (***ii***) repressor (*R*) binds to the URS of gene (*D*1), its protein product (*A*) is the transcriptional activator for gene (*D*2). Protein (*A*) dimerizes with dissociation constant K_1 and dimer (A_2) binds to upstream activation sequence (UAS) of the target gene (*D*2) with dissociation constant K_d,

$$D1 + R = D1R \tag{A-6}$$

$$K_{d1} = \frac{[D1][R]}{[D1R]} \tag{A-7}$$

$$A + A = A_2 \tag{A-8}$$

$$K_1 = \frac{[A][A]}{[A_2]} \tag{A-9}$$

$$D + A_2 = DA_2 \tag{A-10}$$

$$K_d = \frac{[D][A_2]}{[DA_2]} \tag{A-11}$$

The molar balances for DNA and repressor protein are as follows:

$$[D1]_t = [D1] + [D1R] \quad \text{(A-12)}$$

$$[R]_t = [R] + [D1R] \quad \text{(A-13)}$$

$$[D2]_t = [D2] + [D2A_2] \quad \text{(A-14)}$$

$$[A]_t = [A] + 2\times[A_2] + 2\times[D2A_2] \quad \text{(A-15)}$$

The probability of transcription for activator gene ($D1$) is defined as the ratio of concentrations of free $D1$ to total $D1$

$$f = \frac{[D1]}{[D1]_t} \quad \text{(A-16)}$$

and probability for transcriptional expression of target gene ($D2$) is defined as the ratio of A_2 bound $D2$ to total $D2$ concentrations,

$$f_1 = \frac{[D2A_2]}{[D2]_t} \quad \text{(A-17)}$$

$[A]_t$ total concentration of transcriptional activator and is defined as:

$$[A]_t = f_p \times [A_t]^{\max} \quad \text{(A-18)}$$

where $[A]_t^{max}$ is the maximum concentration of transcriptional activator protein, which is fixed for an organism and vary for different organisms and f_p is fractional translation for given fractional transcription as reported by Verma et al., [24].

$$f_p = f^n \quad \text{(A-19)}$$

where 'n' is co-response coefficient [29]. For prokaryotes, average value of n is one and in eukaryotes this value varies from 0.2 to 1.0 for varoius genes [30]. In the current analysis the value of '*n*' is taken as one.

In the mechanism (***iii***) repression occurs through the combination of mechanisms (***i***) and (***ii***) in which repressor (R) binds to the URS of both the genes (activator and target genes) with dissociation constant K_{d1}. The protein product (A) of activator gene ($D1$) dimerizes with dissociation constant K_1 and dimer (A_2) binds to target gene ($D3$) with dissociation constant K_d. The equations for equilibrium interactions are as follows,

$$D1 + R = D1R \quad \text{(A-20)}$$

$$K_{d1} = \frac{[D1][R]}{[D1R]} \quad \text{(A-21)}$$

$$D3 + R = D3R \quad \text{(A-22)}$$

$$K_{d1} = \frac{[D3][R]}{[D3R]} \quad \text{(A-23)}$$

$$A + A = A_2 \quad \text{(A-24)}$$

$$K_1 = \frac{[A][A]}{[A_2]} \quad \text{(A-25)}$$

$$D3 + A_2 = D3A_2 \quad \text{(A-26)}$$

$$K_d = \frac{[D3][A_2]}{[DA_2]} \quad \text{(A-27)}$$

The molar balances for DNA, repressor and activator proteins are as follows:

$$[D1]_t = [D1] + [D1R] \quad \text{(A-28)}$$

$$[R]_t = [R] + [D1R] + [D3R] \quad \text{(A-29)}$$

$$[D3]_t = [D3] + [D3R] + [D3A_2] \quad \text{(A-30)}$$

$$[A]_t = [A] + 2 \times [A_2] + 2 \times [D3A_2] \quad \text{(A-31)}$$

The probability of transcription for activator gene (*D*1) is defined as the ratio of free *D*1 to total *D*1

$$f = \frac{[D1]}{[D1]_t} \quad \text{(A-32)}$$

and probability for transcriptional expression of target gene (*D*3) is defined as the ratio of *D*3 bound to activator to total *D*3 concentration,

$$f_1 = \frac{[D3A_2]}{[D3]_t} \quad \text{(A-33)}$$

As before $[A]_t$, is the total concentration of transcriptional activator and is quantified as:

$$[A]_t = f_p \times [A_t]^{\max} \quad \text{(A-34)}$$

For all the three mechanisms of repression the values for genes concentrations $[D1]_t = [D2]_t = [D3]_t = 2.372 \times 10^{-11}$ M were fixed for an model organism with cellular volume 70 μm^3 (equal to the cellular volume of *Saccharomyces cerevisiae*) [51] having single copy per cell. $[A]_t^{max}$ was fixed at 8.0 nM (equal to maximum concentration of Gal4p in *Saccharomyces cerevisiae*) K_d

and K_{d1}were fixed at 2.0 x 10^{-10} M and 5.0 x 10^{-12} M respectively. The values of 'f_1' were evaluated at various $[R]_t$, total repressor concentrations.

Model Development for Phosphorylation and dephosphorylation of Mig1p

Mig1p exists in two forms, activated free form (*M*1) and inactive phosphorylated form (*M*1-*P*). The Mig1p is phosphorylated by monocyclic cascade mechanism using two enzymes Snf1 protein kinase and dephosphorylated by protein phosphatase. The model for monocyclic cascade phosphorylation is taken from Goldbeter and Koshland, [22, 23]. Input signal "*I*" is quantified as follows,

$$\mathrm{I} = \frac{k_1 \times [Snf1]_{\max}}{k_2 \times [Phosphatase]_{\max}} \qquad \text{(A-35)}$$

where k_1 and k_2 are rate constants for phosphorylation and dephosphorylation of Mig1p.

Fractional phosphorylation of Mig1p (*M*1) is defined by the ratio of phosphorylated to total concentration of Mig1p.

$$f = \frac{[M1...P]}{[M1]_t} \qquad \text{(A-36)}$$

$$[M1]_t = \frac{k_{m1}\left(\frac{f}{1-f} - I \times \frac{k_{m2}}{k_{m1}}\right)}{f \times (I-1)} \qquad \text{(A-37)}$$

where k_{m1} and k_{m2} are Michaelis-Menten constants for Snf1 kinase and phosphatase respectively. The glucose inhibition for phosphorylation of Mig1p (*M1p*) can be quantified by Michaelis-Menten type relationship as follows,

$$[Snf1]_t = [Snf1]_{\max}\left(\frac{K_{glu(Snf1)}}{K_{glu(Snf1)} + Glu}\right) \qquad \text{(A-38)}$$

where, $K_{glu(Snf1)}$ is half saturation constant and its value is fixed equal to 8.0 mM by fitting experimental data.

Mig1p Dependent Glucose Repression

Nucleocytoplasmic Translocation of Mig1p

The translocation of Mig1p (*M1*) between cytoplasm and nucleus is assumed to be a reversible process with respect to unphosphorylated Mig1p with the distribution coefficient of 'K_{Mig1p}'. The value of K_{Mig1p} is equal to the ratio of free Mig1p in the cytoplasm to that in the nucleus.

$$M1c \Leftrightarrow M1n \tag{A-39}$$

$$K_{Mig1p} = \frac{[M1c]}{[M1n]} \tag{A-40}$$

Mig1p (*M1n*) binds to *GAL4* gene encoding transcriptional activator for *GAL* genes, three genes of *GAL* family (*GAL1, GAL3* and *MEL1*) [5, 9, 12, 20] and thirty four more genes of different families, one of them *SUC2* having two binding sites [12, 20] and rest thirty three are denoted by '*D*' having one binding site. The dissociation constant K_{d1} has been taken for binding of Mig1p to *GAL4, GAL1*, *GAL3*, *MEL1* and *SUC2* and other genes having one binding site and K_{d1} for *GAL* genes.

$$GAL4 + M1n = GAL4M1n \tag{A-41}$$

$$K_{d1} = \frac{[GAL4][M1n]}{[GAL4M1n]} \tag{A-42}$$

$$GAL1 + M1n = GAL1Mn \tag{A-43}$$

$$K_{d1} = \frac{[GAL1][M1n]}{[GAL1M1]} \tag{A-44}$$

$$GAL3 + M1n = GAL3M1n \tag{A-45}$$

$$K_{d1} = \frac{[GAL3][M1n]}{[GAL3M1n]} \tag{A-46}$$

$$MEL1 + M1 = MEL1M1n \tag{A-47}$$

$$K_{d1} = \frac{[MEL1][M1n]}{[MEL1M1n]} \tag{A-48}$$

$$SUC2 + M1n = SUC2M1n \tag{A-49}$$

$$K_{d1} = \frac{[SUC2][M1n]}{[SUC2M1n]} \tag{A-50}$$

$$SUC2M1n + M1n = SUC2M1nM1n \tag{A-51}$$

$$K_{d1} = \frac{[SUC2M1n][M1n]}{[SUC2M1nM1n]} \quad \text{(A-52)}$$

$$D + M1n = DM1n \quad \text{(A-53)}$$

$$K_{d1} = \frac{[D][M1n]}{[DM1n]} \quad \text{(A-54)}$$

The molar balances for different genes and Mig1p are as follows:

$$[GAL4]_t = [GAL4] + [GAL4M1n] \quad \text{(A-55)}$$

$$[SUC2]_t = [SUC2] + [SUC2M1n] + [SUC2M1nM1n] \quad \text{(A-56)}$$

$$[D]_t = [D] + [DM1n] \quad \text{(A-57)}$$

$$[M1]_t = [M1c] + [M1n] + [GAL4M1n] + [GAL1M1n] + [GAL3M1n] + [MEL1M1n] + [SUC2M1n] + 2[SUC2M1nM1n] + 33[DM1n] \quad \text{(A-58)}$$

The probability of transcription of *GAL4* can be defined as the ratio of free *GAL4* gene to $[GAL4]_t$. Glucose also causes Mig1p independent repression of *GAL4* transcription [12] and there a factor was multiplied to include this effect by glucose. Thus,

$$f_{GAL4} = \frac{[GAL4]}{[GAL4]_t} \times \left\{ 0.75 + 0.25 \times \left(\frac{K_{s1}}{K_{s1} + Glu} \right) \right\} \quad \text{(A-59)}$$

where K_{s1} is the half saturation constant for Mig1p independent glucose repression. For transforming fractional transcription of *GAL4* to fractional protein expression, the co-response coefficient of 0.9 was used.

$$f_{Gal4p} = f_{GAL4}^{\ 0.9} \quad \text{(A-60)}$$

where factor 0.9 is the co-response coefficient [29] of protein expression and mRNA. The value of co-response coefficient for *GAL4* gene in *S. cerevisae* was recalculated as 0.9 using experimental data for transcriptional expression reported by Nehlin et al., [12] and Gal4p expression data reported by Griggs and Johnston [32].

Similar to *GAL4*, the probability of transcription of *SUC2* can be defined as the ratio of free *SUC2* gene to $[SUC2]_t$. Equation A-61 also includes Mig1p independent repression of *SUC2* transcription by glucose. Thus,

$$f_{SUC2} = \frac{[SUC2]}{[SUC2]_t} \times \left\{ 0.3 + 0.7 \times \left(\frac{K_{s1}}{K_{s1} + Glu} \right) \right\} \quad \text{(A-61)}$$

where K_{s1} is the half saturation constant for Mig1p independent glucose repression. For transforming fractional transcription of *SUC2* to fractional protein expression, the following conversion factor was used

$$f_{Suc2p} = f_{SUC2}^{0.5} \quad \text{(A-62)}$$

The value of co-response coefficient for *SUC2* gene in *S. cerevisiae* is taken to be same as that for *GAL* genes.

Model Development for *GAL* System

Equilibrium model was also developed for the transcriptional mechanism of *GAL* genes as described in Fig. 1a [24]. The transcriptional expression of *GAL* genes depends on the interaction of Gal4p dimer with the DNA binding sites. The *GAL* family of *S. cerevisiae* has two types of genes, genes having one binding site for Gal4p and other with two binding sites for Gal4p [21, 25]. We have considered three genes with one binding site (*GAL3*, *GAL80* and *MEL1*) and seven genes with two binding sites (*GAL1*, *GAL2*, *GAL7*, *GAL10*, *MTH1*, *PCL10* and *FUR4*) [26]. Although *GAL1* and *GAL10* have four binding sites, are shared by the same genes [21]. We have split the four common binding sites with two dedicated binding sites for each gene based on both sides transcriptional expression mechanism [24]. Gal4p dimerizes with dissociation constant 'K_1' before binding to the operator sites as given below:

$$G4 + G4 = G4_2 \quad \text{(A-63)}$$

$$K_1 = \frac{[G4]^2}{[G4_2]} \quad \text{(A-64)}$$

The Gal4p dimer binds to the operator of gene with one binding site ($D1$) with dissociation constant 'K_d'.

$$[D1] + [G4_2] = [D1 - G4_2] \quad \text{(A-65)}$$

$$K_d = \frac{[D1] \times [G4_2]}{[D1 - G4_2]} \quad \text{(A-66)}$$

Gal4p binds to the operators of genes with two binding sites ($D2$) with a dissociation constant of K_d for the first site and K_d/m for the second site. Here 'm' is a factor-quantifying cooperativity. Thus,

$$[D2]+[G4_2]=[D2-G4_2] \quad \text{(A-67)}$$

$$[D2-G4_2]+[G4_2]=[D2-G4_2-G4_2] \quad \text{(A-68)}$$

$$K_d=\frac{[D1]\times[G4_2]}{[D1-G4_2]}=\frac{m[D2-G4_2]\times[G4_2]}{[D2-G4_2-G4_2]} \quad \text{(A-69)}$$

Glucose inhibits binding of Gal4p to UAS of *GAL* genes [24, 26], therefore dissociation constant for binding of Gal4p to *GAL* genes in the presence of glucose is quantified as reported by Ren et al., [26]. Thus,

$$K_{d(Glu)}=K_d\left(1+\frac{Glu}{K_{s2}+Glu}\right) \quad \text{(A-70)}$$

where K_{s2} is half saturation constant for glucose inhibition for the binding of Gal4p to UAS.

Since Gal4p is an activator, the Gal4p bound operators allow RNA polymerase to transcribe. The probability of expression can be defined as the ratio of bound operator to total operator sites. Equation A-71 also includes the Mig1p independent repression of *GAL* genes by glucose [12]. Thus,

$$f_1=\frac{[D1-G4_2]}{[D1]_t}\left\{0.3+0.7\left(\frac{K_{s2}}{K_{s2}+Glu}\right)\right\} \quad \text{(A-71)}$$

$$f_2=\frac{[D2-G4_2]+[D2-G4_2-G4_2]}{[D2]_t}\left\{0.3+0.7\left(\frac{K_{s2}}{K_{s2}+Glu}\right)\right\} \quad \text{(A-72)}$$

where K_{s2} is half saturation constant for Mig1p independent glucose inhibition, f_1 and f_2 are the probabilities of expression for genes with one binding site and two binding sites, respectively. The value of co-response coefficient for *GAL* genes in *S. cerevisiae* was calculated as 0.5 using the experimental data provided in [24].

In a non-inducing non-repressing medium (NINR) like glycerol, the expression of *GAL* genes is not detectable, as Gal80p, a shuttling repressor protein [28] represses the transcription by binding to Gal4p bound with DNA [28]. The translocation of Gal80p between cytoplasm and nucleus is assumed to be a reversible process with the distribution coefficient of 'K_{Gal80p}'. The value of K_{Gal80p} is equal to the ratio of free monomeric Gal80p in the cytoplasm to that in the nucleus.

$$G80c \Leftrightarrow G80n \quad \text{(A-73)}$$

$$K_{Gal80p} = \frac{[G80c]}{[G80n]} \tag{A-74}$$

Gal80p forms dimer [21, 41] before binding to free Gal4p and DNA-(Gal4p)$_2$. Therefore, the following equilibrium relations are considered:

$$[G80n] + [G80n] = [G80n_2] \tag{A-75}$$

$$K_2 = \frac{[G80n]^2}{[G80n_2]} \tag{A-76}$$

$$[G80c] + [G80c] = [G80c_2] \tag{A-77}$$

$$K_2 = \frac{[G80c]^2}{[G80c_2]} \tag{A-78}$$

$$[G4_2] + [G80n_2] = [G4_2 - G80n_2] \tag{A-79}$$

$$[D1 - G4_2] + [G80n_2] = [D1 - G4_2 G80n_2] \tag{A-80}$$

$$K_3 = \frac{[G4_2][G80n_2]}{[G4_2 - G80n_2]} = \frac{[D1 - G4_2][G80n_2]}{[D1 - G4_2 G80n_2]} \tag{A-81}$$

$$[D2 - G4_2] + [G80n_2] = [D2 - G4_2 G80n_2] \tag{A-82}$$

$$K_3 = \frac{[D2 - G4_2][G80n_2]}{[D2 - G4_2 G80n_2]} \tag{A-83}$$

$$[D2 - G4_2 - G4_2] + [G80n_2] = [D2 - G4_2 G80n_2 - G4_2] \tag{A-84}$$

$$K_3 = \frac{[D2 - G4_2 - G4_2][G80n_2]}{[D2 - G4_2 G80n_2 - G4_2]} \tag{A-85}$$

$$[D2 - G4_2 G80n_2 - G4_2] + [G80n_2] = [D2 - G4_2 G80n_2 - G4_2 G80n_2] \tag{A-86}$$

$$K_3 = \frac{[D2 - G4_2 G80n_2 - G4_2][G80n_2]}{[D2 - G4_2 G80n_2 - G4_2 G80n_2]} \tag{A-87}$$

The dissociation constant of binding of $G80n_2$ to $G4_2$ is taken as of same order for free and $G4_2$ complexed with DNA.

In an inducing medium (presence of galactose) Gal3p is activated to Gal3*p [24] and it binds to Gal80p to relieve repression by Gal80p [24, 25]. In this model, we have considered that activated Gal3p does not enter in the nucleus and it induces *GAL* genes expression by monomer binding with nucleocytoplasmic shuttling repressor, Gal80p as a monomer in the cytoplasm [28].

$$[G3]+[G80c]=[G3-G80c] \quad \text{(A-88)}$$

$$K_4=\frac{[G3][G80c]}{[G3-G80c]} \quad \text{(A-89)}$$

Since Gal80p and Gal3p are expressed by the *GAL* genes system, their total concentrations are dependent on the status of the switch. Thus to account for autoregulation of Gal80p and Gal3p [24], the total concentration of these were related to translation status of genes with one binding site (f_1p).

$$[G80]_t = f_{1p}[G80]_{\max} \quad \text{(A-90)}$$

$$[G3]_t = f_{1p}[G3]_{\max} \quad \text{(A-91)}$$

Recently, it has been reported that in a medium containing galactose, the Gal3p protein concentration is five fold of Gal80p [24, 28]. Thus we have set

$$[G3]_t = 5\times[G80]_t \quad \text{(A-92)}$$

it also relates

$$[G3]_{\max} = 5\times[G80]_{\max} \quad \text{(A-93)}$$

Since galactose activates Gal3p and glucose inhibits the galactose activation of Gal3p, the active Gal3p* concentration is assumed to be dependent on a Michaelis-Menten type relationship with respect to galactose and glucose, thus

$$\left[G3^*\right]_t = [G3]_t\left(\frac{Gal}{K_S+Gal}\right)\left(\frac{K_{s3}}{K_{s3}+Glu}\right) \quad \text{(A-94)}$$

where K_S and K_{s3} are half saturation constants for galactose activation and glucose inhibition of Gal3p.

The *GAL* genes with one binding site (*D*1) which denotes *GAL80* (having only Gal4p binding site), *GAL3* and *MEL1* (having one Gal4p and one Mig1p binding site). D2 represents genes with two binding sites, that is *GAL1* (having two Gal4p binding sites and one Mig1p binding site) and *GAL2*, *GAL7*, *GAL10*, *MTH1*, *PCL10* and *FUR4* (having two Gal4p binding sites). The molar balances for the genes having one and two Gal4p binding sites are as follows:

$$[GAL80]_t = [GAL80]+[GAL80-G4_2]+[GAL80-G4_2G80n_2] \quad \text{(A-95)}$$

$$[GAL3]_t = [GAL3]+[GAL3-G4_2]+[GAL3-G4_2G80n_2]+[GAL3-M1n] \quad \text{(A-96)}$$

$$[MEL1]_t = [MEL1]+[MEL1-G4_2]+[MEL1-G4_2G80n_2]+[MEL1-M1n] \quad \text{(A-97)}$$

$$[GAL1]_t = [GAL1] + [GAL1 - G4_2] + [GAL1 - G4_2 G80n_2] + [GAL1 - G4_2 - G4_2] + [GAL1 - G4_2 G80n_2 - G4_2] + [GAL1 - G4_2 G80n_2 - G4_2 G80n_2] + [GAL1 - M1n] \quad \text{(A-98)}$$

$$[D2]_t = [D2] + [D2 - G4_2] + [D2 - G4_2 G80n_2] + [D2 - G4_2 - G4_2] + [D2 - G4_2 G80n_2 - G4_2] + [D2 - G4_2 G80n_2 - G4_2 G80n_2] \quad \text{(A-99)}$$

The molar balances for regulatory proteins Gal4p, Gal80p and Gal3*p are given as follows:

$$[G4]_t = [G4] + 2\times[G4_2] + 2\times[G4_2 - G80n_2] + 2\times[D1 - G4_2] + 2\times[D1 - G4_2 G80n_2] + 2\times[D2 - G4_2] + 2\times[D2 - G4_2 G80n_2] + 4\times[D2 - G4_2 - G4_2] + 4\times[D2 - G4_2 G80n_2 - G4_2] + 4\times[D2 - G4_2 G80n_2 - G4_2 G80n_2] \quad \text{(A-100)}$$

$[G4]_t$ is defined as follows:

$$[G4]_t = f_{GAL4} \times [G4]_{\max} \quad \text{(A-101)}$$

where $[G4]_{max}$ is the maximum concentration of Gal4p expressed in the strain with genotype *mig1::gal80*. The value of $[G4]_{max}$ was recalculated using experimental data for transcription [12] and total Gal4p concentration in wild type strain [24, 36].

$$[G80]_t = [G80n] + [G80c] + 2\times[G80n_2] + 2\times[G80c_2] + 2\times[G4_2 - G80n_2] + [G80c - G3] + 2\times[D1 - G4_2 G80n_2] + 2\times[D2 - G4_2 G80n_2] + 2\times[D2 - G4_2 G80n_2 - G4_2] + 4\times[D2 - G4_2 G80n_2 - G4_2 G80n_2] \quad \text{(A-102)}$$

$$[G3]_t = [G3] + [G80c - G3] \quad \text{(A-103)}$$

$[G80]_t$ and $[G3]_t$ are obtained from equation A-90 and A-93. Thus the value of $[G80]_{max}$ is required to estimate $[G80]_t$ and $[G3]_t$.

All the binding constants were used from reported values in literature and are given in Table A-1, while the total species concentrations were calculated as discussed below. The total concentrations of regulatory proteins and half saturation constants were estimated based on experimental fit. The model equations were solved by f-solve algorithm of MATLAB-12 (The Math Works Inc., U.S.A.). The total molar balances for all the species were verified independently by adding the concentrations of the complexes.

Model Parameters

The dissociation constants for protein-protein and DNA-protein interactions were taken from literature as reported in Table A-1. We have considered three genes with one binding site

(*GAL3*, *GAL80* and *MEL1*) and seven genes with two binding sites (*GAL1*, *GAL2*, *GAL7*, *GAL10*, *MTH1*, *PCL10* and *FUR4*) [26]. Assuming a total volume of 70 μm^3 for a yeast cell [24], the concentration of a gene was determined as 2.372×10^{-11}M. The dimerization constant for Gal4p was assumed to be 1×10^{-7} M to give a total Gal4p concentration of 5.47 nM (about 230 molecules). However, in a wildtype strain in presence of Mig1p, a fraction of it is synthesized (which is about 0.7 of the 230 molecules). Therefore, in a wildtype strain, our analysis actually predicts only about 160 molecules, which results in about 80 dimers, which closely matches the value determined experimentally by Kodadek [50]. The dissociation constant for dimerization of Gal4p was of the same order as for λ-phage repressor [24]. The $[Gal80p]_{max}$ was fixed at a value to yield 5% protein expression in a non-inducing non-repressing (NINR) medium like glycerol. $[Gal3p]_{max}$ was taken to be five times of $[Gal80p]_{max}$ (as reported in [28]). The binding of Gal3p to Gal80p was assumed to be of the same order as that of Gal80p to Gal4p. The analysis indicates a change in Gal80p concentration 0.05 μM to 0.6 μM in the absence and presence of galactose. The value of K_s was fixed based on galactose concentration at 50% expression of wild type.

Table A-1. Parameters values used in the steady state model

Parameters	**Values**	**Source**
K_d	2.0 x 10^{-10} M	[24]
K_{d1}	2.0 x 10^{-10} M	Assumed
$K_{glu(Snf1)}$	8.0 mM	From experimental fit
k_1	1.0	Assumed
k_2	1.0	Assumed
k_{m1}	1.0	Assumed
k_{m2}	1.0	Assumed
$[Snf1]_{max}$	1.21 nM	Assumed
$[Phosphatase]_{max}$	0.91 nM	Assumed
K_{Mig1p}	0.5	Based on parameter sensitivity
K_{Gal80p}	0.4	Based on parameter sensitivity
K_{s1}	5.0 mM	From experimental fit
K_{s2}	0.5 mM	From experimental fit
K_{s3}	2 mM	Assumed
K_s	1.0	From experimental fit
K_1	1.0 x 10^{-7} M	[24]
K_2	1.0 x 10^{-10} M	[41]
K_3	5.0 x 10^{-11} M	[41]
K_4	6.3 x 10^{-11} M	Based on parameter sensitivity
m	30	[21]
$[GAL4]_t$	2.372 x 10^{-11} M	Model calculation
$[D]_t$	33 x 2.372 x 10^{-11} M	Model calculation
$[SUC2]_t$	2.372 x 10^{-11} M	Model calculation
$[GAL80]_t$	2.372 x 10^{-11} M	Model calculation
$[GAL3]_t$	2.372 x 10^{-11} M	Model calculation
$[MEL1]_t$	2.372 x 10^{-11} M	Model calculation
$[GAL1]_t$	2.372 x 10^{-11} M	Model calculation
$[D2]_t$	6 x 2.372 x 10^{-11} M	Model calculation
$[Mig1p]_t$	3.0 x 10^{-9} M	Model calculation
$[Gal4p]_{max}$	5.4 x 10^{-9} M	Model calculation
$[Gal80p]_{max}$	1.0 x 10^{-6} M	Model calculation
$[Gal3p]_{max}$	5.0 x 10^{-6} M	5 x $[Gal80p]_{max}$ [28]

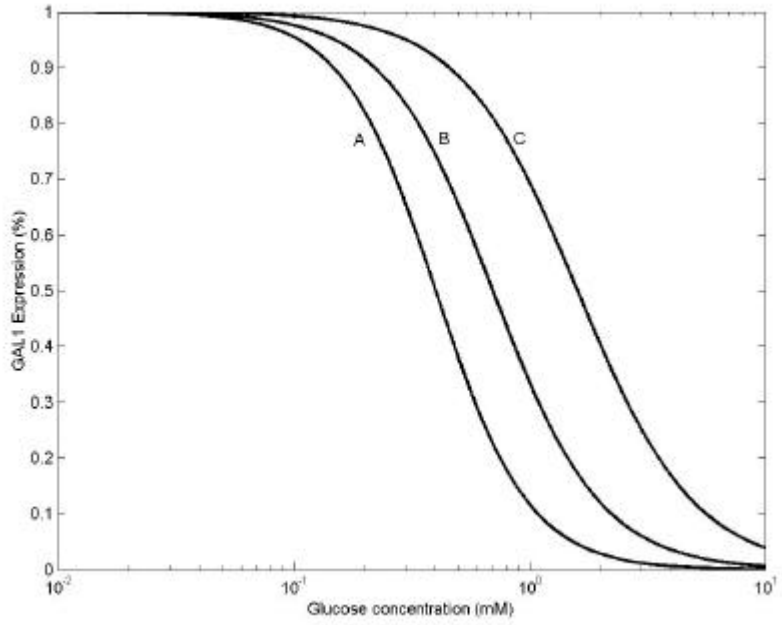

Fig. 1A Repression of *GAL1* at different concentration of glucose to variations in nucleocytoplasmic shuttling constant of Mig1p, (A) K_{Mig1p}= 0.8; (B) K_{Mig1p}= 0.5 (C) K_{Mig1p}= 0.1

Sensitivity analysis of parameters:

Sensitivity analysis of parameters was carried out using the steady state model. The sensitivity of parameters involved in the galactose response that is interactions involving Gal4p, Gal80p and Gal3p has been presented elsewhere (Verma *et. al.* [24]), where it has been demonstrated that the response is sensitive only to the shuttling constant for Gal80p (that is K_{Gal80p}). Fig. 1A demonstrates the effect of variation in shuttling constant of unphosphorylated Mig1p on the repressive response. The figure indicates that due to changes in nucleocytoplasmic shuttling constant of Mig1p (K_{Mig1p}), the amount of glucose required for 50% repression changes and is a direct function of this parameter. It should be noted that the steepness of the response itself does not get affected. This implies that the steepness of the curve is robust to parametric alterations, while the half saturation constant is dependent on the specific parameter values used. However, it should be emphasized here that the hierarchical response does not get affected due to parameters. That is, if the curve for *GAL1* shifts to the right, *SUC2* also shifts to the right, accordingly maintaining the hierarchical expression. The sensitivity analysis for the other oarameters such as Mig1p concentrations and binding of Mig1p to cognate sites also yielded similar results.