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The Effects of Unsaturated Fatty Acid Depletion on the Lipid Composition and Energy Metabolism of a Fatty Acid Desaturase Mutant of *Saccharomyces cerevisiae*

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Abstract

1. The lipid composition of a mutant of *Saccharomyces cerevisiae* which cannot synthesize unsaturated fatty acid (UFA) can be extensively manipulated by growing the organism in the presence of added fatty acids.

2. Growth of the mutant is supported by a wide range of unsaturated fatty acids including oleic, palmitoleic, petroselenic, 11-eicosaenoic, ricinoleic, arachidonic, clupanodonic, linoleic and linolenic acids; 9- and 10-hydroxystearic acids support growth less effectively, but erucic, nervonic, elaidic and saturated fatty acids ($C_{8:0}$–$C_{20:0}$)* are ineffective. All the fatty acids which support growth are incorporated into cell lipids, apparently without further metabolism.

3. The effects of altered lipid composition on the energy metabolism of yeast cells were investigated. Cells containing less than approximately 20 % of their fatty acids as UFA cannot grow on non-fermentable substrates, and their growth on glucose is restricted to that which can be supported by fermentation alone.

4. UFA-depleted cells contain mitochondria which are apparently normal in morphology, furthermore they have normal levels of cytochromes $a + a_3$, b, c_1 and c and respire at normal rates. This suggests that the lesion in energy metabolism produced by UFA-depletion may be the loss of the ability of the mitochondria to couple respiration to phosphorylation.

5. UFA-depleted cells incorporate added UFA into their cell lipids and subsequently regain the ability to grow on non-fermentable substrates, showing that the lesion in energy metabolism is fully reversible.

Introduction

Lipids are a major constituent of all cellular membranes, and it has long been recognized that their physiocochemical properties are important in determining the structure and permeability properties of membranes. Although individual membranes of diverse

* Fatty acids are denoted by the convention C number of carbon atoms: number of unsaturated bonds.

function vary greatly in the nature and amount of their lipid components, the precise role of the lipid is still obscure. A major approach to the investigation of the role of lipids in the function of membrane-integrated enzyme complexes has been the degradation of the membrane into its components by treatment with detergents or organic solvents followed by attempts to recombine the fragments into a functional membraneous unit. This has been used with notable success to investigate the components and organization of the terminal electron transport chain of the inner mitochondrial membrane.[1] Such studies have also helped to establish that some membranes which contain multienzyme complexes may consist in part of lipoprotein subunits, and that lipids are essential for the activity of membrane-bound enzymes (for reviews see references 2–5).

An alternative approach to the study of the involvement of lipids in membrane is to utilize genetic or physiological situations which permit the manipulation of membrane lipid composition *in vivo*. These methods avoid the potential hazards of artifactually induced membrane re-organization which may occur during degradative treatments *in vitro*. The yeast, *Saccharomyces cerevisiae*, is a particularly suitable organism for such an experimental attack, especially in regard to the study of mitochondrial membranes. The growth of this organism on non-fermentable substrates is dependent upon functional mitochondria whereas growth on fermentable substrates is independent of mitochondrial function. Thus genetic and physiological situations which result in the formation of abnormal mitochondrial membranes can be experimentally exploited. *S. cerevisiae* cannot synthesize unsaturated fatty acids or ergosterol under anaerobic conditions,[6,7] but it can incorporate into its membranes from the growth medium a wide variety of added unsaturated fatty acids[8] or sterols.[9] Although the mitochondrial lipids of anaerobically grown *S. cerevisiae* can be manipulated in this way,[10] the system is of limited usefulness in the study of mitochondrial membrane functions as anaerobiosis also prevents the formation of the mitochondrial respiratory enzymes.[11] In the present study it is shown that the membrane lipid composition of a fatty acid desaturase mutant of *S. cerevisiae*[12,13] can be varied under both aerobic and anaerobic conditions by growth of the organism in the presence of added fatty acids. Such manipulation has enabled the definition of an experimental situation in which most cell membranes function normally so that the cell can grow on a fermentable substrate, but the functions of the mitochondrial membranes are specifically affected so that growth on a non-fermentable substrate is restricted. The mitochondrial lesion has been found to be a defect in oxidative phosphorylation and detailed investigations of this phenomenon are described in a companion paper.[14] A preliminary report of some of this work has appeared elsewhere.[15]

Methods

Organisms. The strains of *Saccharomyces cerevisiae* used were (a) An unsaturated fatty acid requiring mutant KD115, obtained from Dr. M. Resnick, Donner Laboratory, University of California, Berkeley, California, U.S.A.[12,13] and (b) a fatty acid desaturase revertant of KD115 designated KD115-1, which was selected from a lawn of UV-irradiated KD115 cells plated on media lacking UFA.

Growth media. Growth media contained: Difco yeast extract, 1%; *Saccharomyces* salts, comprising $(NH_4)_2SO_4$, 0·12%; KH_2PO_4, 0·10%; $MgCl_2$, $6H_2O$, 0·07%; NaCl, 0·05%; $CaCl_2$, 0·01%; $FeCl_3$, 0·0005%, and as source of energy and carbon either

glucose, galactose, glycerol or ethanol at the concentrations indicated. Sterile glucose solution was added to previously autoclaved medium, as partial decomposition of the glucose occurred on autoclaving. The pH of all culture media was adjusted to 5·4 before autoclaving. Fatty acid supplements were added either as the free acid (emulsified in 0·2% Triton X-100) or as the Tween derivative (fatty acid esters of polyoxyethylene sorbitan). Solid media contained: Difco yeast extract, 0·2%; *Saccharomyces* salts; glucose of glycerol, 2%; agar, 2% and Tween 80, 1% (where indicated). Cultures were grown at 28° C. For aerobic cultures of up to one litre in volume a rotary shaker was used; cultures of larger volume were grown in carboys under forced aeration. Anaerobic cultures were grown as described by Wallace *et al.*[16] The initial cell density of all cultures was adjusted to 5–10 μg dry weight cells/ml.

Estimation of cell yield. Cell growth was estimated by the direct weighing of cells dried in an air oven at 110° C for 24 hours. Washed cells, centrifuged out from a suitable volume of culture media, were placed on tared glass planchettes, dried at 110° C for 24 hours, cooled and then weighed. Cell growth was also determined turbidometrically by measuring the optical density at 640 nm of a suitably diluted culture sample, and a calibration curve was prepared by plotting the optical density at 640 nm of suspensions of cells whose concentrations were determined by the direct weighing method. This method was usually used in growth studies, but where accurate cell densities were necessary the direct weighing method was used.

Determination of cell viability. The percentage of viable cells in given culture was determined by counting a suitably diluted sample of cells in a haemocytometer, plating out on solid media (containing 1% Tween 80), and counting the number of colonies after two to three days incubation at 28° C. Contaminant and revertant cells were counted by plating on solid media from which the 1% Tween was omitted. In the absence of added UFA, the mutant grew only to a pinpoint colony; contaminant and revertant cells gave normal sized colonies.

Lipid Analyses

Extraction of lipids from cells (a) *Total lipid.* For the extraction of total lipid, a 1 : 1 suspension of whole cells in water was completely disrupted by three or four passes through a French Pressure cell at 7 tons/in² and kept at 0–2° C. The broken cell preparation was then extracted with twenty volumes of chloroform–methanol (2 : 1 v/v), for 2 hours at room temperature, followed by two further extractions using ten volumes chloroform–methanol (2 : 1 v/v). This method results in virtually complete extraction and minimizes degradation of cell lipids.[10] The extracts were pooled, dried over anhydrous sodium sulphate, and the fatty acids recovered by removal of the ether under reduced pressure on a rotary evaporator below 30° C. The fatty acids were methylated by the method of Stoffel *et al.*[17] with anhydrous methanol–HCl (0·5 M), and analysed by gas liquid chromatography as described below. Where it was necessary to determine the total fatty acid content of cells, a known weight of pentadecanoic acid (15 : 0) was was added to a known weight of cells prior to saponification. In many analyses of this nature, in particular with Tween 80 grown cells, it was necessary to run two determinations, one with the pentadecanoic acid and one without, as the Tween 80 used was found to contain a trace amount of pentadecanoic acid which was incorporated into the cells.

Analysis of the total fatty acid extract. Total fatty acid contents and fatty acid composition

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were determined by gas liquid chromatography using an F and M Chromatogram, Model 810, equipped with dual flame ionization detectors. The operating characteristics of the chromatogram were determined by the method of Horning et al.[18] The fatty acids were identified by their retention times on the columns relative to pentadecanoic acid, and the composition of the mixture was determined by measurement as described by James.[19] Ricinoleic and 9- and 10-hydroxystearic acids were converted to their acetoxy derivatives as described by Light et al.[20] prior to chromatography.

Quantitative fractionation of the lipid extracts. The total lipid extracts were quantitatively separated into their various components using column chromatography, in conjunction with thin-layer chromatography. The identity of the phospholipids was determined by comparing their behaviour in two different solvent systems relative to that of authentic reference phospholipids, by their behaviour to the different staining and detection methods and from previously published R_f values.[21–23] The phosphorus content of each component was determined after digestion with 60% perchloric acid by the method of Chen et al.[24]

Thin layer chromatography. Thin layer chromatography was carried out on Merck "Silica Gel H" plates 0·25 mm thick. The plates were prepared according to Stahl,[25] and activated before use by heating in an oven at 110° C for one hour. Two solvent systems were used: Solvent 1: chloroform–methanol–acetic acid–water (25:15:4:2 v/v);[21] Solvent 2: chloroform–methanol–ammonia (14:6:1 v/v). The lipids on the developed chromatograms were detected by several methods.
(1) Charring with 40% sulphuric acid to detect all organic material.[26]
(2) Stained with iodine vapour to locate all lipid material.[26]
(3) Sprayed with ninhydrin to detect amino-lipids.[27]
(4) Sprayed with the modified Dragendorff reagent to detect choline containing lipids.[28]
(5) Sprayed with acid molybdate to detect phosphorus-containing lipid.[28]
Sterol was determined by a modification of the Liebermann–Burchard reaction.[29]

Reduced cytochrome spectra of whole cells were determined on a Carey model 14 spectrophotometer as described by Clark-Walker and Linnane.[30]

Electron microscopy of whole cells was as described by Wallace et al.[16]

Respiratory activity of whole cells was determined polarographically at 30° in a medium containing 2 mM glucose, 50 mM K–PO_4 (pH 7·4).

Materials. Yeast extract was obtained from Difco Laboratories. Tween 80 was purchased from Chemical Materials Ltd., Melbourne, Australia and was found by gas chromatography to have the following fatty acid composition: oleic acid 71%, palmitoleic acid 13%; myristoleic acid 3%; and saturated fatty acids 13%. Pentadecanoic, elaidic, clupanodonic, eicosaenoic, and arachidonic acids were purchased from Sigma Chemical Co., St. Louis, U.S.A. 9- and 10-hydroxystearic, ricinoleic, linoleic, linolenic, elaidic and petroselenic acids were obtained from K and K Laboratories Inc., Hollywood, California, U.S.A. Erucic and nervonic acids were the gift of Dr. A. W. Rodwell, C.S.I.R.O. Division of Animal Health, Parkville, Victoria, Australia. The phospholipid reference standards, phosphatidyl ethanolamine and phosphatidyl choline were purchased from Calbiochem Inc., Los Angeles, California, U.S.A. Phosphatidyl serine and phosphatidyl inositol were obtained from Koch Light and Co., Colnbrook, England. Where necessary impurities were removed by thin layer chromatography using the solvent system of Skipski et al.[21] Whatman DEAE cellulose (DE 11) was obtained from

Reeve Angel and Co., London E.C.4, England. "Camag" Neutral Alumina (Brockmann Activity = 1) was obtained from May and Baker (Australia), West Footscray, Victoria, Australia. "Unisil" activated silicic acid (mesh 100–200) was obtained from Clarkson Chemical Co. Inc., Williamsport, Pennsylvania, U.S.A. All other reagents were the best commercially available grades.

Results

The Non-lipid Auxotrophic Requirement of KD115 and KD115-1

The UFA-requiring mutant KD115 is unable to grow on a completely synthetic medium even when supplemented with an excess of UFA, but the addition of a small amount of yeast extract allows growth. As the revertant KD115-1 shows a similar requirement it appears that both strains have a second distinct growth requirement unrelated to the UFA-requirement. Table I illustrates the requirement for yeast extract for growth on *Saccharomyces* medium containing 600 μg/ml UFA; in this medium 1·0% yeast extract allows growth of the revertant to about 8 mg/ml, and eliminates the requirement for the non-lipid factor by the mutant which stops growing at 6.5 mg/ml due to UFA-depletion.

TABLE I. The growth requirement by strains KD115 and KD115-1 for an unidentified component of yeast extract

Yeast extract (% w/v)	Growth of KD115 (mg dry weight/ml)	Growth of KD 115-1
0·00	0·05	0·05
0·02	0·64	0·69
0·10	2·50	2·63
0·20	4·12	4·25
0·50	6·50	6·62
1·0	6·51	8·53
2·0	6·56	10·10

Cells were grown to stationary phase on *Saccharomyces* salts medium containing 5% glucose, 600 μg/ml UFA and the concentrations of Difco yeast extract indicated. The results are the average of two experiments.

Attempts to determine the nature of the non-lipid requirements were unsuccessful; all compounds tested for their ability to support growth were found to be inactive whether tested individually or in combination. The following compounds were tested: L-serine, L-threonine, L-valine, L-trytophane, L-isoleucine, L-leucine, L-tyrosine, L-methionine, L-histidine, L-arginine, L-lysine, L-glutamate, L-ornithine and L-phenylalanine (tested at 10 mg/l both individually and together); ergosterol (10 mg/l); α-tocopherol (5 mg/l); adenine (10 mg/l); uracil (10 mg/l); biotin (100 μg/l); calcium pantothenate (10 mg/l); folic acid (10 μg/l); thiamine (5 mg/l); pyridoxine hydrochloride (5 mg/l); nicotinic acid (5 mg/l); riboflavin (500 μg/l); inositol (100 mg/l); and the phospholipids, phosphatidyl

inositol, phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine (500 μg/l). Fractionation of the yeast extract on Sephadex G-75 showed that the factor was of relatively small molecular weight and water soluble. Although the nature of the non-lipid requirement is still unknown, its effect can be eliminated in liquid media by the addition of 1% Difco yeast extract which supports growth to more than 8 mg/ml in UFA-supplemented medium. The Difco yeast extract contained small amounts of oleic and palmitoleic acids (approximately 40 μg per g), but since 1% yeast extract supported growth of KD115 to only 0·1 mg/ml in 5% glucose medium, this amount of unsaturated fatty acid was of negligible significance. A concentration of 0·2% yeast extract was chosen for solid media, as it supported the growth of large colonies in the presence of excess unsaturated fatty acids but only allowed the formation of pinpoint colonies in the absence of fatty acid supplements.

The Manipulation of the UFA Content of Cells of S. cerevisiae *under Aerobic and Anaerobic Conditions*

The unsaturated fatty acids of *S. cerevisiae* can vary in content from 0·4 to 60 mg/g dry weight of cells depending on the metabolic and physiological state of the cells.[10, 31] In particular, *S. cerevisiae* cannot synthesize UFA under anaerobic conditions because of an obligatory requirement for oxygen in the desaturase reaction, and must be supplemented with UFA in order to grow. This enables the fatty acid content of anaerobically grown cells to be extensively manipulated by growing the cells in the presence of different supplements of UFA.[10] The fatty acid desaturase mutant KD115 affords the further possibility of manipulating the UFA content of the cells under aerobic conditions. Resnick and Mortimer[12] have previously shown that the growth of strain KD115 can be supported by free unsaturated fatty acids, their methyl esters or by Tween 80. The naturally occurring unsaturated fatty acids of *S. cerevisiae* are the monoenoic acids, although some strains can synthesize linoleic acid to a very limited extent.[7, 32] The main acids are palmitoleic and oleic with a trace of myristoleic; the relative proportion of these vary from strain to strain. Tween 80, which is a soluble form of UFA of approximate fatty acid composition, saturated fatty acids 13%, unsaturated fatty acids 87% (myristoleic 3%, palmitoleic 13% and oleic 71%), was used in the present experiments as a conveniently available source of UFA similar in composition to the natural yeast unsaturated fatty acids.

Table II compares the fatty acid compositions of the mutant strain KD115 and the revertant strain KD115-1 under aerobic and anaerobic conditions, and in the present of the UFA supplements indicated. Mutant cells grown aerobically on growth-limiting levels of UFA (50 μg/ml), contain very low levels of UFA (about 5% of total fatty acids) and are similar in unsaturated fatty acid composition to both mutant and revertant cells grown anaerobically with limiting UFA (50 μg/ml); they also contain a large proportion of short-chain saturated fatty acids ($C_{8:0}$ to $C_{14:0}$), although the average chain length of these acids is longer than in the anaerobically grown cells. When the mutant is grown aerobically with full supplements of UFA (3 mg/ml), the fatty acid composition at the cells is very similar to that of the revertant cells grown aerobically. The UFA-requiring mutant, therefore, offers the opportunity to study the effects of extensive changes in fatty acid composition on the metabolism of aerobically grown yeast cells.

TABLE II. The fatty acid composition of cells of strains or KD115 and KD115-1 grown under aerobic and anaerobic conditions

Cell type	UFA supplement (μg/ml)	Fatty acid composition (mg/100 mg fatty acid mixture)											
		$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{8:0}-C_{14:0}$	$C_{16:0}+C_{18:0}$	UFA
Aerobic KD115	50	0	1·7	4·0	12·0	0	68·5	1·0	9·2	3·6	17·7	77·7	4·6
Anaerobic KD115	50	1·2	11·2	11·4	5·3	0	52·3	2·0	11·9	4·7	29·1	64·2	6·7
Anaerobic KD115-1	50	1·0	12·0	11·5	4·8	0	50·3	2·1	12·5	5·8	29·3	62·8	7·9
Aerobic KD115-1	0	0	TR	0·6	1·3	TR	18·0	28·2	7·3	34·4	1·0	28·3	72·6
Aerobic KD115	3000	0	TR	1·8	2·1	1·5	22·8	17·4	6·4	48·3	3·6	29·2	67·2

Cells were grown aerobically or anaerobically as described in Methods on *Saccharomyces* salts medium containing 5% glucose and 1% Difco yeast extract. Supplements of unsaturated fatty acids were added where indicated in the form of Tween 80; supplements were calculated as μg of free fatty acid. The results are the average of three experiments. TR represents <0·5%.

The Effect of The Carbon Source on the Growth Yield and Fatty Acid Composition of Cells of Strain KD115 Grown with Increasing Supplements of UFA

The final growth yields of cells of strain KD115 on media containing excess glucose or glycerol and increasing supplements of UFA are shown in Fig. 1. The growth yields of cells are proportional to the concentration of added UFA in the range 0–100 μg of UFA/ml on glucose medium and in the range 0–600 μg of UFA/ml on glycerol medium, indicating that under these conditions growth is strictly limited by the availability of UFA; the yields of cells are 32·5 mg dry weight/mg UFA on glucose and 7·5 mg dry
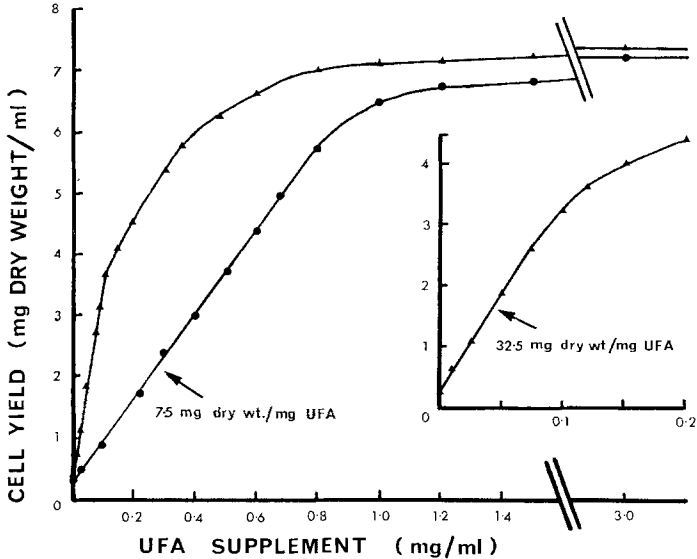

Figure 1. Growth of strain KD115 on glucose and glycerol media with increasing supplements of UFA. Cells were grown aerobically to stationary phase at 28° on 1% yeast extract, *Saccharomyces* salts media containing either 5% glucose or 5% glycerol plus supplements of UFA added as Tween 80; the yield of cells for each UFA supplement was then determined: ▲—▲ glucose, ●—● glycerol. The inset is a magnification of the growth curve of cells grown on glucose media supplemented with 0–0·2 mg UFA/ml of medium. The final growth yields of cells per mg of UFA corresponding to the linear portions of the two growth curves are indicated on the diagram.

weight/mg UFA on glycerol. At higher supplements of UFA the growth yields of cells per mg of UFA on the two substrates progressively decrease and approach one another. In the presence of excess UFA (3 mg/ml) the growth yields of cells reach a maximum value and are the same on both media, being limited by some factor other than the carbon source of UFA.

Cells grown as described in Fig. 1 were harvested and subjected to fatty acid analysis, and the results are summarized in Table III. When cells stop growing due to strict UFA-limitation their fatty acids contain approximately 20% UFA on glycerol media but as low as 5% UFA on glucose media. This is consistent with the growth yields obtained in Fig. 1 which indicate that low supplements of UFA will support four times as much growth on glucose as on glycerol. At higher supplements of UFA the amounts of short chain and long chain saturated fatty acids fall, and the percentages of unsaturated

fatty acids rise to approximately 70% of total fatty acids in both glucose and glycerol-grown cells. At all levels of UFA-supplementation the cells grown on glycerol media contain approximately 30% more total fatty acid than those grown on glucose media.

Under conditions where mitochondrial oxidation, or phosphorylation are defective, yeast cells retain the ability to grow on a fermentable carbon source such as glucose, but are incapable of growth on a non-fermentable carbon source such as glycerol. The ability of the mutant to grow four times more extensively on glucose medium than on glycerol medium with limiting UFA supplements suggests that UFA-depletion specifi-

TABLE III. The fatty acid composition of cells of strain KD115 grown on glucose and glycerol media containing various UFA supplements

UFA supplement (μg/ml)	Substrate	Total fatty acid (mg/g dry weight of cells)	Fatty acid composition (mg/100 mg fatty acid mixture)		
			Saturated fatty acids ($C_{8:0}$–$C_{14:0}$)	Saturated fatty acids ($C_{16:0} + C_{18:0}$)	Unsaturated fatty acids ($C_{16:1} + C_{18:1}$)
50	Glucose	27·9	17·7	77·7	4·6
	Glycerol	38·6	16·1	64·6	19·3
100	Glucose	28·8	15·9	77·1	7·0
	Glycerol	36·7	23·5	60·0	16·5
200	Glucose	29·5	15·2	76·6	8·2
	Glycerol	35·8	17·2	61·5	21·3
400	Glucose	30·4	12·0	66·9	21·1
	Glycerol	37·5	18·0	60·1	21·9
600	Glucose	31·2	10·3	51·4	38·3
	Glycerol	38·0	16·4	59·9	23·0
1000	Glucose	33·0	5·1	36·4	58·5
	Glycerol	43·1	8·4	44·9	44·5
3000	Glucose	36·4	3·6	29·2	67·2
	Glycerol	53·1	2·9	20·2	72·2

Fatty acid supplements were added in the form of Tween 80, and cells were grown aerobically at 28° on media containing 1% yeast extract, *Saccharomyces* salts and either 5% glucose or 5% glycerol as carbon source. Cells were then harvested and analysed for fatty acids.

cally affects mitochondrial functions. Consequently, cells are also capable of growing on glucose medium until their fatty acids contain much lower levels of UFA than on glycerol medium.

The Efficiency of Utilization of Fermentable and Non-fermentable Energy Sources for the Growth of Mutant Cells in the Presence of High and Low Supplements of UFA

Bauchop and Elsden[33] showed that microorganisms grown in a complex medium synthesis approximately 10·5 g dry weight of cells per mole of ATP produced by catabolism of the energy source. Kormancikova et al.[34] have extended this work to show that the phosphorylation efficiencies of growing yeast cells with a number of substrates may be approximately determined from the yields of cells grown in a complex medium containing limiting amounts of the substrates and excess amounts of vitamins, minerals and amino acids.

In order to investigate whether UFA-depletion specifically affects mitochondrial metabolism, the growth yields of strain KD115 on the substrates glucose, glycerol and ethanol were measured in the presence of high (1·5 mg/ml) and low (40 μg/ml) supplements of UFA. Yeast extract (1% w/v) was added to the media as a source of excess vitamins, amino acids and nucleotides, so that the substrates were acting essentially as a source of energy rather than as a source of carbon. The results of these experiments are shown in Fig. 2. In the presence of excess UFA the final cell yield is proportional to glucose concentration and is 1·09 mg dry weight/mg glucose; this indicates a full utiliza-
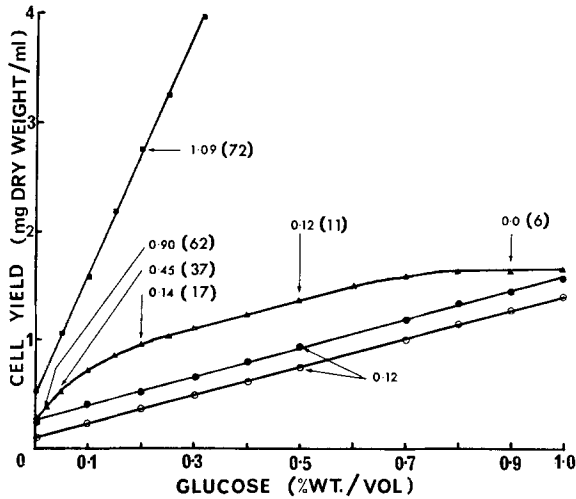

Figure 2. Growth of cells of strain KD115 on glucose media at high and low levels of UFA-supplementation. Cultures were grown on 1% yeast extract, *Saccharomyces* salts medium supplemented with the levels of glucose indicated, harvested at stationary phase, and growth yields determined. Other additions were: ■—■ UFA (1·5 mg/ml); ▲—▲ UFA (40 μg/ml); ●—● UFA (1·5 mg/ml) + chloramphenicol (2 m/gml); ○—○ UFA (40 μg/ml) + chloramphenicol (2 mg/ml). The slopes of the growth curves are indicated by the numbers above the curve, these represent the growth increment at the point indicated in mg dry weight cells/mg glucose. The numbers in parentheses indicate the UFA contents (percentage of total fatty acids).

tion of the substrate by both fermentative and oxidative metabolism.[34] In the presence of the antibiotic chloramphenicol which inhibits the formation of functional mitochondria,[35] the growth increment of 0·12 mg dry weight/mg glucose represents purely fermentative metabolism.[34] When the mutant is grown with limiting UFA the growth response curve shows three distinct phases. In the first phase of the growth response curve, which obtains with 0–0·2% glucose, the growth yield of cells falls from approximately 1 mg to 0·12 mg dry weight/mg glucose as the UFA content of the cellular fatty acids falls from 72% to 17%. The second phase of the growth response curve is obtained with 0·2 to 0·5% glucose where the UFA content of the fatty acids drops from 17 to 11%, but the yield of cells remain steady at 0·12 mg dry weight/mg glucose indicating growth purely by fermentative metabolism. Growth yields further decline at concentrations of

glucose above 0·5% until the cells finally stop growing when the fatty acids contain 6% UFA. These results are interpreted to mean that the ability to obtain energy by oxidative metabolism is progressively lost as the UFA content of the cellular fatty acids drops from the normal level of about 70 to 17%. Subsequently, the ability to grow by fermentative metabolism is unimpaired until the UFA content of the fatty acids drops below 10%, and growth finally ceases when the UFA content falls to approximately 6%.

Figure 3 shows the growth yields of the mutant on the non-fermentable substrates ethanol and glycerol. In the presence of limiting UFA (50 μg/ml) the growth increment of the mutant is negligible as the concentrations of ethanol or glycerol are increased, but in the presence of excess UFA (3 mg/ml) the growth yield of cells increases linearly with addition of substrate being 1·6 mg dry weight/mg ethanol and 1·05 mg dry weight/ mg glycerol. Thus the UFA-depleted cells lose the ability to grow on non-fermentable
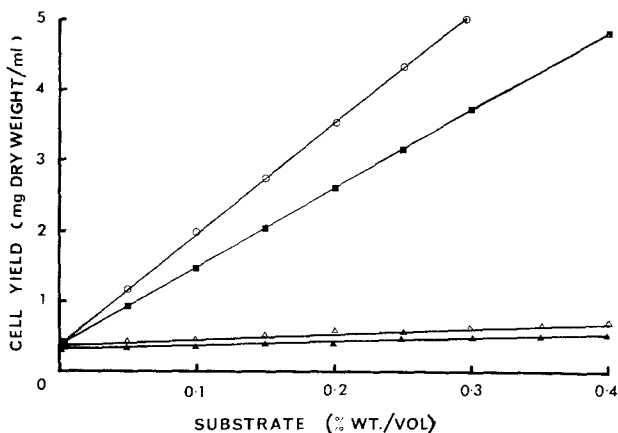

Figure 3. The growth of cells of strain KD115 on non-fermentable substrates at low and high levels of UFA-supplementation. Cells were grown as described in Fig. 1 and the final growth yields were determined. Substrates and UFA supplements were : o—o ethanol + 3 mg/ml UFA; ■—■ glycerol + 3 mg/ml UFA; △—△ ethanol + 50 μg/ml UFA; ▲—▲ glycerol + 50 μg/ml UFA.

substrates, whereas the UFA-supplemented cells appear to utilize these substrates normally by oxidative metabolism.

The Effects of UFA-supplementation on the Total Lipid Content and Composition of Whole Cells

Since the UFA-depleted cells lose the ability to grow on non-fermentable substrates, it was of interest to compare the total lipid content and composition of cells grown in the presence of high and low levels of UFA. For convenience both types of cells were grown to 1·2 mg/ml on 0·4% glucose; this concentration of glucose supports growth by fermentation alone to 0·5 mg/ml, and growth beyond this extent proceeds by oxidative metabolism giving catabolite derepressed cells. Table IV compares the contents of neutral lipids, sterols and phospholipids of the two cell types. When cells are grown on medium containing 50 μg UFA/ml about 26% of their fatty acids are unsaturated, whereas for cells grown on 1·5 mg UFA/ml the UFA content is 73% of the fatty acids. The total

TABLE IV. Lipid composition of cells of strain KD115 grown on 0·4% glucose and high and low supplements of UFA

UFA supplement (μg/ml)	UFA (% total fatty acid)	Total lipid content	Phospho-lipid content	Neutral lipid content	Sterol content	Phospholipids				
			(mg/g dry weight cells)			DPG	PC	PE	PI	PS
						(mg/g dry weight cells)				
50	26	57	12·4	45	7·7	1·84	5·40	2·26	2·52	0·38
1500	73	83	17·6	65	5·8	2·11	6·17	3·15	5·81	0·35

Cultures were grown to 1·2 mg dry weight cells/ml on 1% yeast extract, 0·4% glucose, *Saccharomyces* salts medium plus the level of UFA supplementation indicated. Lipid analyses were performed as detailed in the Methods section; the results are the average of three determinations. Neutral lipid refers to that fraction eluted from silicic acid by chloroform and contained triglycerides, hydrocarbons, free fatty acids, sterol and sterol esters. The following abbreviations have been used: DPG—diphosphatidyl glycerol (cardiolipin); PC—phosphatidyl choline; PE—phosphatidyl ethanolamine; PI—phosphatidyl inositol; PS—phosphatidyl serine. UFA content refers to the % UFA in the whole cell fatty acids as determined by saponification. Phospholipid composition was determined by column chromatography as detailed in Methods.

lipid content of the cells is reduced by approximately 30% on the lower level of UFA, and this comprises a similar percentage decrease in both neutral lipids and phospholipids. The amount of sterol, however, increase somewhat in the UFA-depleted cells. Analysis of the individual phospholipid fractions shows that UFA-depletion causes a reduction of more than 50% in the phosphatidyl inositide content of the cells, whereas the levels of the remaining phospholipids are decreased only slightly. Table V compares the fatty acid compositions of the neutral and phospholipid fractions of the two cell types. Although there are marked differences in the fatty acid composition of the lipids from the cells grown on high and low levels of UFA, these changes are reflected to the same extent in both the neutral lipid and phospholipid fractions.

TABLE V. Fatty acid composition of the neutral and phospholipid fractions of cells of strain KD115 grown on 0·4% glucose and high and low supplements of UFA

UFA supplement (μg/ml)	Lipid fraction	Fatty acid composition (mg/100 mg total fatty acid)		
		$C_{8:0}-$ $C_{14:0}$	$C_{16:0}+$ $C_{18:0}$	UFA
50	Neutral lipids	12·1	61·7	26·2
	Phospholipids	13·6	57·6	28·8
1500	Neutral lipids	2·9	24·8	72·2
	Phospholipids	2·9	25·1	72

Cultures were grown on 1% yeast extract 0·4% glucose, *Saccharomyces* salts plus the UFA supplements indicated. The cultures were harvested at a cell density of 1·2 mg dry weight/ml. The results given are those of typical experiments.

The Specificity of the Fatty Acid Requirement for the Growth of the Mutant on Glucose and Glycerol Media

The UFA-requiring mutant offers the opportunity to study the suitability of different fatty acids for the aerobic growth of *S. cerevisiae*. Furthermore, it is possible to determine whether any special limitations are placed on the nature of the fatty acid for mitochondrial activity by comparing the growth of the mutant with the test fatty acid on fermentable and non-fermentable substrates. The ability of different fatty acids to support the

TABLE VI. Growth of UFA-requiring mutant KD115 with different fatty acids

Fatty acid supplement	Growth on glucose (mg dry weight/ml)	Growth on glycerol
None	0·15	0·04
9-Hexadecenoic (palmitoleic)	2·30	0·68
9- and 10-OH-octadecanoic (hydroxystearic)	1·37	0·40
6-Octadecenoic (petroselenic)	2·35	0·65
9-Octadecenoic (oleic)	2·20	0·69
trans-9-Octadecenoic (elaidic)	0·23	0·04
12-OH-9-octadecenoic (ricinoleic)	1·80	0·55
9,12-Octadecadienoic (linoleic)	2·20	0·63
9,12,15-Octadecatrienoic (linolenic)	2·14	0·67
5,8,11,14-Eicosatetraenoic (arachidonic)	2·10	0·59
11-Eicosaenoic	2·20	0·60
cis-13-Docosaenoic (erucic)	0·11	0·02
cis-15-Tetracosaenoic (nervonic)	0·10	0·02
4,8,12,15,19-Docosapentaenoic (clupanodonic)	2·25	0·63
Tween 80	2·18	0·64
Mixed yeast fatty acids	2·31	0·70

Cells were grown aerobically at 28° on *Saccharomyces* salts, 1% yeast extract media containing either 5% glucose or 5% glycerol. The fatty acids were added at 100 μg/ml of medium emulsified in 0·2% triton-X100. Cells of strain KD115 were adapted to glycerol growth prior to inoculation. Cells were grown for 42 hours and 72 hours on glucose and glycerol media respectively before the determination of the cell yield. The mixed yeast fatty acids were isolated from commercial bakers yeast by the saponification procedure detailed in Methods; its composition was approximately 20% saturated fatty acids, 49% palmitoleic acid and 31% oleic acid. The results are the average of two experiments.

growth of mutant cells was examined in media containing either 5% glucose as a fermentable carbon source or 5% glycerol as a non-fermentable carbon source. These media were supplemented with the test fatty acid at 100 μg/ml as this level of supplementation is growth-limiting on both glucose and glycerol media. Results of these experiments are shown in Table VI. Straight chain saturated fatty acids ($C_{8:0}$–$C_{20:0}$) are inactive in supporting growth. Unsaturated fatty acids with a 9-10 cis double bond support growth, but elaidic acid which contains a 9-10 trans linkage is inactive. Petroselenic (cis 6-7 unsaturated), 11-eicosaenoic, arachidonic (5-6, 8-9, 11-12, 14-15 unsaturated) and clupanodonic (4-5, 8-9, 12-13, 15-16, 19-20 unsaturated) acids also support growth, but erucic (cis 13-14 unsaturated) and nervonic (cis 15-16 unsaturated)

TABLE VII. Fatty acid composition of cells of strain KD115 grown in 5% glucose media supplemented with different fatty acids

Fatty acid supplement	Fatty acid composition (mg/100 mg total fatty acids)										Added fatty acids	Total unsaturated and hydroxy fatty acids
	Saturated						Unsaturated					
	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
9-Hexadecenoic (palmitoleic)	1·7	6·4	15·4	0	58·3	13·2	4·3	0·7	0	0	4·3	5·0
9-Octadecenoic (oleic)	0·8	3·8	17·4	0·5	62·0	10·6	1·0	3·9	0	0	3·9	4·9
6-Octadecenoic (petroselenic)	1·4	5·5	12·8	0·5	62·0	13·4	0·6	TR	0	0	3·8	4·4
9- and 10-Hydroxyoctadecanoic (hydroxystearic)	2·9	3·0	6·9	0·8	61·0	15·2	1·2	1·8	0·8	0·7	5·7	10·2
12-OH-9-Octadecenoic (ricinoleic)	0·4	4·1	12·3	0·4	55·8	10·4	1·1	0·9	1·6	0·4	3·7	7·7
9,12-Octadecadienoic (linoleic)	TR	1·0	5·6	1·2	68·2	18·2	1·1	1·3	3·3	0	3·3	5·7
9,12,15-Octadecatrienoic (linolenic)	TR	1·2	6·2	1·2	67·4	20·4	1·1	2·0	0·6	3·9	3·9	7·6
5,8,11,14-Eicosatetraenoic (arachidonic)	1·7	6·5	18·0	TR	54·0	13·2	1·4	1·6	0·7	0	2·9	6·6
4,8,12,15,19-Docosapentaenoic (clupanodonic)	1·8	6·7	15·7	0·6	58·0	12·3	1·1	1·1	TR	0	2·7	4·9
11-Eicosaenoic	2·1	4·9	11·6	1·3	63·3	9·1	1·2	1·4	0	0	5·1	7·7
Tween 80	0·3	3·2	12·4	1·0	61·6	14·5	2·1	4·9	0	0	7·0	7·0
Mixed yeast fatty acids	0·6	0·6	9·0	0	67·2	16·8	2·4	3·4	0	0	5·8	5·8

Cells were grown aerobically on 5% glucose, 1% yeast extract *Saccharomyces* salts media supplemented with the fatty acids indicated at a concentration of 100 μg/ml of medium. Cells were harvested at 42 hours and the fatty acid composition analysed as described in Methods. Only trace levels of $C_{8:0}$ and $C_{14:1}$ were detected and so are omitted from the table. The 1% yeast extract medium contained traces of palmitoleic and oleic acids. 9-10-Hydroxystearic, ricinoleic, linoleic, linolenic and arachidonic acids added to the media contained significant traces of $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$ fatty acids, and 9-10-hydroxystearic and ricinoleic acids also contained traces of linolenic acid; these impurities were incorporated into the cells together with the test fatty acid. The sample of ricinoleic acid supplied in the medium contained three unknown fatty acids which ran between methyl linolenate and acetoxyricinoleic methyl ester; these were incorporated into the cells and comprised 9-0% of the total fatty acids. The Tween 80 had the following composition; saturated fatty acids 13%; unsaturated fatty acids 87% (palmitoleic 13%, oleic 71%, myristoleic 3%). The yeast fatty acids were as described in Table V. TR represents <0·4%. The results are those of typical experiments.

TABLE VIII. Fatty acid composition of cells of strain KD115 grown in 5% glycerol media supplemented with different fatty acids

Fatty acid supplement	Saturated						$C_{16:1}$	Unsaturated			Added fatty acid	Total unsaturated and hydroxy fatty acids
	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
9-Hexadecenoic (palmitoleic)	TR	4·9	3·7	0	57·8	13·9	19·2	0·5	0	0	19·2	19·7
9-Octadecenoic (oleic)	TR	1·1	4·0	0	58·4	12·0	1·1	23·4	0	0	23·4	24·5
6-Octadecenoic (petroselenic)	0·6	1·7	4·2	5·9	41·6	25·2	4·2	TR	0	0	16·7	20·9
9- and 10-Hydroxyoctadecanoic (hydroxystearic)	TR	2·0	2·0	0·9	26·7	6·6	0·8	2·2	0	0	58·5	61·5
12-OH-9-octadecenoic (ricinoleic)	TR	2·7	9·1	3·6	45·6	9·5	1·0	3·7	3·2	3·4	13·4	24·7
9,12-Octadecadienoic (linoleic)	1·0	1·2	1·6	2·6	54·2	25·3	1·0	3·2	9·4	0·5	9·4	14·1
9,12,15-Octadecatrienoic (linolenic)	1·4	1·0	2·1	0·2	63·3	5·7	2·4	0·2	2·7	21·0	21·0	26·3
5,8,11,14-Eicosatetraenoic (arachidonic)	1·5	2·9	3·7	1·5	52·6	12·5	2·2	2·9	2·9	0	17·1	25·3
4,8,12,15,19-Docosopentaenoic (clupanodonic)	TR	2·2	8·6	TR	56·9	15·0	0	0·6	0	0	16·7	17·3
11-Eicosaenoic Tween 80	5·6	6·3	6·1	2·7	55·2	4·7	3·6	15·8	0	0	19·4	19·4
Mixed yeast fatty acids	TR	TR	TR	0	65·6	10·9	16·0	7·2	0	0	23·2	23·2

Cells were grown on 5% glycerol, 1% yeast extract, *Saccharomyces* salts medium supplemented with the fatty acids indicated at a concentration of 100 μg/ml of medium. Cultures were harvested at 90 hours and the fatty acids analysed as described in Methods. Other details are described in the legend to Table VI. The sample of ricinoleic acid contained three unknown fatty acids which ran between methyl linoleic and acetoxyricinoleic methyl ester. These were incorporated into the cells and comprised 4·8% of the total fatty acids. The results are those of typical experiments.

acids are inactive. A mixture of the 9- and 10-hydroxystearic acids support growth to a lesser extent that is supported by the unsaturated fatty acids. The fatty acid requirement of the mutant can thus be satisfied by a wide range of unsaturated or hydroxy fatty acids similar to those which Bloch and coworkers have shown to fulfil the fatty acid requirement for anaerobic growth.[7, 8, 20, 36, 37] The fatty acids that support growth on glucose also support growth on glycerol, but in all cases growth is approximately four times greater on the fermentable substrate. This indicates that a specific loss of mitochondrial function occurs upon depletion of each of the growth supporting fatty acids.

The fatty acid compositions of mutant cells grown in glucose or glycerol media in the presence of growth-limiting supplements of the effective fatty acids are given in Tables VII and VIII. With each of the unsaturated fatty acids tested growth ceases on glycerol media when approximately 20% of the total fatty acids are UFA, indicating that mitochondrial function is specifically lost. In contrast, cells continue to grow on glucose media until the UFA is depleted to approximately 5% of total fatty acids. 9- and 10-hydroxystearic acids are less effective in supporting growth, which ceases on glycerol medium when approximately 60% of the cellular fatty acids are hydroxystearic acids, and ceases on glucose medium when 5·7% of the fatty acids are hydroxystearic acids (plus 4·5% UFA from impurities present in the medium). Thus, although 9- and 10-hydroxystearic acids are less effective in supporting growth, the mitochondria are again specifically affected by depletion of these fatty acids.

The Reversibility of the Effects of UFA-depletion on the Energy Metabolism of the Cell

In order to study the detailed effects of UFA-depletion on cell metabolism it is important to establish whether these effects are reversible and that the cells do not die as a consequence of UFA-depletion. The viability of UFA-depleted cells of strain KD115 was, therefore, studied. Cells continue growing on glucose until their UFA-content is only 5–7% of the total fatty acids; cells depleted to this extremely low level of UFA show a progressive loss in viability and approximately 50% are dead after 4–6 hours in stationary phase. Indeed, all cells containing fatty acids with less than 10% UFA show a progressive loss in viability, indicating that irreversible changes are occurring in cellular membranes. Cells stop growing on the non-fermentable substrates ethanol and glycerol, and on the slowly fermentable sugar galactose when their UFA-content is approximately 17–25% of the total fatty acids, indicating that this level of UFA-depletion affects mitochondrial functions; these cells remain fully viable for up to 12 hours in stationary phase. Accordingly, cells grown on ethanol or galactose were chosen to investigate the reversibility of the effects of UFA-depletion energy metabolism.

Figure 4 shows the growth curve of cells of strain KD115 on a medium containing 0·2% glucose, 2% ethanol and 40 μg/ml of UFA. Both glucose and added UFA are exhausted when growth reaches 0·25 mg dry weight/ml; beyond this point growth is supported by the oxidation of ethanol, and the UFA-content of the cells starts to diminish. Cells cease growing at approximately 22 hours when their UFA-content is 21% of total fatty acids. At 26 hours excess UFA-supplements (600 μg/ml) are added, and the UFA is rapidly incorporated into the cells. Growth recommences 5–7 hours after the addition of the UFA when the cellular fatty acids contain 39% UFA. At 12 hours after the addition of the UFA the cells are growing rapidly and their fatty acids contain 71% UFA, which is the level normally found in cells grown with excess UFA supplements. This experiment

clearly demonstrates that the ability of cells to grow on a non-fermentable substrate is critically dependent on the UFA-content of the cells, and that the lesion in energy-metabolism produced by UFA-depletion is readily reversible.
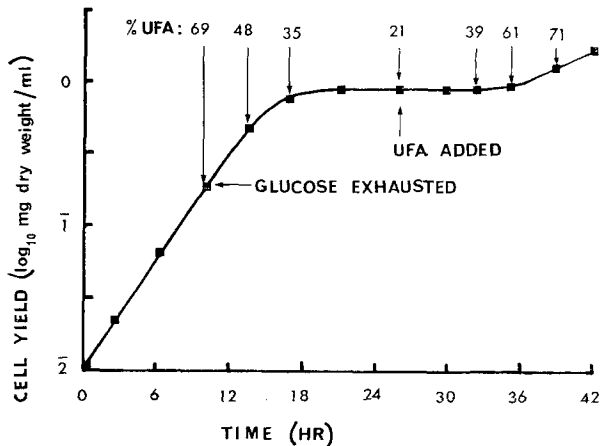

Figure 4. The recovery of cells of strain KD115 from UFA-depletion. Cells of strain KD115 were grown to stationary phase on *Saccharomyces* medium containing 0·2% glucose, 2% ethanol and 40 μg/ml UFA. Cells were harvested at intervals and their UFA-content were determined as described in Methods; the times of harvesting and the percentage of UFA in the cellular fatty acids are indicated by the arrows.

The Effects of UFA-depletion on the Respiratory Activity and Cytochrome Content of Whole Cells of Strain KD115

Since UFA-depletion appears to affect the provision of energy by mitochondrial oxidation, the respiratory activities of UFA-depleted cells were measured. In these experiments the cells were grown to stationary phase in a medium containing low supplements of UFA (50 μg/ml). The extent of growth of the cells and hence the degree of UFA-depletion was varied by progressively increasing the concentration of glucose in the medium from 0 to 1%. Figure 5 shows the respiratory activities and fatty acid compositions of the UFA-depleted cells. As the glucose in the medium is increased from 0 to 0·5% the respiratory activity of the cells remains constant, but the UFA-content of the cellular fatty acids drops from 72 to 11%. At higher concentrations of glucose (0·5 to 1·0%) the respiratory activity of the cell drops by about 40% as the UFA-content of the fatty acids is depleted from 11 to 6%. Further depletion of the unsaturated fatty acids is not possible as the cells grow no further even when the medium contains 5% glucose.

These results appear to suggest that extensive UFA-depletion inhibits the synthesis of respiratory enzymes, but it is also possible that the diminution of respiratory activity and loss of cytochromes at concentrations of glucose greater than 0·5% is due to the catabolite repression of cytochrome synthesis.[38] This was investigated by comparing cells grown on glucose concentrations of up to 5% in the presence of excess and limiting

supplements of UFA. The spectra, respiratory activities and cell yields of cells of KD115 grown on ethanol (2%) and glucose (0·5, 1·0 and 5·0%) in the presence of high (4 mg/ml)
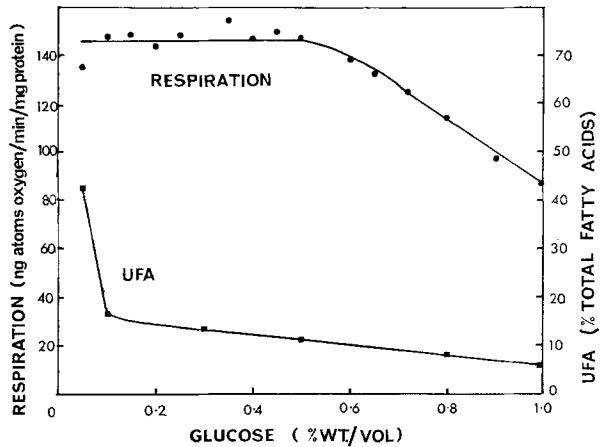

Figure 5. The effect of UFA-content and glucose concentration on the respiratory activity of mutant cells. Cells of KD115 were grown to stationary phase on 1% yeast extract, *Saccharomyces* salts medium containing UFA (50 μg/ml) and the concentrations of glucose indicated. Cells were harvested, and their fatty acid compositions and respiratory activities determined as described in Methods.

and low (50 μg/ml) supplements of UFA are shown in Fig. 6. The cells grown on ethanol and 0·5% glucose with either high or low supplements of UFA have normal cytochromes spectra and high respiratory activities, indicating that the effects of catabolite repression are minimal. The cells grown on 5% glucose with either high or low supplements of UFA have a low content of cytochrome *a*, a new broad absorption band is apparent at 580–590 nm, and their respiratory activities are greatly reduced, indicating a high degree of catabolite repression. In contrast, the spectra and respiratory activities of cells grown on media containing 1% glucose are affected by the level of UFA-supplementation; the cells grown on high UFA have a depressed spectrum and high respiratory activity, but those grown with low UFA have a partially repressed spectrum and a decreased respiratory activity. However, the cells grow much more extensively in the presence of excess UFA, and they may have consequently undergone a greater degree of catabolite derepression. Thus it is not possible to decide from these experiments whether the decrease in respiratory activity and cytochrome *a* content of cells containing less than 11% UFA in their fatty acids is caused by UFA-depletion or by catabolite repression.

Electron Microscopy

Figure 7 shows electron micrographs of whole cells of strain KD115 containing high (72%) and low (19%) levels of UFA. Both types of cells contain typical mitochondria (M) with well developed cristae (CR), and no obvious differences in morphology are apparent.

Discussion

The present study demonstrates that the fatty acid composition of the mutant KD115 can be extensively manipulated under both aerobic and anaerobic conditions by growing the organism in the presence of defined fatty acid supplements. The UFA content of cells can be decreased from approximately 80% to as little as 5% of the total fatty acids
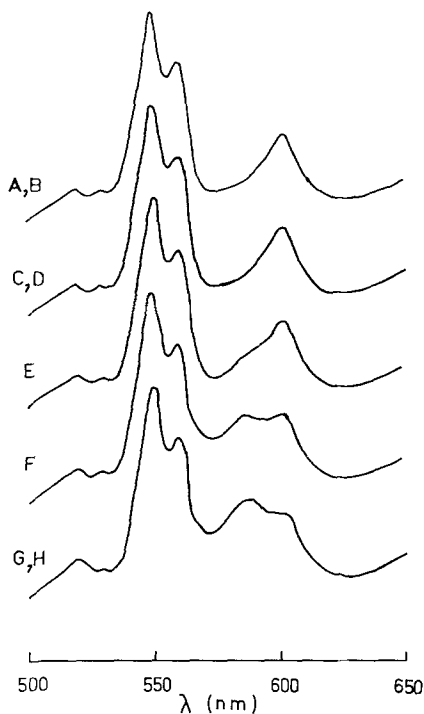
Figure 6. Reduced cytochrome spectra of UFA-depleted cells of strain KD115. Cells were grown to the stationary phase of growth in a medium containing 1% yeast extract, *Saccharomyces* salts and the UFA supplements and energy sources indicated. Cells were harvested, and their growth yields, respiratory activities and reduced cytochrome spectra were determined as described in Methods. In the spectral determinations cells were suspended at a concentration of 20 mg dry weight/ml.
A. 2% ethanol, UFA (4 mg/ml); cell yield 6·3 mg dry weight/ml, respiratory activity 170 ng atoms oxygen/min/mg dry weight.
B. 2% ethanol, UFA (50 µg/ml); cell yield 0·65 mg dry weight/ml respiratory activity 160 ng atoms oxygen/min/mg dry weight.
C. 0·5% glucose, UFA (4 mg/ml); cell yield 5·2 mg dry weight/ml, respiratory activity 155 ng atoms oxygen/min/mg dry weight.
D. 0·5 glucose, UFA (50 µg/ml); cell yield 1·3 mg dry weight/ml, respiratory activity 150 ng atoms/min/mg dry weight.
E. 1% glucose, UFA (4 mg/ml); cell yield 6.4 mg dry weight/ml, respiratory activity 160 ng atoms/min/mg dry weight.
F. 1% glucose, UFA (50 µg/ml); cell yield 1·6 mg dry weight/ml, respiratory activity 85 ng atoms/min/mg dry weight.
G. 5% glucose, UFA (4 mg/ml); cell yield 6·4 mg dry weight/ml, respiratory activity 40 ng atoms/min/mg dry weight.
H. 5% glucose, UFA (50 µg/ml) 1 cell yield 1·6 mg dry weight/ml; respiratory activity 45 ng atoms/min/mg dry weight.

before the cells stop growing aerobically on glucose media. The UFA decrease is accompanied by a rise in the level of the short chain saturated fatty acids ($C_{8:0}$–$C_{14:0}$). A similar increase in these acids in response to the depletion of UFA has been reported to
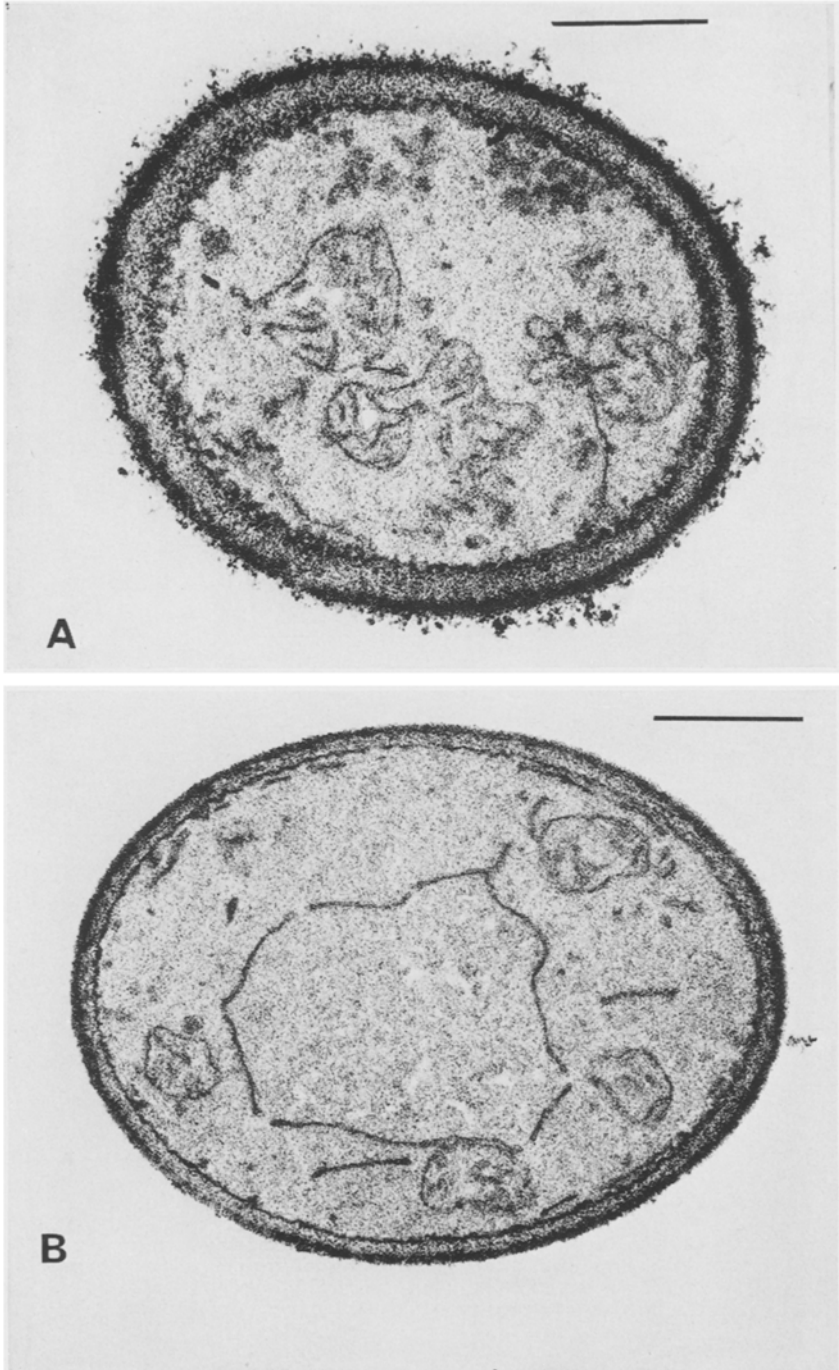
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Figure 7. Electron micrographs of KD115 cells containing high and low levels of UFA. Cells were grown to stationary phase on *Saccharomyces* medium containing 2% galactose and either 50 μg UFA/ml or 4 mg UFA/ml. A. Cell containing 72% UFA ($\times$25,000). B. Cell containing 19% UFA ($\times$26,000). The bars represent 1 μ.

occur in anaerobically grown cells by Bloch and others.[7, 8, 10, 20, 36, 37] Meyer and Bloch[36] found that the short chain saturated fatty acids were exclusively esterified in the 2 position of the phospholipids which is the position normally occupied by the UFA in aerobic cells. The replacement of the UFA by the short chain acid may reflect an attempt by the organism to maintain certain physical properties of the hydrocarbon chains in the phospholipid molecules. Salem[40] has calculated the strength of bonding between adjacent hydrocarbon chains due to London–Van der Waal forces and has found that the inter-action between short chain saturated acids ($C_{10:0}$, $C_{12:0}$, $C_{14:0}$) is of the same order as that between the monounsaturated palmitoleic and oleic acids. However, short chain fatty acids are only able to replace unsaturated fatty acids in some functions as this study shows that the cells have an absolute requirement for some low level of UFA in order to grow aerobically.

The final yields of cells grown on limiting UFA are four to five times greater on glucose than on glycerol media. Furthermore, the mutant continues to grow on glucose until its UFA-content is as low as 5% of the total fatty acids, whereas it stops growing on non-fermentable substrates when its UFA-content is approximately 20% of total fatty acids. These results suggest that UFA-depletion specifically affects mitochondrial functions which are required for growth on non-fermentable substrates. This conclusion is further supported by a detailed examination of the specific growth yields of UFA-depleted and UFA-supplemented cells on different substrates. Bauchop and Elsden[38] showed that in a rich medium microorganisms synthesize approximately 10·5 g dry weight of cell material per mole of ATP produced by the catabolism of an energy source. Assuming that glucose catabolism by *S. cerevisiae* yields 28 ATP/glucose aerobically and 2 ATP/glucose anaerobi-cally,[35] the theoretical yield of cells would be 1·63 and 0·12 mg dry weight/mg glucose respectively. Kormancikova *et al.*[35] have measured specific growth yields of *S. cerevisiae* and obtain experimental cell yields of 0·7–1·6 mg dry weight/mg glucose aerobically (depend-ent on glucose concentration) and 0·12 mg dry weight/mg glucose anaerobically. Our value of 1·1 mg dry weight/mg glucose for UFA-supplemented cells is in close agreement with these results, and suggests an efficient utilization of the substrate by both fermenta-tion and oxidative metabolism. However, the yield of 0·12 mg dry weight/mg glucose obtained in UFA-depleted cells represents a purely fermentative energy metabolism and indicates that the mitochondria in these cells are non-functional. This is confirmed by the observation that in the presence of chloramphenicol, which prevents the formation of functional mitochondria by inhibiting mitochondrial protein synthesis,[35] the yield of cells is also 0·12 mg dry weight/mg glucose. Assuming that in *Saccharomyces* the oxidation of ethanol yields 12 ATP and that of glycerol yields 14 ATP molecules, the theoretical growth yields would be 2·74 mg dry weight/mg ethanol and 1·59 mg dry weight/mg glycerol. Our experimentally determined values of 1·6 mg dry weight/mg ethanol and 1·05 mg/dry weight/mg glycerol for UFA-supplemented cells are somewhat lower than the theoretical values, but are very similar to the growth efficiencies obtained by Kormancikova *et al.*[35] for non-fermentable substrates. In contrast, UFA-depleted cells failed to grow on non-fermentable substrates indicating a mitochondrial lesion.

The fatty acids that support the aerobic growth of the mutant are similar to those required for the anaerobic growth of prototrophic strains of *S. cerevisiae*.[7, 8, 20, 36, 37] A low order of specificity is shown, and growth is supported by either polyunsaturated fatty acids, or monounsaturated fatty acids containing a cis-unsaturated linkage at the 6-, 9- or

11-position. However, elaidic acid, which has a trans-unsaturated band at the 9-position, and monounsaturated fatty acids with cis-unsaturated bonds at the 13- and 15-positions are ineffective in supporting growth. A specific loss of mitochondrial function is observed upon depletion of each of the fatty acids that supports growth, and with each fatty acid cells grow four to five times more extensively on glucose medium than on glycerol medium.

9- and 10-hydroxystearic acids also support growth, but are much less effective than the unsaturated fatty acids. Light *et al.*[20] have reported that 9-hydroxystearic, 10-hydroxystearic and ricinoleic acids support the anaerobic growth of *Saccharomyces*, and that the hydroxyl groups are not removed but are converted to the acetyl derivatives. Accordingly, the significant amounts of oleic acid found in cells grown on 9- and 10-hydroxystearic acids and of linoleic acid found in cells grown on ricinoleic acid are probably impurities present in the supplements that are preferentially incorporated during growth.

The effects of UFA-depletion on total cell lipid composition are surprisingly small. Cells in which 26% of the fatty acids are unsaturated contain approximately 70% of the neutral lipids and phospholipids present in fully-supplemented cells, but slightly more sterols. The amounts of the various classes of phospholipids are virtually unaltered in the UFA-depleted cells with the marked exception of the phosphatidyl inositides which are reduced to less than 50% of their normal levels. The possible significance of phosphatidyl inositide depletion is discussed in the accompanying paper.[14]

The effects of UFA-depletion on the fatty acid composition of the mutant cells is readily reversed by adding excess UFA to non-growing cells; the reversal takes up to 12 hours to be completed and presumably represents the time taken to release the fatty acid components in the various classes of complex lipids. It remains to be established whether the change in the fatty acid composition of the complex lipids represents transacylation or *de novo* synthesis of the lipids. Accompanying the increase in their UFA-content, the cells regain the ability to grow on non-fermentable substrate, indicating that the mitochondrial lesion is not fatal to the cells and is readily reversed by changes in lipid composition.

The cells contain mitochondria that are not greatly changed in appearance. Moreover, the respiratory activities and cytochromes spectra of cells whose fatty acids contain as little as 12% UFA are normal, indicating that the oxidative functions of the mitochondria are unaffected. This suggests that the lesion affecting UFA-depleted mitochondria may be the loss of the ability to couple phosphorylation to respiration, and the accompanying paper which describes the properties, of UFA-depleted mitochondria confirms this possibility.

There is a significant decrease in the respiratory activity and cytochrome *a* content of cells grown on concentrations of glucose greater than 0·5% and containing less than 12% UFA. However, the cells still have sufficient respiratory activity to enable growth on non-fermentable substrates if the respiration were coupled to phosphorylation. It is not possible to decide from the present experiments whether the decrease in respiratory activity at very low UFA-contents is caused by UFA-depletion or is a consequence of catabolite repression caused by high concentrations of glucose in the media.[38] It should be possible to decide between these two alternatives by growing UFA-depleted cells in continuous culture under conditions of catabolite derepression.

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