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Light Effects in Yeast: Evidence for Participation of Cytochromes in Photoinhibition of Growth and Transport in *Saccharomyces cerevisiae* Cultured at Low Temperatures

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Visible light of moderate intensity inhibits growth, respiration, protein synthesis, and membrane transport in bakers' yeast and has a deleterious effect on membrane integrity. The results of this study indicate that these effects require the presence of cytochromes *b* and a/a_3. The light sensitivities of growth rate and [^{14}C]histidine uptake in wild-type *rho*$^+$ Y185 and D225-5A strains of *Saccharomyces cerevisiae* were compared with those in a variety of mutants lacking cytochrome *b* or a/a_3 or both; a close correlation was found between the presence of these respiratory pigments and photosensitivity. Thus, strain TL5-3C, a nuclear petite lacking cytochromes *b*, *a*, and a_3, was resistant to light; strain GL5-6A, another nuclear petite having reduced amounts of cytochromes *a* and a_3, was partially resistant; strains MB127-20C and MB1-6C, nucelar petites lacking only cytochrome *b*, were also only partially resistant to light; whereas mutants containing all three cytochromes but having their respiratory chain either nonfunctional (strain ZK3-6B) or uncoupled (strain 18-27/t12) were fully sensitive to light. Finally, an equal-energy, broad-band action spectrum for the light inhibition of growth and transport indicated that blue light (408 nm) was most effective; these wavelengths correspond to the Soret region of the cytochrome absorption spectrum. The results suggest, therefore, that the yeast cytochromes *b*, *a*, and a_3 are the primary photoreceptors for the inhibitory effects of light and, perhaps, for other processes, such as the entrainment of biological rhythms in this species.

Visible light has been shown to inhibit growth, respiration, protein synthesis, respiratory adaptation, membrane transport, and membrane integrity in bakers' yeast (6, 7, 9, 10, and 23). Several observations suggest that cytochromes participiate in these inhibitory light effects in yeast. Blue light has been identified as the maximally inhibitory wavelength in investigations on growth, protein synthesis, and respiration (6, 7) and on respiratory adaptation of anaerobically grown *Saccharomyces cerevisiae* cells (9). Ninnemann et al. (15) showed that the inhibition of respiration by high-intensity blue light was due to the destruction of cytochrome a/a_3 and partial destruction of cytochrome *b*. Furthermore, they showed that the photodestruction of the cytochomes requires the presence of molecular oxygen. However, cyanide and azide, ligands which complete with oxygen for the active site of cytochrome oxidase, protect against photodestruction. Finally, in the first paper of this series (23), we showed that respiratory-deficient petite yeasts (*rho*) which lack cytochromes *b* and a/a_3 are resistant to light intensities which cause photokilling of wild-type, parent cells.

In this paper we present supporting evidence that the inhibitory effects of light on growth rate and transport in yeast require the presence of cytochromes *b* and a/a_3 by a comparison of the effects of light of wild-type cells and mutants lacking one or more of these respiratory pigments and by an examination of the effects of light of different wavelengths.

MATERIALS AND METHODS

Yeast strains. The genotypes and phenotypes of the strains of *S. cerevisiae* used in this work are listed in Table 1. Strain Y185 was obtained from H. O. Halvorson of Brandeis University (Waltham, Mass.). Strains Y185r *PET rho* and D225-5A *rho* (elsewhere designated as *rho*$^-$) were isolated in this laboratory (see reference 23). All other strains were isolated by S. Ułaszewski and colleagues at the Microbiology Institute of Wrocław University, Wrocław, Poland.

Media and growth conditions. Cells were main-

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TABLE 1. *Characteristics of yeast strains used*

Strain	Genotype	Growth on ethanol	Phenotype						References
			Chytochrome content				Respiration	Oxidative phosphorylation	
			a/a_3	b	c_1	c			
Y185	*PET rho*$^+$ diploid	+	+	+	+	+	Normal	Normal	14
Y185r	*PET rho* diploid	−	−	−	−	+	Deficient		This paper
D225-5A	*PET rho*$^+$ *α adel lys2*	+	+	+	+	+	Normal	Normal	17-19
D225-5Ar	*PET rho α adel lys2*	−	−	−	−	+	Deficient		This paper
TL5-3C	*pet45 rho* a *lys1*	−	−	−	−	+	Deficient		13, 17; Kovac et al.[a]
GL5-6A	*pet26 rho*$^+$ a *his1 try1*	−	±	+	+	+	Deficient		13, 17
MB127-20C	*pet29-2 rho*$^+$ *α his1*	−	+	−	+	+	Deficient		13, 19
MB1-6C	*pet29-1 rho*$^+$ *α his1*	−	+	−	+	+	Deficient		13, 18
ZK3-6B	*pet23 rho*$^+$ *α his1*	−	+	+	+	+	Deficient		13
18-27	*PET rho*$^+$ a *ade3 try1 met1*	+	+	+	+	+	Normal	Normal	12, 18, 19
18-27/t12	*op1 rho*$^+$ a *ade3 try1 met1*	−	+	+	+	+	Normal	Deficient	12, 18; Kovac et at.[a]

[a] L. Kovac, J. Subik, J. Kolarov, S. Kuzela, and V. Kovacova, internal publication, Departments of Organic Chemistry and Biochemistry, Pezinok Psyh. Hospital, Pezinok, Czechoslovakia, 1968.

tained on Sabouraud (Difco Laboratories, Detroit, Mich.) or YPG (1% yeast extract, 1% peptone [Difco], 2% glucose, and 2% agar) agar slants and were grown in filter-sterilized Wickerham synthetic medium (21) containing 5% glucose and proline (1 mg/ml) as a nitrogen source. This minimal medium was supplemented with appropriate amino acids, adenine, and uracil. For the photoeffect experiments, cultures (100 ml) were inoculated with 2-ml inoculants (previously grown in liquid YPG at 28°C for 1 day in the dark) and then were grown at 12 ± 0.1°C in growth cabinets without stirring, except during sampling for cell number once per day. After dilution of the aliquots, cell concentration was determined with a Coulter model B electronic particle counter (Coulter Electronics Inc., Hialeah, Fla.), using a 50-μm-aperture tube.

Uptake of amino acids. Amino acid transport was measured as [^{14}C]histidine uptake as described previously (22). Uptakes were carried out at 12°C over a period of 30 min. The [^{14}C]histidine was used at a specific activity of 0.5 μCi/μmol.

Cytochrome absorption spectra. The cytochrome absorption spectra of cells harvested from YPG agar plates grown at 12 or 28°C were determined with a cell paste. The cells were scraped off the plates and reduced by mixing with powdered $Na_2S_2O_4$, pressed into specially prepared cuvettes giving a final cell paste thickness of 2 mm, and chilled in liquid nitrogen. The spectra were determined in a recording spectrophotometer (Cary 17) using a Lucalox disk (General Electric Co., Schenectady, N.Y.) as a reference blank. The spectra presented in the figures were reproduced by tracing over actual recordings.

Action spectra. Cells were pregrown in Wickerham medium at 12°C as described previously. The cells were inoculated into 100-ml cultures in 250-ml flasks containing magnetic stirring bars. Flasks were masked with black tape and aluminum foil so that light could only enter through sharp-cutoff filters (Corning Glass Works, Corning, N.Y.) taped to the fronts of the flasks. Each filter allowed all light above a given wavelength to be transmitted but permitted virtually no transmittance below the cutoff value. The distance of the flasks from the light source was adjusted so that the total energy reaching the cultures was identical at all wavelengths used in any given experiment. Transmitted energies for each filter were determined with a radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

RESULTS

Growth and uptake in Y185 cells. The growth and transport patterns for the wild-type *rho*$^+$ Y185 strain are shown in Fig. 1. Cells were grown in the dark or illuminated with cool white fluorescent light (5,400 lx incident at surface of vessel). During growth in the dark, amino acid transport showed a transient derepression followed by a sharp decrease during the stationary phase of growth (Fig. 1b). These results are similar to those reported previously (23) by this laboratory. Exposure of cultures of Y185 *rho*$^+$ cells to light resulted in severe inhibition of growth and transport (Fig. 1a and b). The low-temperature spectrum (Fig. 1c) shows that *rho*$^+$ cells had a normal complement of respiratory cytochromes.

Plating of cells from light-inhibited Y185 *rho*$^+$ cultures showed very low viability; surviving cells were almost exclusively *rho* petites which failed to grow on ethanol. The effect of light on ethidium bromide-treated, light-resistant Y185 *rho* cells (23) was also studied (Fig. 1). In the dark, the *rho* cells grew as well as *rho*$^+$ cells did (Fig. 1a) and exhibited a normal transport pattern (Fig. 1b). Cultures exposed to light (5,400 lx) appeared to go through a phase of adaptation to light for 2 to 3 days, after which they grew almost as quickly as dark-grown cells. This period of adaptation to light was also reflected in the transport pattern in light-treated cells, the peak in transport being delayed by 1 day (Fig.

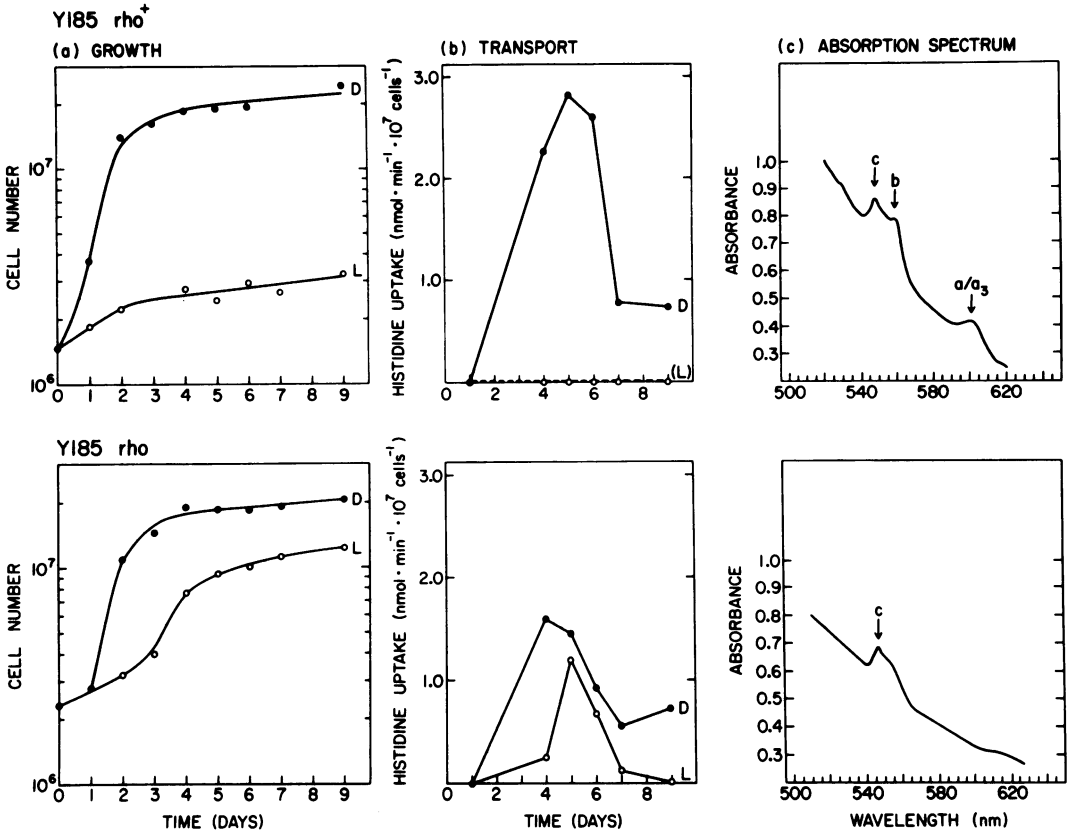

FIG. 1. *Growth, transport, and low-temperature absorption spectra of S. cerevisiae strain Y185 rho⁺ and rho cells. Cells were grown at 12°C in proline-supplemented medium either in the dark (●) or in the light (○) (5,400 lx of white fluorescent light). Aliquots were removed at intervals, and cell number (a) was determined after suitable dilution with a model B Coulter electronic particle counter. Histidine transport (b) was assayed as described in the text. Low-temperature spectra of the cellular cytochromes (c) were carried out with a Cary dual-beam spectrophotometer by comparing the spectrum of a 0.2-mm-thick paste of reduced cells frozen in liquid nitrogen with reference to a Lucalox disk.*

1b). The absorption spectrum of *rho* cells suggests that this partial resistance to light may be due to the absence of cytochromes a/a_3 and *b*. Identical results were obtained with a Y185 *rho* strain isolated from light-inhibited *rho*⁺ cells not treated with ethidium bromide (data not shown).

The differential killing effect of light on *rho*⁺ versus *rho* cells resulted in the overgrowth of *rho* cells when a *rho*⁺ population was grown in the presence of light (Fig. 2). Figure 2a shows the changes in viable count of dark-grown and light-grown (5,400 lx) cultures of *rho*⁺ and *rho* strains of Y185. The viable count of the *rho*⁺ culture fell dramatically during day 1 of illumination and did not begin to increase until after day 4. The cells which ultimately grew in the illuminated cultures were *rho* variants. By day 7 of growth, the *rho* cells had increased from less than 1% of the inoculum population to 96% of the population. The resistance to photokilling of *rho* cells is shown in Fig. 2a, in which the kinetics of increase of viable count were essentially identical with that of the total cell count for Y185 *rho* shown in Fig. 1a.

Similar results were obtained with the haploid, wild-type strain D225-5A with respect to the differential effects of light on growth and amino acid transport of *rho*⁺ and *rho* cells (data not shown) and the overgrowth of *rho* cells in illuminated *rho*⁺ cultures (Fig. 2b). In this strain, however, the rate of overgrowth of the *rho* variants was faster so that the lag period before net growth was observed in the illuminated *rho*⁺ culture was shorter (cf. *rho*⁺ cells in Fig. 2b and a). (As for strain Y185, the cells which grew in the illuminated culture had the *rho* phenotype.) When *rho* cells isolated from an

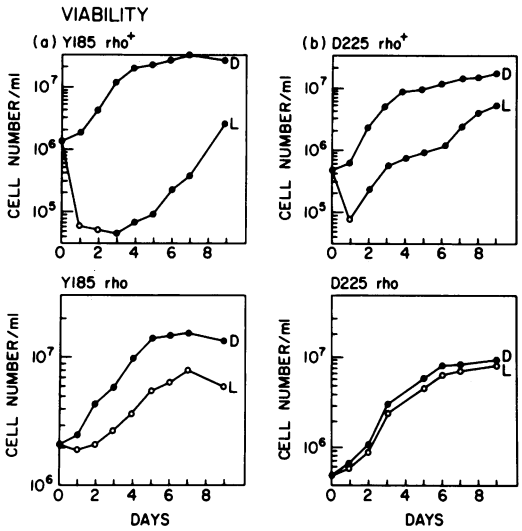

FIG. 2. *Viability of cells plated at different times from dark-grown (●) and light-grown (○) (5,400 lx) yeast cultures. (a) Strain Y185 rho⁺ and rho cells; (b) strain D225-5A rho⁺ and rho cells. Appropriately diluted 0.1-ml cell aliquots were plated on solid YPG plates and incubated in the dark for 3 to 4 days at 28°C; the numbers of colonies formed were then counted with a colony counter. Note that in both rho⁺ strains, the viable count fell dramatically during day 1 of illumination of the light-grown cultures and then later began to increase; the cells that ultimately produced this regrowth were rho variants (◑), as ascertained by their morphology, petite colony size, and inability to grow on medium containing glycerol or ethanol.*

illuminated *rho*⁺ culture were grown in the light, they were totally resistant (Fig. 2b). These cells lacked cytochromes *b*, *a*, and a_3, as revealed by their absorption spectra. Once again, the peak in transport in dark-grown *rho* cultures (not shown) occurred at least 1 day earlier than in illuminated *rho* cells (cf. Fig. 1b for Y185 *rho*).

The correlation between the resistance of *rho* cells to light and the absence of cytochromes *b*, *a*, and a_3, together with the observation of Ninnemann et al. (14) that the inhibition of respiration by high-intensity blue light is due to the destruction of cytochromes *a* and a_3 and partial destruction of cytochrome *b*, suggests that these cytochromes are the photoreceptors for the ultimate photokilling of yeast. This suggestion is confirmed by the effect of light on selected yeast strains (Table 2) with various defects in the respiratory chain (Table 1): there is a close correlation between the presence of cytochromes *b*, *a*, and a_3 and photosensitivity.

Thus, strain TL5-3C, a nuclear petite which lacks cytochromes *b*, *a*, and a_3, was resistant to light; strain GL5-6A, a nuclear petite with reduced amounts of cytochromes *a* and a_3, was partially resistant to light; strains MB127-20C and MB1-6C, nuclear petites which lack only cytochrome *b*, were also partially resistant to light; and strain ZK3-6B, a nuclear petite which is respiratory deficient but contains all the cytochromes, was fully sensitive to light. Finally, strain 18-27, a wild-type strain, and its oxidative phosphorylation-deficient derivative, 18-27/t12, both of which contain all three cytochromes, were fully sensitive to light inhibition. The light sensitivity of strains ZK3-6B and 18-27/t12, which contain all three cytochromes, shows that the presence of cytochromes *b*, *a*, and a_3 is enough to confer sensitivity to light even though the respiratory chain is not functional (ZK3-6B) or is uncoupled (18-27/t12).

Nevertheless, the data suggest that processes other than those mediated by the cytochromes may play some role in light inhibition. Thus, dark-grown *rho* cultures of strains Y185 and D225-5A typically showed peaks in cell growth (Fig. 1a) and transport (Fig. 1b) that occurred earlier than in light-grown *rho* cells. Similar results were obtained for the TL5-3C and GL5-6A strains, which both lack normal cytochrome complements. Additionally, light-grown Y185 *rho* cells never achieved quite the same titer (Fig. 1a) or demonstrated the same viability (Fig. 2a) as dark-grown Y185 *rho* cultures, suggesting the presence either of some undetected residual cytochromes *a* and a_3 or a nonheme photoreceptor. (This was not the case for *rho* cells of the D225-5A strain: illuminated and dark-grown cultures behaved almost identically [Fig. 2b] with regard to growth and viability.)

Action spectra of light inhibition of growth and histidine transport. The action spectrum for the inhibition of growth by light of various wavelengths (equal energies, 4.0×10^3

TABLE 2. *Effect of light on growth and transport in various strains of* S. cerevisiae

Strain[a]	Inhibition by light	
	Growth	Transport
Y-185	+++	+++
Y185r	+	+
D225-5A	++	++
D225-5Ar	−	+
TL5-3C	−	+
GL5-6A	+	+
MB127-20C	+	+
MB1-6C	+	+
ZK3-6b	+++	+++
18-27	+++	+++
18-27/t12	+++	+++

[a] See Table 1 for characteristics.
[b] +, Inhibition of process relative to dark-grown controls; −, lack of effect.

ergs·cm^{-2}·s^{-1}) is shown in Fig. 3. Light in the 400- to 420-nm region of the visible spectrum was as effective as white light in causing inhibition of growth. Little or no inhibition was seen at wavelengths above 500 nm.

Comparable results were obtained for the action of light on transport. The effect of light of different wavelengths (at equal energies of either 4.0×10^3 or 5.2×10^3 ergs·cm^{-2}·s^{-1}) on the rate of histidine transport during growth is shown in Fig. 4. Note that light at wavelengths above 500 nm had little effect on histidine transport. As the light utilized, however, included progressively more irradiation from the blue region, inhibition increased. Light at 408 nm was as effective as white light. Clearly, the inhibitory action of white light on growth and transport in yeast can be attributed primarily to irradiation in the blue region.

DISCUSSION

Visible light has long been known to inhibit cell growth, protein synthesis, respiration, and a number of other functions in the yeast *S. cerevisiae* (8–10, 14, 15, 23). Recent studies in this laboratory have added inhibition of membrane transport activity to the extensive list of damaging effects of visible light (23). The inhibition of sugar and amino acid transport activity was found to be due to the development of cell membrane leakiness. Respiratory-deficient (*rho*) cells which are devoid of cytochromes *b*, *a*, and a_3, however, were found to be resistant to light damage. This suggested that the site of action of light was in the mitochondrion and involved these cytochromes. In this paper we confirm that the presence of these cytochromes is required for photoinhibition of cell growth, viability, and amino acid transport. Furthermore, cell growth, the loss of cell viability, and transport activity are resistant to light only if all three cytochromes are absent from the mitochondrion (Fig. 1 and 2). Consistent with this conclusion is the fact that the absence of cytochrome *b* alone or of cytochromes *a* and a_3 confers only partial resistance (Table 2), although a more rigorous quantitative relationship between the amount of chromophore and the level of photosensitivity awaits further analysis. Finally, the conferral of light sensitivity by the cytochromes is independent of their participation in normal mitochondrial function: respiratory-deficient mutants which have the usual complement of cytochromes and oxidative phosphorylation-deficient mutants are fully sensitive to light (Table 2). The results suggest that the heme chromophores of the three cytochromes are the endogenous photosensitizers responsible for the photodynamic damage ultimately observed in our experiments. This suggestion is supported by the increase in the inhibitory effects of light of constant energy successively enriched with wavelengths (Fig. 3 and 4) that

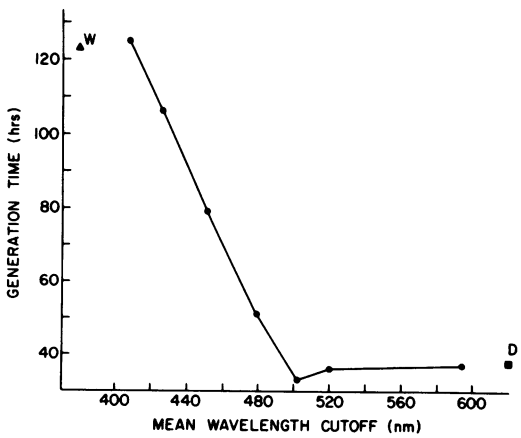

FIG. 3. *Action spectrum for the inhibition of growth in 100-ml cultures of S. cerevisiae strain Y185 grown at 12°C. All cultures received equal energy (4.0 × 10^3 ergs·cm^{-2}·s^{-1}) through sharp-cutoff Corning filters as described in the text. Progressively shorter wavelengths are added to the spectrum received by any given culture as one moves left along the abscissa. Symbols: ▲, white-light control; ■, dark-grown control.*

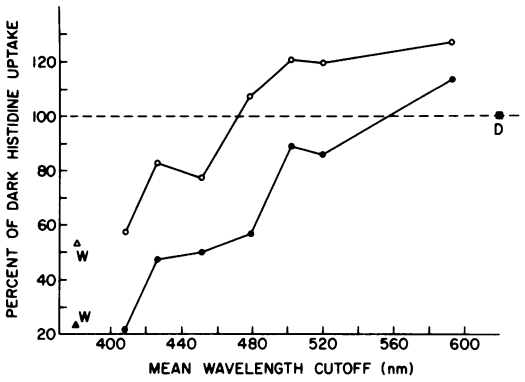

FIG. 4. *Action spectra (at two different energy levels) for the inhibition of histidine transport in 100-ml cultures of S. cerevisiae strain Y185 grown at 12°C. The mean vlaues for transport at a given wavelength at the same stage of population growth were determined from individual time course rate studies (30 min at 12°C) and are plotted as percentage of transport rate of the dark control (■, dashed line) against the lowest wavelength received by the culture (cf. Fig. 3). Energies received: 4.0 × 10^3 (○) and 5.2 × 10^3 (●) ergs·cm^{-2}·s^{-1} (white-light [no filters] controls: △ and ▲, respectively).*

constitute the Soret region of the cytochrome absorption spectrum.

These conclusions are consistent with those of Ninnemann et al. (14), who showed that "moderate" white light (10^4 ergs·cm^{-2}·s^{-1}) and high-intensity blue light (10^6 ergs·cm^{-2}·s^{-1}) inhibit respiration in yeast by the destruction of cytochromes a/a_3 and the partial destruction of cytochrome b. From the action spectrum for the destruction of cytochrome a in yeast, Ninnemann et al. (15) concluded that the action of blue light was mediated via the Soret band of cytochrome oxidase. Because the photodestruction of cytochrome a/a_3 requires the presence of oxygen and because these cytochromes are protected from photodestruction by azide and cyanide, respectively, Ninnemann et al. (15) proposed that the mechanism of photodestruction involves a close association between the O_2 and Fe^{3+} of the cytochromes. Ninnemann et al. (16) extended these observations for yeast cells to the light inhibition of respiration of isolated beef heart mitochondria and reported that this inhibition is also due to an oxygen-dependent photodestruction of cytochrome a_3 (i.e., cytochrome oxidase).

Studies by Aggarwal et al. (1), however, on isolated rat liver mitochondria showed that whereas visible light inhibits the respiration of these mitochondria, cytochrome c oxidase activity (and, therefore, cytochromes a and a_3) was unaffected. Instead, they observed the photoinactivation of the inner membrane-bound flavoproteins, NADH, and succinic dehydrogenases. They reported that light damage to the mitochondria involves at least two phases. The first was characterized by the loss of energy coupling, reflected by stimulation of both electron transport and adenosine triphosphatase, and the second was characterized by inhibition of respiration and eventual dissolution of mitochondrial integrity. They attributed the photoinactivation of respiration and the loss of mitochondrial integrity to a flavin photosensitization process in which singlet oxygen and the superoxide ion are produced. These might inactivate the dehydrogenases directly and induce membrane lipid peroxidation, thereby generating additional reactive molecules which, in turn, would affect mitochondrial enzymes and cause the "dissolution of mitochondrial integrity." The ability of flavins to act as endogenous photosensitizers in the manner described was supported by the enhancement of a qualitatively similar damage to the mitochondria when mitochondria were exposed to visible light in the presence of riboflavin. It is possible, of course, that the blue light effects observed in yeast, though requiring cytochromes, may also require flavins; energy transfer from flavins to cytochromes can occur. This can be determined only by the use of appropriate mutants and from a high-resolution action spectrum.

Irrespective of whether flavins also contribute to mitochondrial damage, loss of mitochondrial activity alone cannot account for the observed inhibition of cell growth, the loss of cell viability, the development of membrane leakiness, and the inhibition of amino acid transport, because the selective photodestruction only of cytochromes b, a, and a_3 produces petites which are light-resistant. The events that cause the loss of mitochondrial activity must ultimately cause irreversible damage to the cell membrane—either directly, by singlet oxygen or the superoxide ion, or indirectly, by the action of these reactive species on the vacuolar membrane that would cause release of hydrolytic enzymes (20).

If, indeed, yeast cytochromes b, a, and a_3 are the primary photoreceptors for the inhibitory effects of light (especially by blue wavelengths) observed, they may fall into the larger class of blue light receptors that are being reported with increasing frequency for a large number of biological phenomena (2) and may constitute photoreceptors for the entrainment of biological rhythms in this species (4, 5). Of course, our results do not exclude effects induced by wavelengths of light other than blue light. Near-UV light of 365 nm also inactivates a quinone in the electron transport chain as well as cytochrome oxidase in yeast (14). Recently, Doyle and Kubitschek (3) showed that near-UV light of 365 to 366 nm inactivates the energy-independent sugar transport system in yeast without causing cell membrane leakiness. Additionally, Koch et al. (11) report direct inactivation of the β-galactoside permease of *Escherichia coli* by near-UV light. The possible participation of these wavelengths in the photoeffects of visible light described in this report must await the future determination of a high-resolution action spectrum.

Finally, we have demonstrated that light does not inhibit Y185 *rho*$^+$ cells grown at 20°C (23), whereas it does inhibit this strain at lower temperatures. This phenomenon can be explained by several factors: the presence of less dissolved oxygen, singlet oxygen, and peroxides (as well as less cytochrome) at the higher temperature; concomitantly, the lower activity of protective catalases and superoxide dismutases at lower temperatures; and, lastly, the differences in cell generation times and membrane composition at different temperatures.

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