Supporting Methods

Fermentor control. The basic medium consisted of D-glucose (20 g/l), $(NH_4)_2SO_4$ (5 g/l), KH_2PO_4 (2 g/l), $MgSO_4.7H_2O$ (0.5 g/l), $CaCl_2.2H_2O$ (0.1 g/l), $FeSO_4.7H_2O$ (20 mg/l), $ZnSO_4.7H_2O$ (10 mg/l), $CuSO_4.5H_2O$ (5 mg/l), $MnCl_2.4H_2O$ (1 mg/l), 70% H_2SO_4, 1 ml/l), Difco yeast extract (1 g/l) and Sigma Antifoam A (0.2 ml/l). For this study we used modified jar fermentors (Eyela, Japan). Unless otherwise stated the cultures temperature was controlled at 30°C (Fig 5B; ± 0.02°C), pH was maintained at 3.4 (± 0.03) by the automatic addition of 2.5 N NaOH (Fig 5C), were agitated at 750 rpm (Fig. 5D; ±3 rpm), aerated at 0.150 L/min by mass flow control (Fig 5E; ±0.02 L/min; B.E. Marubishi, Japan). At all times the reactor pressure was kept below 101700 Pa (atmospheric 101325 Pa) by monitoring using a manometer (DM-760, Comfix, Japan; Fig. 5D) installed on a split outlet flow stream, and replacing blocked outlet filters when required (Hepa-vent™). Temperature control utilized an external sensor connected to a circulating waterbath (F25-ME, Julabo, Japan). The heat production during the respiratory phase of the fermentation can be observed by the oscillatory bath temperature, whereas the reactor temperature is controlled. Local control of agitation and pH was carried out by Labo controllers (B.E. Marubishi, Japan). The dilution rate was maintained at 0.087 h^{-1} (Fig 5F; ±0.004 h^{-1}) was calculated by dividing the flow rate (Fig 5G) by the media volume (measured using a balance; SB16001, Mettler Toledo, Japan; Fig 5F). The flow rate was manually adjusted by altering media addition *via* through 1 mm tubing inserted (inner diameter; Masterflex, Cole Palmer, USA) using a peristaltic pump (AC2110, ATTA, Japan). The pump was a six roller planetary design which minimizes pulsing during rotation (about 10

rpm). Furthermore media was introduced into the fermentor *via* 23 gauge steel needle, this insured that the media was introduced in a stream of <20 µL droplets or just under a droplet per second at the operating dilution rate. Flow rate was calculated by placing the media reservoir on a balance (PMK-16, Mettler Toledo, Japan) and measuring the time taken to undergo a 10.0 g (±0.05 g) weight. Balances were setup to read from unstable environments and shielded from direct breezes. Data acquisition and remote control of all instruments was carried out using in-house software and samples were acquired every 10 s.

Gas Analyses. Continuous partial pressure of oxygen (P_{O_2}) and partial pressure of carbon dioxide (P_{CO_2}) off-gas measurements were carried out using an Enoki-III (Figaro engineering, Japan) analyzer. The partial pressure of hydrogen sulfide (P_{H_2S}) in the off-gas was measured continuously using an electrode based gas monitor (HSC-1050HL, GASTEC, Japan). Instruments were calibrated as per manufacturer's instruction. Assuming there is little contribution H_2S in the air input, O_2 uptake rates (q_{O_2}), CO_2 production rates (q_{CO_2}), H_2S production rates (q_{H_2S}) were derived from the following equations:

$$q_{O_2} = \frac{F_{in}}{RTV_R}\left(P_{O_{2(in)}} - P_{O_{2(out)}}\left(\frac{1-P_{O_{2(in)}}}{1-\left(P_{O_{2(out)}}+P_{CO_{2(out)}}+P_{H_2S_{(out)}}\right)}\right)\right)$$

$$q_{CO_2} = \frac{F_{in}}{RTV_R}\left(P_{CO_{2(out)}}\left(\frac{1-P_{O_{2(in)}}}{1-\left(P_{O_{2(out)}}+P_{CO_{2(out)}}+P_{H_2S_{(out)}}\right)}\right) - P_{CO_{2(in)}}\right)$$

$$q_{H_2S} = \frac{F_{in}}{RTV_R}\left(P_{H_2S(out)}\left(\frac{1-P_{O_{2(in)}}}{1-\left(P_{O_{2(out)}}+P_{CO_{2(out)}}+P_{H_2S_{(out)}}\right)}\right)\right)$$

Where F_{in} was the gas flow into the system, R was the universal gas constant (0.0820575 L atm mol^{-1} K^{-1}) and V_R was the volume of the reactor (0.65 L).

GC-MS measurements. Samples (500µL) were rapidly quenched in 500µL methanol at -70°C (dry ice/ acetone bath) and ~500µL glass beads. Samples were then frozen at -80°C. The samples then underwent 3 freeze/thaw/vortex cycles (-80°C/-20°C; beat for 30 s) and were dried *in vacuo* prior to derivitization. Two-step derivitization of dried samples were done by protecting the carbonyl moieties by methoximation using 100µL of a 20 mg mL^{-1} solution of methoxyamine hydrochloride (Fluka) in pyridine (Fluka) at 30°C for 90 min. Acidic protons were subsequently derivatized with 100µL

N-methyl-*N*-trimethylsilyltrifluoride (MSTFA, M&N) at 37°C for 30 min. 60µL were transferred into 200µL glass vials (Chromacol) and 1µL were injected split-less into a Leco Pegasus III GC-tof-MS system (St. Joseph, USA) consisting of a Focus autosampler (ATAS), an Agilent 6890N gas chromatograph equipped with a 20m DB5-MS column (20m x 0.25mm ID x 0.25µm film). Injector temperature was 250°C, the interface was set to 260°C and the ion source temperature held at 230°C. Helium flow was 1.2 mL min^{-1}. After 1min at 80°C, oven temperature was increased by 30°C min^{-1} to 330°C, held at 330°C for 3min and cooled to 80°C. Automated deconvolution and peak finding was performed using ChromaTof software (Leco, St. Joseph, USA) and peak alignment was carried out in MATLAB (V6.5.1, The MathWorks).

Interaction network. Protein-DNA binding data (1) was downloaded for 102 transcription factors and filtered so that only significant interactions conserved amongst at least 2 other yeast species were included ($p<0.001$), these were further supplemented by cited interactions from SGD (2). Each edge was colored in dark cyan and was directional and had the interaction descriptor "macTF", "eTF" or "cTF" the prefix mac describes high throughput, e describes literature evidence and c describes cited interactions respectively. Protein-protein interactions that were not originally described in the complex information were downloaded from the GRID database (3) and consisted of immuno precipitation, curated 2-hybrid interactions and cited interactions. If a protein-protein interaction had only one line of evidence then it was ignored. These were further manually curated by constructing an interaction map of protein-localization (4) and omitting interactions that were made dubious by lack of proximity, e.g., a nuclear protein probably does not interact with a protein solely located in the mitochondria. Each edge was non-directional and had the descriptor "pp" and was represented by a dark magenta line. The reaction network was constructed from a modified reaction list of Förster (5), cross-referenced with yeast reaction networks from yeastcyc (2) and KEGG (6). A reaction was represented by a series of metabolites reacting with a protein ("mp"; red) followed by the release of products from the protein ("pm"; green). The edges for these nodes were directional and bi-directional reactions were shown by metabolites having both product and reactant edges. A sub-network of metal, transporter and carrier proteins was also constructed from this reaction list, proteins listed as metal binding in SGD and the yeast transport protein database (7), these interactions were non-directional and were represented by a gray line. The derived networks are available in .sif and .gml format from (http://www.symbio.jst.go.jp/dougie/yROS.html). We have been

conservative in our network construction because in any large throughput datasets the networks will contain a number of false positives, especially regarding protein-protein interaction datasets (8). Therefore we have tried to focus on interactions that have a number of lines of evidence for their existence or are likely because of species-species conservation.

Signal processing. The phase (θ) of each sample (k) was calculated for each cycle (m) for each dataset.

$$\theta_k = 360° \left(\frac{t_k - t_{\left(\frac{d[O_2]}{dt}_{\min}\right)_m}}{t_{\left(\frac{d[O_2]}{dt}_{\min}\right)_{m+1}} - t_{\left(\frac{d[O_2]}{dt}_{\min}\right)_m}} \right)$$

Where t was the sample time and the start point for each cycle was minimum first derivative of the dissolved oxygen concentrations ($\frac{d[O_2]}{dt}_{\min}$). Samples were then phase adjusted to reconstruct three cycles where θ_1 was closest to 0° (Fig. 6).

Discrete Fast Fourier transforms ($\mathcal{F}$(x)) and subsequent calculations were carried out using MATLAB. The amplitude spectra ($A_{i,j}$) were derived from the real ($a_{i,j}$) and imaginary ($b_{i,j}$) coefficient series for each transcript (i) as follows:

$$A_{i,j} = 2\sqrt{{a_{i,j}}^2 + {b_{i,j}}^2}$$
$$1 < j < \frac{k}{2}$$

Where j was the frequency; for example when a *circa* 40 minute periodicity was sampled every 4 minutes, j = 3 was chosen to focus the analysis (0.39mHz; 42.7min). The oscillation strength (O) was calculated by dividing $A_{i,3}$ by the arithmetic power for each cycle:

$$\text{If } A_{i,3} = A_{i,\max}\text{, then } O_i = \frac{A_{i,3}}{O_\tau} \frac{m_{\max}}{\sum_{i=1}^{i_{\max}} (I_{i,k} - I_{\min_m})}\text{, else}$$

$$O_i = 0$$

Where $I_{i,k}$ is the raw data value and $I_{\min_m}$ is the local minimum for cycle (m). O_τ was an edge effect correction factor to compensate for differences in the observed period and the period of

sampling. O_τ was calculated by generating a sine waveform using the sample phase angle with respect to the observed periodicity. It was calculated by the following equation:

$$A_{\tau,3} = 2\sqrt{a_{\tau,3}{}^2 + b_{\tau,3}{}^2}$$

$$O_\tau = A_{\tau,3} \frac{m_{\max}}{\sum_{i=1}^{i_{\max}} (\sin(\theta_k) + 1)}$$

Where $A_{\tau,3}$ is the FFT amplitude of period (τ) at 0.39 mHz derived from the real ($a_{\tau,3}$) and imaginary ($b_{\tau,3}$) coefficients of the generated waveform. The phase angle ($\varphi_{i,j}$) was calculated from the FFT coefficients using the following equation:

$$\varphi_i = \frac{180°}{\pi} \tan^{-1}\left(-\frac{b_{i,3}}{a_{i,3}}\right) + \theta_1 + \varphi_\tau$$

Where edge effects caused by the FFT were compensated by the addition of φ_τ:

$$\varphi_\tau = \frac{180°}{\pi} \tan^{-1}\left(-\frac{b_{\tau,3}}{a_{\tau,3}}\right) + \theta_1$$

Waveforms that were spiked or triangular in shape (produced mainly by transcripts produced in the oxidative phase or metabolites produced in the early oxidative phase) produced $O > 1$. As a computational control the data matrix was then randomly permuted and φ, and O were calculated for each data set.

References

1. MacIsaac, K. D., Wang, T., Gordon, D. B., Gifford, D. K., Stormo, G. D. & Fraenkel, E. (2006) *BMC Bioinformatics* **7,** 113.
2. Cherry, J. M., Ball, C., Weng, S., Juvik, G., Schmidt, R., Adler, C., Dunn, B., Dwight, S., Riles, L., Mortimer, R. K. & Botstein, D. (1997) *Nature* **387,** 67-73.
3. Breitkreutz, B. J., Stark, C. & Tyers, M. (2003) *Genome Biol* **4,** R23.
4. Kumar, A., Agarwal, S., Heyman, J. A., Matson, S., Heidtman, M., Piccirillo, S., Umansky, L., Drawid, A., Jansen, R., Liu, Y., Cheung, K. H., Miller, P., Gerstein, M., Roeder, G. S. & Snyder, M. (2002) *Genes Dev* **16,** 707-19.
5. Forster, J., Famili, I., Fu, P., Palsson, B. O. & Nielsen, J. (2003) *Genome Res* **13,** 244-53.
6. Kanehisa, M. & Goto, S. (2000) *Nucleic Acids Res* **28,** 27-30.
7. Andre, B. (1995) *Yeast* **11,** 1575-611.

8. Edwards, A. M., Kus, B., Jansen, R., Greenbaum, D., Greenblatt, J. & Gerstein, M. (2002) *Trends Genet* **18,** 529-36.
9. Satroutdinov, A. D., Kuriyama, H. & Kobayashi, H. (1992) *FEMS Microbiol Lett* **77,** 261-7.
10. Keulers, M., Suzuki, T., Satroutdinov, A. D. & Kuriyama, H. (1996) *FEMS Microbiol Lett* **142,** 253-8.
11. Keulers, M., Satroutdinov, A. D., Suzuki, T. & Kuriyama, H. (1996) *Yeast* **12,** 673-82.
12. Murray, D. B., Engelen, F., Lloyd, D. & Kuriyama, H. (1999) *Microbiology* **145 (Pt 10),** 2739-45.
13. Murray, D. B., Roller, S., Kuriyama, H. & Lloyd, D. (2001) *J Bacteriol* **183,** 7253-9.
14. Murray, D. B., Klevecz, R. R. & Lloyd, D. (2003) *Exp Cell Res* **287,** 10-5.
15. Klevecz, R. R., Bolen, J., Forrest, G. & Murray, D. B. (2004) *Proc Natl Acad Sci U S A* **101,** 1200-5.