Supplement for "Perturbation-based analysis and modeling of combinatorial regulation in the yeast sulfur assimilation pathway"

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Differential equation model in Figure 5A

While equations 1-4 in the main text were derived from standard descriptions of transcription, translation, and mass-action kinetics, the derivation of Equation 5 deserves more detail. Consider three protein species, an activator (A), repressor (R), and non-specific factor (X) which recognize the same promoter binding site. If these proteins are in equilibrium with DNA, then:

(S1) $$A + DNA_{free} \rightleftharpoons A:DNA$$

(S2) $$R + DNA_{free} \rightleftharpoons R:DNA$$

(S3) $$X + DNA_{free} \rightleftharpoons R:DNA$$

The dissociation constants of A, R, and X are $K_{d,A}$, $K_{d,R}$, and $K_{d,X}$, respectively, and this results in:

(S4) $$[A]\cdot[DNA]_{free} = K_{d,A}\cdot[A:DNA]$$

(S5) $$[R]\cdot[DNA]_{free} = K_{d,R}\cdot[R:DNA]$$

(S6) $$[X]\cdot[DNA]_{free} = K_{d,X}\cdot[X:DNA]$$

The occupancy of A at the binding site is given by:

(S7) $$O_A = \frac{[A:DNA]}{[DNA]_{free} + [A:DNA] + [R:DNA] + [X:DNA]}$$

By definition, $K_d = \frac{1}{K_a}$. Using this relation and substituting (S4-S6) into (S7) we obtain:

(S8) $$O_A = \frac{K_{a,A}[A]}{1 + K_{a,A}[A] + K_{a,R}[R] + K_{a,X}[X]}$$

We can then write the rate of change of target transcription, $\frac{d[M]_{TARGET}}{dt}$, as

(S10) $$\frac{d[M(t)]_{TARGET}}{dt} = \mu_A O_A(t) + \mu_R O_R(t) + \mu_X O_X(t) - \frac{1}{\tau}[M(t)]_{TARGET},$$

where we have assumed first-order degradation of transcripts. We then set $\mu_R = 0$ and $K_{a,X}[X] = x_0$ where x_0 is an effective parameter of the affinity and concentration of the non-specific factor. This results in

$$\frac{d[M(t)]_{TARGET}}{dt} = \frac{\mu_A K_{a,A}[A(t)] + \mu_X x_0}{1 + x_0 + K_{a,A}[A(t)] + K_{a,R}[R(t)]} - \frac{1}{\tau}[M(t)]_{TARGET} \quad \text{(S11)}$$

which is the functional form of Equation 5 in the main text. Descriptions of parameters for the full model in the text are contained in Table S1.

Table S1: Parameter values and descriptions for simulations in Figure 5A

Parameter	Description	Value
$M_{CBF1}(t)$	number of co-factor mRNAs	0 (at t = 0)
$P_{CBF1}(t)$	number of co-factor proteins	0 (at t = 0)
$P_{CBF1,MET4,COMPLEX}(t)$	number of trans-activator complexes	0 (at t = 0)
$M_{TARGET}(t)$	number of target transcripts produced	0 (at t = 0)
$P_{MET4,TOTAL}$	number of activator proteins	0 or 2000
$\mu_1(t)$	rate of transcription of *CBF1* downstream of P_{GAL1}	0 min^{-1} prior to pulse, 4 min^{-1} after pulse
μ_2	strength of activation of $M_{TARGET}(t)$ by $P_{CBF1,MET4,COMPLEX}(t)$	3 min^{-1}
μ_3	strength of activation of $M_{TARGET}(t)$ by x_0	1 min^{-1}
τ_1	mRNA half-life	10 min
τ_2	protein half-life	20 min
τ_3	effective parameter (time delay between activator complex formation and finding the correct binding site, recruiting RNA polymerase, and transcription initiation)	5 min
β_1	translation rate	3 min^{-1}
γ_1	effective parameter that accounts for fast formation of Cbf1p dimers, formation of active complexes with Met4p, and a slow dissociation of these complexes	3 min^{-1}
K_a	strength of TF binding	1
x_0	effective parameter of the affinity and amount of non-specific factor	1

Differential equation model of a Met4p/Met32p feed-forward loop

(S12) $$\frac{dM_{MET4}(t)}{dt} = \mu_1(t) - \frac{1}{\tau_1} M_{MET4}(t)$$

(S13) $$\frac{dP_{MET4}(t)}{dt} = \beta_1 M_{MET4}(t) - \gamma_1 P_{MET4}(t) P_{MET32}(t) - \frac{1}{\tau_2} P_{MET4}(t)$$

(S14) $$\frac{dM_{MET32}(t)}{dt} = \phi_1 \left(\frac{K_{a,1} P_{MET4}(t-\tau_3)}{1 + K_{a,1} P_{MET4}(t-\tau_3)} \right) - \frac{1}{\tau_1} M_{MET32}(t)$$

(S15) $$\frac{dP_{MET32}(t)}{dt} = \beta_1 M_{MET32}(t) - \gamma_1 P_{MET4}(t) P_{MET32}(t) - \frac{1}{\tau_2} P_{MET32}(t)$$

(S16) $$\frac{dP_{MET4,MET32,COMPLEX}(t)}{dt} = \gamma_1 P_{MET4}(t) P_{MET32}(t) - \frac{1}{\tau_2} P_{MET4,MET32,COMPLEX}(t)$$

(S17) $$\frac{dM_{OUTPUT}(t)}{dt} = \phi_2 \left(\frac{K_{a,2} P_{MET4,MET32,COMPLEX}(t-\tau_3)}{1 + K_{a,2} P_{MET4,MET32,COMPLEX}(t-\tau_3)} \right) - \frac{1}{\tau_1} M_{OUTPUT}(t)$$

Table S2: Parameter values and descriptions for simulations in Figure S4.

Parameter	Description	Value
$M_X(t)$	number of mRNA molecules of species X	0 (at t = 0)
$P_X(t)$	number of proteins of species X	0 (at t = 0)
ϕ_1	strength of activation of $M_{MET32}(t)$ by $P_{MET4}(t)$	varies
ϕ_2	strength of activation of $M_{OUTPUT}(t)$ response to $P_{MET4,MET32,COMPLEX}(t)$	varies
$\mu_1(t)$	rate of transcription of *MET4* downstream of P_{GAL1}	0 min^{-1} prior to pulse, 4 min^{-1} after pulse
τ_1	mRNA half-life	10 min
τ_2	protein half-life	20 min
τ_3	effective parameter accounting for the time delay between the activator localizing to the nucleus, finding the correct binding site, recruiting RNA polymerase, and initiating transcription	5 min
β_1	translation rate	3 min^{-1}
K_a	strength of TF binding	1
γ_1	effective parameter of Met4p-Met32p complex formation with slow dissociation	3 min^{-1}

Supplemental Figure Legends

Figure S1: Gene expression data overview. (A) Clustered heatmap of methionine-limited GEV experiments collected in this study. 872 genes were floored to a log2-transformed value of 0 in each array and removed prior to clustering the remaining 5384 genes. (B) The number of genes that are upregulated >2-fold (red), repressed >2-fold (green), and the sum of both (black) at each time point when GEV is induced in DBY12142 (Control).

Figure S2: Cbf1p switches between a repressor and a co-activator of Cluster 9 genes based on choice of nutrient limitation. Cells were grown under either 20 mg/L phosphate-limited (P) or 7.5 mg/L methionine-limited (M) growth. Cbf1p overexpression experiments are sampled out to 90 minutes. The phosphate-limited Cbf1p experiment samples are at t = 0, 5, 15, 30, 45, and 90 minutes following β-estradiol addition to the culture. The methionine-limited Cbf1p experiment samples are at t = 0, 2.5, 5, 15, 30, 45, 60, and 90 minutes. The GEV-only controls have an additional time-point at 2 hours. The phosphate-limited GEV-only experiment samples are at t = 0, 2.5, 5, 18, 30, 45, 60, 90 and 120 minutes. The methionine-limited GEV experiment samples are at t = 0, 2.5, 5, 15, 30, 45, 60, 90, and 120 minutes. Previously identified targets of Cbf1p are indicated in orange ($p < 0.005$; MacIsaac *et al*, 2006) and cyan (Lee *et al*, 2010).

Figure S3: The transcriptional responses of Cluster 3 and Cluster 5 genes to Cbf1p induction under phosphate (P) or methionine (M) limitation.

Figure S4: Simulations of feed-forward architecture. (A) Met4p stimulates the production of *MET32* transcript (and thus, Met32p protein). Met4p then forms a complex with Met32p and stimulates the production of a target gene (marked *OUTPUT*). (B) Numerical modeling of schematic in (A). The model can be found in Equations S12-S17 in the Supplement. The parameters ϕ_1 and ϕ_2 are the strength of stimulation of *MET32* transcription by Met4p and the strength of stimulation of *OUTPUT* transcription by the Met4p-Met32p complex, respectively.

Figure S5: Heatmap of clustered transcription factor activities calculated with MatrixREDUCE. The activities are shown for t = 2.5, 5, 15, 30, 45, 60, and 90 minutes following β-estradiol pulse from the time-zero transformed data in Fig 2.

Figure S6: Comparison of expression data with biochemically-determined TF targets. (A) The fraction of biochemically-determined (YEASTRACT) direct targets induced or repressed at least 2-fold in response by a TF. (B) The number of targets activated (A), repressed (R), or both activated and repressed (B) at least 2-fold by a single TF.

Figure S7: Construction of GEV-inducible alleles. Linear fragments of KanMX4-P_{GAL1} DNA were PCR amplified with homology to genomic targets.

Homology is chosen to place the new promoter and drug cassette between the first ATG of the *TF* open reading frame and the *TF*'s native promoter.

Figure S8: Growth of *met6*∆ strain with different levels of methionine. (A) Final optical density (measured in Klett units) of methionine auxotroph strain grown in YNB + varying amounts of methionine for 2 days. (B) Histograms of cell volumes from steady-state (chemostat) cultures with 3.75, 7.5, or 15 mg/L of methionine. (C) Density of steady-state cultures determined by Klett and Coulter Counter.