Supplemental Materials

Mathematical model

We model the Sre1 regulatory system in sre1N yeast with three rate equations describing the production and degradation of sre1N+ mRNA ([mXₜ]), total Sre1N protein ([Xₜ]), and total Ofd1 protein ([Fₜ]). All other chemical species follow the notation in Figure 1B.

\[
\frac{dmX}{dt} = k_dmnX \left( 1 + \frac{k_{minX1}N}{K_{XND1}N} [mX] + \frac{k_{minX2}N^2}{K_{XND1}N^2} [N]^2 - [mX] \right)
\] (S1)

\[
\frac{d[XT]}{dt} = k_{pXN}[mX] + k_{dXN}[XN] - k_{dXNF}[XNF] - k_{dXNUF}[XNUF] - \beta[XT]
\] (S2)

\[
\frac{d[F]}{dt} = k_{pF} - k_{dF}[F]
\] (S3)

To model Sre1N upregulating transcription of its own mRNA (Eq. S1), we use a simple ligand-receptor model in which Sre1N can bind to two independent sites on the sre1N+ promoter (Todd et al., 2006). This model is consistent with our observations of this phenomenon (Figure S2). We model the rate of mRNA production as a function of free Sre1N (i.e. not bound to Ofd1); the amount of Sre1N bound to DNA is assumed negligible. For simplicity, we describe sre1N+ mRNA in units relative to its basal concentration when no free Sre1N is present. sre1N+ concentration decreases with a rate coefficient \( k_{dXN} \) due to degradation as well as dilution with exponential cell growth (Porter et al., 2010). We assume that the rate of Sre1N translation is directly proportional to the concentration of sre1N+ mRNA (Eq. S2). The rate coefficients for Sre1N degradation differ depending on whether Sre1N is unbound ([Xₜ]), bound to non-oxygenated Ofd1 ([XNF]), or bound to oxygenated Ofd1 ([XNUF]). Total concentration of Sre1N decreases with rate coefficient \( \beta \) due to dilution. We model Ofd1 as being produced at a rate \( k_{pF} \) (Eq. S3); its concentration decreases with a rate coefficient \( k_{dF} \), which includes the effects of degradation and dilution.

The total concentrations of Sre1N ([Xₜ]), Ofd1 ([Fₜ]), and Nro1 ([Rₜ]) are summed as follows:

\[
[Xₜ] = [XN] + [XNF] + [XNUF]
\] (S4)

\[
[Fₜ] = [F] + [UF] + [XNF] + [XNUF] + [RF] + [URF]
\] (S5)

\[
[Rₜ] = [R] + [RF] + [URF]
\] (S6)

We model total Nro1 ([Rₜ]) as constant.

Since binding and unbinding happen rapidly compared to production and degradation, we model each binding interaction at steady state with dissociation constants as follows:

\[
K_{UF} = \frac{[U][F]}{[UF]}
\] (S7)

\[
K_{XNF} = \frac{[XN][F]}{[XNF]}
\] (S8)

\[
K_{XNUF} = \alpha K_{XNF} = \frac{[XN][UF]}{[XNUF]}
\] (S9)

\[
K_{RF} = \frac{[R][F]}{[RF]}
\] (S10)

\[
K_{URF} = \gamma K_{RF} = \frac{[R][UF]}{[URF]}
\] (S11)

While ofd1+ is a target gene of Sre1N (Todd et al., 2006), for simplicity we model increased Ofd1 production during hypoxia as a smooth function of oxygen levels:

\[
k_{pF}([U]) = \frac{1}{2} (k_{pF}^{low} + k_{pF}^{high} U) + \frac{1}{2} (k_{pF}^{high} - k_{pF}^{low} U) \tanh (U_{scale} ([U] - U_{th}))
\] (S12)
In several instances (Figure 6B in Lee et al. (2011) and our timecourse experiments, Figure 2D-F) we observed a time delay between when cells are subjected to hypoxia and when their response begins. While the source of this delay is not understood, we incorporate it into the model by letting \([U]\), the input to the model, be a low-pass-filtered version of \([U_{\text{env}}]\), the level of oxygen in the cells’ environment:

\[
\frac{d[U]}{dt} = k_{dU} ([U_{\text{env}}] - [U])
\]  

We used Eqs. S1–S13 to compute the dynamics and the steady state of the Sre1 regulatory system in sre1N yeast. Parameters for these equations are given in Table S1 (known, fixed parameters) and Table S2 (unknown, variable parameters).

**Determination of Nro1:Ofd1:Sre1 ratio**

To determine the relative abundance of Sre1, Ofd1, and Nro1 protein in S. pombe cells, we grew wild-type, sre1-13xMyc, ofd1-13xMyc, and nro1-13xMyc cells to exponential phase in rich medium at 30°C. We harvested cells at a density of \(1 \times 10^7\) cells/ml and used \(2 \times 10^7\) cells for immunoblot analysis. First, we immunoblotted extracts from wild-type cells for Sre1, Ofd1, and Nro1 using antibodies to each protein. We then immunoblotted extracts from sre1-13xMyc cells using both anti-Sre1 and anti-Myc antibodies. Similarly, we immunoblotted extracts from ofd1-13xMyc cells using both anti-Ofd1 and anti-Myc antibodies, and we immunoblotted extracts from nro1-13xMyc cells using both anti-Nro1 and anti-Myc antibodies. Signals from each lane of western blots were quantified using a VersaDoc Imaging System with Quantity One software (Bio-Rad). With the quantified western blots, we computed the relative levels of Sre1, Ofd1, and Nro1 in wild-type cells as follows:

\[
\begin{align*}
\frac{\text{Ofd1 in wild-type}}{\text{Sre1 in wild-type}} &= \frac{\text{Ofd1 in wild-type}}{\text{Ofd1 in ofd1-13xMyc}} \times \frac{\text{Ofd1 in ofd1-13xMyc}}{\text{Sre1 in ofd1-13xMyc}} \times \frac{\text{Sre1 in wild-type}}{\text{Sre1 in sre1-13xMyc}} \\
\frac{\text{Nro1 in wild-type}}{\text{Sre1 in wild-type}} &= \frac{\text{Nro1 in wild-type}}{\text{Nro1 in nro1-13xMyc}} \times \frac{\text{Nro1 in nro1-13xMyc}}{\text{Sre1 in nro1-13xMyc}} \times \frac{\text{Sre1 in wild-type}}{\text{Sre1 in sre1-13xMyc}} \\
\end{align*}
\]

We found that the ratio of Nro1 : Ofd1 : Sre1 precursor is approximately 17.1 : 9.7 : 1 in untreated wild-type cells.

**Determination of fixed model parameters**

Since Sre1 is a transcription factor, we estimate its abundance to be about 1000 molecules per cell (Ghaemmaghami et al., 2003) and assume that it is mostly dimerized (Parraga et al., 1998) for an effective Sre1 count of 500 dimers per cell. In wild-type cells under atmospheric oxygen conditions, most of this Sre1 is found in the ER membrane. Assuming a doubling time of 2.3 h, the concentration of Sre1 decreases due to dilution with a rate coefficient of 0.3 h\(^{-1}\), and Sre1 is transported out of the ER and cleaved to form the nuclear form Sre1N with a rate coefficient of about 1.1 h\(^{-1}\) (Porter et al., 2010). Thus, maintaining a steady-state level of Sre1 dimer requires a production rate of 700 dimers/h. In sre1N cells, we take this to be the basal production rate of Sre1N, the nuclear form of Sre1, and we assume that Sre1N is predominantly localized in the nucleus. Using a nuclear volume of 10 \(\mu m^3\) (Neumann and Nurse, 2007), we compute the basal production rate of Sre1N dimer (which we assume is the functional unit of Sre1N) to be \(k_{pXN} = 0.12 \mu M/h\).

Based on our measurements of Sre1, Ofd1, and Nro1, we estimate the abundance of Ofd1 and Nro1 proteins in wild-type cells to be 4850 molecules per cell and 8550 molecules per cell, respectively. Since Ofd1 and Nro1 are both predominantly localized in the nucleus (Hughes and Espenshade, 2008; Lee et al., 2009), we estimate their respective concentrations there to be 0.8 \(\mu M\) and 1.4 \(\mu M\). For Ofd1, given a
degradation rate coefficient $k_{df} = 0.5 \text{ h}^{-1}$ (determined by trial and error), this requires a high-oxygen production rate $k_{pf}^{\text{high}} = 0.4 \mu\text{M/h}$ and a low-oxygen production rate $k_{pf}^{\text{low}} = 0.6 \mu\text{M/h}$. For Nro1, we assume a constant concentration $[R_T] = 1.4 \mu\text{M}$.

While the dissociation constant between Nro1 and Ofd1’s C-terminal degradation domain had been measured to be 4.0 \mu M (Yeh et al., 2011), it was not clear whether this measurement applied best to oxygenated or non-oxygenated Ofd1 ($K_{URF}$ or $K_{RF}$). To check this, we performed the seven tests for model parameter sets (Materials and Methods) using $K_{RF} = 4.0 \mu\text{M}$ and $K_{URF} = \gamma K_{RF}$; no parameter sets passed all seven tests. We then repeated the tests with $K_{URF} = 4.0 \mu\text{M}$ and $K_{RF} = K_{URF}/\gamma$; in this case 86,011 parameter sets passed all seven tests (Results). All results reported in this study refer to the latter case ($K_{URF} = 4.0 \mu\text{M}$).

**Computational procedures**

Steady states of the model were computed by an algorithm that roughly estimates the trajectory of the model from a given starting point until a steady state is reached. Trajectories of the model in time were computed by solving Eqs. S1-S13 using the CVODE differential equation solver (Hindmarsh et al., 2005). Parameters for the positive feedback model (Eq. 1) were fit by minimizing the squared error between the logarithms of calculated and measured $sre1N^+$ mRNA using the Nelder-Mead simplex method (Hutt, 2011) with parameters $k_{pmXN1}$, $(k_{pmXN2} - k_{pmXN1})$, $K_{XND1}$, and $K_{XND2}$ squared to ensure nonnegativity. To pick the starting point for this minimization, we ran the seven tests on a uniform subset of the parameter space with starting point $(k_{pmXN1}, (k_{pmXN2} - k_{pmXN1}), K_{XND1}, K_{XND2}) = (1, 1, 1, 1)$. We then repeated the seven tests on the same subset of the parameter space, taking the starting point to be the median $(k_{pmXN1}, (k_{pmXN2} - k_{pmXN1}), K_{XND1}, K_{XND2})$ of the parameter sets that passed all seven tests the first time. We repeated this process until the number of parameter sets passing all seven tests stopped increasing with successive iterations. All positive feedback models fit the data with $0.945 \leq R^2 \leq 0.975$ computed from logarithms of calculated and measured $sre1N^+$ mRNA.

All computations were performed using MATLAB 7.4.0 (MathWorks, Natick, MA) running on Dell Precision T5400 computers with Red Hat Linux 5.1. Routines for computing steady states, evaluating Eqs. S1–S13, and finding optimal parameters for the positive feedback model were written in C and compiled as MEX-files to improve execution speed. Running the full suite of tests for every parameter combination considered took 32.4 computer-days.
Figure S1. Pairwise histograms of model parameters found in the parameter sets that passed all seven tests. Red regions denote parameter pairs that appeared together frequently; blue regions denote pairs that rarely appeared together; white regions denote pairs that never appeared together. Sample Pearson correlation coefficients $r$ were computed between pairs of parameters (logarithms of log-spaced parameters) to assess pairwise dependence; $|r| \geq 0.1$ are shown.
Figure S2. Experiments with yeast lacking Ofd1 show the positive feedback of free Sre1N promoting $sre1^+$ transcription. Sre1N protein and $sre1^+$ (or $sre1N^+$) mRNA were measured in several strains of yeast lacking the $ofd1^+$ gene (Table S3) as described in Materials and Methods. Data points are normalized to that in $sre1N ofd1\Delta$ yeast.
Table S1. Fixed parameters in the model.

| Parameter   | Meaning                                                                 | Value                        |
|-------------|--------------------------------------------------------------------------|------------------------------|
| $k_{pXN}$   | basal Sre1N production rate                                              | $0.12 \mu M/h$ $^a$         |
| $k_{dmXN}$  | rate coefficient for $sre1N^+$ mRNA degradation                          | $7.0 \text{ h}^{-1}$ $^b$    |
| $k_{pF}^{\text{low}}$ | Ofd1 production rate at low oxygen                                  | $0.6 \mu M/h$ $^a$          |
| $k_{pF}^{\text{high}}$ | Ofd1 production rate at high oxygen                                    | $0.4 \mu M/h$ $^a$          |
| $k_{dF}$    | rate coefficient for Ofd1 degradation                                    | $0.5 \text{ h}^{-1}$ $^b$    |
| $[R_T]$     | total Nro1 concentration                                                 | $1.4 \mu M$ $^a$            |
| $K_{URF}$   | dissociation constant between Nro1 and oxygenated Ofd1                  | $4.0 \mu M$ (Yeh et al., 2011) |
| $\beta$     | coefficient for dilution of a chemical species due to exponential cell growth | $0.3 \text{ h}^{-1}$ (Porter et al., 2010) |
| $k_{dU}$    | filter coefficient for oxygen delay model                               | $20 \text{ h}^{-1}$ $^b$    |
| $U_{th}$    | oxygen threshold at which Ofd1 production is upregulated                | $5\% \text{ O}_2$ $^b$      |
| $U_{scale}$ | scales oxygen range over which Ofd1 production is upregulated           | $0.5$ $^b$                   |

$^a$ Calculation described in Experimental Procedures.

$^b$ Our estimate.
Table S2. Ranges of unknown parameters tested with the model. The example parameter set used in this paper (right column) is that which passed all seven tests and had the highest minimum $R^2$ value between simulation and data for $sre1N^+$ mRNA and Sre1N protein in the steady-state and hypoxia experiments (i.e. data in Figure 2A, B, D, E).

| Parameter | Meaning | Range tested | Value used in examples |
|-----------|---------|--------------|------------------------|
| $k_{dXN}$ | rate coefficient for Sre1N degradation, not bound to Ofd1 | 0–2.5 h$^{-1}$ | $n = 11^a$ 1.0 h$^{-1}$ |
| $k_{dXNF}$ | rate coefficient for Sre1N degradation, bound to Ofd1, not oxygenated | 0–21 h$^{-1}$ | $n = 22^a$ 3.0 h$^{-1}$ |
| $k_{dXNUF}$ | rate coefficient for Sre1N degradation, bound to Ofd1, oxygenated | 0–21 h$^{-1}$ | $n = 22^a$ 4.0 h$^{-1}$ |
| $K_{UF}$ | dissociation constant of Ofd1 and oxygen | 0.001–5% O$_2$ | $n = 29^b$ 0.0385% O$_2$ |
| $K_{XNF}$ | dissociation constant of Sre1N and Ofd1, not oxygenated | 0.001–100 µM | $n = 41^b$ 0.0042 µM |
| $K_{XNUF}$ | dissociation constant of Sre1N and Ofd1, oxygenated | 0.001–1 µM | $n = 25^b$ 0.01 µM |
| $\gamma$ | ratio of Ofd1-Nro1 dissociation constants with/without oxygen ($K_{URF}/K_{RF}$) | 1–10,000 | $n = 33^b$ 316 |
| $k_{pmXN1}$ | $sre1N^+$ transcription rate, one Sre1N molecule bound to promoter | fit by optimization | 14.6 |
| $k_{pmXN2}$ | $sre1N^+$ transcription rate, two Sre1N molecules bound to promoter | fit by optimization | 24.0 |
| $K_{XND1}$ | dissociation constant of Sre1N and first $sre1N^+$ binding site | fit by optimization | 0.0243 µM |
| $K_{XND2}$ | dissociation constant of Sre1N and second $sre1N^+$ binding site (i.e. with molecule already bound to the other site) | fit by optimization | 8.01 µM |

$^a$ Linearly spaced. $n$ refers to the number of different values considered.

$^b$ Logarithmically spaced.
Table S3. *S. pombe* strains used in this work.

| *S. pombe* strain       | Genotype                                      | Reference or source                  |
|-------------------------|-----------------------------------------------|--------------------------------------|
| Wild-type (KGY425)      | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1  | Burke and Gould (1994)               |
| *sre1N* (PEY875)        | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 sre1N | Hughes and Espenshade (2008)         |
| *sre1-13xMyc* (PEY670)  | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 sre1-13xMyc::kanMX6 | This study                           |
| *ofd1-13xMyc* (BTY42)   | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 ofd1-13xMyc::kanMX6 | This study                           |
| *nro1-13xMyc* (CLY92)   | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 nro1-13xMyc::kanMX6 | This study                           |
| *ofd1∆* (PEY872)        | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 \(\Delta\) ofd1::kanMX6  | Hughes and Espenshade (2008)         |
| *sre1N ofd1∆* (PEY873)  | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 sre1N \(\Delta\) ofd1::kanMX6  | Hughes and Espenshade (2008)         |
| *sre1N ofd1∆ ubr1∆* (CLY269) | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 sre1N \(\Delta\) ofd1::kanMX6 ubr1::kanMX6 | Lee et al. (2011)                    |
| *scp1∆ ofd1∆* (CLY335)  | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 \(\Delta\) scp1::kanMX6 \(\Delta\) ofd1::kanMX6 | This study                           |
| *scp1∆ ofd1∆ + pAH5* (1 CaMV-Sre1N) | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 \(\Delta\) scp1::kanMX6 \(\Delta\) ofd1::kanMX6 pCaMV-Sre1N(1-440)-ura4 pCaMV-Sre1N(1-440)-leu2 | This study, Stewart et al. (2011) |
| *scp1∆ ofd1∆ + pAH2* + pAH5 (2 CaMV-Sre1N) | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 \(\Delta\) scp1::kanMX6 \(\Delta\) ofd1::kanMX6 pCaMV-Sre1N(1-440)-ura4 pCaMV-Sre1N(1-440)-leu2 | This study, Stewart et al. (2011) |
| *ofd1∆ ubr1∆* (CLY337)  | \(h^−\) leu1-32 ura4-D18 ade6-M210 his3-D1 \(\Delta\) ofd1::kanMX6 \(\Delta\) ubr1::natMX6 | This study                           |
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