The flux of fatty acids toward \( \beta \)-oxidation was analyzed in \textit{Saccharomyces cerevisiae} by monitoring polyhydroxyalkanoate synthesis in the peroxisome from the polymerization, by a bacterial polyhydroxyalkanoate synthase, of the \( \beta \)-oxidation intermediates 3-hydroxyacyl-CoAs. Synthesis of polyhydroxyalkanoate was dependent on the \( \beta \)-oxidation enzymes acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase multifunctional protein, which are involved in generating 3-hydroxyacyl-CoAs, and on the peroxin \textit{PEX5}, which is involved in the import of proteins into the peroxisome. In wild type cells grown in media containing fatty acids, the polyhydroxyalkanoate monomer composition was largely influenced by the nature of the external fatty acid, such that even-chain monomers are generated from oleic acid and odd-chain monomers are generated from heptadecenoic acid. In contrast, polyhydroxyalkanoate containing predominantly 3-hydroxyoctanoate, 3-hydroxydecanoate, and 3-hydroxydodecanoate was synthesized in a mutant deficient in the peroxisomal 3-ketothiolase (\textit{fox3}) growing either on oleic acid or heptadecenoic acid as well as in wild type and \textit{fox3} mutants grown on glucose or raffinose, indicating that 3-hydroxyacyl-CoAs used for polyhydroxyalkanoate synthesis were generated from the degradation of intracellular short- and medium-chain fatty acids by the \( \beta \)-oxidation cycle. Inhibition of fatty acid biosynthesis with cerulenin blocked the synthesis of polyhydroxyalkanoate from intracellular fatty acids but still enabled the use of extracellular fatty acids for polymer production. Mutants affected in the synthesis of lipoic acid showed normal polyhydroxyalkanoate synthesis capacity. Together, these results uncovered the existence of a substantial futile cycle whereby short- and medium-chain intermediates of the cytoplasmatic fatty acid biosynthetic pathway are directed toward the peroxisomal \( \beta \)-oxidation pathway.

Polyhydroxyalkanoate (PHA) is a family of polyesters composed primarily of \( R \)-3-hydroxyalkanoic acids (1–4). PHA is synthesized as intracellular inclusions by a wide variety of bacteria, including Gram-positive and Gram-negative species, and is used as a carbon and electron sink. PHAs have properties of thermoplastics and elastomers, and are regarded as an attractive source of renewable and biodegradable polyesters (1–4).

Pseudomonads, such as \textit{Pseudomonas oleovorans} and \textit{Pseudomonas aeruginosa}, typically synthesize PHA containing 3-hydroxyacids ranging from 6 to 14 carbons in length (2, 3). \textit{P. oleovorans} can synthesize PHA when cells are grown on alkanoic acids as the carbon source (2, 3). The nature of the PHA produced is related to the substrate used for growth and is typically composed of monomers that are 2n (where \( n \geq 0 \)) carbons shorter than the substrate. For example, growth of \textit{P. oleovorans} on dodecanoate generates PHA containing 3-hydroxydecanoic acid, 3-hydroxydecanoic acid, 3-hydroxystearic acid, and 3-hydroxyhexanoic acid (5). In these cells, PHA is synthesized by the PHA synthase from the polymerization of 3-hydroxyacyl-CoA intermediates generated by the \( \beta \)-oxidation of alkanoic acids. PHAs have also been produced in a number of recombinant eukaryotes, including insect cells (6), yeast (7–9), and plants (10–13).

Beyond its value as a biopolymer for commercial applications, PHA can be used as a valuable indicator of the flux of carbon through various metabolic pathways providing substrates for its synthesis. To date, this has been mostly exploited in transgenic \textit{Arabidopsis thaliana} expressing the PHA synthase from \textit{P. aeruginosa} in the peroxisome (14–18). In these plants, PHA containing saturated and unsaturated 3-hydroxyalkanoic acids ranging from 6 to 16 carbons is synthesized during germination using intermediates of the \( \beta \)-oxidation of fatty acid derived from the degradation of triacylglycerides (14). Manipulation of the plant fatty acid composition, either through the use of mutants in fatty acid desaturases or by feeding external fatty acids, demonstrated that the PHA monomer composition adequately reflects both the nature and quantity of fatty acid directed toward peroxisomal \( \beta \)-oxidation (15). From these initial studies, peroxisomal PHA was subsequently used to reveal the existence of a futile cycle of fatty acids in transgenic plants expressing a medium-chain fatty acyl-carrier protein thioesterase in leaves or developing seeds (15, 16) as well as in developing \textit{A. thaliana} embryos deficient in the synthesis of triacylglycerides (16). The PHA monomer composition has also been used to analyze the pathway of degradation of unsaturated fatty acids in plants (17).

Recently, the synthesis of PHA from intermediates of \( \beta \)-oxidation has been demonstrated in \textit{Saccharomyces cerevisiae} expressing the PHA synthase from \textit{P. aeruginosa} in the peroxisome (7). Growth of these recombinant \textit{S. cerevisiae} in media containing oleic acid or heptadecenoic acid resulted in the accumulation of PHA containing either even-chain monomers from 6 to 14 carbons in length, or odd-chain monomers from 5 to 13 carbons, respectively (7). In an effort aimed at understanding the factors influencing the flux of fatty acids toward \( \beta \)-oxidation, we have examined PHA synthesis in mutants of...
S. cerevisiae affected in various enzymes involved in fatty acid metabolism. In this study, we show that the synthesis of PHA in the fox3Δ0 mutant deficient in the peroxisomal 3-oxoacyl-CoA thiolase reveals the presence of a substantial futile cycle of intermediates of the cytoplasmic fatty acid biosynthetic pathway toward peroxisomal β-oxidation in yeast growing in media containing various carbon sources.

**EXPERIMENTAL PROCEDURES**

**Strains and Culture Conditions**—Plasmids were maintained and propagated in *Escherichia coli* DH5α according to Sambrook et al. (19). Wild type *S. cerevisiae* strain BY4742 (matα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) as well as the mutants fox3Δ0 (YGL205w::kanMX4, fox2Δ0 (YKR096c::kanMX4), and pes5Δ0 (YDR244W::kanMX4), all derived from the strain BY4742, were obtained from EUROSCARF (www.uni-frankfurt.de/bi15/mikro/euroscarf/index.html). *S. cerevisiae* harboring the PHA synthesis gene from *P. aeruginosa* was maintained in leucine-deficient media (0.07% yeast nitrogen base without amino acids (Difco, Detroit, MI), 0.5% ammonium sulfate, 2% glucose, and 0.69 grams/liter leucine drop-out supplement (Clontech) with or without 200 μg/ml gentamycin). For experiments analyzing PHA synthesis in cells growing on media containing fatty acids, a stationary phase culture was harvested by centrifugation, and cells were washed once in water and resuspended at a 1:10 dilution in fresh media containing 1% (w/v) glucose, 2% Pluronic F-127 (w/v) (Sigma), and 0.1% (w/v) fatty acids. Cells were grown for an additional 3–4 days before harvesting for PHA analysis. A similar protocol was used for experiments growing cells in media containing carbohydrates, except that the cells were diluted 1:40 in leucine-deficient media supplemented with either 2% (w/v) glucose or 3% (w/v) raffinose. In some experiments, cerulenic (Sigma) was added at a final concentration of 25–50 μM, and fatty acids (oleic acid, palmitic acid, and tridecanoic acid) were added at concentrations between 0.005–0.01%.

**DNA Constructs**—The PHAC1 synthase from *P. aeruginosa* was modified for peroxisomal targeting by the addition, at the carboxyl end of the protein, of the last 34 amino acids of the *Brassica napus* isocitrate lyase, as described previously (18). The plasmid Yiplac128-PHA containing the modified PHAC1 gene was under the control of the *S. cerevisiae* catalase A (CTA1) promoter and terminator region, as described previously (7). To construct the plasmid CTA-PHA, the cassette containing the CTA1 promoter-PHAC1 synthase-CTA1 terminator was excised from Yiplac128-PHA and cloned into the replicative shuttle plasmid Yiplac111 (20). In the plasmid GPD-PHA, the PHAC1 gene is under the control of the constitutive glycerol-3-phosphate dehydrogenase promoter (21). This plasmid was constructed by first excising the PHA synthase along with the CTA1 terminator from the CTA-PHA plasmid with EcoRI and blunt-ended with Klenow DNA polymerase. The fragment was then subcloned into the vector pRS415-GPD (21) at the SmI site. Plasmids were transferred into the various *S. cerevisiae* strains by the lithium acetate procedure (22), and transformants were recovered on media without leucine.

**Analysis of Fatty Acids and PHA**—Analysis of fatty acids and PHA was performed essentially as described previously (7, 14).

**RESULTS**

*Synthesis of PHA in Wild Type and β-Oxidation Mutants of S. cerevisiae*—Synthesis of PHA in cells expressing a peroxisomal PHA synthase from the plasmid CTA-PHA and grown on medium containing oleic acid was examined in wild type BY4742 cells as well as in three mutants having a deletion in the genes encoding the enzymes of the core peroxisomal β-oxidation cycle. These mutants were deficient either in the acyl-CoA oxidase (FOX1), the multifunctional protein (FOX2) possessing the 2-enzyme-CoA hydratase II and R-3-hydroxacyl-CoA dehydrogenase activities, or the 3-ketothiolase (FOX3). Also used was a mutant deficient in the peroxin PEX5, which is involved in the import, into the peroxisomes, of proteins having a type 1 peroxisomal targeting signal, including FOX1, FOX2, and the modified PHA synthase. Wild type cells grown for 3 days on oleic acid produce ~0.06% PHA (weight/dry weight) containing even-chain monomers from 6 to 14 carbons that are generated by the degradation of oleic acid via the peroxisomal β-oxidation cycle, namely the monomers H14:1, H14:0, H12:0, H10:0, H8:0 and H6:0 (3-hydroxy acid monomers are identified with the prefix H, followed by the number of carbons and the number of unsaturated bonds) (Fig. 1A). The presence of both 3-hydroxytetradecenoic acid (H14:1) and 3-hydroxytetradecanoic acid (H14:0) agrees with the generation of the corresponding acyl-CoA by the β-oxidation of fatty acids having a cis-unsaturated bond at an odd-numbered carbon (23). No PHA was detected in the fox1Δ0, fox2Δ0, and pes5Δ0 mutants expressing the PHA synthase (data not shown). These results are in agreement with the fact that the 3-hydroxyacyl-CoAs used by the PHA synthase are generated by the peroxisomal β-oxidation pathway. Expression of the PHA synthase in the fox3Δ0 mutant resulted in the accumulation of ~0.006% (weight/dry weight) of a PHA that contained only the monomers H8:0, H10:0, and H12:0 (Fig. 1B). These results were unexpected, because the only 3-hydroxyacyl-CoA that could be generated in the fox3Δ0 mutant from the degradation of the external oleic acid was 3-hydroxyoctadecenoyl-CoA, which is itself not a substrate for the PHA synthase because this enzyme in yeast accepts only 3-hydroxyacyl-CoAs between 5 and 14 carbons in length (7).

*PHA Synthesized in the fox3Δ0 Mutant Is Derived from Intracellular Short- and Medium-chain Fatty Acids*—The presence of PHA in the fox3Δ0 mutant raised the question as to the source of the carbon that can generate peroxisomal 3-hydroxyacyl-CoAs from 8 to 12 carbons in length. Analysis of the synthetic media used to grow cells combined with experiments wherein external fatty acids were added to the growth media
revealed that the level of contaminating fatty acids present in the growth media was at least one order of magnitude below the minimum level of fatty acids required to influence PHA synthesized fox3Δ0 cells (data not shown). Thus, contaminating fatty acid cannot explain the PHA synthesized in fox3Δ0 cells. The presence of an alternative 3-ketothiolase that could functionally complement the FOX3 enzyme and generate shorter-chain 3-hydroxyacyl-CoAs from oleic acid was examined. Wild type and fox3Δ0 cells expressing the peroxisomal PHA synthase were grown in media containing 0.1% oleic acid and 0.1% lauric acid. Wild type cells grown on both oleic and lauric acid produced 5-fold more PHA compared with cells grown only on oleic acid (Fig. 1C). The monomer composition of the PHA synthesized from the co-feeding was shifted toward shorter saturated monomers, with the H14:1 and H14 monomers being undetectable. This shift in monomer composition is on the bottom line (weight of PHA/cell dry weight) × 10^−4. Values are mean ± S.D. (n = 3). The PHA monomer composition is on the y-axis.

**Synthesis of PHA in fox3Δ0 Cells Grown on Carbohydrates—**In contrast to wild type, the fox3Δ0 mutant cannot utilize external fatty acids as a carbon source to grow because the β-oxidation pathway is blocked. Thus, fox3Δ0 cells grown in media containing 0.1% glucose and 0.1% oleic acid are essentially starved for carbon once the glucose is consumed. To assess whether the synthesis of PHA in fox3Δ0 cells was dependent on these particular growth conditions, the synthesis of PHA was monitored in cells grown on carbohydrates as the principal carbon source. Because the CTA1 promoter used in the CTA-PHA vector is activated by fatty acids but repressed by glucose (7, 25, 26), a new vector named GPD-PHA was constructed by putting the PHA synthase under the control of the constitutive glyceral-3-phosphate dehydrogenase promoter. Western analysis of wild type cells transformed with the plasmid GPD-PHA revealed the expression of the PHA synthase to approximately the same level in cells grown in media containing either glucose, raffinose, or oleic acid (data not shown). Synthesis of PHA in wild type cells transformed with GPD-PHA and grown in media containing Δ10cis-heptadecenoic acid was essentially the same as that reported for the plasmid CTA-PHA, with the PHA being composed mainly of the odd-chain monomers H5:0, H7:0, H9:0, and H11:0 (Fig. 2C). In contrast, fox3Δ0 cells transformed with GPD-PHA and grown in media containing Δ10cis-heptadecenoic acid produced a PHA containing only the H8, H10, and H12 monomers (Fig. 2D).

Wild type and fox3Δ0 cells transformed with the plasmid GPD-PHA and grown for 4 days in media containing 2% glucose synthesize ~0.01% (weight/dry weight) PHA (Fig. 3, A and B). The monomer composition for these two strains was similar and included the monomers H6:0, H8:0, H10:0, and H12:0. In contrast to glucose, raffinose does not repress the genes involved in β-oxidation (27, 28). Growth of cells for 4 days in media containing raffinose resulted in an ~4-fold increase in PHA synthesized in fox3Δ0 and wild type cells compared with growth in media containing glucose, although the monomer composition remained similar for all treatments (Fig. 3, C and D). No PHA was detected in fox1Δ0, fox2Δ0, or pex5Δ0 cells transformed with the GPD-PHA plasmid and grown on raffinose. Together, these results demonstrate that, in cells growing on carbohydrates as the main carbon source, synthesis of PHA

**Fig. 2.** Synthesis of PHA in wild type and fox3Δ0 cells grown in media containing Δ10cis-heptadecenoic acid (17:1). Cells were transformed with either the CTA-PHA or GPD-PHA constructs and grown in media containing 0.1% glucose and 0.1% Δ10cis-heptadecenoic acid (17:1). The amount of PHA synthesized is indicated on the bottom line (weight of PHA/cell dry weight) × 10^−4. Values are mean ± S.D. (n = 3). The PHA monomer composition is on the y-axis.
Fig. 3. Synthesis of PHA in wild type and fox3Δ0 cells grown on carbohydrates. Cells were transformed with the GPD-PHA construct and grown in media containing either 2% glucose (GLC) or 3% raffinose (RAF). The amount of PHA synthesized is indicated on the bottom line (weight of PHA/cell dry weight) \( \times 10^{-1} \). Values are mean ± S.D. (n ≥ 3). The PHA monomer composition is on the y-axis.

from intracellular fatty acids of 6, 8, 10, and 12 carbons occurs and requires a functional peroxisome and \( \beta \)-oxidation cycle.

The Contribution of Pathways of Lipoic Acid and Fatty Acid Biosynthesis to Peroxisomal PHA—The synthesis of PHA in fox3Δ0 cells grown in media containing raffinose was monitored over 5 days (Fig. 4). The quantity of PHA per unit dry weight of cells remained relatively constant during the initial lag phase, decreased during the exponential growth phase, and increased during the stationary phase. These data indicate that PHA synthesis in fox3Δ0 still occurs when cells have ceased to divide and are in nutrient-limiting conditions. Although some PHA could also be synthesized during the exponential phase, the rate of PHA accumulation is lower than the rate of increase of the cell mass.

The impact of inhibiting fatty acid biosynthesis on PHA production was tested in the fox3Δ1 strain. Cerulenin, an inhibitor of the fatty acid synthase (FAS) (29), was added at 48 h in the exponential growth phase of a fox3Δ0 culture growing on raffinose. The addition of cerulenin limited growth of cells as observed by the lower density of the culture (Fig. 4). Furthermore, the addition of cerulenin quickly abolished the increase in PHA that was observed after 48 h in cultures growing without cerulenin.

The effect of cerulenin on the monomer composition of synthesized PHA was also examined in fox3Δ0 cells that were grown in media containing 3% raffinose, 0.01% oleic acid, 0.005% palmitic acid, and 0.01% tridecanoic acid as well as with and without cerulenin. Oleic and palmitic acids were added as a source of fatty acids necessary for the synthesis of membrane lipids and the maintenance of cell growth after the addition of cerulenin. As shown in Fig. 5A, growth of fox3Δ0 cells in this complex medium containing a low amount of tridecanoic acid and without cerulenin lead to the synthesis of PHA containing the H13 monomer as well as the H6, H8, H10, and H12 monomers. The addition of cerulenin resulted in a 9-fold increase in the proportion of the H13 monomer as well as a 4-fold reduction in the amount of PHA relative to cultures grown without cerulenin (Fig. 5B). These results are in agreement with the hypothesis that even-chain PHA monomers are derived from intermediates of the cerulenin-sensitive FAS, whereas the odd-chain H13 monomer is derived from the degradation of the external tridecanoic acid via the cerulenin-insensitive \( \beta \)-oxidation cycle. Furthermore, the overall decrease in PHA produced in cells grown in media with cerulenin and 0.01% tridecanoate would indicate that, in the absence of inhibitor, the contribution of the internal fatty acids derived from FAS to PHA synthesis was larger than the contribution of the external fatty acid.

Lipoic acid is an eight-carbon fatty acid modified with sulfur insertion and synthesized via the FASII system present in the mitochondria. The contribution of the lipoic acid pathway to the generation of intracellular substrates for peroxisomal PHA synthesis was examined in the oar1 and cem1 mutants deficient in the mitochondrial FASII enzymes essential for lipoic acid synthesis (30, 31). PHA synthesis in wild type and the oar1 and cem1 mutants transformed with the GPD-PHA construct and grown in media containing raffinose was similar in both quantity and monomer composition (data not shown), indicating that the mitochondrial FASII does not contribute to the flux of short- and medium-chain fatty acids toward the peroxisome \( \beta \)-oxidation pathway.

DISCUSSION

S. cerevisiae cells expressing a PHA synthase from P. aeruginosa modified for peroxisomal targeting synthesize PHA derived from the R-3-hydroxyacyl-CoA intermediates of the \( \beta \)-oxidation pathway (7). This is supported by the observation that deletion mutants in the acyl-CoA oxidase and the multifunctional protein, the first two enzymes of the \( \beta \)-oxidation pathway that are required for synthesis of R-3-hydroxyacyl-CoA, do not produce PHA. The requirement of a functional peroxisome is also indicated by the absence of PHA in the pex5Δ0 mutant deficient in the import, into the peroxisome, of proteins having a type 1 peroxisome targeting signal, including proteins such as FOX1, FOX2, and the PHA synthase. In cells grown in media containing external fatty acid as the main source of carbon, PHA monomer composition is directly influenced by the nature of the external fatty acids. Thus, feeding with oleic acid yields a PHA containing even-chain monomers exclusively (Fig. 1A), whereas feeding with \( \Delta 10cis \)-heptadecenoic acid yields a PHA containing primarily odd-chain monomers (Fig. 2, A and C). These results indicate that, under these conditions, the main 3-hydroxyacyl-CoAs going into PHA are primarily derived from the \( \beta \)-oxidation of external fatty acids.

The synthesis of PHA containing the H8:0, H10:0, and H12:0 monomers in the fox3Δ0 mutant grown on oleic acid (Fig. 1B) was surprising, because the only 3-hydroxyacyl-CoA that could be generated from oleic acid entering a \( \beta \)-oxidation cycle that is truncated at the 3-ketothiolase step would be 3-hydroxyoctadecenoyl-CoA, which is itself not a substrate for the PHA synthase. In contrast to wild type cells, PHA synthesized in fox3Δ0 cells grown in media containing \( \Delta 10cis \)-heptadecenoic acid does not contain any odd-chain monomers but rather the monomers H8:0, H10:0, and H12:0, indicating that the substrates for PHA synthesis in the fox3Δ0 mutants are not derived from the external fatty acids (Fig. 2, B and D). However, in fox3Δ0 cells grown in the presence of both oleic acid and lauric acid, PHA
containing the H12:0 monomer exclusively is generated, a result expected if the main substrate entering the truncated β-oxidation cycle is derived from the external lauric acid (Fig. 1D). This demonstrates that, similar to wild type cells, fox3Δ0 mutant cells are still competent to acquire fatty acids from the external media, target them to the peroxisome, and initiate their degradation through β-oxidation. Together, these results indicated that the 8-, 10-, and 12-carbon monomers found in PHA synthesized in fox3Δ0 cells grown on oleic and Δ10cis-heptadecenoic acids are derived from intracellular fatty acids (or fatty acyl-esters) of 8, 10, and 12 carbons.

The monomer composition of the PHA synthesized in fox3Δ0 cells grown on media containing either fatty acids, glucose, or raffinose changed little with the exception of the presence of the additional H6:0 monomer only in cells grown on carbohydrates in addition to the H8:0, H10:0, and H12:0 monomer (Figs. 2 and 3). Furthermore, the monomer composition and quantity of PHA synthesized in wild type and fox3Δ0 cells grown on carbohydrates were remarkably similar. The absence of both the H14:1 and H14:0 in PHA from wild type cells grown on carbohydrates is important, because H14:1 is an indicator of the degradation of palmitoleic and oleic acids, whereas H14 is an indicator of the degradation of palmitic and stearic acids. These four fatty acids account for >98% of the fatty acid found in cells at either the exponential or stationary growth phase, whereas fatty acids of ≤14 carbons account for <2% (data not shown, and Ref. 32). This indicates that, in wild type cells as well as fox3Δ0 grown on carbohydrates, the fatty acids that enter the peroxisomal PHA are not derived from the turnover of all fatty acids found in membranes but rather are enriched in short- and medium-chain fatty acids. The observation that the addition of cerulenin, a powerful inhibitor of FAS, inhibits the accumulation of PHA in cells grown on raffinose as well as increases the proportion of odd-chain monomers at the expense of even-chain monomers in cells grown in media containing tridecanoic acid indicates that the premature release of short- and medium-chain fatty acids from the FAS complex contributes to the overall carbon flux toward peroxisomal β-oxidation. Furthermore, the demonstration that a mutation in the pathway of mitochondrial lipoic acid biosynthesis did not affect PHA synthesis from glucose indicates that it is the cytoplasmic FAS complex that mainly contributes to this carbon flux.

The amount of PHA synthesized in fox3Δ0 cells was influenced by the carbon source used for growth with the maximal amount obtained with raffinose (0.055% weight/dry weight), whereas glucose and oleic acid gave lower amounts (0.012 and 0.006% weight/dry weight, respectively). The increase of PHA in raffinose compared with glucose is likely to be due, at least in part, to the fact that in contrast to glucose, raffinose does not repress transcription of the genes of the β-oxidation cycle (27, 28). Although the β-oxidation genes should be fully activated in media containing oleic acid, the amount of PHA synthesized was lowest. One explanation for this could be that the 3-hydroxyoctadecenoyl-CoA produced in fox3Δ0 cells grown on oleic acid may inhibit the flux of intracellular short- and medium-chain fatty acids through the truncated β-oxidation cycle and thus decrease carbon flux to PHA.

It is striking that the amount of PHA synthesized in wild type cells was inhibited by cerulenin (25 μM, final concentration) was added (diamond) or omitted (square) from the media.
type cells grown on raffinose (0.05% weight/dry weight; Fig. 3C), wherein the PHA is largely derived from intracellular short- and medium-chain fatty acids, is similar to or slightly lower than the amount of PHA synthesized in wild type cells grown either on oleic acid (0.06% weight/dry weight; Fig. 1A) or Δ10 cis-heptadecenoic acid (0.14–0.18% weight/dry weight; Fig. 2, A and C), wherein the bulk of β-oxidation intermediates going into PHA are derived from the external fatty acids and where carbon flux through β-oxidation cycle is expected to be maximal because cells depend on it for growth. Although PHA synthesized in yeast peroxisomes may not be directly proportional to the carbon flux to the β-oxidation cycle under all growth conditions, these results nevertheless indicate that the flux of short- and medium-chain fatty acids derived from FAS toward peroxisomal β-oxidation is substantial and clearly larger than the turnover of long-chain fatty acids.

The molecular mechanism responsible for this futile cycling of short- and medium-chain fatty acids in yeast is unclear at present. Fatty acids lower than 14 carbons are present only in trace amounts in yeast lipids, with the bulk of fatty acids being C16:0, C16:1, C18:0, and C18:1 (32). In yeast, fatty acids are released from the FAS complex as acyl-CoAs by the malonyl/palmitoyl transferase domain of the β-subunit of FAS, which plays the dual role by loading malonyl substrate moieties in chain elongation and translocating the palmitoyl moieties to a CoA acceptor in chain termination (33). Experiments with purified FAS from yeast indicated that the primary products released from the FAS complex in vitro are long-chain acyl-CoAs (34, 35). However, it has also been demonstrated that the presence of the acyl-CoA binding protein ACBP1 in an in vitro reaction mixture results in an enhanced release of medium-chain acyl-CoAs from the FAS complex, indicating that the presence of other proteins or co-factors can greatly influence the distribution of fatty acyl-CoAs released from FAS (35). The presence of a futile cycle of short- and medium-chain fatty acids in yeast may thus indicate that the release of short- and medium-chain acyl-CoAs from FAS in vivo is considerably larger than would be suspected from either in vitro experiments or fatty acid composition of lipids. Further experiments are required to determine whether this is specific to the FAS of yeast or whether it also applies to the FAS of plants and animals.

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