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Influence of light on growth and pigmentation of the yeast *Phaffia rhodozyma*

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Abstract

Light and antimycin markedly affected growth and carotenoid synthesis by *Phaffia rhodozyma*. Exposure of the yeast to high light intensities on agar plates resulted in growth inhibition and decreased carotenoid synthesis. The carotenoid compositions of the yeast were also notably changed by light. β-zeacarotene increased, whereas β-carotene and xanthophylls decreased including astaxanthin, phoenicoxanthin, and 3-hydroxy-3′,4′-didehydro-β,ψ-caroten-4-one (HDCO). In liquid medium, growth of the wild-type strain (UCD-FST-67-385) was inhibited by antimycin, but this inhibition was relieved by exposure to light. Light also stimulated carotenoid synthesis about twofold in these antimycin-treated cells. Light may have rescued growth by induction of an alternative oxidase system which facilitated electron disposal when the main respiratory chain was inhibited by antimycin. Isolation and characterization of the oxidase enzymes should be useful in strain development for increased carotenoid production.

Abbreviations: DCIP – 2,6-dichlorophenol-indophenol, HDCO – 3-hydroxy-3′,4′-didehydro-β,ψ-caroten-4-one, PG – n-propyl gallate, SHAM – salicylhydroxamic acid, TTFA – thenoyltrifluoroacetone

Introduction

Phaffia rhodozyma is a pigmented yeast which could be valuable as an astaxanthin source for aquacultured animals (Johnson et al. 1977, 1980). The principal carotenoid in *P. rhodozyma* is trans-astaxanthin with lesser quantities of phoenicoxanthin, 3-hydroxy-echinenone, echinenone, γ-carotene, neurosporene, lycopene and 3-hydroxy-3′,4′-didehydro-β,ψ-caroten-4-one (HDCO) (Andrewes et al. 1976). *P. rhodozyma* may accumulate β-zeacarotene in stressful conditions (Johnson & Lewis 1979). Cis-astaxanthin and two unknown xanthophylls that were postulated to have the structures 3,3′-dihydroxy-β,ψ-caroten-4,4′-dione (DCD) and 4-hydroxy-3′,4′-didehydro-β,ψ-carotene (HDC) accumulate in mutants with increased sensitivity to antimycin (An et al. 1989). The enzymes and pathway for biosynthesis of astaxanthin in *P. rhodozyma* has not been determined. The presence in the yeast of several carotenoids that may be transformed to astaxanthin including β-zeacarotene, HDCO, HDC and DCD implies that either alternate pathways of astaxanthin biosynthesis are present in the yeast or that an enzyme complex catalyzes desaturation and oxygenation of carotene precursors in a nonspecific order. Biochemical and genetic evidence has been reported for enzyme

complexes involved in biosynthesis of carotenoids in other fungi (Dogbo et al. 1988; Ruddat & Garber 1983).

Recently, we isolated mutants of *P. rhodozyma* on yeast-malt (YM) plates containing 50 μM antimycin A that produced up to 5-fold more carotenoids than the parent (An et al. 1989). These strains were found to be more sensitive than the wild-type to several respiration inhibitors, and we postulated that an alteration in respiration was associated with increased astaxanthin formation, possibly due to increased disposal by an alternate pathway. Many fungi produce antimycin-insensitive respiratory systems (Henry & Nyns 1975), which enable them to grow when the main respiratory chain is inhibited. These oxidative systems may be located in the mitochondria of fungi (Henry & Nyns 1975), while their expression is influenced by nuclear gene products (Edwards & Kwiencienski 1973; Edwards & Rosenberg 1976). The alternative oxidase systems have not been characterized but they may involve oxygenases such as peroxidases, cytochrome P-450, or flavin-linked enzymes.

Although several pigmented yeasts including species of *Rhodotorula, Cryptococcus,* and *Sporobolomyces* possess antimycin-resistant respiration systems (Henry & Nyns 1975; Shiraishi & Fujii 1986), carotenoid synthesis has not been reported to be associated with expression of these oxidative pathways. Recently, Moore et al. (1989) reported that carotenoids protected *Rhodotorula mucilaginosa* against oxidative stress, and that high concentrations of carotenoids in this yeast may be involved in cyanide-insensitive respiration. Moore et al. (1989) proposed that induction of cytochrome P-450 leads to increased oxygen radical formation, and that carotenoids scavenge these and protect the yeasts. Batra (1967) and Batra et al. (1969) found that light and antimycin stimulated carotenoid formation in the prokaryote *Mycobacterium marinum*. This study shows that light influences growth and carotenogenesis in the yeast *P. rhodozyma* and provides evidence that disposal of oxygen radicals is important in xanthophyll formation.

Materials and methods

Chemicals

The sources and purities of chemicals and other materials used in study of *P. rhodozyma* have been described (An et al. 1989). Antimycin A (a mixture of antimycins A1 and A3; catalog # 2006), salicylhydroxamic acid (SHAM), propylgallate (PG), N,N,N′,N′-tetramethyl-p-phenylenedia mine (TMPD) and 2,6-dichlorophenol-indophenol (DCIP) were purchased from Sigma Chemical Co.

Yeast strains and growth media

The *P. rhodozyma* strains used were the natural isolate UCD-FST-67-385 (Phaff et al. 1972; Miller et al. 1976) and mutants derived from this strain (Table 1). They were grown in yeast extract/malt extract/peptone/glucose medium (YM broth, Difco Co., Detroit, MI) as previously described (An et al. 1989). Growth was determined by the optical density (660 nm) of a washed cell suspension; 1 mg dry cell weight per ml corresponds to an O.D. of 1.35. Dry weights are reported throughout. All

Table 1. Strains of *P. rhodozyma* used in this study.

Strain	Method of isolation	Parent	Carotenoid content (μg/g yeast)[1]
67-385	Natural isolate	–	300–450
18	EMS mutagenesis[2]	67-385	440
18-13	EMS mutagenesis	18	550
18-13-6	EMS mutagenesis	18-13	630
ant-1	Antimycin agar[3]	67-385	960
ant-1-4	NTG mutagenesis	ant-1	1050

[1] Because carotenoid content is dependent on culture conditions, strain 67-385 was also grown in all determinations as a control except for experiments with mutant 18-13-6, in which case strain 18-13 was grown for comparison. Yeast yields are reported on a dry weight basis.

[2] Mutagenesis procedures with ethylmethane sulfonate (EMS) and N-methyl-N′-nitro-N-nitrosoguanidine (NTG) were described (An et al. 1989).

[3] Isolated on YM agar containing 50 μM antimycin (An et al. 1989).

strains were stored at −70° C in 40% YM medium/60% glycerol.

Growth in light

The influence of light on yeast growth and pigmentation was examined by culturing cells in a temperature controlled incubator/shaker (Environ-Shaker model 3597, Lab-Line Instruments, Inc., Melrose Park, IL). Flasks (300 ml baffled flasks containing 30 ml of media) were inoculated with 10^6–10^7 cells and usually incubated for 5 days at 20° C with shaking at 150 rpm. Light was supplied by two Sylvania 20 watt Coolwhite fluorescent tubes held 20–40 cm from the flask media surfaces. Agar media (10 cm diameter) were inoculated with approx. 10^2–10^6 cells of actively growing yeast, and the light sources were varied 5 to 10 cm from the agar surface. To estimate the effects of various wavelengths of light, petri dish covers or flasks were coated with blue or red inks with fiber pencils (Faber Castell 3000). When scanned for light transmittance in a spectrophotometer (Gilford Response), it was found that red coating permitted 85% transmittance in the 600–750 nm range; whereas at other wavelengths of visible light there was < 10% transmittance. Blue coating permitted 85% transmittance in the 400–500 nm range and transmittance was < 10% at other wavelengths. For dark controls, flasks or petri plates were wrapped in aluminum foil. When insoluble chemicals were included in the media, they were first dissolved in a small quantity of ethanol (< 0.3% final conc.), which did not affect yeast growth or pigmentation.

Carotenoid extraction and analysis

P. rhodozyma was grown for 5–7 days in flasks or for 7–10 days on agar media before extraction. Yeasts were harvested from liquid media by centrifugation and from agar media by scraping the agar surfaces with a microscope slide. The yeast cells were suspended in distilled water, washed in water, and extracted and analyzed for carotenoids

Table 2. Carotenoids compositions of *P. rhodozyma* strains UCD-FST-67-385 and ant-1-4 grown with varying wavelengths of light.

Strain	67-385				ant-1-4			
Light	White	Blue	Red	Dark	White	Blue	Red	Dark
	Carotenoid (μg/g yeast)							
unknown #1[1]	6	12	13	7	ND[2]	7	20	34
cis-astaxanthin	27	23	42	26	29	42	72	82
trans-astaxanthin	66	131	92	201	83	175	325	313
phoenicoxanthin	ND	ND	ND	3	ND	7	7	20
HDCO[3]	29	37	35	46	9	21	124	122
unknown #2[4]	10	12	18	26	ND	14	33	34
3-hydroxy-echinenone	5	ND	4	3	23	35	20	27
β-zeacarotene	11	5	4	ND	29	42	ND	ND
β-carotene	ND	2	9	10	ND	ND	46	41
others	6	9	2	7	7	7	7	7
Total	160	231	219	329	180	350	654	680
	Yeast growth (mg yeast/plate)							
	89	117	110	128	39	38	126	170

[1] Tentatively identified as 3,3′-dihydroxy-β, ψ-caroten-4,4′-dione (An et al. 1989).
[2] ND; not determined because of low content (< 1%).
[3] HDCO; 3,3′-dihydroxy-3′,4′-didehydro-β, ψ-caroten-4-one.
[4] Tentatively identified as 4-hydroxy-3′,4′-didehydro-β, ψ-carotene (An et al. 1989).

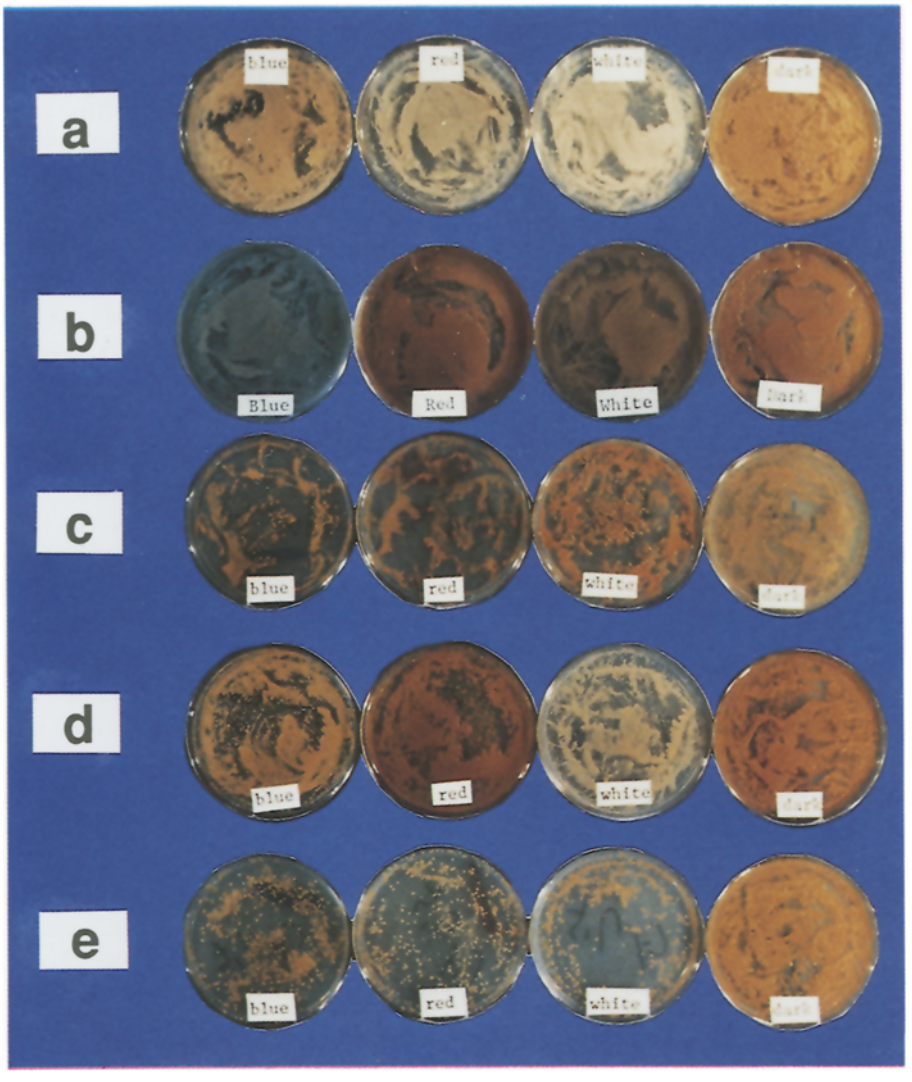

Fig. 1. Influence of light and respiratory inhibitors on pigmentation of *P. rhodozyma* grown on YM agar plates for 10 days. Row *a*: UCD-FST-67-385; Row *b*: 18-13-6; Row *c*: 18-13-6 with antimycin 5 μM; Row *d*: 18-13-6 with thenoyltrifluoroacetone 10 μM; Row *e*: 18-13-6 with KCN 7.5 mM.

by thin-layer chromatography and absorption spectroscopy as previously described (An et al. 1989).

Chemical assays

Yeast lipid content was determined according to Stewart (1975). Glucose was determined with the dinitrosalicylic acid reagent by the method of Miller (1959).

Results

Influence of light on yeast growth on agar plates

It was previously reported that light did not substantially affect growth or carotenoid formation by *P. rhodozyma* in shake flasks (Johnson & Lewis 1979). It was possible that the lack of a response to light in the previous study was due to the low light intensities used. The influence of higher intensities of light on growth and carotenoid formation was

examined in this study. About one million yeast cells were inoculated onto the surface of agar plates and the plates were exposed to two 20 watt fluorescent bulbs placed 5 cm from the agar surface in a temperature controlled incubator. Under these conditions, light inhibited growth and altered pigment composition (Table 2). The natural isolate, UCD-FST-67-385, and its antimycin-sensitive mutants ant-1-4 and 18-13-6 (An et al. 1989), grew better and had increased pigmentation in the dark, but were differently affected by light (Fig. 1). Red and blue wavelengths of light slightly inhibited growth and carotenoid formation in the wild strain UCD-67-385. In contrast, growth was markedly inhibited by blue light in the mutant compared to the parent. Carotenoid formation in ant-1-4 was not inhibited by red light but was strongly decreased by blue light. These results indicate that ant-1-4 has increased concentrations or altered contents of a blue light-sensitive component(s) compared with the parent.

Inclusion of various respiratory inhibitors in the agar media dramatically affected synthesis of pigments by the yeast (Fig. 1). In particular, antimycin (5 μM) stimulated pigment synthesis under blue and white lights in mutant 18-13-6 (rows b and c). TTFA (10 μM) increased carotenoid formation or changed carotenoid composition under red light (row d). Cyanide (7.5 mM) inhibited growth in light but only slightly changed the color of the cultures (row e).

Effect of light on carotenoid composition

Illuminated and dark-grown yeast cells were removed from the surface of agar plates and examined for their carotenoid compositions. Compared to dark-grown controls, white and blue light substantially increased the formation of β-zeacarotene, particularly in ant-1-4, and decreased the content of β-carotene, astaxanthin, and certain other xanthophylls in the wild-type and ant-1-4 (Table 2). In red wavelengths of light or in the dark, the mutant accumulated 3-hydroxy-3′,4′-didehydro-β, ψ-caroten-4-one (HDCO), a pigment first isolated by Andrewes et al. (1976). These results imply that enzymes or cofactors involved in xanthophyll synthesis in the mutant are more sensitive to blue light than they are in the parent.

Photosensitivity of P. rhodozyma

Light inhibited growth of the antimycin-sensitive mutant to a greater degree than the wild-type (Table 2). This finding implied that the mutant might be more sensitive to photokilling, which was further examined by determining the survival in light of wild-type and mutant cells in the presence of hydrogen peroxide and paraquat. These compounds are known to enhance photokilling in several fungi. The antimycin-sensitive mutant ant-1 was more sensitive to light in the presence of paraquat than was the wild strain (Table 3). Light alone inhibited growth but did not increase killing. These results indicated that the mutant was more sensitive than the parent to radicals generated through photoreduction of paraquat.

Effect of antimycin and light on yeast growth and carotenogenesis in YM broth

As discussed above, when grown on agar plates the antimycin-sensitive mutants were more sensitive than the parent to paraquat-mediated photokilling and carotenogenesis was also strongly influenced by light. Since antimycin A inhibits the primary

Table 3. Sensitivity of *P. rhodozyma* to photokilling.

Strain	67-385		ant-1	
Light	Dark	Light	Dark	Light
Chemicals added	% Survival			
Control	100	100	100	100
H_2O_2, 1 mM	90	87	88	91
Paraquat, 0.1%	73	47	80	0
Paraquat + H_2O_2	75	5	56	0

10^2–10^4 yeast cells were spread on YM agar (10 cm diameter) containing the various chemicals and exposed to two 20 watt fluorescent bulbs 5 cm from the agar surfaces. After 2 weeks of incubation at 20° C the colonies were analyzed.

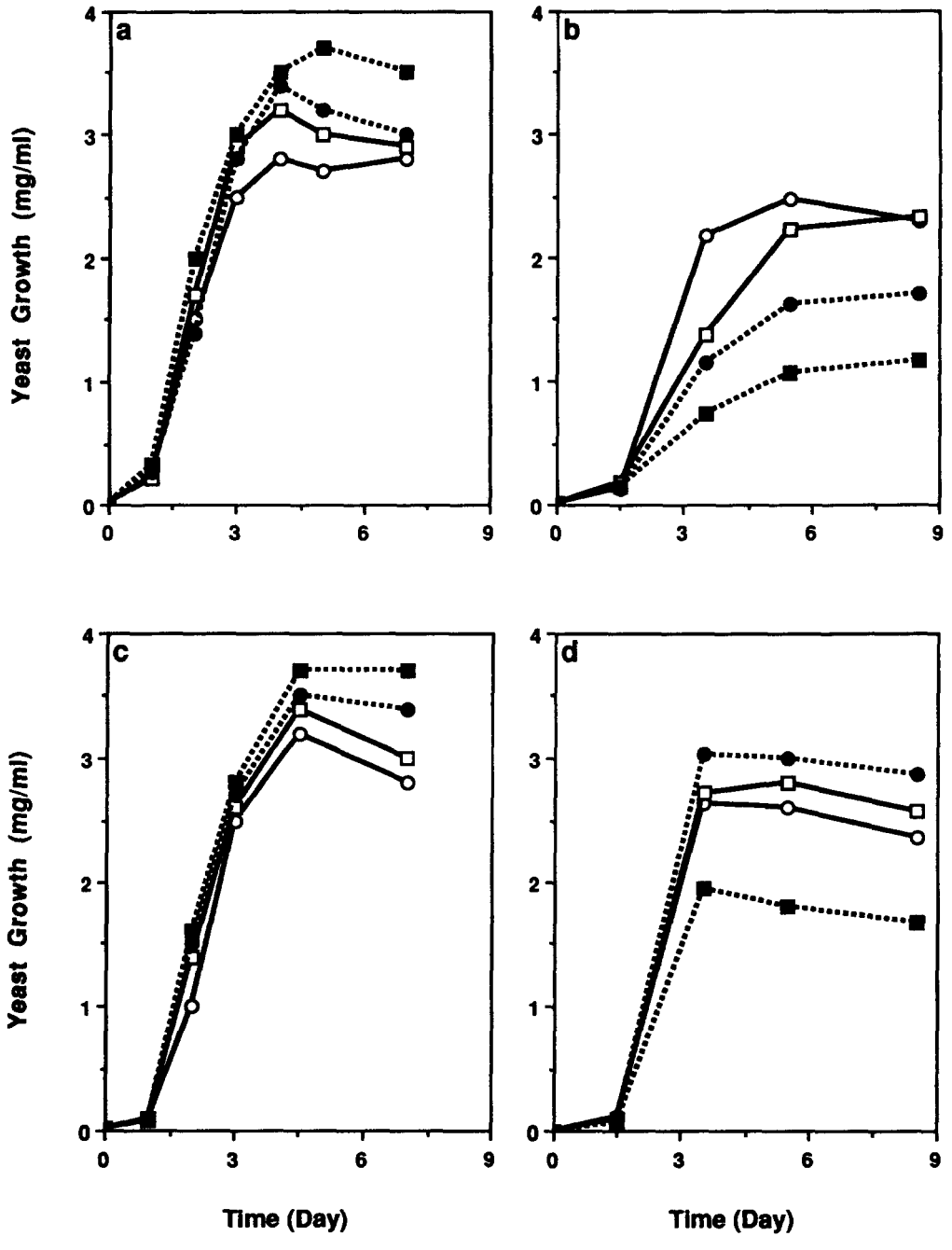

Fig. 2. Influence of light and antimycin on growth of *P. rhodozyma* in YM medium in shake flasks (antimycin 0.2 μM and light were provided from the time of inoculation). Symbols: white light (——○——); blue light (——□——); red light (---●---); and dark (---■---). Panels: (*a*) Growth of 67-385 without antimycin; (*b*) Growth of 67-385 with 0.2 μM antimycin; (*c*) Growth of ant-1-4 without antimycin; (*d*) Growth of ant-1-4 with 0.2 μM antimycin.

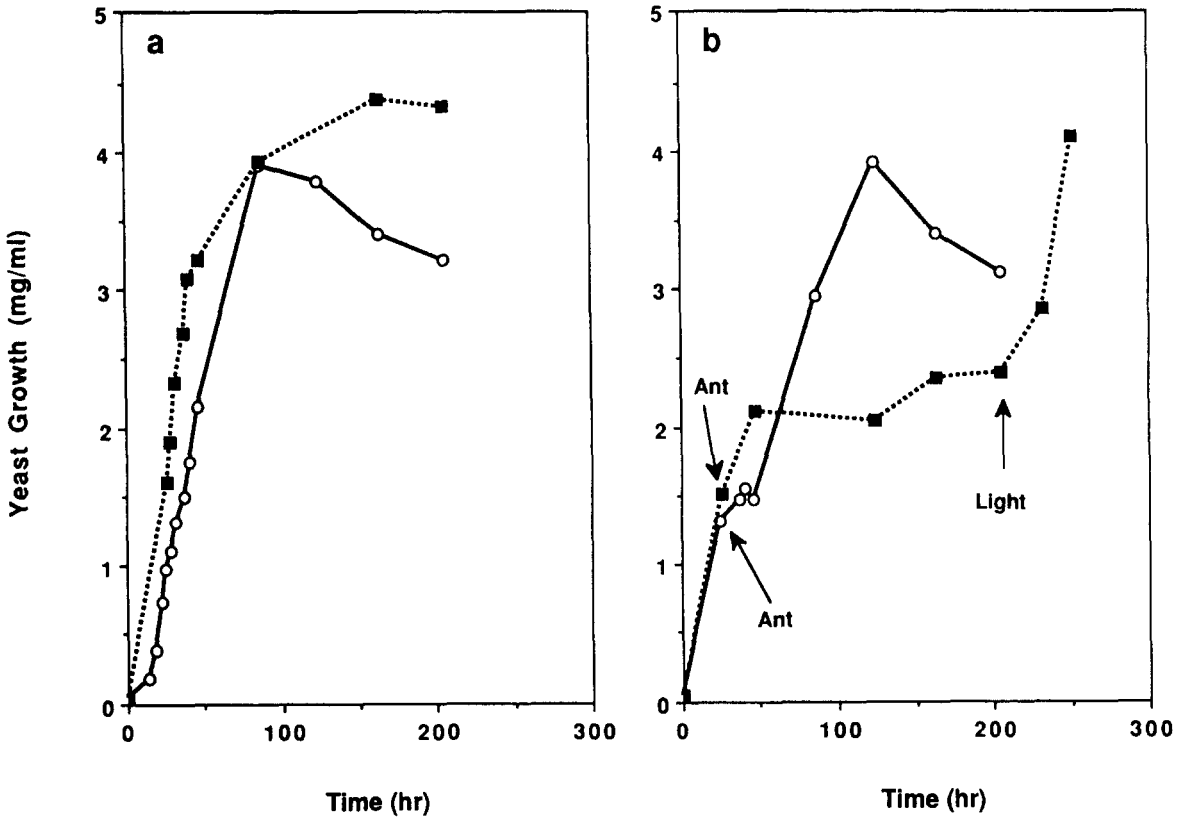

Fig. 3. Effect of light and antimycin on yeast (67-385) growth. Symbols: light provided throughout growth (——○——); dark (– – –■– – –). Panel *a*: growth of *P. rhodozyma* in the absence of antimycin. Panel *b*: effect of additions of antimycin and light on yeast growth; antimycin 0.2 μM was added as indicated by left arrows; Light was provided to dark growing yeast from the time as indicated by right arrow.

respiratory chain at cytochrome b, these results implied that light-sensitive components associated with the main respiratory chain are involved in carotenoid biosynthesis. The influence of light and antimycin was examined further with the natural isolate UCD-FST-67-385 and with ant-1-4 in shake flasks. Illumination of the wild-type strain in a broth containing 0.2 μM antimycin A gave improved growth compared to antimycin-inhibited yeast in the dark (Fig. 2). Increased final dry weights were also found for the mutants ant-1-4 (Fig. 2) and 18-13-6 (data not shown) in light + antimycin compared to yeast exposed to antimycin in the dark. The increased yields of yeast were not due to photodestruction of antimycin since yeasts exposed to antimycin + light were no longer inhibited by antimycin in the dark (see Fig. 6 below) and since other respiratory inhibitors including myxothiazole gave the same growth patterns in light and dark as did antimycin.

Evidence for the presence of an alternative oxidase system in P. rhodozyma

The stimulation by light of growth in cells inhibited by antimycin indicated that light might induce or feed an alternate respiratory oxidase system that facilitates NADPH oxidation and continued growth. The capacity for light to relieve antimycin inhibition is shown in Fig. 3. *P. rhodozyma* grew well in light or dark (Fig. 3a), but addition of antimycin to a growing culture caused cessation of yeast growth (Fig. 3b). Illumination relieved this

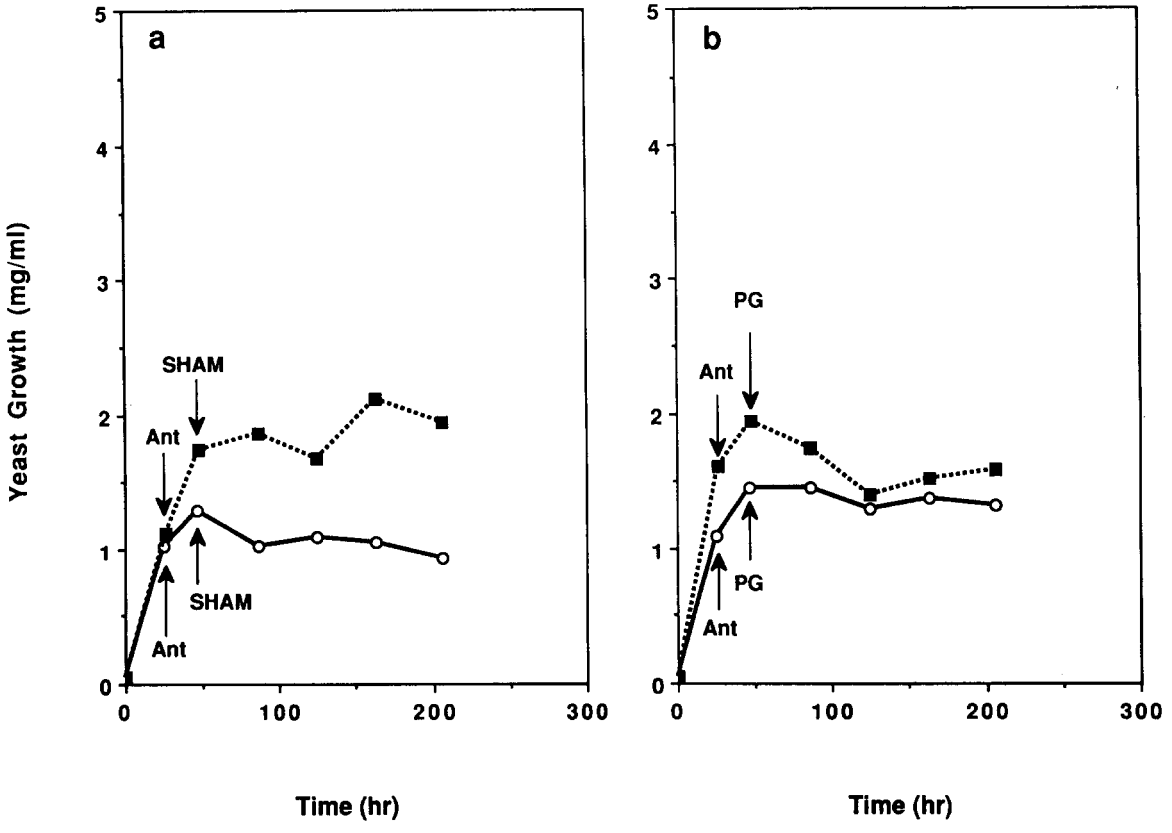

Fig. 4. Effect of propyl gallate and salicylhydroxamic acid on yeast (67-385) growth. Symbols: light provided throughout growth (——○——); dark (---■---). Panel *a*: antimycin 0.2 μM was added as indicated by left arrows; SHAM 3.0 mM was added as indicated by right arrows. Panel *b*: antimycin 0.2 μM was added as indicated by left arrows; PG 2.0 mM was added as indicated by right arrows.

inhibition after a 12–24 hour lag. A culture kept in the dark did not overcome antimycin inhibition; however when illuminated after 200 hours, the yeasts grew after about a 12 hour lag (Fig. 3b).

Salicylhydroxamic acid (SHAM) and propyl gallate (PG) are inhibitors of antimycin-insensitive respiration (Schonbaum et al. 1971; Siedow & Girvin 1980; Benichou et al. 1988). Addition of these inhibitors (SHAM at 3.0 mM and PG at 2.0 mM) to *P. rhodozyma* in YM broth blocked induction of growth in antimycin by light (Fig. 4a and b). These inhibitors did not influence growth of the yeast in the absence of antimycin at the concentrations they were used. Hence, it is not likely that they were nonspecifically chelating metals or causing other nonspecific inhibitions.

Evidence for the involvement of an antimycin-insensitive oxidase pathway was supported by use of N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) and 2,6-dichlorophenol-indophenol (DCIP) which are electron carriers that can bypass the antimycin block of cytochrome b and facilitate transfer of electrons from succinate to cytochrome c (Alexandre & Lehninger 1984). *P. rhodozyma* was not inhibited by antimycin in the dark when these compounds were included in the medium (Fig. 5a and b).

To determine whether continuous light exposure was necessary to bypass antimycin inhibition, cells were exposed to light + antimycin for 24 hours, and then cells were transferred to broth containing antimycin in the dark. Growth was only mildly inhibited by antimycin in the dark after the previous exposure (Fig. 6). Therefore, a brief exposure to light is sufficient to overcome antimycin inhibition.

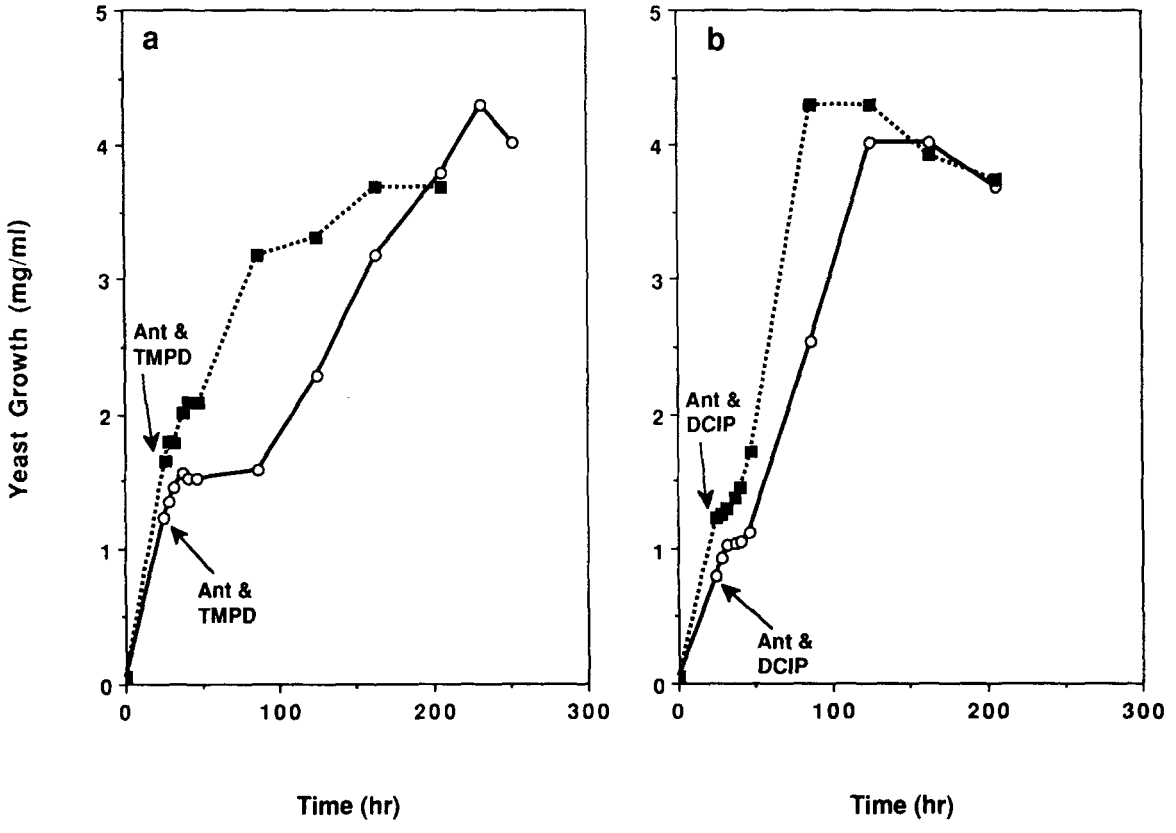

Fig. 5. Effect of N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) and 2,6-dichlorophenol-indophenol (DCIP) on yeast (67-385) growth. Symbols: light provided throughout growth (——○——); dark (- - - ■ - - -). Panel *a*: antimycin 0.2 μM and TMPD 100 μM were added as indicated by the arrows. Panel *b*: antimycin 0.2 μM and DCIP 50 μM were added as indicated by the arrows.

mRNA encoding light-induced antimycin resistance may be synthesized in cytoplasm

The results above indicate that stimulation of growth by light may involve induction or increased use of an alternative oxidase system. Inhibitors of mitochondrial and cytoplasmic mRNA and protein synthesis were used to investigate oxidase induction. If the primary respiratory chain is inhibited and also the synthesis of the oxidase is inhibited, then yeast growth should be prevented. After induction, partial inhibition of protein and mRNA synthesis is less critical. Addition of α-amanitin (an inhibitor of chromosomal mRNA synthesis) or cycloheximide (an inhibitor of cytoplasmic protein synthesis) at levels that did not affect growth of the yeast in YM prevented light-relief of antimycin-inhibited growth (Fig. 7a and b). When these inhibitors were added one day following the addition of antimycin, then slight growth did occur. These results indicate that cytoplasmic protein synthesis was necessary for light relief of antimycin inhibition. In contrast, addition of chloramphenicol (an inhibitor of mitochondrial protein synthesis) did not inhibit light-stimulated growth in antimycin. Addition of rifampicin (an inhibitor of mitochondrial mRNA synthesis) at the same time as antimycin allowed slight growth. When rifampicin was added one day after antimycin, the yeasts grew well. These results indicated that the oxidase system is made in the cytoplasm or is regulated by factors produced in the nucleus or cytoplasm. However, it is also possible that chloramphenicol and rifampicin were poorly taken up by the yeasts.

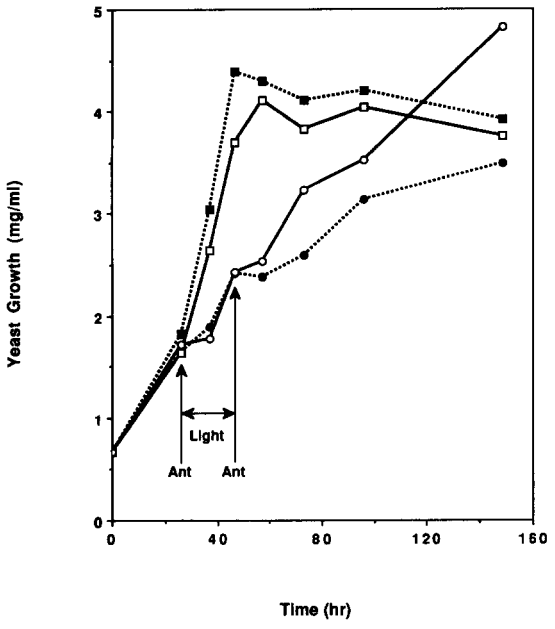

Fig. 6. Effect of one day of illumination on adaptation of *P. rhodozyma* (67-385) to antimycin inhibition in YM medium. Antimycin was provided to certain cultures at left and right arrows. Light was provided to dark cultures between the two arrows (approx. 24 hours). Symbols: (---■---), dark grown yeasts; (——□——), light grown yeasts; (---●---), dark + two additions of antimycin 0.2 μM; (——○——), light + two additions of antimycin 0.2 μM.

Induction of an alternative oxidase increases carotenoid production

The inhibition of yeast growth by antimycin seemed to be due to specific inhibition of the principal respiratory chain and not to secondary changes that might be affected including changes in pH of the media [the final pH in the media ranged from 4.8 to 5.0, which are well above the growth limit of which are well above the growth limit of *P. rhodozyma* (Johnson & Lewis 1979)]. The use of sodium phthalate buffer at pH 5.0 (Johnson & Lewis 1979) did not affect light-induction of growth and carotenogenesis. Also, antimycin + light did not appear to affect the utilization of glucose (data not shown).

Analysis of yeasts grown in light indicated that they had a slightly lower lipid content than cells cultured in the dark (Table 4). The carotenoid content of *P. rhodozyma* grown in antimycin plus

Table 4. Effect of antimycin and illumination on lipid content, growth and carotenoid synthesis in *P. rhodozyma* UCD-FST-67-385.

Conditions	Growth (mg/ml)	Carotenoid (μg/g y)	Total lipid (% g lipid/ g yeast)
Dark	4.4	520	22
Dark + Antimycin 0.2 μM	1.6	480	23
Light	3.3	440	20
Light + Antimycin 0.2 μM	2.2	1000	17

Antimycin was added at the time of inoculation. Light source used was two 20 watt fluorescent tube placed at a distance of 20 cm. 10^6 yeast cells were inoculated in 30 ml YM broth and grown for 5 days.

Table 5. Effect of antimycin and light on carotenoid composition in *P. rhodozyma* UCD-FST-67-385.

	Light	Light + ant[1]	Dark	Dark + ant
	% Carotenoid			
unknown #1[2]	9	11	6	7
cis-astaxanthin	10	11	8	6
trans-astaxanthin	41	50	57	55
phoenicoxanthin	ND[3]	ND	2	2
HDCO[4]	15	10	9	9
unknown #2[5]	6	6	4	5
3-hydroxy-echinenone	5	5	5	6
β-zeacarotene	2	ND	ND	ND
β-carotene	3	ND	3	4
others	8	8	6	7

Antimycin was added 2 days after inoculation (10^7 cells/30 ml YM broth) and yeast was grown for 7 days in buffered (phthalate buffer pH 5.0) YM broth.

[1] Ant: Antimycin 0.2 μM

[2] Tentatively identified as 3,3′-dihydroxy-β,ψ-caroten-4,4′-dione (An et al. 1989).

[3] ND; not determined because of low content (< 1%).

[4] HDCO; 3-hydroxy-3′,4′-didehydro-β, ψ-caroten-4-one.

[5] Tentatively identified as 4-hydroxy-3′,4′-dihedydro-β, ψ-carotene (An et al. 1989).

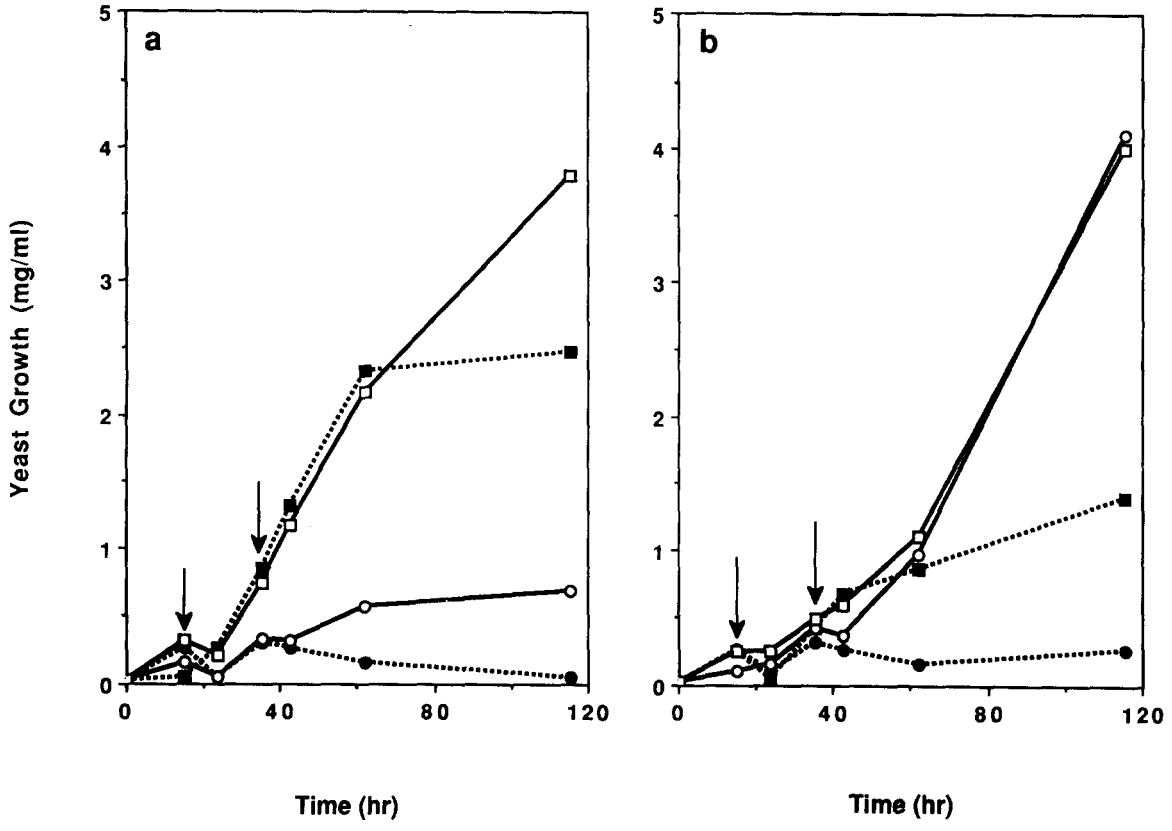

Fig. 7. Influence of protein and mRNA synthesis inhibitors on adaptation of P. rhodozyma 67-385 to antimycin inhibition (see text for details). Antimycin 0.2 μM was added to all samples as indicated by left arrows. Symbols: Panel *a*: (---●---), α-amanitin 10 μM added as indicated by left arrow; (---■---), α-amanitin 10 μM added as indicated by right arrow; (——○——), rifampicin 100 μM added as indicated by left arrow; (——□——), rifampicin 100 μM added as indicated by right arrow. Panel *b*: (---●---), cycloheximide 1 μM added as indicated by left arrow; (---■---), cycloheximide 1 μM added as indicated by right arrow; (——○——), chroamphenicol 100 μM added as indicated by left arrow; and (——□——), chroamphenicol 100 μM added as indicated by right arrow.

light increased by approximately 2–fold. Carotenoid formation in mutant 18-13-6 was also increased 2-3-fold (from 540 μg/g to 1410 μg/g) in antimycin + light compared to light exposure alone.

Extraction of the pigments and characterization from the yeasts grown in liquid medium + antimycin and light (Table 5) indicated that the overall effect of illumination was similar to the results shown in Table 2. Under the dark condition, addition of antimycin did not markedly change carotenoid composition. However, addition of antimycin under illumination increased cis and trans-astaxanthin. These data (Tables 2 and 5) also indicate that the formation of astaxanthin precursors, particularly phoenicoxanthin production, was susceptible to light.

The importance of light and antimycin in carotenoid synthesis was further supported by the finding that xanthophyll synthesis was stimulated in a mutant (yan-1) isolated in our laboratory that produces β-carotene but not xanthophyll pigments. Presumably, this mutant is defective in regulation of expression of the oxygenating enzymes, and light induction alleviated the repression that occurs in the mutant.

Discussion

Growth and carotenoid formation by *P. rhodozyma* is clearly influenced by light. Antimycin-sensitive mutants that produce higher levels of pigment showed increased sensitivity to photokilling, particularly by blue light. Carotenoids are well-known to react with light and with oxygen radicals (Krinsky 1971) and have been postulated to provide protection against UV, singlet oxygen (Krinsky 1971), and visible light (Will III et al. 1987). However, the results of this study and that of An et al. (1989) have shown that more highly pigmented natural isolates or mutants have increased sensitivity to ultraviolet or visible light. Also, the more highly pigmented strains have increased sensitivity to hydrogen peroxide (An et al. 1989). These data indicate that photoreactive molecules other than carotenoids such as flavins or porphyrins are more associated with light sensitization in *P. rhodozyma,* as has been postulated for certain other microorganisms (Krinsky 1971).

Interestingly, the *P. rhodozyma* strains that are most photosensitive also synthesize the highest levels of carotenoids. These data indicate that endogenous photosensitizers, e.g., flavins or porphyrins, are involved in carotenoid biosynthesis. Oxygenation of carotenes to xanthophylls is probably rate limiting in astaxanthin biosynthesis since carotenes accumulate in the yeasts under certain culture conditions. Furthermore, the mutant yan-1, which produces β-carotene under normal culture conditions, forms xanthophylls when treated with antimycin + light. These data strongly suggest that induction of an alternative oxidase system, possibly a flavin-oxygenase or specific cytochrome P-450, allows growth in antimycin-inhibited yeasts and also stimulates carotenoid biosynthesis. Based on preliminary inhibitor experiments with piperonyl butoxide and metapyrone, in the presence of which *P. rhodozyma* grows well but is nonpigmented (Parreiras & Johnson, unpublished data), indicates a role for cytochrome P-450. More work is needed to isolate and characterize the proteins induced by light in the presence of antimycin and to show their involvement in vitro in the biosynthesis of oxygenated carotenoids.

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