PORPHYRIN MUTANTS OF SACCHAROMYCES CEREVISIAE: CORRELATED LESIONS IN STEROL AND FATTY ACID BIOSYNTHESIS

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SUMMARY

The ole2, 3 and 4 mutants of yeast require an unsaturated fatty acid and methionine for growth and do not synthesise ergosterol. They have very similar sterol compositions and all accumulate lanosterol. The mutants lack cytochrome pigments and have negligible respiratory activity. Porphyrin intermediates alleviate the lipid requirement of ole2 and ole3 and restore respiratory competence. It is concluded that the primary defects in these mutants are lesions in porphyrin biosynthesis.

Mutants of the genes ole2, 3 and 4 are unable to grow on non-fermentable substrates and require unsaturated fatty acids (UFA) for growth on glucose media containing yeast extract (1, 2). Bard (3) subsequently showed that they were nystatin-resistant, blocked in sterol biosynthesis and require methionine for growth on defined medium. Sterols extracted from ole2 and the double mutant ole3/ole4 gave a lanosterol-like colour in the Liebermann-Burchard reaction. G.L.C. analysis of the sterols from ole2 indicated the absence of ergosterol (3).

Since haematin enzymes are required for mitochondrial respiration and fatty acid desaturation (4) and have been implicated in sterol metabolism (5), the primary lesion in these mutants could be a block in porphyrin metabolism leading to the loss of haematin enzymes. We report here experiments designed to test the following predictions of this hypothesis; (1) An indirect lesion in sterol biosynthesis should result in the ole mutants having very similar sterol compositions; (2) The mutants should lack mitochondrial respiration and mitochondrial cytochromes; (3) Inter-

mediates in porphyrin biosynthesis might alleviate the pleiotropic consequences of the primary lesion.

METHODS

Yeast strains The mutants ole2, 3 and 4 and the parent strain S288C have been described previously (1, 2, 3).

Sterol analyses Cultures were grown, harvested and the sterols extracted as described by Woods (7). The medium was supplemented with Tween 80 (1% v/v). T.L.C. analyses were performed on plates of silica gel G impregnated with 4.5% $AgNO_3$ and developed in benzene : ethyl acetate (5:1). The sterols were visualised with a Liebermann-Burchard reagent spray. For G.L.C. the extracts were acetylated and analysed on the following columns: SE-30, 1% on Gas-Chrom Q (60/80 mesh) 7' at 240°; OV-17, 1% on Diatomite CLQ (100/120 mesh) 9' at 270°; OV-225, 3% on Gas-Chrom Q (100/120 mesh) 7' at 250°. The carrier gas was argon, 60 ml/min. and retention times (RRT) were calculated relative to cholestane or cholestanol acetate.

Growth and respiratory activity. Cells were grown aerobically at 28° in a medium containing 1% Difco yeast extract, 0.5% bacteriological peptone and either 5% glucose (YEPG) or 1% ethanol (YEPE) as carbon source. This medium was supplemented Tween 80, 1% v/v; ergosterol, 10 µg/ml; δ-amino levulinic acid, 30 µg/ml; haematoporphyrin, 30 µg/ml and haematin, 15 µg/ml as indicated. Culture yield and cytochrome spectra determinations were as described by Clark-Walker and Linnane (6). Respiratory activity of whole cells (1-10 mg dry weight) in 3.0 ml of buffer containing 2mM glucose and 50mM K PO_4, pH 7.4, was measured in a closed Clark oxygen electrode cell at 30°. Antimycin A (5 µg/ml) was added where indicated. All cultures were plated on lipid free medium to assay for revertants and the results from those with >0.1% were discarded.

All biochemicals were purchased from Sigma (London) Ltd.

RESULTS

Cell Sterols Analysis of nonsaponifiable extracts by TLC demonstrated the presence of ergosterol in S288C and its absence in the ole mutants. The sterol patterns of the mutants were identical and included a component with an R_f very close to that of lanosterol. The results of GLC analysis on SE-30 are shown in Fig. 1. S288C gives peaks at 29.0, 31.4, 34.0 and 36.2 mins

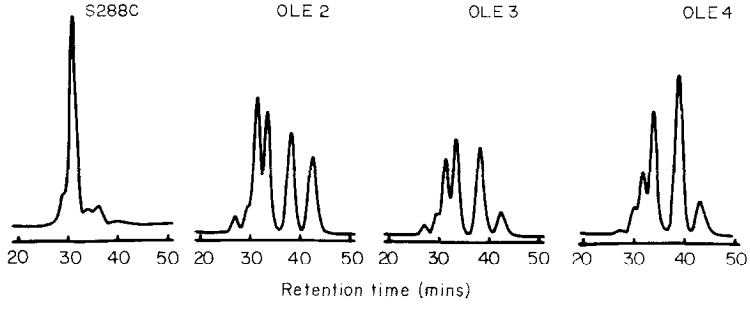

Figure 1. Gas-liquid chromatograms on SE-30 (1%) of sterol acetates from S288C, ole2, ole3 and ole4.

which respectively co-chromatograph with authentic samples of zymosterol, ergosterol, fecosterol and episterol. All three ole mutants give peaks at 27.0, 29.6, 31.4, 33.4, 38.2 and 42.4 mins. Lanosterol co-chromatographs with the peak at 38.2 mins. Since the mutants do not contain ergosterol the peak at 31.4 mins must be due to another sterol. Analysis on OV-17 and OV-225 confirmed that the sterols of the mutants were qualitatively identical and that they included lanosterol.

Effects of porphyrin precursors on growth and respiratory activity. The effects of δ-amino levulinic acid (ALA), haematoporphyrin (HP) and haematin (HN) on the growth of ole2, 3 and 4 are listed in Table 1. The requirement for UFA is absolute for ole3 and 4 but not for ole2. Maximal yield is obtained in Tween 80 + ergosterol. These supplements do not allow growth on YEPE. ALA, HP and HN all alleviate the UFA requirement of ole3 on YEPG and ALA and

Table 1. Growth responses of ole mutants on supplemented YEPG and YEPE

Supplement	ole2		ole3		ole4	
	YEPG	YEPE	YEPG	YEPE	YEPG	YEPE
None	+	0	0	0	0	0
Tween 80	++	0	++	0	++	0
Tween 80 + Ergosterol	+++	0	+++	0	+++	0
ALA	+	0	+++	+++	0	0
HP	+++	+	+++	+	0	0
HN	+++	0	+++	+	0	0

Cultures were inoculated at 1 µg dry weight/ml and sampled after 48 h. Growth responses (dry weight) were scored as follows:
0 = <0.05 mg/ml; + = 0.1 - 0.5 mg/ml; ++ = 0.5 - 2.0 mg/ml;
+++ = >2.0 mg/ml.

HP allow growth on YEPE. HP and HN stimulate ole2 on YEPG but only HP allows any growth on YEPE. Ole4 does not respond to these compounds. The effects of ALA, HP and HN on respiratory activity are shown in Table 2. All three mutant strains have very low respiratory activity and lack antimycin-sensitive respiration, indicating loss of the mitochondrial terminal electron transport chain. Ole3 shows high respiratory activity in the presence of ALA and rather less with HP. Ole2 only responds to HP. Although HN alleviates the the lipid requirement of ole2 and 3 on YEPG it has very little effect on respiration. In the presence of ALA ole3 has activities several fold higher than S288C.

Spectral analysis of cell pigments showed the total absence

Table 2. Respiratory activities of the ole mutants

Strain	Medium	Supplement	Respiratory Activity	
			No antimycin	Antimycin
ole2	YEPG	T80+Erg	1.1	1.1
		ALA	1.2	1.2
		HP	13.0	4.6
		HN	2.3	1.6
	YEPE	HP	56.0	7.6
ole3	YEPG	T80+Erg	1.3	1.3
		ALA	246.0	2.9
		HP	72.0	8.1
		HN	1.3	1.0
	YEPE	ALA	586.0	11.7
		HP	111.0	4.3
ole4	YEPG	T80+Erg	1.2	1.2
		T80+Erg+ALA	1.5	1.2
		T80+Erg+HP	1.7	1.7
		T80+Erg+HN	1.4	1.3
S288C	YEPG	-	61.6	11.2
	YEPE	-	201.0	4.7

Activities are expressed in nanogram atoms O_2/min/mg dry weight.

of cytochromes and porphyrins in ole2, 3 and 4. In the presence of ALA ole3 develops the characteristic absorption bands of cytochromes a + a_3, b and c. The intensity of absorption is greater than for cells of S288C grown under the same conditions.

DISCUSSION

The results indicate that the complex phenotypes of ole2 and ole3 are due to blocks in porphyrin biosynthesis. In ole3 all effects of the primary lesion are reversed by ALA, suggesting that the mutation affects ALA synthetase. The responses of ole2 are consistent with a partial block later in the pathway, presumably after ALA but before haematoporphyrin. The mutant ole4 has an identical phenotype but does not respond to the compounds tested. We suggest that it is either blocked at a later stage in porphyrin biosynthesis or that it is impermeable to the intermediates tested.

The loss of the prosthetic groups of the haematin enzymes in the mutants can account for the observed lipid lesions since cytochrome b_5 is involved in fatty acid desaturation (4) and cytochrome P_{450} has been implicated in the conversion of lanosterol to C_{27} sterols (5). Since haematin reverses the lipid requirement but not respiratory deficiency in ole2 and 3 whereas haematoporphyrin does both it seems likely that the biosynthetic pathway for the prosthetic groups involved in lipid synthesis and cytochromes diverge prior to the insertion of iron into the porphyrin ring.

Karst and Lacroute (8) have reported the isolation of a series of mutants, olergl-6, which differ from the ole set in that they require ergostero but are otherwise identical to them. We have shown that ole2 and ole4 are allelic to olerg2 and olerg4. In view of this we suggest that some or all of the olerg genes are concerned in porphyrin biosynthesis.

The ole mutants will be useful not only in studies of the sequence and control of porphyrin biosynthesis but will also illuminate the role of the haematin group of enzymes in yeast. Moreover ole3 is likely to be a valuable tool in the investigation of the formation and function of mitochondrial membranes as its response to ALA allows the manipulation of both lipids and cytochromes.

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