Annu. Rev. Nutr. 2001. 21:97–119

THE GENETICS OF FATTY ACID METABOLISM IN *SACCHAROMYCES CEREVISIAE*

Pamela J Trotter

The Division of Nutritional Sciences and the Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, Texas 78712; e-mail: trotter@mail.utexas.edu

Key Words β-oxidation, peroxisome, biosynthesis, regulation, yeast

■ **Abstract** Long-chain fatty acids are a vital metabolic energy source and are building blocks of membrane lipids. The yeast *Saccharomyces cerevisiae* is a valuable model system for elucidation of gene-function relationships in such eukaryotic processes as fatty acid metabolism. Yeast degrades fatty acids only in the peroxisome, and recently, genes encoding core and auxiliary enzymes of peroxisomal β-oxidation have been identified. Mechanisms involved in fatty acid induction of gene expression have been described, and novel fatty acid–responsive genes have been discovered via yeast genome analysis. In addition, a number of genes essential for synthesis of the variety of fatty acids in yeast have been cloned. Advances in understanding such processes in *S. cerevisiae* will provide helpful insights to functional genomics approaches in more complex organisms.

CONTENTS

INTRODUCTION: Yeast As a Model System and Tools of the Trade

The budding yeast *Saccharomyces cerevisiae* has long been the model eukaryote of choice for the investigation of basic, yet complex, cellular processes, including the cell cycle, protein targeting and secretion, transcription, and metabolism. Yeast combines an array of characteristics desirable in a model system, including a well-described biochemistry and ease of genetic experimentation. Additionally, the official disclosure of the genome sequence of *S. cerevisiae* in April 1996 (41) provided the first complete sequence of a eukaryotic genome. As observed by Bussey in 1997 (13), with this achievement came "a complete parts list for a eukaryotic cell" and an opportunity to work toward "a comprehensive understanding of how a yeast cell works."

Many genes encoding enzymes in *S. cerevisiae*, such as those encoding core enzymes of fatty acid oxidation and biosynthesis (see below), were characterized using classical genetic approaches. These advances were made possible because *S. cerevisiae* is easily cultivated in a chemically defined medium. Thus, the investigator can precisely control the experimental conditions, both physically and biochemically. Generation, isolation, and analysis of mutant strains can be performed with comparable ease because yeast can be grown in either the haploid or diploid form. Identification of genes [i.e. your favorite gene 1 (*YFG1*)] by complementation of a strain with a mutation in the *yfg1* gene is facilitated by a large collection of plasmids and genomic libraries available for use in *S. cerevisiae* (88). In addition, yeast is especially adept at gene conversion or recombination, so replacement of the genomic copy of *YFG1* with a null allele (*yfg1*Δ) or an allele carrying a specific mutation (*yfg1-1*) is relatively straightforward (90).

The completion of the entire *S. cerevisiae* genome sequence has added several new dimensions to the utility of this organism as a model eukaryote. The yeast genome encodes about one gene every 2 kb, in contrast to the estimated 30 kb or more of DNA required per gene in the human (41). In addition, only 4% of yeast genes contain introns. This relative simplicity of the yeast genome has facilitated the systematic analysis of the sequence data, allowing the identification of probable protein-encoding reading frames. Having this information has led to a new approach to the investigation of gene-function relationships, or functional genomics (13, 53), using "reverse genetics" (86). In the classical genetic approach, one begins with a function, inferred from a mutant phenotype, for which the gene involved is determined. In reverse genetics, one begins with the sequence of a gene and must, based on sequence analysis, devise experiments to determine its biological function. For example, homology information may place a protein into a functional group such as transcription factors, but the challenge lies in determining which genes it might regulate. Databases such as the *Saccharomyces* genome database (15), the Yeast proteome database (23), and the MIPS Yeast Genome

Database (78) provide comprehensive compilations of available functional information on open reading frames in yeast. At the writing of this review, the Yeast Proteome Database listed 6145 proteins: 3717 characterized by biochemical or genetic methods, 587 with predicted function based on homology, and 1841 without a known function (23). It is expected that nearly every gene in *S. cerevisiae* will have a homolog in other eukaryotic organisms (11, 41). Thus, progress toward the elucidation of how the 6000 proteins encoded in the genome work to make a functional yeast cell should prove valuable for functional genomics investigations in more complex organisms (86).

Many areas of eukaryotic cell biology have been positively impacted by the utilization of the *S. cerevisiae* as a model organism. As in other eukaryotes, fatty acids serve important roles as a source of metabolic energy and as building blocks of membrane lipids. An increase in the past decade in the number of investigative tools available has led to a greater understanding of gene-function associations and of the regulation of gene expression in fatty acid metabolism in *S. cerevisiae*. This review discusses recent progress in yeast on the peroxisomal β-oxidation of fatty acids and its regulation, and the biosynthesis and remodeling of endogenous fatty acids.

PEROXISOME BIOGENESIS

Yeast can grow in medium containing fatty acids as the sole carbon source, as the glyoxylate cycle allows for gluconeogenesis. When de novo fatty acid synthesis is blocked, cells can be rescued by addition of fatty acids to the growth medium. In contrast to mammalian cells, in which β-oxidation occurs in both the mitochondria and the peroxisome, yeast lack the enzymes required for mitochondrial β-oxidation (71). Examination of fatty acid oxidation in yeast is thus limited to one organelle and metabolic pathway.

Transfer of *S. cerevisiae* from a glucose-containing medium to a medium containing oleic acid as the sole carbon source is accompanied by a proliferation of peroxisomes and an increase in the activity of β-oxidation enzymes (112). Although peroxisome proliferation had been observed in several other fungi, demonstration that this process occurs in *S. cerevisiae* permitted its investigation using this genetically well-characterized model system. Peroxisome assembly mutants were isolated by screening for an inability to grow on oleic acid, cytosolic localization of peroxisomal matrix enzymes, and absence of the organelles by electron microscopy analysis (34). These initial studies led to an ongoing extensive investigation of the biogenesis of peroxisomes and of the targeting and transport of proteins to the organelle as well as to the identification of more than 20 required proteins, termed peroxins. Targeting of proteins to the peroxisome matrix has been particularly well described. Synthesized by cytosolic ribosomes, these proteins usually contain either a C-terminal (PTS1) or N-terminal (PTS2) signal sequence that is recognized by PTS receptors in the peroxisomal membrane. They are then moved across the membrane to the matrix by a mechanism requiring

a number of proteins. Insertion of peroxisomal membrane proteins, which are also translated in the cytosol, proceeds via a less-well-characterized mechanism. Several recent reviews provide further discussion of peroxisome biogenesis and protein targeting (50, 70, 116). As is discussed below, similar screens for strains unable to grow on fatty acids have led to significant progress in the metabolism of exogenous fatty acids by *S. cerevisiae*.

FATTY ACID TRANSPORT

Uptake and Activation

Before a fatty acid can be catabolized by the yeast cell, it must be transported across the plasma and peroxisomal membranes along with its conversion to a coenzyme A (CoA) derivative. The mechanism of extracellular fatty acid transport across the plasma membranes of cells is a point of debate (1, 47). On one hand, in vitro studies have demonstrated that free fatty acids can very rapidly ($t_{1/2} < 1$ s) traverse synthetic lipid bilayers at physiological pH (47). Others argue that although such a diffusion mechanism may be important at relatively high fatty acid concentrations, at physiological levels saturable, facilitated transport appears to predominate (5). Several membrane proteins have been proposed as possible fatty acid transporters or receptors (1, 27, 55), and acyl-CoA synthetases have been shown to enhance fatty acid uptake into cells (27). In *E. coli*, import of fatty acids requires the coupling of FadL, an outer membrane binding and translocation protein, and acyl-CoA synthetase, located in the inner membrane (27). In addition, a putative eukaryotic transporter, FATP, as well as acyl-CoA synthetase were identified in a screen for cDNAs that, when expressed in cultured murine 3T3-L1 adipocytes, increase fatty acid uptake (92).

Saturable uptake of exogenous fatty acids has also been demonstrated in several yeasts, including *S. cerevisiae* (65, 68, 108). A yeast homolog to the adipocyte FATP was identified by comparison of its predicted amino acid sequence to the open reading frames in the *Saccharomyces* genome database (35). The protein encoded by the yeast *FAT1* gene had 54% overall similarity to FATP and contained an AMP-binding motif common to such proteins as acyl-CoA synthetases. When the fatty acid synthase inhibitor cerulenin is added to the medium, normal yeast can be rescued by supplementation with fatty acids. Cells carrying a disruption of the *FAT1* gene (*fat1*Δ), however, have difficulty growing in the presence of cerulenin even in the presence of fatty acid. Incorporation of fatty acids into lipids was also impaired in the *fat1*Δ cells. Yet, acyl-CoA synthetase activity for 14:0, 16:0, and 18:1 fatty acids in *fat1*Δ cells did not differ from the wild-type *FAT1* cells. Thus, in this initial report, Fat1p was proposed to function as a fatty acid transporter protein, as had been proposed for the murine FATP (35, 92).

Recent observations, however, indicate that the impaired fatty acid uptake observed in *fat1*Δ cells is secondary to a defect in the metabolism of the fatty acid. Subcellular localization experiments suggest that Fat1p is associated with the

endoplasmic reticulum and peroxisomal membranes, rather than the plasma membrane (18). Also, the initial *FAT1* sequence in the database contained an error, and the correction significantly improved the homology to rat very-long-chain acyl-CoA synthetase (VLCS) (18, 117). Significant accumulation of fatty acids with greater than 22 carbons and severely reduced VLCS activity was observed in *fat1*Δ cells (18, 117). Furthermore, heterologous expression of the *FAT1* gene indicates that Fat1p is a VLCS enzyme (18). Fat1p appears to be involved in the maintenance of very-long-chain fatty acid homeostasis, only indirectly affecting utilization of exogenous fatty acids. It is interesting that the murine FATP has also recently been reported to be a VLCS (21).

Five additional genes (*FAA1-4* and *FAT2*) encoding proteins with homologies to acyl-CoA synthetases have been described in *S. cerevisiae* (7, 117). The *FAA1* and *FAA4* genes encode acyl-CoA synthetases (see Figure 1) required for activation of imported exogenous fatty acids (30, 58, 65). Faa1p exhibits a preference for fatty acids with between 12 and 16 carbons (64), and genetic evidence indicates that it is functionally exchangeable with Faa4p or rat liver acyl-CoA synthetase (65). Faa1p and Faa4p account for 99% of the total 14:0 and 16:0 activation activity in *S. cerevisiae*, and when endogenous fatty acid synthesis is blocked, at least one is required for rescue on medium containing exogenous fatty acids (65). Cells carrying disruptions in both genes, *faa1*Δ *faa4*Δ, appear to have normal initial rates of free fatty acid import (65), but bulk accumulation in cell lipids is negligible (18). Thus, it seems that these cells are defective in the activation, but not the transport, of the fatty acids.

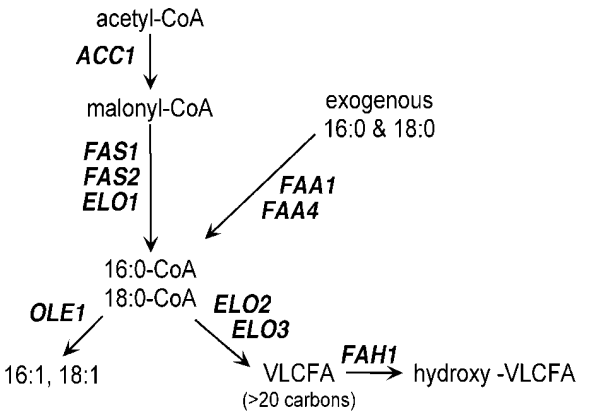

Figure 1 Schematic of fatty acid biosynthesis in *Saccharomyces cerevisiae*. Processes of de novo synthesis, desaturation, elongation, and hydroxylation are shown. The names of genes encoding enzymes that catalyze each step appear in bold italics. Abbreviations: 16:0, palmitic acid; 16:0-CoA, palmitoyl-coenzyme A; 16:1, palmitoleic acid; 18:0, stearic acid; 18:0-CoA, stearoyl-coenzyme A; 18:1, oleic acid; VLCFA, very-long-chain fatty acid. Further details are in the text.

Disruption of the *FAA2* and *FAA3* genes has no affect on the ability of cells to use exogenously supplied fatty acids, indicating that the acyl-CoA synthetases encoded by these genes can access only fatty acids synthesized within the cell (59). Analysis of the fatty acyl specificity of these enzymes indicates that Faa2p can utilize a wide range of fatty acids but that the greatest activity is toward fatty acids with 9–13 carbons. Faa2p has been localized to the matrix side of peroxisomal membranes (52) and accounts for the residual VLCS activity present in cells lacking Fat1p (18). Faa3p has comparatively lower enzyme activity than Faa2p, favors fatty acids with 16–18 carbons, and displays activity toward very-long-chain fatty acids (64). The *FAT2* gene product (Fat2p, previously named Psc60p) is localized in the peroxisomal matrix but is not necessary for growth on oleic acid (7). The cellular roles of Faa3p and Fat2p remain uncertain.

Cytoplasmic Pools

S. cerevisiae expresses at least one acyl-CoA binding protein encoded by the *ACB1* gene (89). Acyl-CoA binding proteins are polypeptides of 86–103 amino acids that bind acyl-CoAs of 14–22 carbons, but not free fatty acids, and are thought to function in acyl-CoA pool formation as well as in regulation of the metabolic and regulatory functions of long-chain fatty acids (66, 67). Disruption of the *ACB1* gene causes cellular acyl-CoA levels to rise 1.5- to 2.5-fold, primarily because of a sevenfold accumulation of newly synthesized steaoryl-CoA, without affecting the overall fatty acid composition of the cell (93). The yeast Acb1p is thought to play a role in the release of newly synthesized stearoyl-CoA from fatty acid synthase for utilization throughout the cell (93). Because disruption of the gene fails to affect cell survival and membrane fatty acid composition, it is predicted that other mechanisms for acyl-CoA trafficking exist (93).

Peroxisomal Import

X-linked adrenoleukodystrophy is a human disease characterized by accumulation of very-long-chain fatty acids, and the affected gene has been identified as a member of the ATP binding cassette transporter family (82, 83). Genes encoding two ATP binding cassette half transporters with significant homology to the human adrenoleukodystrophy protein are present in yeast: *PAT1* (or *PXA2*) and *PAT2* (or *PXA1* or *PAL1*) (10, 51, 52, 98, 105). Disruption of either gene results in decreased growth of the cells on medium containing long-chain fatty acid as the sole carbon source, without any affect on the biogenesis of the peroxisomal organelle (52, 97, 105). Long-chain fatty acid oxidation is impaired in whole cells lacking the proteins, but when cells and organelles are disrupted, oxidation is normal. Thus, these proteins appear to be involved in fatty acid import across the peroxisomal membrane. Moreover, immunoprecipitation studies indicate a physical interaction between the encoded proteins, Pat1p and Pat2p (97), which suggests they are two components of the same complex. Finally, the substrate of the transporter is apparently an acyl-CoA derivative because when peroxisomal acyl-CoA

synthetase activity is removed by disruption of the *FAA2* gene, oxidation of long-chain fatty acids is completely dependent on Pat1p/Pat2p. The mechanism by which the Pat1p/Pat2p transporter functions remains unclear. It has been suggested that it may act on membrane-associated fatty acyl-CoAs to facilitate the flip of the polar CoA group across the membrane (8, 51).

Cells lacking the Pat1p/Pat2p transporter are capable of growth on such medium-chain fatty acids as laurate, 12:0 (52). In addition, the peroxisomal acyl-CoA synthetase Faa2p (see above) is required for oxidation of medium- but not long-chain fatty acids. Thus, it appears that fatty acids of medium length enter the peroxisome before their activation, which explains their independence of the Pat1p/Pat2p long-chain acyl-CoA transporter. Mislocalization of Faa2p to the cytosol causes medium-chain fatty acids to be activated to their CoA derivatives outside the peroxisome, and their oxidation becomes entirely dependent on the presence of Pat1p/Pat2p (52). In summary, to enter β-oxidation, long-chain fatty acyl-CoAs are formed in the cytosol and enter the peroxisome via Pat1p/Pat2p, whereas medium-chain fatty acids first reach the peroxisomal lumen, where they are then activated by Faa2p.

FATTY ACID OXIDATION

Saturated Fatty Acids

The core reactions of peroxisomal β-oxidation in *S. cerevisiae* (see Figure 2) are comparable to nonmitochondrial pathways in other organisms (71). The process begins with oxidation of acyl-CoA to *trans*-Δ^2-enoyl-CoA by acyl-CoA oxidase, encoded by the *POX1* (or *FOX1*) gene (29), producing hydrogen peroxide that is detoxified by peroxisomal catalase, encoded by the *CTA1* gene (22, 101). The *trans*-Δ^2-enoyl-CoA hydratase and NAD^+-dependent D-3-hydroxyacyl-CoA dehydrogenase steps producing 3-ketoacyl-CoA are catalyzed by a bifunctional protein encoded by the *FOX2* gene (54). The NAD^+ required for this reaction is not obtained from the cytosol, as the peroxisomal membrane is impermeable to NAD(H) (110). Rather, NADH is reoxidized by a peroxisomal malate dehydrogenase (Mdh3p) as part of a predicted redox shuttle mechanism across the peroxisomal membrane (51, 110). The final cleavage of the ketoacyl-CoA to yield acetyl-CoA and the shortened acyl-CoA is catalyzed by ketoacyl-CoA thiolase encoded by the *FOX3* (or *POT1*) gene (32, 57). Disruption of *POX1*, *FOX2*, or *FOX3* results in an inability of cells to grow with fatty acids as sole carbon source (29, 54, 57).

The final product of fatty acid oxidation in yeast is acetyl-CoA, which is exported to the mitochondria for utilization in the citric acid cycle. Two pathways for export of acetyl-CoA from peroxisomes have been described (110). In one pathway, acetyl-CoA produced in the peroxisome is transported to the mitochondria as an acetyl-carnitine derivative. One nuclear gene, *CAT2*, encodes the acetyl-carnitine acyltransferases found in both the peroxisome and the mitochondria (33). Alternatively, the acetyl-CoA may be metabolized via the glyoxylate cycle, which serves

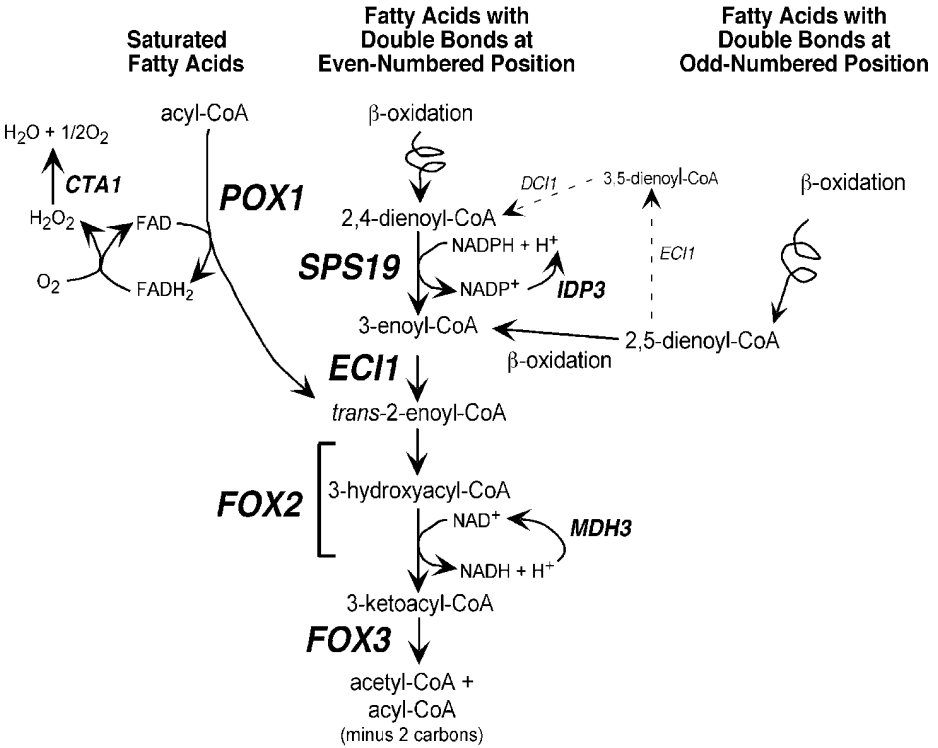

Figure 2 Schematic of fatty acid degradation in *Saccharomyces cerevisiae*. Reactions for β-oxidation of saturated fatty acids (left), and fatty acids containing double bonds at even (middle) or odd-numbered (right) carbons are shown. The names of genes encoding enzymes that catalyze each step appear in larger bold italics, and genes encoding auxiliary enzymes are in smaller bold italics. The dispensable alternative pathway for 2,5-dienoyl-CoA metabolism is depicted by dotted arrows. Further details are in the text.

to produce four carbon units from the two-carbon acetyl-CoA. Intermediates of this pathway include isocitrate and/or succinate, which can be imported into the mitochondria (110). Genetic experiments indicate that at least one of these pathways must be active to support fatty acid oxidation in yeast peroxisomes (110).

Unsaturated Fatty Acids

S. cerevisiae also catabolizes an array of unsaturated and polyunsaturated fatty acids with *cis* double bonds at odd or even positions (see Figure 2). A recent report indicates yeast can also degrade *trans*-unsaturated fatty acids (43). During β-oxidation of fatty acids that are *cis* unsaturated at an odd position, such as oleic acid (18:1Δ9), a 2,5-dienoyl-CoA intermediate is formed. After an additional round of oxidation, a 3-*cis* double bond is created and isomerized by

Δ^3-*cis*-Δ^2-*trans*-enoyl-CoA isomerase, allowing oxidation to continue. A peroxisomal form of this enzyme is encoded by the *ECI1* gene in *S. cerevisiae* (39, 44). Cells carrying an *eci1*Δ disruption are unable to utilize oleic acid (18:1Δ9) as their sole carbon source. Heterologous expression of a cDNA for the rat mitochondrial enzyme was effective in rescuing the *eci1*Δ mutant (44). Eci1p is also required for degradation of fatty acids with odd-numbered *trans*-double bonds, indicating it is actually a Δ^3-*cis/trans*-Δ^2-*trans*-enoyl-CoA isomerase (43). An additional enzyme, $\Delta^{3,5}$, $\Delta^{2,4}$-dienoyl-CoA isomerase, involved in an alternate and dispensable pathway of 2,5-dienoyl-CoA metabolism (Figure 2) is encoded by the *DCI1* gene in *S. cerevisiae* (45).

Oxidation of fatty acids with *cis*-double bonds in an even position gives rise to a 2-*trans*-4-*cis*-dienoyl-CoA intermediate (Figure 2). Further oxidation requires reduction of this intermediate to 3-enoyl-CoA by an NADPH-dependent 2,4-dienoyl-CoA reductase, followed by isomerization to 2-enoyl-CoA by the Δ^3-*cis*-Δ^2-*trans*-enoyl-CoA isomerase (Eci1p). As expected, growth of *eci1*Δ mutants on fatty acids with a *cis*-double bond in an even or odd position is impaired (44). In contrast, the gene for yeast 2,4-dienoyl-CoA reductase, *SPS19*, is required for growth on petroselinic acid (18:1Δ6), but not oleic acid (18:1Δ9), confirming its essential role in metabolism of fatty acids with a double bond at an even position (46). Degradation of fatty acids with *trans*-double bonds at even positions does not require Eci1p, Sps19p, or Dci1p (43).

It is unlikely that the 2,4-dienoyl-CoA reductase Sps19p obtains NADPH from the cytosol (110). The necessary NADPH (Figure 2) appears to be provided by an oleic acid–inducible, peroxisomal isocitrate dehydrogenase activity encoded by the *IDP3* gene (49, 111). Cells containing an *idp3*Δ disruption are unable to grow on fatty acids with double bonds at even positions (i.e. 18:1Δ6 or 18:2Δ9,12) but retain growth on stearic acid (18:0) and oleic acid (18:1Δ9) (49, 111). It has been suggested that Idp3p functions in a redox shuttle across the peroxisomal membrane to maintain the levels of NADPH within the organelle (111).

FATTY ACID INDUCTION OF GENE EXPRESSION

As mentioned above, peroxisome biogenesis and function is induced by exposure to fatty acids. In yeast, expression of such peroxisomal proteins as β-oxidation enzymes is subject to several types of regulation, primarily at the level of transcription (72). Transcription of most of these genes is repressed by glucose, which requires several proteins, including Abf1p, RP-A, and Car80p. Derepression by removing glucose and growing cells on a nonfermentable carbon source, such as acetate or glycerol, allows a modest increase in expression. Adr1p, Snf1p, and Snf4p are proteins required for general derepression of glucose repressible genes. Expression of many genes encoding peroxisomal proteins is not only derepressed by the removal of glucose, it is also further induced by such fatty acids as oleic acid. For instance, when cells are cultured in medium containing $\geq$2% glucose,

the activities of acyl-CoA oxidase (Pox1p) and thiolase (Fox3p) are nearly undetectable. Growth in a nonfermentable carbon source causes these activities to rise by 20 times. Exposure to oleic acid increases the activities of these enzymes a further 20-fold (72). Efficient fatty acid induction of genes encoding peroxisomal enzymes is contingent on removal of glucose repression. Thus, the derepression proteins Adr1p and Snf1p are also necessary for complete oleic acid induction of several genes encoding peroxisomal proteins (72, 84, 99, 100). Mechanisms of glucose repression and derepression are not considered further here, and the reader is referred to recent reviews (14, 38, 72).

Regulation Via Oleate Response Elements

The promoters of many yeast genes encoding peroxisomal proteins contain an upstream activating sequence responsible for oleic acid regulation of transcription, dubbed the oleate response element (ORE) (31, 32, 36, 115). The element, which contains palindromes of CGG with a spacer of 15–18 bp that contains conserved T and A nucleotides, may be present in one or two copies, depending on the gene (91, 115). The ORE consensus has been deduced as either CGG-$N_{15–18}$-CCG (91) or CGGNNNTNA-$N_{9–12}$-CCG (63). Initial footprint and gel retardation analysis demonstrated binding of a protein to the element that coincided with transcriptional activation. Mutational analysis within the ORE motif interfered with protein binding as well as gene activation (31, 36, 115). One half of the palindromic element of *FOX3* was found to bind a small amount of protein and allow some transcriptional activation. Thus, it was proposed that activation of transcription via the ORE occurs via binding of an activator dimer complex (31).

Genes encoding two proteins that interact with OREs have been identified. The *PIP2*(*OAF2*) gene, identified in screens for peroxisome induction mutants (62, 91), is induced by oleic acid and encodes a protein that exhibits DNA binding activity and is capable of oleic acid–specific transcriptional activation of a reporter gene. Cells containing a *pip2*Δ disruption display no defect in derepression but are unable to grow on oleic acid or induce peroxisome biogenesis because of impaired oleic acid induction of gene expression. A second protein named Oaf1p was purified using an affinity column to which oligonucleotides encoding an ORE had been coupled (74). Microsequence analysis of Oaf1p allowed the identification of the gene by database searching. Cells containing the *oaf1*Δ null allele are unable to grow on oleic acid, presumably because gene induction by oleic acid and binding of protein(s) to OREs is impaired in *oaf1*Δ cells. Expression analysis of more than 20 oleic acid–induced genes containing putative OREs found that most are dependent on Oaf1p and Pip2p for complete induction (63, 91). However, not all genes containing a consensus ORE are regulated in a manner requiring Oaf1p/Pip2p, and at least one Oaf1p/Pip2p-regulated gene appears to lack an ORE (63).

Immunoprecipitation experiments indicate that Oaf1p interacts physically with Pip2p, leading to the suggestion that the two transcription factors interact with the ORE and activate transcription as a heterodimer (62). The domain structure of the

two proteins is similar; each contains an N-terminal Zn_2Cys_6 DNA binding domain, two inhibitory domains and an auxiliary domain near the middle of the protein, and a C-terminal 26–27 amino acid activation domain (3). The individual role of each protein in the oleic acid activation of transcription has been addressed using protein fusions and reporter constructs. The data led to a proposed model (3) in which Pip2p activity is inhibited under derepressing conditions via its interaction with inactive Oaf1p. When oleic acid is present, Oaf1p becomes activated and the Oaf1p/Pip2p complex binds the ORE and activates transcription. When glucose is added back, the activity of both Oaf1p and Pip2p is repressed directly. As yet, what the precise molecular nature is by which the Oaf1p/Pip2p complex is activated by oleic acid and repressed by glucose remains unknown.

Identification of Novel Fatty Acid–Responsive Genes

The availability of the *S. cerevisiae* genomic database has facilitated the identification of novel genes induced by oleic acid using genome sequence analysis. The *Saccharomyces* genome database has been searched for genes containing a possible Oaf1p/Pip2p–binding site using a consensus oleate response element (ORE) sequence (63). Forty genes contained the putative binding site within the first 500 bp upstream of the start codon, and 22 were actually induced by oleic acid. Many of the genes encoded known peroxisomal proteins required for β-oxidation. Seven novel oleic acid–regulated open reading frames were identified, one of which was later identified as peroxisomal Δ^3-*cis*-Δ^2-*trans*-enoyl-CoA isomerase (*ECI1*) (39, 44).

A similar search utilizing a computer algorithm called CoSMoS (for context-sensitive motif searches), in which motif searches are combined with position or context specifications within the open reading frame, has recently been used to identify novel peroxisomal proteins (40). CoSMoS was used to scan the yeast genome for genes encoding proteins with 100 or more amino acids containing peroxisomal targeting sequence type 1 at the C terminus or a peroxisomal targeting sequence type 2 within the first 25 amino acids. The tool was also used to identify open reading frames with 100 or more codons with an ORE consensus within 500 bp upstream. CoSMoS identified most of the proteins known to contain peroxisomal targeting sequences (7 of 8) or to be induced by oleic acid (13 of 14). Additionally, genes encoding 18 candidate peroxisomal proteins as well as 300 putative oleic acid–regulated genes were identified. Subcellular localization and mutational analysis has confirmed the role of some of these proteins in peroxisomal function. The *DCI1* gene encoding $\Delta^{3,5,}\Delta^{2,4}$-dienoyl-CoA isomerase was originally identified in this screen (40, 45).

The *Saccharomyces* genome database has also facilitated the genome-wide analysis of mRNA expression. SAGE (serial analysis of gene expression) can be used to generate a profile of gene expression for cells under different conditions (113, 114). Short, oligonucleotide sequence tags (10–14 bp) containing unique sequences corresponding to certain transcripts are used to assay the abundance of

mRNAs in the mixture. The number of times each sequence tag occurs in a SAGE library is generally proportional to the level of expression of the mRNA. This technique has recently been used to compare the profile of mRNA expression (termed the transcriptome) of glucose and oleic acid–grown yeast cells (60). As expected, transcripts of genes encoding β-oxidation enzymes were more highly expressed on oleic acid than on glucose. The induction of mRNAs for enzymes involved in putative redox shuttles across peroxisomal and mitochondrial membranes was also high. A transcript profile was also determined for cells carrying the *oaf1*Δ and *pip2*Δ null alleles. Comparison of this profile to that of wild-type cells identified genes dependent on these transcription factors; it also identified genes induced as the cell's response to this defect. Data from this type of analysis provides the basis for detailed knowledge of the metabolic changes underlying utilization of different carbon sources, as well as ascribing possible function to novel genes identified via the genome sequencing project.

FATTY ACID BIOSYNTHESIS

Fatty acids provided to *S. cerevisiae* in the medium are readily incorporated into complex lipids, especially when fatty acid synthesis is inhibited (19). Thus, altering the membrane lipid composition to study its effects on function is quite feasible. The profile of fatty acids actually synthesized de novo by *S. cerevisiae*, however, is relatively uncomplicated. The vast majority are saturated and monounsaturated fatty acids containing 16 and 18 carbons (24, 118). In addition, 1%–2% of the total fatty acids synthesized are very-long-chain fatty acids from 20–30 carbons, of which 26:0 is reported to be most abundant (118).

Saturated Fatty Acids

Like other eukaryotes, de novo biosynthesis of saturated fatty acids in *S. cerevisiae* (see Figure 1) requires acetyl-CoA carboxylase and the fatty acid synthase complex. The gene encoding acetyl-CoA carboxylase, called *ACC1* (or *FAS3*), encodes a protein of approximately 250 kDa (2, 48). The deduced amino acid sequence of the encoded protein displays approximately 34% homology to the rat enzyme and contains typical biotin carboxylase, biotin binding, and transcarboxylase domains (2). Haploid yeast containing an *acc1* mutation are incapable of vegetative growth, even in the presence of exogenous fatty acids, which indicates that the gene is necessary for function(s) in addition to fatty acid biosynthesis (48). Fatty acid synthase in yeast is composed of two nonidentical, multifunctional subunits, α and β, organized as a hexamer ($\alpha_6\beta_6$). The 208-kDa α subunit, encoded by the *FAS2* gene, is trifunctional, containing the β-ketoacyl synthase, β-ketoacyl reductase, and acyl carrier protein functions (81, 95). The pentafunctional, 220-kDa β subunit is encoded by the *FAS1* gene and possesses acetyl-, malonyl-, and palmitoyl-transferase, as well as dehydratase and enoyl reductase activities (17, 95, 96). Haploid yeast strains containing mutations in the fatty acid synthase complex are not viable

unless the medium is supplemented with exogenous myristic (14:0), palmitic (16:0), stearic (18:0), or oleic (18:1) acid (94).

Like other eukaryotes, fatty acid biosynthesis in yeast is repressed by the presence of fatty acids (16, 61). Growth of yeast in the presence of exogenous fatty acids decreases both acetyl-CoA carboxylase and fatty acid synthase activities by 50%–70%. Transcription of the *FAS1*, *FAS2*, and *ACC1* are all decreased by fatty acids. If *FAS2* is overexpressed in cells, the expression of *FAS1* and *ACC1* is increased, indicating coordinate regulation of the three genes. In addition to fatty acid repression, these three genes are coordinately regulated by an inositol/choline response element, along with a number of genes involved in phospholipid biosynthesis (16, 48).

Desaturation

S. cerevisiae is only able to synthesize monounsaturated fatty acids containing a Δ9 double bond, primarily palmitoleic (16:1) and oleic (18:1) acids. The Δ9 desaturase that produces these fatty acids in the endoplasmic reticulum is encoded by the *OLE1* gene (79, 104). In order to grow, yeast strains carrying an *ole1* mutation require supplementation with exogenous monounsaturated or polyunsaturated fatty acids containing either a Δ9 unsaturation or, in certain fatty acids, a Δ10, Δ11, or Δ5 double bond (76, 103). *OLE1* was also independently cloned as a gene involved in mitochondrial movement and transfer into daughter cells during mitosis, indicating a role for unsaturated fatty acids in this process (102). *OLE1* encodes a protein with an internal region that is 60% homologous to rat stearoyl-CoA desaturase. A fusion protein containing the N-terminal 27 amino acids of Ole1p fused to the rat stearoyl-CoA desaturase is able to complement the defect of an *ole1* mutant (104). Despite this functional interchangeability and unlike the mammalian enzyme, the yeast desaturase fails to exhibit a dependence on cytochrome *b5*, which is thought to be the electron donor for fatty acid desaturases. Rather, the yeast protein has an essential C-terminal extension with high homology to cytochrome *b5* that may serve to facilitate electron transfer (79). Ole1p is considered to be the only desaturase required for synthesis of monounsaturated fatty acids in yeast (103).

Regulation of *OLE1* expression has received a significant amount of attention recently. Transcription of *OLE1* is weakly induced by saturated fatty acids (1.6-fold) and severely repressed (up to 60-fold) by unsaturated fatty acids (19, 76), which is also reflected in desaturase enzyme activity (9). In fact, when yeast cells are grown in the presence of exogenous unsaturated fatty acids, such as linoleic acid (18:2 Δ9, 12), the cells readily incorporate them into membrane lipids, replacing nearly all the monounsaturated fatty acids (9, 103). *OLE1* gene regulation is dependent on acyl-CoA formation by acyl-CoA synthetases Faa1p and Faa4p as well as the acyl-CoA binding protein Acb1p, which indicates that metabolism of the fatty acid is required (19). Regulation of *OLE1* mRNA expression occurs both at the level of transcription (76) and via mRNA stability (42). A number

of mutants insensitive to *OLE1* repression by unsaturated fatty acids (37, 77) or unable to derepress *OLE1* in response to saturated fatty acids (37) have been identified. Two redundant transcription factors, encoded by the *SPT23* and *MGA2* genes, were found to be required for *OLE1* transcription. Expression of *OLE1* on a plasmid or supplementation with oleic or palmitoleic acids suppresses the lethality of the *spt23*Δ *mga2*Δ double mutant (120). A recent report suggests that Spt23p processing from an inactive membrane-bound form to an active soluble form is regulated by the cellular fatty acid pool via a novel ubiquitin/proteosome–dependent process (56). Further studies of Spt23p activation will likely enrich our understanding of the role of the biophysical state of membranes in regulation of gene expression.

Elongation

S. cerevisiae has enzyme systems capable of elongating fatty acyl-CoAs formed from de novo synthesis or acquired from the medium up to 26 carbons (see Figure 1). Elongation activity in homogenates is dependent on malonyl-CoA as the two-carbon donor, NADPH, and a medium- or long-chain fatty acyl-CoA primer with greater than 10 carbons (28). Several different membrane-bound elongation systems localized to the endoplasmic reticulum or mitochondria have been described, including systems capable of elongation of medium-chain fatty acyl-CoAs (12 and 14 carbons to 16 and 18 carbons) and a system that converts 18-carbon fatty acids to fatty acids with 20 or more carbons (6, 28, 107).

Recent genetic approaches in yeast have identified several genes involved in fatty acid elongation. Two groups have recently independently identified a gene essential for elongation of medium- (12 carbons) to long-chain (16–18 carbons) fatty acyl-CoAs (28, 107). Strains unable to elongate 12- to 14-carbon fatty acids were isolated by mutagenizing strains lacking fatty acid synthase (*fas*-) and identifying mutants that could be rescued with fatty acids of 16 or greater carbons but not with fatty acids with $\leq$15 carbons. The *ELO1* gene was identified as encoding an enzyme involved in lengthening fatty acids from 14 to 16 carbons. Strains carrying an *elo1*Δ disruption in the presence of functional fatty acid synthase (*FAS*+) exhibit no phenotype, indicating that the FAS complex is also capable of producing the required 16-carbon species. Sequence analysis by Toke & Martin (107) shows that the *ELO1* gene encodes a protein containing a putative NADPH binding site, a motif (HXXHH) characteristic of nonheme, iron deoxy cluster enzymes such as desaturases, and a cluster of lysine residues that may represent a sequence for retrieval of the protein from the Golgi to the endoplasmic reticulum.

Comparison of the deduced amino acid sequence of Elo1p to the *Saccharomyces* genome database revealed two additional genes, *ELO2* and *ELO3,* involved in formation of very-long-chain fatty acids (85). The predicted amino acid sequences of *ELO2* (previously cloned as *GNS1* and *FEN1*) and *ELO3* (previously identified as *APA1*, *SUR4*, and *SRE1*) exhibit 76% and 72% homology to Elo1p, respectively.

Both Elo2p and Elo3p contain the deoxy iron cluster motif, but neither has a recognizable NADPH binding site. Disruption of both genes in cells is lethal, but loss of either one alone results in a loss or large decrease in the formation of 26:0 fatty acid from 16- or 18-carbon fatty acids. Analysis of fatty acid profiles in *elo2*Δ and *elo3*Δ mutants indicate that Elo2p is involved in elongation up to 24 carbons, whereas Elo3p has a broader specificity and is required for elongation from 24 carbons to 26 carbons.

Sphingolipids in yeast contain a ceramide composed of a long-chain base such as phytosphingosine or dihydrosphingosine N-acylated with either a 26-carbon (26:0) or hydroxylated 26-carbon (HO-26:0) fatty acid (73). As one might expect, in *elo2*Δ or *elo3*Δ strains unable to synthesize 26:0, there is an accumulation of long-chain bases and an absence of ceramide (85). An additional gene, *TSC13*, has recently been identified as encoding an enoyl-CoA reductase required for very-long-chain fatty acid synthesis (T Dunn, S Kohlwein, personal communication). Strains containing a *tsc13* defect also accumulate long-chain bases and exhibit decreased levels of very-long-chain fatty acids. This phenotype is exacerbated by the introduction of a mutation in either *ELO2* or *ELO3*. Finally, hydroxylation of 26:0 to HO-26:0 requires the *FAH1* gene (80). Thus, the *ELO2*, *ELO3*, and *TSC13* gene products appear to play essential roles in the formation of very-long-chain fatty acids (see Figure 1) vital in sphingolipid biosynthesis.

FUNCTIONAL GENOMICS AND THE FUTURE

The new era of yeast functional genomics brought on by the availability and analysis of the genome sequence has already facilitated the characterization of many novel genes, including several involved in fatty acid metabolism (i.e. *FAT1*, *ECI1*, *DCI1*, *IDP3*, *OAF1*, *ELO2*, *ELO3*). This, however, only represents a first step toward developing a working model for the eukaryotic cell (13). The genome project and international cooperation has led to the development of an array of new genomics tools and experimental approaches that will aid in the continued effort (12, 87). A collection of yeast strains carrying disruptions in almost all open reading frames encoding 100 or more amino acids (∼6000) became available for purchase in July 2000 (15, 119). Collections of strains carrying transposon-tagged or fusion-tagged alleles, which should prove useful for biochemical studies, have also been created (69, 75). Large-scale screens for novel protein-protein interactions in yeast have also become possible and can provide new clues as to the biological function of previously unclassified proteins (109).

Comprehensive analysis of gene expression via serial analysis of gene expression (SAGE) (60, 113, 114) or microarray technology (26) can provide a profile of the expression of the complete set of yeast genes under specific conditions (the transcriptome). Also, an effort using two-dimensional gel electrophoresis is being used to describe the proteome or the complete set of proteins synthesized in specific situations (87). Finally, quantitative metabolite profiles (the metabolome)

of wild-type versus deletion strains may aid in elucidating the cellular roles of proteins with unknown function (106).

Experience and knowledge gained from the large effort in yeast functional genomics should prove valuable for analysis of more-complex genomes by providing a basis for comparative genomics (20, 86). A clue to the function of a novel gene from a more-complex organism may come via "in silico" experiments on the computer, revealing homology to a gene or a domain of a gene more completely studied in *S. cerevisiae*. In addition, indication of function for a new gene might come from the phenotype of yeast carrying a mutation in a similar gene, from the ability of the new gene to complement the corresponding yeast mutant when expressed on a plasmid, or from comparable patterns of gene expression or protein subcellular localization. To cite an example from fatty acid metabolism, advances made toward elucidating the roles of Pat1p and Pat2p in yeast peroxisomal fatty acid import shed light on the function of the homologous protein defective in X-linked adrenoleukodystrophy (52). Finally, yeast can be utilized as a living test tube for heterologous reconstitution of processes specific to higher organisms by introducing the necessary gene(s); recent examples include the biosynthesis of *n*-3 and *n*-6 polyunsaturated fatty acids (4) and editing of the mRNA of apolipoprotein B (25). Through these and other creative approaches, yeast will continue as a valuable tool during future analysis of more-complex genomes. Thus, continued effort toward understanding how the genes of yeast work together to make a functioning cell is critical. With better understanding of yeast biology comes greater potential for insight into the functional analysis of novel genes from higher organisms.

ACKNOWLEDGMENTS

I thank Teresa Dunn and Sepp Kohlwein for generously sharing data prior to its publication. I am also indebted to Chuck Martin, Dean Appling, and Anne Tibbetts for their critical reading of the manuscript and helpful comments. Research in my laboratory on genes required for yeast growth on fatty acids is supported by the National Institutes of Health.

LITERATURE CITED

1. Abumrad N, Coburn C, Ibrahimi A. 1999. Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm. *Biochim. Biophys. Acta* 1441:4–13
2. Al-Feel W, Chirala SS, Wakil SJ. 1992. Cloning of the yeast *FAS3* gene and the primary structure of yeast acetyl-CoA carboxylase. *Proc. Natl. Acad. Sci. USA* 89:4534–38
3. Baumgartner U, Hamilton B, Piskacek M, Ruis H, Rottensteiner H. 1999. Functional analysis of the Zn_2Cys_6 transcription factors Oaf1p and Pip2p. *J. Biol. Chem.* 274:22208–16
4. Beaudoin F, Michaelson LV, Hey SJ, Lewis

MJ, Shewry PR, et al. 2000. Heterologous reconstitution in yeast of the polyunsaturated fatty acid biosynthetic pathway. *Proc. Natl. Acad. Sci. USA* 97:6421–26

5. Berk PD, Stump DD. 1999. Mechanisms of cellular uptake of long-chain free fatty acids. *Mol. Cell. Biochem.* 192:17–31
6. Bessoule JJ, Lessire R, Rigoulet M, Guerin B, Cassagne C. 1988. Localization of the synthesis of very-long-chain fatty acid in mitochondria from *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 177:207–11
7. Blobel F, Erdmann R. 1996. Identification of a yeast peroxisomal member of the family of AMP-binding proteins. *Eur. J. Biochem.* 240:468–76
8. Borst P, Schinkel AH. 1997. Genetic dissection of the function of mammalian P-glycoproteins. *Trends Genet.* 13:217–22
9. Bossie MA, Martin CE. 1989. Nutritional regulation of yeast Δ-9 fatty acid desaturase activity. *J. Bacteriol.* 171:6409–13
10. Bossier P, Fernandes L, Vilela C, Rodrigues-Pousada C. 1994. The yeast YKL741 gene situated on the left arm of chromosome XI codes for a homologue of the human ALD protein. *Yeast* 10:681–86
11. Botstein D, Chervitz SA, Cherry JM. 1997. Yeast as a model organism. *Science* 277:1259–60
12. Brown AJP, Tuite MF. 1998. Yeast gene analysis in the next millennium. *Methods Micro.* 26:451–61
13. Bussey H. 1997. 1997 ushers in an era of yeast functional genomics. *Yeast* 13:1501–3
14. Carlson M. 1999. Glucose repression in yeast. *Curr. Opin. Microbiol.* 2:202–7
15. Cherry JM, Ball C, Dolinski K, Dwight S, Harris M, et al. 2000. *Saccharomyces Genome Database*. http://genome-www.stanford.edu/Saccharomyces/
16. Chirala SS. 1992. Coordinated regulation and inositol-mediated and fatty acid mediated-repression of fatty acid synthase genes in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 89:10232–36
17. Chirala SS, Kuziora MA, Spector DM, Wakil SJ. 1987. Complementation of mutations and nucleotide sequence of *FAS1* gene encoding β subunit of yeast fatty acid synthase. *J. Biol. Chem.* 262:4231–40
18. Choi J-Y, Martin CE. 1999. The *Saccharomyces cerevisiae FAT1* gene encodes an acyl-CoA synthetase that is required for maintenance of very long-chain fatty acid levels. *J. Biol. Chem.* 274:4671–83
19. Choi J-Y, Stukey J, Hwang S-Y, Martin CE. 1996. Regulatory elements that control transcription activation and unsaturated fatty acid-mediated repression of the *Saccharomyces cerevisiae OLE1* gene. *J. Biol. Chem.* 271:3581–89
20. Clark MS. 1999. Comparative genomics: the key to understanding the Human Genome Project. *BioEssays* 21:121–30
21. Coe NR, Smith AJ, Frohnert BI, Watkins PA, Bernlohr DA. 1999. The fatty acid transport protein (FATP1) is a very long-chain acyl-CoA synthetase. *J. Biol. Chem.* 274:36300–4
22. Cohen G, Fessl F, Traczyk A, Rytka J, Ruis H. 1985. Isolation of the catalase A gene of *Saccharomyces cerevisiae* by complementation of the *cta1* mutation. *Mol. Gen. Genet.* 200:74–79
23. Costanzo MC, Hogan JD, Cusick ME, Davis BP, Fancher AM, et al. 2000. The yeast proteome database (YPD) and *Caenorhabditis elegans* proteome database (WormPD): comprehensive resources for the organization and comparison of model organism protein information. *Nucleic Acids Res.* 28:73–76
24. Cottrell M, Viljoen BC, Kock JLF, Lategan PM. 1986. The long-chain fatty acid compositions of species representing the genera *Saccharomyces, Schwanniomyces and Lipomyces*. *J. Gen. Microbiol.* 132:2401–3
25. Dance GS, Sowden MP, Yang Y, Smith HC. 2000. APOBEC-1 dependent cytidine to uridine editing of apolipoprotein B RNA in yeast. *Nucleic Acids Res.* 28:424–29
26. DeRisi JL, Iyer VR, Brown PO. 1997.

Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–86
27. DiRusso CC, Black PN. 1999. Long-chain fatty acid transport in bacteria and yeast. Paradigms for defining the mechanism underlying this protein-mediated process. *Mol. Cell. Biochem.* 192:41–52
28. Dittrich F, Zajonc D, Hühne K, Hoja U, Ekici A, et al. 1998. Fatty acid elongation in yeast: biochemical characteristics of the enzyme system and isolation of elongation-defective mutants. *Eur. J. Biochem.* 252:477–85
29. Dmochowska A, Dignard D, Maleszka R, Thomas DY. 1990. Structure and control of the *Saccharomyces cerevisiae POX1* gene encoding acyl-coenzyme A oxidase. *Gene* 88:247–52
30. Duronio RJ, Knoll LJ, Gordon JI. 1992. Isolation of a *Saccharomyces cerevisiae* long-chain fatty acyl:CoA synthetase gene (*FAA1*) and assessment of its role in protein N-myristoylation. *J. Cell Biol.* 117:515–29
31. Einerhand AWC, Kos WT, Distel B, Tabak HF. 1993. Characterization of a transcriptional control element involved in proliferation of peroxisomes in yeast in response to oleate. *Eur. J. Biochem.* 214:323–31
32. Einerhand AWC, Voorn-Brouwer TM, Erdmann R, Kunau W-H, Tabak HF. 1991. Regulation of transcription of the gene coding for peroxisomal 3-oxoacyl-CoA thiolase of *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 200:113–22
33. Elgersma Y, van Roermund CWT, Wanders RJA, Tabak HF. 1995. Peroxisomal and mitochondrial carnitine acetyltransferases of *Saccharomyces cerevisiae* are encoded by a single gene. *EMBO J.* 14:3472–79
34. Erdmann R, Veenhuis M, Mertens D, Kunau W-H. 1989. Isolation of peroxisome-deficient mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 86:5419–23
35. Faergeman NJ, DiRusso CC, Elberger A, Knudsen J, Black PN. 1997. Disruption of the *Saccharomyces cerevisiae* homologue to murine fatty acid transport protein impairs uptake and growth on long-chain fatty acids. *J. Biol. Chem.* 272:8531–38
36. Filipits M, Simon MM, Rapatz W, Hamilton B, Ruis H. 1993. A *Saccharomyces cerevisiae* upstream activating sequence mediates induction of peroxisome proliferation by fatty acids. *Gene* 132:49–55
37. Fujimori K, Anamnart S, Nakagawa Y, Sugioka S, Ohta D, et al. 1997. Isolation and characterization of mutations affecting expression of the Δ9-fatty acid desaturase gene, *OLE1*, in *Saccharomyces cerevisiae*. *FEBS Lett.* 413:226–30
38. Gancendo JM. 1998. Yeast carbon catabolite repression. *Micro. Mol. Biol. Rev.* 62: 334–61
39. Geisbrecht BV, Zhu D, Schulz K, Nau K, Morrell JC, et al. 1998. Molecular characterization of *Saccharomyces cerevisiae* Δ^3,Δ^2-enoyl-CoA isomerase. *J. Biol. Chem.* 273:33184–91
40. Geraghty MT, Bassett D, Morrell JC, Gatto GJ Jr, Bai J, et al. 1999. Detecting patterns of protein distribution and gene expression *in silico*. *Proc. Natl. Acad. Sci. USA* 96:2937–42
41. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, et al. 1996. Life with 6000 genes. *Science* 274:546–67
42. Gonzalez CI, Martin CE. 1996. Fatty acid-responsive control of mRNA stability: unsaturated fatty acid-induced degradation of the *Saccharomyces OLE1* transcript. *J. Biol. Chem.* 271:25801–9
43. Gurvitz A, Hamilton B, Ruis H, Hartig A. 2001. Peroxisomal degradation of trans-unsaturated fatty acids in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* In press
44. Gurvitz A, Mursula AM, Firzinger A, Hamilton B, Kilpeläinen SH, et al. 1998. Peroxisomal Δ^3-*cis*-Δ^2-*trans*-enoyl-CoA isomerase encoded by *ECI1* is required for growth of the yeast *Saccharomyces cerevisiae* on unsaturated fatty acids. *J. Biol. Chem.* 273:31366–74

45. Gurvitz A, Mursula AM, Yagi AI, Hartig A, Ruis H, et al. 1999. Alternatives to the isomerase-dependent pathway for the β-oxidation of oleic acid are dispensable in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 274:24514–21
46. Gurvitz A, Rottensteiner H, Kilpeläinen SH, Hartig A, Hiltûnen JK, et al. 1997. The *Saccharomyces cerevisiae* peroxisomal 2,4-dienoyl-CoA reductase is encoded by the oleate-inducible gene *SPS19*. *J. Biol. Chem.* 272:22140–47
47. Hamilton JA. 1998. Fatty acid transport: difficult or easy? *J. Lipid Res.* 39:467–81
48. Hasslacher M, Ivessa AS, Paltauf F, Kohlwein SD. 1993. Acetyl-CoA carboxylase from yeast is an essential enzyme and is regulated by factors that control phospholipid metabolism. *J. Biol. Chem.* 268:10946–52
49. Henke B, Girzalsky W, Berteaux-Lecellier V, Erdmann R. 1998. *IDP3* encodes a peroxisomal NADP-dependent isocitrate dehydrogenase required for the β-oxidation of unsaturated fatty acids. *J. Biol. Chem.* 273:3702–11
50. Hettema EH, Distel B, Tabak HF. 1999. Import of proteins into peroxisomes. *Biochim. Biophys. Acta* 1451:17–34
51. Hettema EH, Tabak HF. 2000. Transport of fatty acids and metabolites across the peroxisomal membrane. *Biochim. Biophys. Acta* 1486:18–27
52. Hettema EH, van Roermund CWT, Distel B, van den Berg M, Vilela C, et al. 1996. The ABC transporter proteins Pat1 and Pat2 are required for import of long-chain fatty acids into peroxisomes of *Saccharomyces cerevisiae*. *EMBO J.* 15:3813–22
53. Hieter P, Boguski M. 1997. Functional genomics: It's all how you read it. *Science* 278:601–2
54. Hiltûnen JK, Wenzel B, Beyer A, Erdmann R, Fosså A, Kunau W-H. 1992. Peroxisomal multifunctional β-oxidation protein of *Saccharomyces cerevisiae*: molecular analysis of the *FOX2* gene and gene product. *J. Biol. Chem.* 267:6646–53
55. Hirsch D, Stahl A, Lodish HF. 1998. A family of fatty acid transporters conserved from mycobacterium to man. *Proc. Natl. Acad. Sci. USA* 95:8625–29
56. Hoppe T, Matuschewski K, Rape M, Schlenker S, Ulrich HD, Jentsch S. 2000. Activation of a membrane-bound transcription factor by regulated ubiquitin/-proteosome-dependent processing. *Cell* 102:577–86
57. Igual JC, Matallana E, Gonzalez-Bosch C, Franco L, Perez-Ortin JE. 1991. A new glucose-repressible gene identified from the analysis of chromatin structure in deletion mutants of yeast *SUC2* locus. *Yeast* 7:379–89
58. Johnson DR, Knoll LJ, Levin DE, Gordon JI. 1994. *Saccharomyces cerevisiae* contains four fatty acid activation (*FAA*) genes: an assessment of their role in regulating protein N-myristoylation and cellular lipid metabolism. *J. Cell Biol.* 127:751–62
59. Johnson DR, Knoll LJ, Rowley N, Gordon JI. 1994. Genetic analysis of the role of *Saccharomyces cerevisiae* acyl-CoA synthetase genes in regulating protein *N*-myristoylation. *J. Biol. Chem.* 269:18037–46
60. Kal AJ, van Zonneveld AJ, Benes V, van den Berg M, Koerkamp MG, et al. 1999. Dynamics of gene expression revealed by comparison of serial analysis of gene expression transcript profiles from yeast grown on two different carbon sources. *Mol. Biol. Cell* 10:1859–72
61. Kamiryo T, Parthasarathy S, Numa S. 1976. Evidence that acyl coenzyme A synthetase activity is required for repression of yeast acetyl coenzyme A carboxylase by exogenous fatty acids. *Proc. Natl. Acad. Sci. USA* 73:386–90
62. Karpichev IV, Luo Y, Marians RC, Small GM. 1997. A complex containing two transcription factors regulates peroxisome proliferation and the coordinate induction of

β-oxidation enzymes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 17:69–80

63. Karpichev IV, Small GM. 1998. Global regulatory functions of Oaf1p and Pip2p (Oaf2p), transcription factors that regulate genes encoding peroxisomal proteins in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 18:6560–70
64. Knoll LJ, Johnson DR, Gordon JI. 1994. Biochemical studies of three *Saccharomyces cerevisiae* acyl-CoA synthetases, Faa1p, Faa2p, and Faa3p. *J. Biol. Chem.* 269:16348–56
65. Knoll LJ, Johnson DR, Gordon JI. 1995. Complementation of *Saccharomyces cerevisiae* strains containing fatty acid activation gene (*FAA*) deletions with a mammalian acyl-CoA synthetase. *J. Biol. Chem.* 270:10861–67
66. Knudsen J, Jensen MV, Hansen JK, Faergeman NJ, Neergaard TBF, Gaigg B. 1999. Role of acylCoA binding protein in acyl-CoA transport, metabolism and cell signaling. *Mol. Cell. Biochem.* 192:95–103
67. Knudsen J, Neergaard TBF, Gaigg B, Jensen MV, Hansen JK. 2000. Role of acyl-CoA binding protein in acyl-CoA metabolism and acyl-CoA-mediated cell signalling. *J. Nutr.* 130:294S–98S
68. Kohlwein SD, Paltauf F. 1983. Uptake of fatty acids by the yeasts, *Saccharomyces uvarum* and *Saccharomycopsis lipolytica*. *Biochim. Biophys. Acta* 792:310–17
69. Kumar A, Cheung K-H, Ross-Macdonald P, Coelho PSR, Miller P, Snyder M. 2000. TRIPLES: a database of gene function in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 28:81–84
70. Kunau W-H. 1998. Peroxisome biogenesis: from yeast to man. *Curr. Opin. Microbiol.* 1:232–37
71. Kunau W-H, Bühne S, de la Garza M, Kionka C, Mateblowski M, et al. 1988. Comparative enzymology of β-oxidation. *Biochem. Soc. Transact.* 16:418–20
72. Lazarow PB, Kunau W-H. 1997. Peroxisomes. In *The Molecular and Cellular Biology of the Yeast Saccharomyces*, Vol. III. *Cell Cycle and Cell Biology*, ed. JR Pringle, JR Broach, EW Jones, pp. 547–605. Cold Spring Harbor, NY: Cold Spring Harbor Lab.
73. Lester RL, Wells GB, Oxford G, Dickson RC. 1993. Mutant strains of *Saccharomyces cerevisiae* lacking sphingolipids synthesize novel inositol glycerophospholipids that mimic sphingoid structures. *J. Biol. Chem.* 268:845–56
74. Luo Y, Karpichev IV, Kohanski RA, Small GM. 1996. Purification, identification, and properties of a *Saccharomyces cerevisiae* oleate-activated upstream activating sequence-binding protein that is involved in the activation of POX1. *J. Biol. Chem.* 271:12068–75
75. Martzen MR, McCraith SM, Spinelli SL, Torres FM, Fields S, et al. 1999. A biochemical genomics approach for identifying genes by the activity of their products. *Science* 286:1153–55
76. McDonough VM, Stukey JE, Martin CE. 1992. Specificity of unsaturated fatty acid-regulated expression of the *Saccharomyces cerevisiae OLE1* gene. *J. Biol. Chem.* 267:5931–36
77. McHale MW, Kroening KD, Bernlohr DA. 1996. Identification of a class of *Saccharomyces cerevisiae* mutants defective in fatty acid repression of gene transcription and analysis of the *frm2* gene. *Yeast* 12:319–31
78. Mewes HW, Heumann K, Kaps A, Mayer K, Pfeiffer F, et al. 1999. MIPS: a database for genomes and protein sequences. *Nucleic Acids Res.* 27:44–48
79. Mitchell AG, Martin CE. 1995. A novel cytochrome b5-like domain is linked to the carboxyl terminus of the *Saccharomyces cerevisiae* Δ-9 fatty acid desaturase. *J. Biol. Chem.* 270:29766–72
80. Mitchell AG, Martin CE. 1997. Fah1p, a *Saccharomyces cerevisiae* cytochrome b_5 fusion protein, and its *Arabidopsis thaliana* homolog that lacks the cytochrome b_5

domain both function in the α-hydroxylation of sphingolipid very long-chain fatty acids. *J. Biol. Chem.* 272:28281–88

81. Mohamed AH, Chirala SS, Mody NH, Huang W-Y, Wakil SJ. 1988. Primary structure of the multifunctional α subunit protein of yeast fatty acid synthase derived from *FAS2* gene sequence. *J. Biol. Chem.* 263:12315–25
82. Mosser J, Douar A-M, Sarde C-O, Kioschis P, Feil R, et al. 1993. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature* 361:726–30
83. Mosser J, Lutz Y, Stoeckel ME, Sarde C-O, Kretz C, et al. 1994. The gene responsible for adrenoleukodystrophy encodes a peroxisomal membrane protein. *Hum. Mol. Genet.* 3:265–71
84. Navarro B, Igual JC. 1994. *ADR1* and *SNF1* mediate different mechanisms in transcriptional regulation of yeast *POT1* gene. *Biochem. Biophys. Res. Commun.* 202:960–66
85. Oh C-S, Toke DA, Mandala S, Martin CE. 1997. *ELO2* and *ELO3*, homologues of the *Saccharomyces cerevisiae ELO1* gene, function in fatty acid elongation and are required for sphingolipid formation. *J. Biol. Chem.* 272:17376–84
86. Oliver SG. 1997. Yeast as a navigational aid in genome analysis; 1996 Kathleen Barton-Wright Memorial Lecture. *Microbiology* 143:1483–87
87. Oliver SG, Winson MK, Kell DB, Baganz F. 1998. Systematic functional analysis of the yeast genome. *Trends Biotechnol.* 16:373–78
88. Rose MD, Broach JR. 1991. Cloning genes by complementation in yeast. *Methods Enzymol.* 194:195–238
89. Rose TM, Schultz ER, Todaro GJ. 1992. Molecular cloning of the gene for the yeast homology (*ACB*) of diazepam binding inhibitor/endozepine/acyl-CoA-binding protein. *Proc. Natl. Acad. Sci. USA* 89:11287–91
90. Rothstein R. 1991. Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. *Methods Enzymol.* 194:281–301
91. Rottensteiner H, Kal AJ, Filipits M, Binder M, Hamilton B, et al. 1996. Pip2p: a transcriptional regulator of peroxisome proliferation in the yeast *Saccharomyces cerevisiae*. *EMBO J.* 15:2924–34
92. Schaffer JE, Lodish HF. 1994. Expression cloning and characterization of a novel adipocyte long-chain fatty acid transport protein. *Cell* 79:427–36
93. Schjerling CK, Hummel R, Hansen JK, Børsting C, Mikkelsen JM, et al. 1996. Disruption of the gene encoding the acyl-CoA-binding protein (*ACB1*) perturbs acyl-CoA metabolism in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 271:22514–21
94. Schweizer E, Bolling H. 1970. A *Saccharomyces cerevisiae* mutant defective in saturated fatty acid biosynthesis. *Proc. Natl. Acad. Sci. USA* 67:660–66
95. Schweizer M, Lebert C, Höltke J, Roberts LM, Schweizer E. 1984. Molecular cloning of the yeast fatty acid synthetase genes, *FAS1* and *FAS2*: illustrating the structure of the *FAS1* cluster gene by transcript mapping and transformation studies. *Mol. Gen. Genet.* 194:457–65
96. Schweizer M, Roberts LM, Höltke H-J, Takabayashi K, Höllerer E, et al. 1986. The pentafunctional *FAS1* gene of yeast: its nucleotide sequence and order of the catalytic domains. *Mol. Gen. Genet.* 203:479–86
97. Shani N, Valle D. 1996. A *Saccharomyces cerevisiae* homolog of the human adrenoleukodystrophy transporter is a heterodimer of two half ATP-binding cassette transporters. *Proc. Natl. Acad. Sci. USA* 93:11901–6
98. Shani N, Watkins PA, Valle D. 1995. *PXA1*, a possible *Saccharomyces cerevisiae* ortholog of the human adrenoleukodystrophy gene. *Proc. Natl. Acad. Sci. USA* 92:6012–16
99. Simon M, Adam G, Rapatz W, Spevak W,

Ruis H. 1991. The *Saccharomyces cerevisiae ADR1* gene is a positive regulator of transcription of genes encoding peroxisomal proteins. *Mol. Cell. Biol.* 11:699–704

100. Simon M, Binder M, Adam G, Hartig A, Ruis H. 1992. Control of peroxisome proliferation in *Saccharomyces cerevisiae* by *ADR1, SNF1* (*CAT1, CCR1*) and *SNF4* (*CAT3*). *Yeast* 8:303–9
101. Skoneczny M, Chelstowska A, Rytka J. 1988. Study of the coinduction by fatty acids of catalase A and acyl-CoA oxidase in standard and mutant *Saccharomyces cerevisiae* strains. *Eur. J. Biochem.* 174:297–302
102. Stewart LC, Yaffe MP. 1991. A role for unsaturated fatty acids in mitochondrial movement and inheritance. *J. Cell Biol.* 115:1249–57
103. Stukey JE, McDonough VM, Martin CE. 1989. Isolation and characterization of *OLE1*, a gene affecting fatty acid desaturation from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 264:16537–44
104. Stukey JE, McDonough VM, Martin CE. 1990. The *OLE1* gene of *Saccharomyces cerevisiae* encodes the $\Delta 9$ fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. *J. Biol. Chem.* 265:20144–49
105. Swartzman EE, Viswanathan MN, Thorner J. 1996. The *PAL1* gene product is a peroxisomal ATP-binding cassette transporter in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* 132:549–63
106. Teusink B, Baganz F, Westerhoff HV, Oliver SG. 1998. Metabolic control analysis as a tool in the elucidation of the function of novel genes. *Methods Micro.* 26:297–336
107. Toke DA, Martin CE. 1996. Isolation and characterization of a gene affecting fatty acid elongation in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 271:18413–22
108. Trigatti BL, Baker AD, Rajaratnam K, Rachubinski RA, Gerber GE. 1992. Fatty acid uptake in *Candida tropicalis*: induction of a saturable process. *Biochem. Cell Biol.* 70:76–80
109. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, et al. 2000. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 403:623–27
110. van Roermund CWT, Elgersma Y, Singh N, Wanders RJA, Tabak HF. 1995. The membrane of peroxisomes in *Saccharomyces cerevisiae* is impermeable to NAD(H) and acetyl-CoA under *in vivo* conditions. *EMBO J.* 14:3480–86
111. van Roermund CWT, Hettema EH, Kal AJ, van den Berg M, Tabak HF, Wanders RJA. 1998. Peroxisomal β-oxidation of polyunsaturated fatty acids in *Saccharomyces cerevisiae*: isocitrate dehydrogenase provides NADPH for reduction of double bonds at even positions. *EMBO J.* 17:677–87
112. Veenhuis M, Mateblowski M, Kunau W-H, Harder W. 1987. Proliferation of microbodies in *Saccharomyces cerevisiae*. *Yeast* 3:77–84
113. Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. 1995. Serial analysis of gene expression. *Science* 270:484–87
114. Velculescu VE, Zhang L, Zhou W, Vogelstein J, Basrai MA, et al. 1997. Characterization of the yeast transcriptome. *Cell* 88:243–51
115. Wang T, Luo Y, Small GM. 1994. The *POX1* gene encoding peroxisomal acyl-CoA oxidase in *Saccharomyces cerevisiae* is under control of multiple regulatory elements. *J. Biol. Chem.* 269:24480–85
116. Waterham HR, Cregg JM. 1997. Peroxisome biogenesis. *BioEssays* 19:57–66
117. Watkins PA, Lu J-F, Steinberg SJ, Gould SJ, Smith KD, Braiterman LT. 1998. Disruption of the *Saccharomyces cerevisiae FAT1* gene decreases very long-chain fatty acyl-CoA synthetase activity and elevates intracellular very long-chain

fatty acid concentrations. *J. Biol. Chem.* 273:18210–19

118. Welch JW, Burlingame AL. 1973. Very long-chain fatty acids in yeast. *J. Bacteriol.* 115:464–66
119. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, et al. 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285:901–6
120. Zhang S, Skalsky Y, Garfinkel DJ. 1999. *MGA2* or *SPT23* is required for transcription of the Δ9 fatty acid desaturase gene, *OLE1*, and nuclear membrane integrity in *Saccharomyces cerevisiae*. *Genetics* 151:473–83