Supporting Information

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SI Materials and Methods

YRO Exposed to Natural Sunlight. A yeast respiratory oscillation (YRO was established as described near a west-facing window in the dark (blinds closed). After sunset, the blinds were raised to allow the following day's sunlight to enter the window and strike the oscillating culture. Light intensity was measured periodically throughout the day using a LI-COR LI-250A quantum light meter placed at 90° increments around the vessel's exterior. (See Table S1 for representative light measurements.)

Quantification of Colored Light on the YRO and Absorption Spectrum of Cytochrome Oxidase. The fluence of filtered light applied to the YRO was determined by averaging the light intensity at eight positions around the interior of the light-filtered vessel as measured by a LI-COR 250A light meter with a LI-190S quantum probe. Because the probe was not equally sensitive across the visible spectrum, each of the measured light intensities was multiplied by a correction value for spectral response (provided by LI-COR for the LI-190S quantum probe). The correction values were 1.56 for the blue filter, 1.31 for the green filter, and 1.11 for the red filter.

The transmission spectra for unfiltered and filtered cool white fluorescent (CWF) light were measured using a QuantaMaster QM-7/SE (Photon Technology International). The percentage of the area under each transmission curve for blue, green, and red light (binned into 25-nm groups across the spectrum) was multiplied by the total fluence for each of the filtered light treatments on the YRO to estimate the number of photons in each 25-nm bin that struck the culture during the filtered light treatments.

The 25-nm–binned absorption spectra for reduced and oxidized cytochrome oxidase were created from the published transmission spectra of pig heart muscle cytochrome oxidase (1). Figures 3 and 4 from ref. 1 were converted to absorption spectra, and each 250-nm bin (from 400–650 nm) represents the average absorption across 25 nm.

Dot products for blue, green, and red light treatments with respect to cytochrome oxidase absorbance spectra were calculated by adding the absorbances for oxidized and reduced cytochrome oxidase for each 25-nm bin in Fig. S3*B* and then separately multiplying this sum by the light intensity for each color (red, green, or blue) for each 25-nm bin. Dot products from all bins were summed for each color.

Creation of Yeast Strain yBR-ura3ΔCEN.PK113-7D. A uracil auxotrophic strain of CEN.PK113-7D was created by knocking out the *URA3* gene of CEN.PK113-7D by replacing its coding sequence with an extra copy of the yeast *CUP1-1* gene. The *CUP1-1* gene (plus 470 bp upstream and 300 bp downstream) was amplified by PCR from yeast genomic DNA of strain S288C using the following primers, which also add an NcoI restriction site (underlined) to both ends of the product:

TTCACTGAGCTCCATGGATAATTAGTAAGCCGATCCC

ATGATTGAGCTCCATGGCATTTTAGGATTAATACATATAGC

The PCR product was inserted into the middle of the *URA3* gene of pRS306 (2) at its NcoI site, thereby disrupting URA3′s coding sequence and providing 230 bp of *URA3* homology upstream and downstream from the *CUP1* PCR product. Yeast strain CEN.PK113-7D was transformed with PstI/StuI-digested DNA from the constructed plasmid and was selected for *URA3* knockout on 5-fluoroorotic acid (5-FOA) yeast extract/peptone/dextrose (YPD) plates (0.1% 5-FOA, 1% yeast extract, 2% peptone, 2% D-glucose, 2% agar). Positive transformants were assayed for their inability to grow on complete supplement mix-ura (CSM-ura) plates [0.67% yeast nitrogen base without amino acids (Difco), 770 mg/L CSM Drop-Out ura (Formedium), 2% D-glucose, 2% agar]. Candidates for ura3Δ were grown in YPD (without selection pressure) overnight and were plated on CSM-ura plates to identify reversion-incapable strains of *ura3*Δ CEN.PK113-7D. The strain *yBR-ura3ΔCEN.PK113-7D* showed no evidence of *URA3* reversion after multiple generations of growth in nonselective medium; however, it retained the ability to grow on 5-FOA YPD. Furthermore, this strain does not oscillate under growth conditions that normally produce the YRO in the WT strain CEN.PK 113–7D.

Quantitative RT-PCR. The CEN.PK strain yBR-ura3ΔCEN.PK113-7D was grown in continuous culture under nonoscillating conditions in a Bioflo 115 reactor as described (3) with the exception that 17 mg/L of uracil was added to the medium. This strain was used because the auxotrophic limitation prevents the formation of oscillations under continuous culture. After 24 h of batch growth in darkness, yeast was grown in continuous culture in the dark for another 24 h before three 1.5-mL samples of yeast were collected from the bioreactor and processed independently for their RNA. The culture was illuminated at 280 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 12 h by two 65-W CWF lamps (Lithonia Lighting). Then three more samples of yeast were collected from the bioreactor and processed independently for their RNA. RNA was isolated by glass bead disruption followed by phenol/chloroform extraction and ethanol precipitation (4). The quantitative RT-PCR protocol (5) and primers (6) for *TRX2* and *RPO21* (reference gene) were followed as described elsewhere (5) using the GoTaq 1-Step RT-qPCR System (Promega) and a C1000/CFX96 real-time thermocycler (Bio-Rad).

Sodium Azide Treatment of YRO. Initially, various concentrations (4–20 μM) of sodium azide were added as a single injection into an oscillating culture the CEN.PK strain at various times to determine an optimal range of concentrations for possible YRO perturbation. However, single injections of NaN_3 elicited only transient effects on the dissolved oxygen oscillation. Because the culture medium is acidic (pH 4), it is likely that the sodium azide was volatilized into gaseous azoic acid that rapidly left the culture after a single injection. To compensate for the gaseous loss of azoic acid and the constant dilution of the continuous culture, we introduced sodium azide into the oscillating culture at a steady drip of 3.4 μmol/h or 10 μmol/h using a syringe pump.

1. Horie S (1964) On the absorption spectrum of cytochrome a-3. *J Biochem* 56:57–66.
2. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* 122(1):19–27.
3. Robertson JB, Stowers CC, Boczko E, Johnson CH (2008) Real-time luminescence monitoring of cell-cycle and respiratory oscillations in yeast. *Proc Natl Acad Sci USA* 105(46):17988–17993.
4. Pikielny CW, Rosbash M (1985) mRNA splicing efficiency in yeast and the contribution of nonconserved sequences. *Cell* 41(1):119–126.
5. Auchère F, Santos R, Planamente S, Lesuisse E, Camadro JM (2008) Glutathione-dependent redox status of frataxin-deficient cells in a yeast model of Friedreich's ataxia. *Hum Mol Genet* 17(18):2790–2802.
6. Lefevre S, et al. (2012) The yeast metacaspase is implicated in oxidative stress response in frataxin-deficient cells. *FEBS Lett* 586(2):143–148.

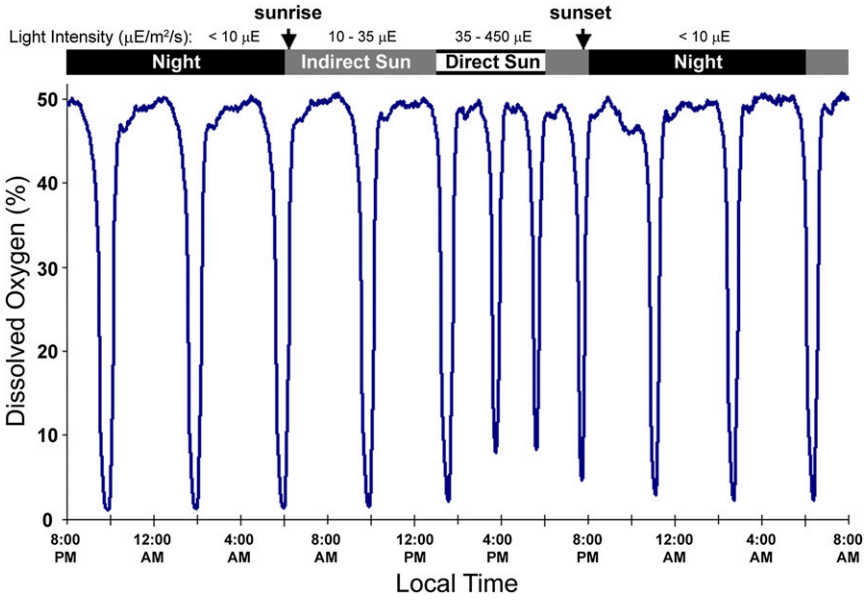

Fig. S1. Natural sunlight can affect the YRO similarly to artificial CWF light. An oscillation with a 4-h period was established 30 cm from a west-facing window at night. Between sunrise and 1:00 PM local time, indirect sunlight came through the window with light intensities never greater than 35 $\mu E \cdot m^{-2} \cdot s^{-1}$. This dim, indirect sunlight had little, if any, noticeable effect on the period or amplitude of the YRO. Between 1:00 PM and 6:00 PM local time, direct full sunlight (with intermittent cloud cover) struck the oscillating culture with light intensities that ranged from 35 to 450 $\mu E \cdot m^{-2} \cdot s^{-1}$. Direct sunlight noticeably shortened the period and amplitude of the YRO, and the effect diminished upon the return to indirect sunlight as the sun began to set.

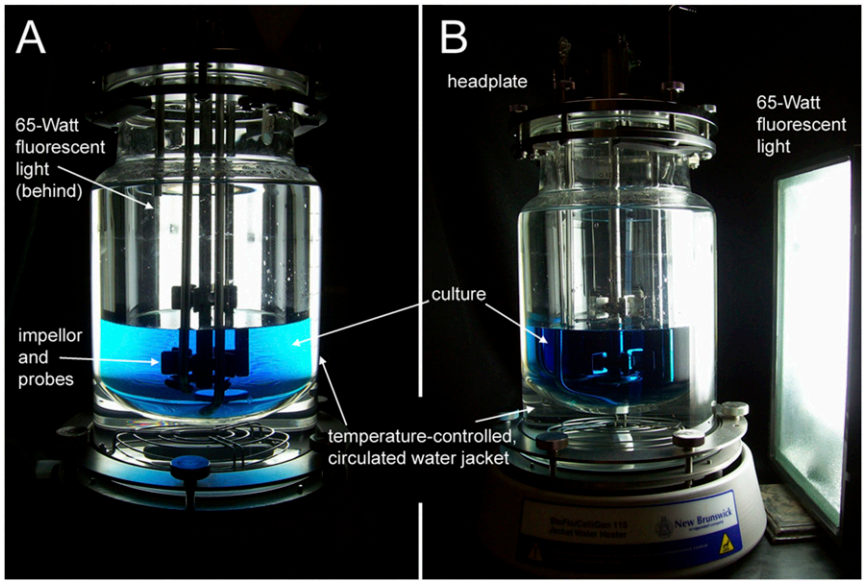

Fig. S2. A depiction of the Bioflo 115 bioreactor and a single 65-W fluorescent light source as used for experiments involving light applied to continuous cultures. *A* and *B* show the bioreactor and light source from two different angles. To generate this figure, 900 mL of blue-stained water was used in place of culture so that the culture vessel and the temperature-controlled water jacket could be visually distinguished.

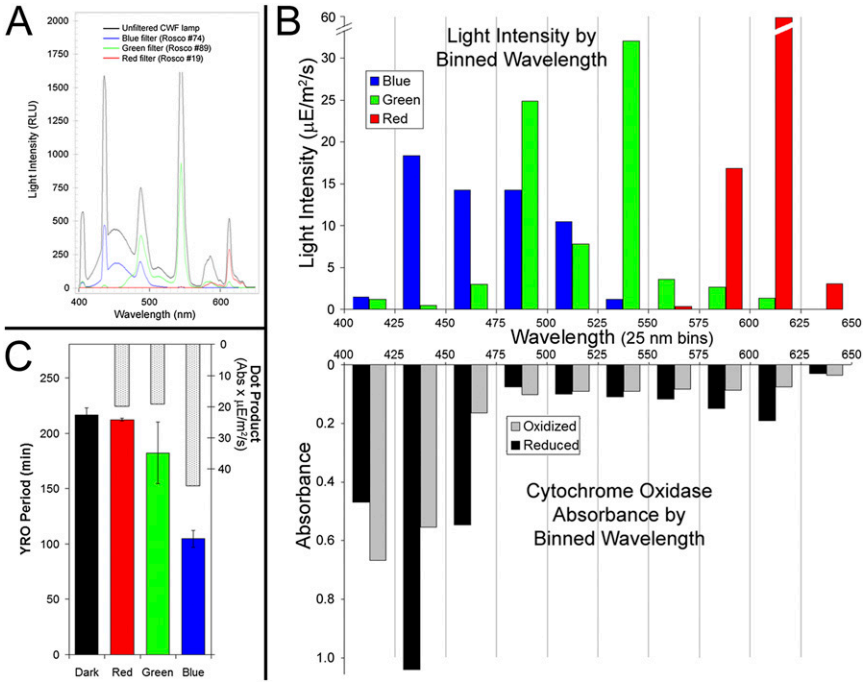

Fig. S3. Quantification of light on the YRO and a candidate photoreceptor. (*A*) Transmission spectra of visible light from filtered (colored traces) or unfiltered (black trace) compact CWF light measured by a QuantaMaster QM-7/SE fluorescence spectrophotometer. (*B*) Estimated light intensity that was applied to the YRO is shown across the visible spectrum in 25-nm bins (*Upper*) compared with the absorption spectrum for a candidate light-absorbing complex (cytochrome oxidase) divided into similar 25-nm bins (*Lower*). Reduced cytochrome oxidase has particularly strong absorption for blue light in the range of 425–475 nm, corresponding to the strongest intensities of filtered blue light. (*C*) Similar intensities of red-, green-, and blue-filtered CWF light have noticeably different effects on the YRO (colored bars). Red and green light at 80 $\mu E \cdot m^{-2} \cdot s^{-1}$ have little to no effect on the period of the YRO compared with dark treatment; however blue light at 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ has a noticeably greater effect. Error bars indicate SD for periods within the same treatment. Dot products (stippled bars) show the combined influence of colored light intensity with cytochrome oxidase absorption across all bins in *B* for red, green, and blue light assuming light intensity and absorbance have even and equal influential relationships. Given these assumptions, this analysis predicts that if cytochrome oxidase is the sole light absorber, blue light should have the strongest effect and that red and green light should have lesser and roughly equal effects. Correlation of results shows cytochrome oxidase's absorption is sufficient to account for the effect seen in blue light; however there may be a separate/additional light absorber that accounts for the effect seen in green light.

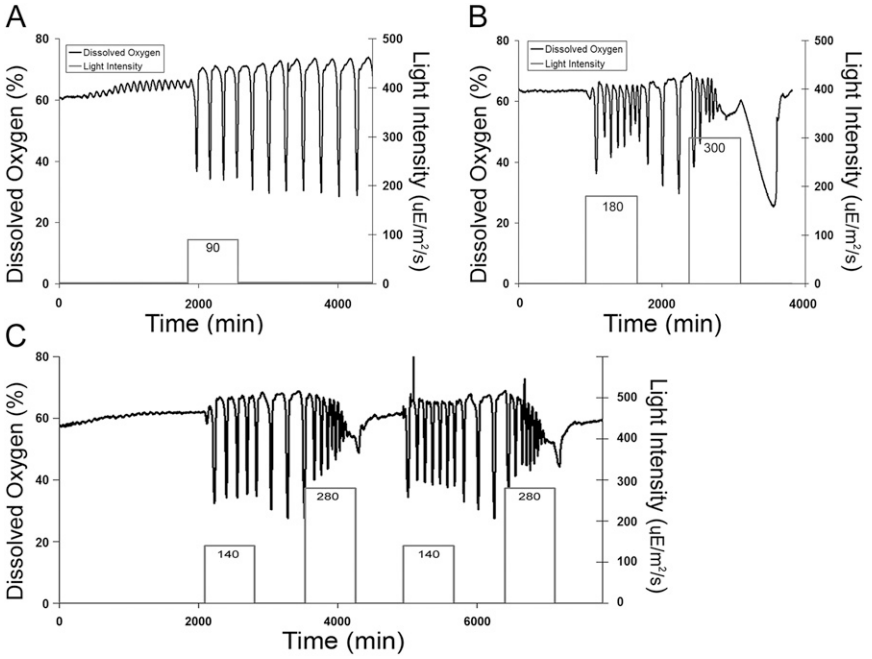

Fig. S4. The *yeast activator protein-1* (*yap1*) knockout strain was treated for 12 h with light of different intensities. (*A*) The effect of a single 12-h light treatment to a continuous culture of *yap1*-knockout yeast. The *yap1*-knockout culture fails to oscillate with robust amplitude when initiated in darkness; however, after exposure to light, the knockout strain oscillates similarly to WT even when transferred back to darkness. (*B*) Another example of *yap1*-knockout strain in continuous culture failing to oscillate with robust amplitude until first exposed to light. Two 12-h light treatments were applied at different intensities than in *A*. Periods of *A* and *B* compared with WT are shown in Fig. 2*C*. (*C*) If the oscillation is damped by exposure to bright light (as at time 4,000 min), the culture requires a moderate light treatment to reinitiate the YRO. For all panels, 12-h light treatments are indicated by rectangular bars for which height corresponds to light intensity and width corresponds to time of treatment. The number inside each bar is the light intensity in $\mu E \cdot m^{-2} \cdot s^{-1}$.

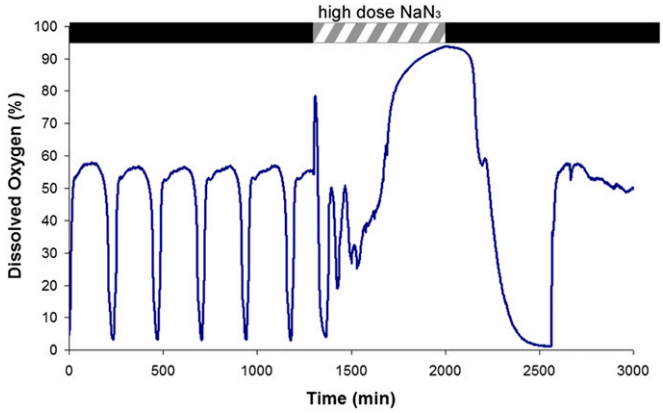

Fig. S5. A more concentrated dose of sodium azide applied to an oscillating yeast culture produces an effect similar to very bright light. An oscillating culture was given a 12-h treatment of 10 μmol/h sodium azide (striped bar, top). During treatment, the period and amplitude of the YRO initially shortened; then, with rising dissolved oxygen, the oscillation destabilized altogether. When the treatment was terminated, the dissolved oxygen level dropped, but the oscillation never reinitiated.

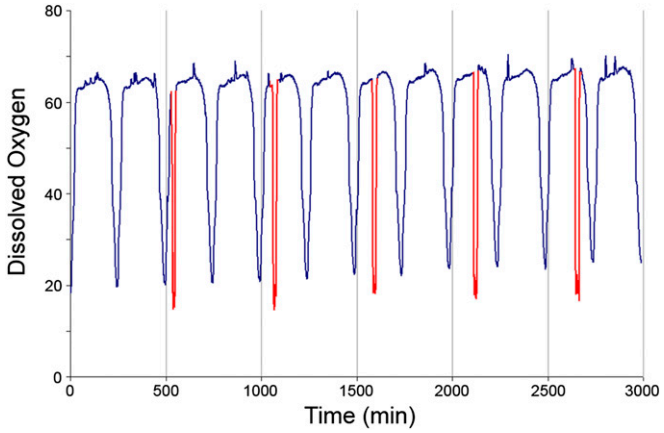

Fig. S6. Effects of artificially produced dissolved oxygen troughs on the YRO. The YRO with a period of 250 min (blue trace) does not show evidence of phase resetting when the oxygen level in the culture is artificially lowered (red trace) by infusion of nitrogen to levels and durations similar to those produced naturally by the high oxygen consumption phase of the YRO. Regardless of when the artificial trough was applied, the YRO maintains a period of 250 min without phase-shifting.

Table S1. Light-meter readings for various conditions

	Artificial light treatment intensity measurements			Representative natural sunlight intensity measurements			
Position	One CWF lamp	Two CWF lamps	Three CWF lamps	Sunlight 11:00 AM indirect	Sunlight 2:30 PM cloudy	Sunlight 2:45 PM clear sky	Sunlight 4:00 PM clear sky
1	120, 120	120,120	260, 260				
2	4, 3	7, 6	500*, 520*	15	40	240	290
3	4, 4	120, 120	260, 270				
4	3.5, 2.5	480*, 470*	550*, 550*	6	17	30	125
5	5, 4	140, 120	150, 120				
6	2, 2	5, 4	8, 8	4	14	20	320
7	110, 100	110, 100	120, 120				
8	480*, 460*	480*, 480*	530*, 520*	45*	123*	460*	1,040*
Average	91, 87	182, 177	297, 296	17	49	187	444

Positions 1–8 are 45° intervals around the bioreactor (positions 8 and 1 are adjacent). Light intensity values are in $\mu E \cdot m^{-2} \cdot s^{-1}$. For CWF sources, duplicate readings were taken, and their averages were used for light intensity in Fig. 1 *A* and *B*. For natural sunlight, single readings were taken at 90° intervals. Values shown in the table represent a sample of light-meter readings taken throughout the day shown in Fig. S1.
*Positions for which the light source was directly facing.