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Effect of Unsaturated Fatty Acids on the Development of Respiration and on Protein Synthesis in an Unsaturated Fatty Acid Mutant of *Saccharomyces cerevisiae*

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The extent of development of respiratory function induced by aeration of an anaerobically grown unsaturated fatty acid auxotroph of *Saccharomyces cerevisiae* is determined by the availability, endogenous or externally supplied, of unsaturated fatty acid. The synthesis of mitochondrial and cytoplasmic enzymes during aeration appears to have a similar basis of regulation by available unsaturated fatty acid. Levels of unsaturated fatty acid that permit the synthesis of mitochondrial enzymes also result in a substantial stimulation of cellular protein synthesis.

In the absence of oxygen, the yeast *Saccharomyces cerevisiae* is unable to form unsaturated fatty acids, ergosterol, and certain small molecules essential for electron transport, such as heme and ubiquinone (3). When a source of essential lipids (long-chain unsaturated fatty acids and ergosterol) is made available, cells of this organism are able to grow anaerobically, but functional mitochondria are not formed (1, 9). Aeration of these anaerobically grown cultures results in the synthesis of components of the electron transport chain, and the development of mitochondrial respiratory function. The biogenesis of mitochondria under these conditions, involving the formation of constituent lipid-protein complexes, presumably requires the coordinated synthesis of both lipid and protein components. We have examined the degree of coordination or integration between the formation of functional lipids, on the one hand, and protein synthesis on the other, in a mutant of *S. cerevisiae* which lacks the normal capacity to form unsaturated fatty acids in the presence of oxygen (6).

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MATERIALS AND METHODS

The unsaturated fatty acid auxotroph KD115 was kindly supplied by B. Wisnieski and R. K. Mortimer, University of California, Berkeley.

Conditions of growth, disruption and fractionation of cells, assay of enzymes and cytochromes, and determination of lipids have all been described previously (4, 5).

Cells were aerated in synthetic medium constituted as follows (per cent, w/v): KH_2PO_4 (0.4), K_2HPO_4 (0.05), $(NH_4)_2SO_4$ (1.15), NaCl (0.01), $CaCl_2 \cdot 2H_2O$ (0.01), and $MgSO_4 \cdot 7H_2O$ (0.05). Present also were ferric citrate (2 mmole), solution A (5 ml/liter), and solution B (1 ml/liter). Solution A contained (mg/liter): $CuSO_4 \cdot 5H_2O$ (40), KI (100), $ZnSO_4 \cdot 7H_2O$ (100), $Na_2MoO_4 \cdot 2H_2O$ (100), $Na_2B_4O_7 \cdot 10H_2O$ (100), and $MnSO_4 \cdot 4H_2O$ (200). Solution B contained (mg/liter): calcium pantothenate (100), thiamine (100), inositol (200), pyridoxine hydrochloride (100), nicotinic acid (50), and biotin (20). During aeration, glucose (0.6 M) was pumped continuously into the cell suspensions (4-6 mg dry wt of cells/ml) at the rate of 5.3 ml per hr per 200 ml of culture. This procedure maintained glucose concentration at 2 to 5 mM during the course of the aeration. As a source of unsaturated fatty acid, this aeration medium was supplemented with Tween 80 (5 ml/liter) as indicated.

Incorporation of L-leucine-$^{14}C(U)$ into cellular protein was measured as follows. Samples (2.0 ml) of cells were removed from the aeration flasks, collected by centrifugation, and resuspended in 2.0 ml

of 50 mM potassium phosphate (pH 7.0)-5 mM glucose; 0.4 μCi of L-leucine-$^{14}C(U)$ (0.5 μCi/μmole) was added, and cells were shaken at 28 C for 10 min. Labeling was terminated by the addition of 4 ml of 7.5% trichloroacetic acid containing 1 mg of unlabeled DL-leucine per ml. The cells were washed once with trichloroacetic acid-leucine, incubated at 85 C for 15 min in trichloroacetic acid-leucine, washed with acetone, heated in ethanol-ether (2:1) at 65 C for 15 min, and washed finally with ether. After drying, the cells were resuspended in water and samples were taken for determination of dry weight and radioactivity.

The incorporation of ^{14}C-leucine into mitochondrial and cytosol protein was measured as follows. Cells were incubated with ^{14}C-leucine for 20 min as described above, chilled, and disrupted mechanically, and a mitochondrial fraction was prepared by the method described previously (5). A soluble fraction was prepared by centrifuging the post-mitochondrial supernatant fluid at 150,000 $\times$ g for 60 min. Protein in the mitochondrial and soluble fraction was precipitated with cold 7% trichloroacetic acid, washed, defatted, and resuspended for determination of radioactivity as described above for whole cells.

RESULTS

Effect of unsaturated fatty acid on the development of respiration and the synthesis of cytochromes in KD115. Cells grown anaerobically without unsaturated fatty acid had broad hemoprotein absorption peaks at 505, 555, and 585 nm (curve A, Fig. 1a). When these lipid-depleted cells were aerated in the presence of unsaturated fatty acid (Tween 80), a rapid development of mitochondrial respiratory function resulted, as shown by an increase in respiration to normal aerobic levels and the formation of cytochrome aa_3, b, and $c+c_1$ (curve C). With no unsaturated fatty acid in the aeration medium, respiratory development was poor, and although the absorption spectrum changed there was little indication of formation of normal aerobic pigments (curve B).

When cells were grown anaerobically in the presence of unsaturated fatty acid and sterol, a different type of anaerobic spectrum resulted (curve A, Fig. 1b), with a broad major peak centered around 560 nm (cf. reference 9). Aeration of these cells resulted in the development of respiration and of aerobic cytochromes whether or not unsaturated fatty acid was present in the aeration medium, though there was some augmentation when Tween 80 was included (Fig. 1b, curves B and C).

These results indicate that a supply of unsaturated fatty acids, either endogenous or external, is necessary for development of respiration by anaerobically grown KD115. However it could not be established from these experiments whether the restriction of unsaturated fatty acid during aeration affected the synthesis of only those enzymes directly associated with membrane (mitochondrial cytochromes) or whether there was a more general effect, to include soluble enzymes of the organelle.

Effect of unsaturated fatty acids on the synthesis of soluble and particulate mitochondrial enzymes. Cells were grown to differing extents of depletion of endogenous lipid by varying the duration of anaerobic growth. The longer cells are grown anaerobically, the greater is the extent of depletion of unsaturated fatty acid. Cells grown in this way were aerated in the presence and absence of Tween 80, and enzyme activities were determined in cell-free homogenates (Table 1). The extent of induced enzyme formation in each cell type depended on the extent of lipid depletion during anaerobic growth, and on the presence of unsaturated fatty acid during aeration. The presence of unsaturated fatty acid during aeration resulted in large increases in the enzyme levels whether completely or partially depleted cells were used. In the absence of unsaturated fatty acid, however, induced enzyme formation was greater in cells which had been only partially depleted. There was no consistent difference between particulate and soluble mitochondrial enzymes in this respect. Catalase, used in these experiments as a marker for nonmitochondrial enzyme induction, showed similar dependence on initial lipid levels in the cells. The synthesis of ergosterol in these cells was partially dependent on the availability of endogenous lipid during aeration.

Effect of unsaturated fatty acid restriction on protein synthesis in aerated cells. The results obtained with catalase and with the soluble mitochondrial enzymes (Table 1) indicated that restriction of lipid availability affected not only the synthesis of membrane-bound enzymes, but also soluble enzymes whether mitochondrial or extra-mitochondrial. This suggested that restriction of unsaturated fatty acid supply to cells may have caused a general inhibition of protein synthesis. This was examined directly in cells aerated in the presence or absence of unsaturated fatty acid and then pulsed with labeled leucine (Table 2). The specific activity of the mitochondrial fraction from cells aerated in the presence of unsaturated fatty acid was approximately twice that of the soluble protein. Omitting unsaturated fatty acids from the aeration medium caused a large inhibition (approximately 60%) of the specific activity of both fractions, so

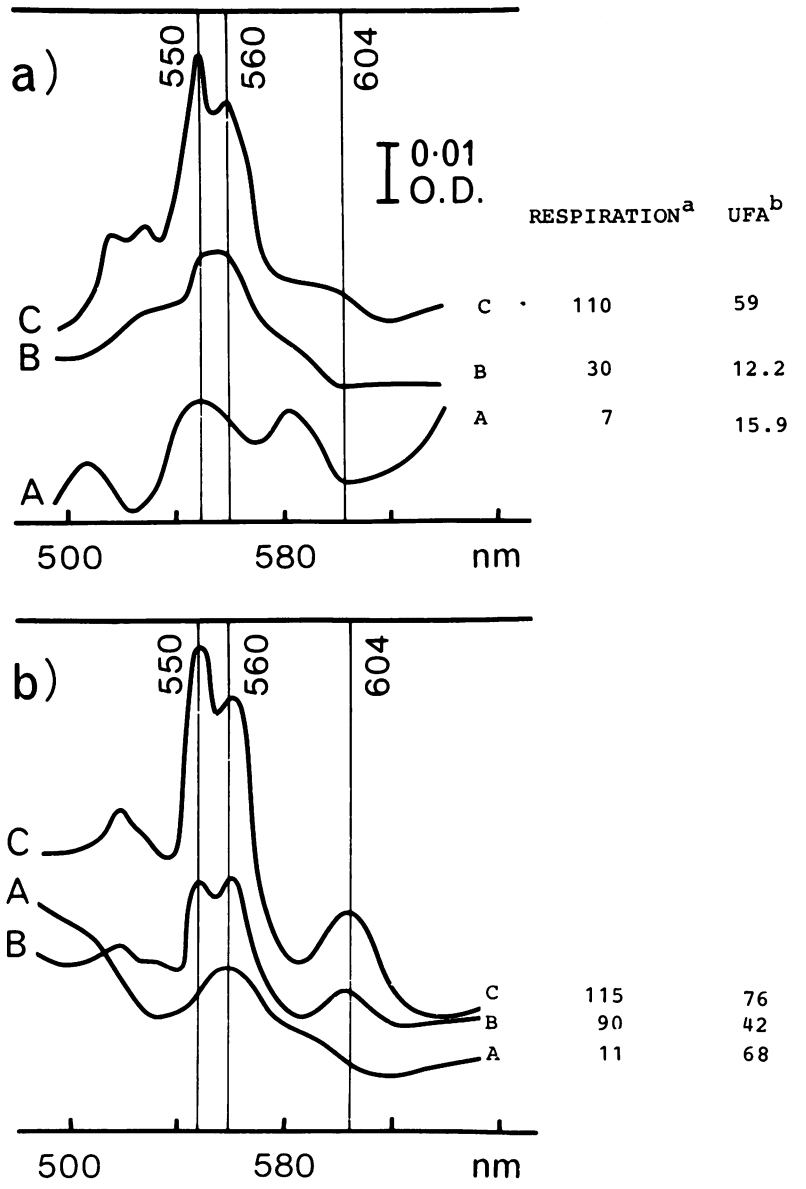

FIG. 1. *Cytochrome content of anaerobic and aerated cultures of KD115. Cells were grown anaerobically (a) without lipid supplement; (b) with Tween 80 (5 ml/liter) and ergosterol (20 mg/liter). After harvesting, cells were aerated in synthetic medium with and without unsaturated fatty acid (Tween 80, 5 ml/liter) for 9 hr. Curve A: anaerobic cells; curve B: cells aerated in absence of unsaturated fatty acid; curve C: cells aerated in presence of unsaturated fatty acid. (a) Respiratory activity of cells as nanogram atoms of oxygen per minute per milligram dry weight. (b) Unsaturated fatty acid content of cells as milligrams of palmitoleic plus oleic acids per 100 mg of total fatty acids.*

that the ratio of labeling was affected only slightly. By contrast, the addition of chloramphenicol during aeration decreased the ratio considerably, and there was little preferential labeling of mitochondrial protein; again there was a large inhibition in both cellular compartments.

DISCUSSION

The capacity to synthesize mitochondrial proteins (particulate or soluble) or cytoplasmic enzymes in the unsaturated fatty acid auxotroph is related to the availability (endogenous or external) of unsaturated fatty acid during

TABLE 1. *Effect of unsaturated fatty acid (UFA) on induced formation of enzymes during aeration of KD115*[a]

Anaerobic growth (hr)	Aeration time (hr)	UFA in aeration medium	Cell UFA (mg of UFA/100 mg of total fatty acids)	Ergosterol (mg/g dry wt)	Malate dehydrogenase[b]	Fumarase[b]	Succinate dehydrogenase[b]	Cytochrome oxidase[b]	Catalase[b]
15	0	–	31	0.4	0.98	0.090	0.025	0.005	0.26
21	0	–	15	0.3	0.92	0.082	0.023	0.005	0.23
15	10	–	18	3.8 (92)[c]	3.0 (51)	0.15 (31)	0.040 (15)	0.096 (68)	2.32 (24)
21	10	–	12	2.4 (42)	1.1 (7)	0.11 (12)	0.042 (6)	0.020 (11)	1.34 (17)
15	10	+	60	4.1	5.0	0.29	0.125	0.141	8.9
21	10	+	59	5.3	3.5	0.27	0.32	0.136	6.8

[a] Cells were grown anaerobically without lipid supplement for 15 and 21 hr to yield cells with different degrees of lipid depletion. After aeration in the presence or absence of UFA (Tween 80, 5 ml/liter), enzyme activities were determined in cell-free homogenates, and fatty acids and sterol were extracted from whole cells.

[b] Expressed as micromoles of substrate consumed per minute per milligram of protein.

[c] Values in parentheses indicate percentage increase in cells aerated in the absence of UFA relative to increase observed in cells aerated in the presence of UFA.

TABLE 2. *Effect of unsaturated fatty acid and chloramphenicol on the labeling of cell fractions prepared from cells pulsed with ^{14}C-leucine*[a]

Addition to aeration medium	Radioactivity incorporated (counts per min per mg of protein)		Relative labeling (MIT/SOL)
	MIT	SOL	
UFA	1,760 (100)	850 (100)	2.07
	1,340 (100)	890 (100)	1.51
UFA + CAP	520 (30)	480 (56)	1.08
	420 (31)	370 (42)	1.13
NIL	730 (41)	450 (53)	1.62
	450 (34)	270 (30)	1.67

[a] Cells were grown anaerobically in nonsupplemented media for 20 hr and then aerated for 7 hr in synthetic medium. Unsaturated fatty acid (UFA) was added to the aeration medium as Tween 80 (5 ml/liter of medium); chloramphenicol (CAP) was present at 9 mM; NIL indicates neither unsaturated fatty acid nor chloramphenicol was added to the aeration medium. After aeration, 0.5-g samples of cells were incubated with ^{14}C-leucine for 20 min and harvested, and cell fractions (mitochondria, MIT; soluble, SOL) were prepared after cells were broken mechanically. Results shown represent the range obtained over four independent experiments. Values in parentheses are expressed relative to those obtained after aeration with UFA present.

aeration. Two possible explanations for this dependence might be advanced. In the first place, a lipid-containing matrix (membrane) may be required as an acceptor for newly synthesized mitochondrial enzymes. The formation of this matrix, inhibited in the absence of essential unsaturated fatty acid, may regulate the synthesis of enzymatic proteins with which the matrix becomes associated. While this explanation may seem likely in the case of the particulate cytochromes and of membrane-bound enzyme such as succinate dehydrogenase, it presents difficulties as an explanation for the results obtained with malate dehydrogenase, fumarase, and catalase. However, it is conceivable that, in the cell, the so-called soluble enzymes are integrated into structures, though of lesser stability than the membranes of mitochondria or other discrete organelles, and are nevertheless susceptible to the same type of regulatory phenomena.

The second possibility is that some or all of the ribosomes on which protein synthesis occurs in these cells are required to be bound to a membrane matrix, similar to the endoplasmic reticulum in cells of more complex forms. In mammalian cells, for instance, it is suggested that free and membrane-bound ribosomes are the sites of synthesis of different classes of proteins (8, and references therein). In terms of this proposal, the experiments described above on the effect of lipid availability on protein synthesis could indicate an effect on those cytoplasmic ribosomes dependent on functional membrane (representing about 60% of the total activity). The residual activity seen would be accounted for as protein synthesis on unbound ribosomes. In that case the effects on enzyme formation during aeration suggest that their synthesis takes place on these membrane-bound ribosomes. Attempts to identify active, membrane-bound cytoplasmic ribosomes directly in *Saccharomyces* have not so far been successful, and electron microscope observation of ribosomes in intact yeast cells suggest that only a very small fraction are attached to cytoplasmic membrane. A further

possibility, associating lipid and protein synthesis, is that ribonucleic acid synthesis is regulated by the availability of functional membrane, as may be the case for mitochondrial ribonucleic acid (2).

Proudlock, Haslam, and Linnane (7), using the mutant KD115, found that in batch cultures partial depletion of unsaturated fatty acid resulted in lowered growth yields. This effect was ascribed to an uncoupling of phosphorylation and respiration and suggests that, in addition to regulating protein synthesis in the cell, unsaturated fatty acid levels may also affect the energy-transducing function of mitochondrial membrane.

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