Visible light alters yeast metabolic rhythms by inhibiting respiration

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Exposure of cells to visible light in nature or in fluorescence microscopy often is considered to be relatively innocuous. However, using the yeast respiratory oscillation (YRO) as a sensitive measurement of metabolism, we find that non-UV visible light has a significant impact on yeast metabolism. Blue/green wavelengths of visible light shorten the period and dampen the amplitude of the YRO, which is an ultraslow rhythm of cell metabolism and transcription. The wavelengths of light that have the greatest effect coincide with the peak absorption regions of cytochromes. Moreover, treating yeast with the electron transport inhibitor sodium azide has similar effects on the YRO as visible light. Because impairment of respiration by light would change several state variables believed to play vital roles in the YRO (e.g., oxygen tension and ATP levels), we tested oxygen’s role in YRO stability and found that externally induced oxygen depletion can reset the phase of the oscillation, demonstrating that respiratory capacity plays a role in the oscillation’s period and phase. Light-induced damage to the cytochromes also produces reactive oxygen species that up-regulate the oxidative stress response gene TRX2 that is involved in pathways that enable sustained growth in bright visible light. Therefore, visible light can modify cellular rhythmicity and metabolism through unexpectedly photosensitive pathways.

Many organisms are exposed to visible light in their environment. Full sunlight can deliver up to 10 quadrillion photons of visible light-cm\textsuperscript{-2}s\textsuperscript{-1} (i.e., 2,000 \textmu Einsteins-m\textsuperscript{-2}s\textsuperscript{-1}), and cloud cover reduces this exposure only by a factor of 10. Even though sunlight provides photosynthetic energy to plants and a medium for the vision of many animal species, its high-intensity rays damage living cells when light-absorbing molecules cannot safely disperse the energy that photons bring. Although the destructive capacity of UV light is widely appreciated, photons of visible light can be deleterious, e.g., by destroying cytochromes and thus affecting cellular respiration (1) or by producing reactive oxygen species (ROS) that cause damage to DNA, membranes, and other cellular components (2). To cope with the damaging effects of light, organisms have evolved different strategies ranging from the expression of shielding pigments, such as melanin and carotenoids (3), to active mechanisms that sense light and respond quickly to mitigate/repair damage, such as iris constriction to protect the retina (4, 5), light-avoidance movements by chloroplasts and mitochondria (6, 7), and the induction/activation of genetic pathways that enable sustained growth in bright visible light. Therefore, visible light can modulate cellular rhythmicity and metabolism through unexpectedly photosensitive pathways.

\textbf{Significance}

In some organisms, respiration fluctuates cyclically, and these rhythms can be a sensitive gauge of metabolism. Constant or pulsatile exposure of yeast to visible wavelengths of light significantly alters and/or initiates these respiratory oscillations, revealing a further dimension of the challenges to yeast living in natural environments. Our results also have implications for the use of light as research tools—e.g., for excitation of fluorescence microscopically—even in organisms such as yeast that do not express specialized photoreceptive molecules. Moreover, the growth of yeast strains that are null for the yeast activator protein-1 gene that regulates oxidative stress genes is exquisitely sensitive to visible light, indicating that light can both modulate respiration and induce oxidative stress.


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rhythm does not persist in constant conditions. These data are consistent with the absence of a light-entrainable circadian oscillator in yeast. These results are significant both in identifying factors that influence metabolism in nature and in showing that experimental light excitation, as used in fluorescence microscopy, has the potential to impact cellular metabolism negatively.

Results and Discussion

Yeast Response to Light in Continuous Culture. We established a stable YRO in the CEN.PK yeast strain and administered light of increasing intensities from cool white fluorescence (CWF) bulbs for 12-h intervals that alternated with 12 h of darkness (Fig. 1). The YRO had a period of about 250 min in total darkness, but white light caused the period of the oscillation to shorten and the amplitude to decrease (Fig. 1A and B). These changes became more apparent with greater intensities of light (90–300 μE·m⁻²·s⁻¹). The lowest intensity of light applied (90 μE·m⁻²·s⁻¹) had only minor effects on period and amplitude; however, the brightest light applied (300 μE·m⁻²·s⁻¹) caused higher frequency (~85 min) and unstable oscillations. Treating the culture with 300 μE·m⁻²·s⁻¹ of light for longer than 16 h destroyed the oscillation (Fig. 1A), but returning the culture to total darkness allowed the oscillation to recover spontaneously about 20 h later. Typical light intensities that are found outdoors on a sunny day can exceed 2,000 μE·m⁻²·s⁻¹, whereas indoor light often is around 5–15 μE·m⁻²·s⁻¹. In fact, direct natural sunlight through a window affects the YRO similarly to artificial light (Fig. S1). In our experiments, the application of light did not change the temperature of the culture, because its temperature was maintained at 30 ± 0.2 °C by a temperature-controlled water jacket that surrounded the entire culture and was between the culture and the light source (Fig. S2).

Determining the wavelength(s) of light that mediate biological responses has proved valuable for identifying the underlying light-absorbing pigments (26, 27), so we tested the effect that red, green, and blue light had on the YRO by placing colored filters in the light path. An oscillating culture with a period of about 210 min was established, and light of different colors was administered in a light/dark cycle with 12 h of light (of different spectra) alternating with 12 h of darkness (see Fig. S3 for the spectra and transmittance of the filters). Red light (80 μE·m⁻²·s⁻¹) had no effect on the YRO’s period and only very minor effects on amplitude, whereas stronger green light (120 μE·m⁻²·s⁻¹) and dimmer blue light (60 μE·m⁻²·s⁻¹) significantly affected the YRO in a manner similar to that of moderately bright white light (180 μE·m⁻²·s⁻¹) (Fig. 1C and D). Dim green light (80 μE·m⁻²·s⁻¹) similar in intensity to blue and red light caused only minor effects (Fig. S3C).

To test if the light/dark cycle might entrain/synchronize a putative circadian oscillation in the yeast (28), the three 12-h treatments of colored light (red, green, blue) were followed by two 12-h treatments of unfiltered white light of sufficient intensity to cause noticeable effects to the YRO (for total of five 24-h light/dark cycles) followed by constant darkness to see if any persisting circadian patterns of period or amplitude changes were evident in the YRO. As shown in Fig. 1C and D, the YRO

Fig. 1. The effects of visible light on the YRO. (A) Different light intensities affect the YRO differentially. Oscillations were initiated in a culture grown in the dark until stable DO oscillations formed (black line, left y-axis). Then 12-h treatments of white light were administered at intensities of 90, 180, and 300 μE·m⁻²·s⁻¹ (gray line, right y-axis) with 12 h of darkness between light treatments. The final 300 μE·m⁻²·s⁻¹ light treatment was maintained for 35 h. Culture temperature was 30 °C in light and dark. (B) The average periods for oscillations during the initial dark phase or during each of the different light treatments for the YRO shown in A (± SD). The x-axis is arranged by light intensity in μE·m⁻²·s⁻¹, and the bar labeled “300**” represents the average period for the second and longer 300 μE·m⁻²·s⁻¹ light treatment. (C) The effects of different spectra of light on the YRO. Oscillations were initiated in the dark until stable DO oscillations formed (black line, left y-axis). Then 12-h treatments of red, blue, or green light were administered (colored lines matching color of light, right y-axis) with 12 h of darkness between treatments. After the application of colored light, two 12-h white light treatments were given. Light intensities of each treatment are shown on the right y-axis and are indicated by numbers under each of the colored or gray lines showing light treatment. (D) The average periods for oscillations occurring during the initial dark phase or during each of the different colored light treatments for the YRO shown in C (± SD). The subjective day bar pertains to the portion of the YRO indicated by dashed black lines of C and represents the times when light would have recurred in accordance with the 12-h light/dark cycle maintained for the previous 5 d.
showed stable periods and amplitudes during this free run similar to those seen before light treatment, thus providing no evidence that a circadian clock influences this phenomenon. Moreover, Fig. 1C shows that the light-induced changes in the period and amplitude of the YRO are rapidly reversible.

The photoreceptors WC-1 and WC-2 mediate blue-light responses in some fungi (27), but the S. cerevisiae genome lacks the WC-1 gene, the WC-2 gene, and any homologs (10). However, cytochromes are pigmented cellular components that are known to absorb blue-green photons (29, 30) and are an integral part of the electron transport chain in the mitochondria of practically all eukaryotes, including yeast. Observations from 1969 to 1979 indicated that visible light can destroy or deactivate cytochromes in mammalian cells, algae, and yeast (1, 25), and this action was suggested to be responsible for impaired yeast growth at low temperatures (31). On the basis of these reports implicating cytochromes as candidate absorbers of light that can inhibit respiratory electron transport, we compared the wavelengths of our light treatments with the absorbance spectra of cytochrome oxidase (Fig. S3B). Our blue-light treatment has modest emission at wavelengths for which reduced cytochrome oxidase has the greatest absorption. This comparison, however, does not exclude the possibility that other pigments (including other cytochromes) may contribute to these effects of light on yeast.

Visible Light Induces the ROS Stress Response, but ROS Does Not Modulate the YRO Period. We hypothesize that there are two likely mechanisms by which light might impinge upon cytochromes to affect the YRO. One is that photoinhibition of electron transport produces ROS that may affect protein/enzyme integrity and cellular oxidative balance. The other is that the absorption of blue light by cytochromes stalls respiratory electron transport and ATP production.

To distinguish between these alternatives, we studied a strain of yeast lacking the gene yeast activator protein-1 (yap1). YAPI encodes a basic leucine zipper transcription factor that localizes to the yeast nucleus in the presence of H₂O₂ and up-regulates transcription of oxidative stress genes such as thioredoxin reductase (TTR1), cytosolic thioredoxin (TRX2), and cytochrome-c peroxidase (CCP1) (32). If visible light impairs electron transport, high-energy electrons react prematurely with O₂ and form superoxide radicals (O₂⁻·), H₂O₂, and hydroxyl radicals (•OH) (32, 33). Therefore, yeast with an impaired ROS defense might be more susceptible to the deleterious effects of visible light. We predicted that a yeast strain that is deficient for Yap1p would show inhibited growth compared with WT when exposed to visible light, so we grew both a yap1-knockout strain (34) and its WT strain at various dilutions side-by-side on plates at different light intensities. Both strains grew equally well when grown in darkness; however, as little as 10 μE·m⁻²·s⁻¹ of white light had an obvious inhibitory effect on the growth of the yap1-knockout strain (yap1) compared with the WT (Fig. 2A). Similar growth inhibition was not seen in WT cells until light intensities reached 200 μE·m⁻²·s⁻¹ (Fig. 2B).

Because the loss of the ROS-responsive transcription factor Yap1p had such a noticeable effect on yeast survival in the presence of visible light, we reasoned that WT yeast grown in continuous culture under strong white light should express an elevated level of TRX2 (the cytosolic thioredoxin gene that is up-regulated by Yap1p in the presence of ROS) compared with cells grown in the dark. To test that prediction, we grew a non-oscillating strain of CEN.PK (yBR-ura3CEN.PK113-7D, to exclude the influence of an oscillation in Yap1p expression) in continuous culture, taking yeast samples from the culture after 24 h of darkness and then again after 12 h of 280 μE·m⁻²·s⁻¹ light. RNA levels (measured by quantitative RT-PCR) collected from these samples showed a roughly threefold difference in TRX2 expression in light-treated yeast and yeast from the same culture in darkness (Fig. 2B).

Therefore, the yap1 strain of yeast is more sensitive to ROS than is WT. If ROS production plays a significant role in light-induced YRO modulation, then the YRO period of yap1 yeast should respond to light differently from WT yeast. However, the YRO period in the yap1-knockout strain of CEN.PK was not significantly different from that in WT when exposed to similar light intensities (Fig. 2C and Fig. S4 A and B). Unexpectedly, however, oscillations initially were absent or at very low amplitude until the exposure to light, whereupon large-amplitude rhythms quickly initiated and remained strong, even with return to darkness (Fig S4). Similarly, bright light abolished oscillations, and this nonoscillatory status persisted in darkness until rhythmicity was reinstated by light of an intermediate intensity (Fig. 2C).

Taken together, these data have several implications. First and most importantly, light-induced ROS production alone cannot account for the changes in YRO period or amplitude that are seen during light exposure, because the YRO light sensitivities are equivalent in WT yeast and the yap1 strain (Fig. 2C). Second, the Yap1p transcription factor that responds to oxidative stress plays a role in yeast’s ability to tolerate the harmful effects of visible light (Fig. 2 A and B). Third, an impaired ROS-scavenging

![Fig. 2.](https://example.com/fig2.png)

**Fig. 2.** The Yap1p oxidative stress-responsive transcription factor plays a role in yeast’s ability to tolerate harmful effects of visible light. (A) A yap1-knockout strain (yap1) and a WT strain of the same background were exposed to varying intensities of visible white light (0–200 μE·m⁻²·s⁻¹) at room temperature and were imaged after 4 d. Both strains grew equally well in the dark, but the yap1-deletion strain showed impaired growth even at 10 μE·m⁻²·s⁻¹. (B) Quantitative RT-PCR shows that transcription of the gene TRX2 (for the peroxide scavenger thioredoxin enzyme) is up-regulated when yeast cells are exposed to 280 μE·m⁻²·s⁻¹ visible light compared with dark (n = 3 ± SD). (C) YRO modulation in a yap1-knockout strain (yap1) oscillating in continuous culture does not differ from that in WT when exposed to the same intensities of visible light. Each bar represents the average period (± SD) in minutes of complete oscillations occurring during the treatments shown in Fig. S4 A and B.
Inhibition of Respiration Is the Mechanism of YRO Modulation. Because light-induced ROS production alone cannot account for the changes in YRO period or amplitude (Fig. 2C), we considered the alternative hypothesis of photoinhibition of electron transport. If the shortened period and reduced amplitude of the YRO are caused by light inhibiting or destroying mitochondrial cytochromes, then it is likely that another method of inhibiting or destroying cytochromes (or their effectiveness as transporters of electrons) would have an effect similar to that of light. Sodium azide is a chemical that inhibits respiration by binding and inhibiting cytochrome c (35) and cytochrome oxidase (36) of the electron transport chain. We introduced sodium azide into the oscillating culture at a steady drip (3.4 μmol/h). Perfusion of azide in 12-h treatments separated by 12 h with no azide showed effects on the YRO similar to those of 12-h light/dark treatments (Fig. 3). That is, sodium azide shortened the period and reduced the amplitude of the dissolved oxygen (DO) oscillation during treatment, and the oscillation returned to its longer period and amplitude once the delivery of the chemical was stopped. Treating the culture with a higher concentration of sodium azide (10 μmol/h) had an effect similar to treatment with very bright light, in that it destroyed the YRO and caused the DO levels to rise during treatment and to fall after the end of treatment (Fig. S5). These data support the conclusion that light affects the YRO by inhibiting the activity of light-absorbing cytochromes, thereby inhibiting electron transport and oxidative phosphorylation.

Oxygen Shortage to the Respiration Pathway Can Reset the YRO. The redox state of the culture is believed to play a vital role in the YRO as it alternates between a high oxygen consumption (HOC) phase and a low oxygen consumption (LOC) phase (13, 21, 22). As shown above, damage to or impairment of the respiratory pathway interferes with the continuous culture’s ability to synchronize subpopulations of oscillating cells spontaneously (Fig. S4). Finally, sudden ROS production by light may provide a signal for individual cells that are oscillating out of phase to synchronize their YROs (Fig. S4).

Using the YRO as a sensitive indicator of the status of intracellular metabolism, we have shown that S. cerevisiae can respond to light with physiological and metabolic consequences that are obvious almost immediately after irradiation. Because of the similar observations with azide treatment, this effect of light probably is mediated by inhibiting heme-containing cytochromes that participate in electron transport for oxidative phosphorylation. Simply reproducing these troughs in amplitude and duration (as a consequence of the inhibition of respiration) is likely the YRO when DO is low (or dropping) and reduced the time before the culture returned to an LOC mode of energy metabolism when DO is high or rising. In addition, the introduction of chemicals (metabolites) such as ethanol and acetaldehyde has been shown previously to result in differing degrees of YRO phase resetting, depending upon the phase of the oscillation at which those substances were introduced (14). This phase resetting in response to the artificial introduction of metabolites prematurely switches the YRO from an LOC phase to an HOC phase characterized by an immediate drop in DO in the culture. Moreover, our results suggest that light and azide may affect the YRO by inhibiting cytochromes. Because azide is known to bind cytochrome oxidase, thereby preventing oxygen from terminally accepting electrons from the electron transport chain, we questioned whether rapidly depriving the culture of oxygen could have a similar phase-resetting effect without the addition of metabolites. We conjectured that the oscillating oxygen level in the culture might be an environmental signal that allows the YRO to remain synchronized among the cells in the population.

To test this prediction, the air supply was replaced with a blend of air and nitrogen to replicate artificially the characteristic DO troughs of the YRO at various phases of the YRO. Simply reproducing these troughs in amplitude and duration had no phase-resetting effect on the oscillation (Fig. S6). However, extreme deprivation of oxygen in the culture by bubbling 100% nitrogen (in place of air) into the culture at 0.9 L/min for 3 min did result in phase resetting at particular phases during the YRO. Fig. 4A shows three representative examples of this phase resetting.

By testing hypoxia-induced phase resetting across different phases of the YRO, we were able to determine which portions of the YRO are most susceptible to the disruption of respiration. Given that light interferes with electron transport (1, 25) and that oxygen shortage resets the YRO at certain phases, we expected the phases of YRO sensitivity to light to be similar to those seen during oxygen deprivation. We tested this prediction by delivering bright light pulses for 30 min at different phases of the YRO. Fig. 4B shows three representative examples of phase resetting caused by light.

Fig. 4C compares the phase-resetting effects for both light (red trace) and hypoxia (blue trace), highlighting that light and hypoxia treatments have similar phases for phase-shifting (between 135° and 315°) and similar phases of relative unresponsiveness (between 315° and 450°). These phase–response curves (PRCs) show that light and oxygen deprivation affect the YRO in similar ways. In both hypoxia and light treatment the cells are forced to alter their metabolic strategy (either immediately or gradually), resulting in phase resetting at certain phases of the YRO. Together, these results imply that absolute DO levels are not the environmental synchronizing signal that maintains the YRO’s population synchrony; rather, the relative change from one metabolic state to another (as a consequence of the inhibition of respiration) is likely to be responsible for resetting the phase of the YRO.

Concluding Remarks
Using the YRO as a sensitive indicator of the status of intracellular metabolism, we have shown that S. cerevisiae can respond to light with physiological and metabolic consequences that are obvious almost immediately after irradiation. Because of the similar observations with azide treatment, this effect of light probably is mediated by inhibiting heme-containing cytochromes that participate in electron transport for oxidative phosphorylation. Ultimately this effect on respiration has consequences for the YRO and ROS-responding genes.
Materials and Methods

See SI Materials and Methods for additional methods.

Illumination During Continuous Culture. The YRO was established with the CEN.PK113-7D strain of S. cerevisiae in continuous culture in a Bioflo 115 reactor as described previously (14). White light was applied to the culture vessel by placing one, two, or three 65-W compact CWF floodlights (Lithonia Lighting) around the vessel’s water jacket (Fig. S2). For colored light treatment, a single layer of a Roscolux color filter (#74 for blue, #89 for green, #19 for red; Rosco Laboratories) was wrapped around the water jacket of the Bioflo 115 reactor, and three CWF lamps were used as described above. For the red-light treatment, in addition to the three CWF lamps, a 60-W incandescent lamp was positioned 15 cm from the red-filtered vessel. Light intensity from these arrangements was measured in the empty vessel using a Li-COR quantum radiometer/photometer (LI-250A) and is shown as the average of eight measurements taken at 45° increments around the vessel’s interior (Table S1).
