# Fatty acid synthesis in mitochondria from *Saccharomyces cerevisiae*

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The ability of purified mitochondria isolated from *S. cerevisiae* to synthesize fatty acids and especially very long chain fatty acids (VLCFA) has been investigated. The VLCFA synthesis requires malonyl-CoA as the $C_2$ unit donor and NADPH as the reducing agent. Moreover the yeast mitochondrial elongase is able to accept either exogenous long chain fatty acyl-CoAs as substrates or elongate endogenous substrates. In the latter case, ATP is required for full activity. Besides this important VLCFA formation, the mitochondria from *S. cerevisiae* were also able to synthesize $C_{16}$ and $C_{18}$.

Very long-chain fatty acid; Fatty acid synthesis; Mitochondria; Lipid synthesis; (*Saccharomyces cerevisiae*)

## 1. INTRODUCTION

Fatty acid synthesis takes place in various cell compartments of the eukaryotic cells. In mammalians, the cytosol is chiefly responsible for the fatty acid de novo synthesis, whereas the further steps concerning either the desaturation or the elongation of the fatty acids are located in the endomembrane system, endoplasmic reticulum and Golgi apparatus. However, mitochondria are also able to form fatty acids by de novo synthesis [1–3] or by elongation of endogenous or exogenous precursors [3–7]. It is classically admitted that the elongation of acyl-CoAs taking place in mitochondria requires acetyl-CoA as the preferred $C_2$ unit donor, and both NADH and NADPH as the

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*Abbreviations:* VLCFA, very long chain fatty acids ($CH_3$-$(CH_2)_n$-COOH with $n \geq 18$); DTT, dithiothreitol; BSA, bovine serum albumin; radio-GLC, radio gas liquid chromatography

reductants, while microsomal elongases are specific for malonyl-CoA and NADPH (review [7]). Consequently it is conceivable that this mitochondrial activity, the physiological significance of which remains to be elucidated, represents a simple reversal of the β-oxidation [8,9]. The validity of such an assumption, however, is based upon very few examples. Moreover, a careful analysis of the available literature shows that the claimed requirement for acetyl-CoA and NADH is in itself an over-simplification of numerous data. In rat liver mitochondria, Boone and Wakil [4] showed the existence of a de novo synthesis from acetyl-CoA and malonyl-CoA and an elongation of long chain acyl-CoAs by acetyl-CoA or malonyl-CoA. The same was true in the case of mitochondria from rat brain [2]. Aeberhard and Menkes [2] and Boone and Wakil [4] have suggested the possibility of an involvement of malonyl-CoA decarboxylase in converting malonyl-CoA into acetyl-CoA, which could then serve as the immediate precursor of the active two-carbon unit used in the elongation of acyl-CoAs. More recently, Bourre et al. [6] and Paturneau-Jouas et al. [3] have shown that mitochondria from brain

elongate exogenously added long chain acyl-CoAs in the presence of acetyl-CoA, whereas in the absence of long chain acyl-CoAs, malonyl-CoA and not acetyl-CoA is the $C_2$ unit donor. These results have been confirmed by Murad and Kishimoto [7] in mitochondria from rat brain. In this paper we demonstrate for the first time, that yeast mitochondria are able to elongate acyl-CoAs or endogenous precursors, but that in no case is acetyl-CoA the $C_2$ unit donor.

## 2. MATERIALS AND METHODS

### 2.1. Substrates and reagents

[1-$^{14}$C]Acetyl-CoA (46.6 Ci/mol) was obtained from NEN. [2-$^{14}$C]Malonyl-CoA (55 Ci/mol) and [1-$^{14}$C]stearoyl-CoA (56 Ci/mol) were purchased from Amersham. All other chemical products were obtained from Sigma.

### 2.2. Preparation of mitochondria

Cells of the diploid wild strain Saccharomyces cerevisiae (yeast foam) were grown aerobically at 28°C in a complete medium: 1% yeast extract, 0.1% potassium phosphate, 0.12% ammonium sulphate (pH 4.5) supplemented with 2% lactate as carbon source. The cells were harvested in logarithmic phase. Mitochondria were isolated as described in [10]. This mitochondrial fractionation procedure was checked by electron microscopy and sucrose density gradient centrifugation [11].

### 2.3. Fatty acid synthesis measurements

Routinely 20 to 50 $\mu$l of the preparation of mitochondria (20 to 50 $\mu$g of mitochondrial proteins) were incubated at 30°C in the presence of [2-$^{14}$C]malonyl-CoA (17 $\mu$M), NADPH (0.5 mM), NADH (0.5 mM), ATP (1 mM), $MgCl_2$ (1 mM), DTT (2 mM) and 0.65 M mannitol, 10 mM Tris

Table 1

Incorporation of radioactive precursors into fatty acids by mitochondria from S. cerevisiae

| Conditions | nmol/mg per h | Conditions | nmol/mg per h | Relative activity |
|---|---|---|---|---|
| [1-$^{14}$C]Acetyl-CoA + ATP | 0.04 ± 0.03 (3) | [2-$^{14}$C]malonyl-CoA + ATP | 3.63 ± 2.38 (5) | 1 |
| [1-$^{14}$C]Acetyl-CoA | 0.02 ± 0.01 (3) | [2-$^{14}$C]malonyl-CoA | 0.70 ± 0.5 (5) | 0.22 ± 0.06 (5) |
| [1-$^{14}$C]Acetyl-CoA + malonyl-CoA | 0.01 ± 0.003 (3) | [2-$^{14}$C]malonyl-CoA + acetyl-CoA | 3.24 ± 1.64 (5) | 0.85 ± 0.07 (5) |
| [1-$^{14}$C]Acetyl-CoA + malonyl-CoA + ATP | 0.01 ± 0.001 (3) | [2-$^{14}$C]malonyl-CoA + acetyl-CoA + ATP | 4.0 ± 2.5 (5) | 1.14 ± 0.08 (5) |
| [1-$^{14}$C]Acetyl-CoA + $C_{18}$-CoA | 0.01 ± 0.004 (3) | [2-$^{14}$C]malonyl-CoA + $C_{18}$-CoA | 3.98 ± 2.59 (4) | 1 |
| [1-$^{14}$C]Acetyl-CoA + $C_{20}$-CoA | 0.01 ± 0.003 (3) | [2-$^{14}$C]malonyl-CoA + $C_{20}$-CoA | 5.58 ± 4.3 (4) | 1.41 ± 0.25 (5) |
| [1-$^{14}$C]Acetyl-CoA + $C_{18}$-CoA + ATP | 0.02 ± 0.001 (3) | [2-$^{14}$C]malonyl-CoA + $C_{18}$-CoA + ATP | 5.0 ± 1.42 (4) | 1.28 ± 0.22 (5) |
| [1-$^{14}$C]Acetyl-CoA + $C_{20}$-CoA + ATP | 0.02 ± 0.004 (3) | [2-$^{14}$C]malonyl-CoA + $C_{20}$-CoA + ATP | 8.93 ± 7.09 (3) | 1.92 ± 0.19 (4) |

Incubations were carried out for 1 h in the presence of protein quantities ranging from 29 to 70 $\mu$g. Acetyl-CoA, when present, was at a final concentration of 0.15 mg/ml. Other conditions are as described in section 2. Numbers in brackets represent the number of experiments

maleate, 0.36 mM EGTA buffer (pH 6.7) in a total volume of 0.1 ml. When acyl-CoAs were used as substrates, ATP was generally omitted and the concentration of each acyl-CoA was 9 $\mu$M. [1-$^{14}$C]Acetyl-CoA, when used, was 17 $\mu$M. The reaction was stopped by the addition of 0.1 ml of 20% KOH in $H_2O$/methanol (9:1). Fatty acids were extracted and the label was determined as described [12]. The distribution of the label among the fatty acids was determined by radio-GLC under the conditions described in [13].

## 2.4. Protein determination

Proteins were estimated by the method of Bradford [14], using BSA (1 mg/ml) as the standard.

## 3. RESULTS

### 3.1. Incorporation of labeled precursors into fatty acids by mitochondria from S. cerevisiae

The ability of yeast mitochondria to synthesize fatty acids from various substrates was investigated and the results are reported in table 1. [1-$^{14}$C]Acetyl-CoA, used as the only substrate, was not incorporated into fatty acids and the addition of ATP did not increase this incorporation. The addition of malonyl-CoA or malonyl-CoA plus ATP had no effects. Moreover, when acyl-CoAs

Table 2

Cofactor requirements for fatty acid synthesis

| Conditions | [2-$^{14}$C]-Malonyl-CoA | [2-$^{14}$C]-Malonyl-CoA + $C_{18}$-CoA | [2-$^{14}$C]-Malonyl-CoA + $C_{20}$-CoA |
|---|---|---|---|
| Complete | 100 | 100 | 100 |
| − ATP | 26 | 91 | 57 |
| − NADPH | 28 | 26 | 15 |
| − NADH | 94 | 121 | 80 |
| − NADPH, − NADH | 24 | 29 | 23 |
| − MgCl$_2$ | 52 | 62 | 55 |
| $t = 0$ | 2 | 0.3 | 0.2 |

The complete incubation system contained [2-$^{14}$C]-malonyl-CoA (17 $\mu$M), NADPH (0.5 mM), NADH (0.5 mM), ATP (1 mM), MgCl$_2$ (1 mM), DTT (2 mM). Incubations were carried out for 1 h with 45 $\mu$g mitochondrial proteins. $t = 0$ means incubation times were zero

($C_{18}$-CoA or $C_{20}$-CoA) were used (even in the presence of ATP), no significant incorporation of [1-$^{14}$C]acetyl-CoA into fatty acids was observed. All these results demonstrate that, under our conditions, acetyl-CoA is neither a primer nor a $C_2$ unit donor for the fatty acid synthesis in mitochondria from S. cerevisiae.

In contrast with the results observed for [1-$^{14}$C]acetyl-CoA, [2-$^{14}$C]malonyl-CoA was incorporated into fatty acids. The basal activity ([2-$^{14}$C]malonyl-CoA used as the only substrate) was stimulated ∼4-fold when ATP or acetyl-CoA was added to the reaction mixture.

In the absence of ATP, the addition of either $C_{18}$-CoA or $C_{20}$-CoA increased respectively the basal activity about 6–7-fold. Upon addition of ATP (1 mM), the elongation of $C_{18}$-CoA was slightly stimulated (∼25%), while elongation of $C_{20}$-CoA was more greatly increased (40%).

### 3.2. Cofactor requirements

The cofactor requirements for fatty acid synthesis from labeled malonyl-CoA are shown in table 2. As may be observed, the presence of ATP (1 mM) increased, on the one hand, the incorporation of [2-$^{14}$C]malonyl-CoA in the absence of primers and, on the other, stimulated the elongation of acyl-CoAs when $C_{20}$-CoA is the primer used. Using $C_{18}$-CoA as the primer, the presence of ATP in the incubation mixture did not significantly increase the incorporation of [2-$^{14}$C]malonyl-CoA into fatty acids. The addition of Mg$^{2+}$ (1 mM) increased each activity about 2-fold. The omission of NADPH from the incubation mixture resulted in a drastic decrease of the fatty acid synthesis: whatever the precursor used, practically 80% of the activity was lost. In the absence of NADH, activities were not severely disturbed. These results indicated that NADPH, and not NADH, is the preferred reducing reagent for the fatty acid synthesis in mitochondria from S. cerevisiae.

### 3.3. Label distribution

The label distribution among the various fatty acids synthesized after malonyl-CoA incorporation was further investigated and results are reported in table 3. In the absence of ATP and long chain acyl-CoAs, the main label incorporation was observed in stearic acid and palmitic acid,

Table 3

Label distribution into fatty acids

| Conditions | % fatty acids | | | | | | | | | % VLCFA |
|---|---|---|---|---|---|---|---|---|---|---|
| | $C_{10}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{22}$ | $C_{24}$ | $C_{26}$ | |
| [2-^{14}C]Malonyl-CoA | 1.8 (0.03) | 4.8 (0.07) | 8.0 (0.12) | 27.9 (0.41) | 40.2 (0.59) | 11.8 (0.17) | 5.5 (0.08) | – | – | 17.3 (0.25) |
| [2-^{14}C]Malonyl-CoA + ATP | – | – | – | 7.3 (0.43) | 20 (1.18) | 18.5 (1.09) | 12.5 (0.74) | 11.4 (0.67) | 30.4 (1.79) | 72.8 (4.29) |
| [2-^{14}C]Malonyl-CoA + $C_{18}$-CoA | – | – | – | 5.6 (0.44) | 11.2 (0.88) | 19.2 (1.51) | 13.6 (1.06) | 17.7 (1.39) | 32.6 (2.56) | 83.1 (6.53) |
| [2-^{14}C]Malonyl-CoA + $C_{20}$-CoA | – | – | 0.9 (0.11) | 2.6 (0.31) | 2.7 (0.32) | 1.7 (0.2 ) | 21.0 (2.52) | 31 (3.72) | 40.1 (4.81) | 93.8 (11.3) |

The distribution of label has been determined as described in section 2. The numbers in brackets are the nmol fatty acids synthesized/h per mg mitochondrial proteins. Incubations were carried out for 1 h with 30 μg proteins. Other conditions are described in section 2

representing respectively 27.9 and 40.2% of the total radioactivity. Under these conditions, less than 0.3 nmol VLCFAs were synthesized/h per mg mitochondrial proteins.

The addition of ATP-$Mg^{2+}$ stimulated the synthesis of VLCFA about 16-fold, which amounted to about 75% of the total label, while the synthesis of $C_{16}$ remained unchanged and that of $C_{18}$ increased 2-fold.

In the presence of $C_{18}$-CoA (without ATP), the synthesis of palmitic and stearic acids was only slightly modified and the synthesis of VLCFA was greatly stimulated (25-fold). The replacement of $C_{18}$-CoA by $C_{20}$-CoA led to an almost complete disappearance of the label found in the $C_{20}$ fatty acid, which is in good agreement with an elongation of the exogenous $C_{20}$-CoA.

Moreover, whatever the conditions used for the VLCFA synthesis, the longer the acyl chain, the more the VLCFAs were labeled. In any case, $C_{26}$, with 30 to 40% of the total incorporation was the most labeled fatty acid.

## 4. DISCUSSION

The fatty acid biosynthesis by mitochondria isolated from various types of cells has been reported repeatedly [1–7,15]. With mitochondria from animals, as the source of enzyme, a unique feature of this synthesis is that it accepts acetyl-CoA as the $C_2$ unit donor, though malonyl-CoA may also be used [4]. Both NADH and NADPH are used as reductants [2–4].

In mitochondria isolated from higher plants fatty acid synthesis has also been described [16]; such synthesis requires NADPH and any one of the following labelled precursors: [2-^{14}C]malonyl-CoA, [1-^{14}C]acetyl-CoA, [1-^{14}C]acetate, [2-^{14}C]-malonate was incorporated into fatty acids. Using mitochondria from Euglena gracilis SM-ZK (a permanent chloroplast-lacking mutant), Inui et al. [17] have shown a mitochondrial malonyl-CoA-independent fatty acid synthesis requiring NADH and $NADP^+$ as cofactors [17,18].

The yeast mitochondria differ markedly from the other mitochondria so far reported: malonyl-CoA is the only $C_2$ unit donor, and NADPH is required for full activity, whereas the omission of NADH does not greatly change the synthesis.

In the absence of ATP, [2-^{14}C]malonyl-CoA is chiefly incorporated into $C_{16}$ and $C_{18}$ acids; this is compatible with a de novo synthesis in yeast mitochondria, as already hypothesized for the mitochondria from brain [2,3]. This possibility is supported by the experiments which showed that, in the presence of acetyl-CoA, there was a greater incorporation of [2-^{14}C]malonyl-CoA than in its absence. But this hypothesis was definitely ruled out by the reverse experiments with [1-^{14}C]acetyl-CoA and unlabeled malonyl-CoA which resulted

in no fatty acid labeling at all. Moreover, since [1-$^{14}$C]acetyl-CoA is not incorporated into fatty acids, mitochondrial fatty acid synthesis is not a mere reversal of the $\beta$-oxidation.

It has already been observed, for example in mitochondria from brain, that ATP is required for full fatty acid synthesizing activity [2]. Its role in yeast mitochondrial fatty acid synthesis is not clear. With mitochondria from *S. cerevisiae* as a source of enzyme, the ATP addition does not result in a mere increase of the overall synthesis, but in a marked modification of the label distribution among the fatty acids, with more than 50% of the label contained in $C_{24}$ and $C_{26}$ acids. One hypothesis which will be further analysed is that ATP allows a second elongation system to be operative, in addition to that already functioning in its absence.

The first system (non-ATP requiring system) synthesizing long chain fatty acids (almost $C_{16}$ and $C_{18}$) would be different from the cytosolic fatty acid synthetase which incorporated [1-$^{14}$C]acetyl-CoA into palmitic and stearic acids.

The second system would require ATP to synthesize VLCFA. These molecules were also synthesized in the absence of ATP but in the presence of acyl-CoAs as primers. It was noticed that whatever the conditions used, when VLCFAs are synthesized, the first system continues to synthesize long chain fatty acids, irrespective of the incorporation of [2-$^{14}$C]malonyl-CoA related to the presence or absence of long chain acyl-CoAs in the incubation mixture.

Our results demonstrate, for the first time, the existence of VLCFA synthesis in yeast mitochondria. This synthesis required malonyl-CoA, instead of acetyl-CoA. It has been assumed that the fatty acid synthesis observed already in plant mitochondria could be due to a contamination of these organelles by plastids [19,20]. However, the use of a permanent chloroplast-lacking mutant of *E. gracilis* [17,18] has demonstrated that plant mitochondria have the enzymatic machinery required for autonomous fatty acid synthesis. This work shows in addition that the yeast mitochondria are also able to synthesize fatty acids, especially VLCFA. Thus, as is the case for chloroplast and

fatty acid synthesis, the endoplasmic reticulum can no longer be considered the unique site of VLCFA formation in plants.

## REFERENCES

[1] Quagliariello, E., Landriscina, C. and Coratelli, P. (1968) Biochim. Biophys. Acta 164, 12–24.

[2] Aeberhard, E. and Menkes, J.H. (1968) J. Biol. Chem. 243, 3834–3840.

[3] Paturneau-Jouas, M., Baumann, N. and Bourre, J.M. (1976) Biochimie 58, 341–349.

[4] Boone, S.C. and Wakil, S.J. (1970) Biochemistry 9, 1470–1479.

[5] Paturneau-Jouas, M., Baumann, N. and Bourre, J.M. (1976) Biochem. Biophys. Res. Commun. 71, 1326–1334.

[6] Bourre, J.M., Paturneau-Jouas, M., Daudu, O.L. and Baumann, N. (1977) Eur. J. Biochem. 72, 41–47.

[7] Murad, S. and Kishimoto, Y. (1978) Arch. Biochem. Biophys. 185, 300–306.

[8] Colli, W., Minkle, P.C. and Pulmann, M.E. (1969) J. Biol. Chem. 214, 6432–6443.

[9] Englard, S. and Siegel, L. (1969) Methods Enzymol. 13, 99–106.

[10] Guerin, B., Labbe, P. and Somlo, M. (1979) Methods Enzymol. 55, 149–159.

[11] Velours, J., Guerin, B. and Duvert, M. (1977) Arch. Biochem. Biophys. 182, 295–304.

[12] Agrawal, V.P., Lessire, R. and Stumpf, P.K. (1984) Arch. Biochem. Biophys. 230, 580–589.

[13] Lessire, R., Juguelin, H., Moreau, P. and Cassagne, C. (1985) Arch. Biochem. Biophys. 239, 260–269.

[14] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.

[15] Gonzales-Buitrago, J.M., Megias, A., Municio, A.M. and Perez-Albarsanz, M.A. (1979) Comp. Biochem. Physiol. 64B, 1–10.

[16] Mazliak, P., Oursel, A., Ben Abdelkader, A. and Grosbois, M. (1972) Eur. J. Biochem. 28, 399–411.

[17] Inui, H., Miyatake, K., Nakano, Y. and Kitaoka, S. (1984) Eur. J. Biochem. 142, 121–126.

[18] Inui, H., Miyatake, K., Nakano, Y. and Kitaoka, S. (1985) Arch. Biochem. Biophys. 237, 423–429.

[19] Stumpf, P.K. (1980) in: The Biochemistry of Plants (Stumpf, P.K. ed.) vol.4, pp.177–204, Academic Press, New York.

[20] Douce, R. (1985) in: Mitochondria in Higher Plants, pp.243–250, Academic Press, London.