A Yeast Acetyl Coenzyme A Carboxylase Mutant Links Very-Long-Chain Fatty Acid Synthesis to the Structure and Function of the Nuclear Membrane-Pore Complex

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The conditional mRNA transport mutant of Saccharomyces cerevisiae, acc1-7-1 (mtr7-1), displays a unique alteration of the nuclear envelope. Unlike nucleoporin mutants and other RNA transport mutants, the intermembrane space expands, protuberances extend from the inner membrane into the intermembrane space, and vesicles accumulate in the intermembrane space. MTR7 is the same gene as ACC1, encoding acetyl coenzyme A (CoA) carboxylase (Acc1p), the rate-limiting enzyme of de novo fatty acid synthesis. Genetic and biochemical analyses of fatty acid synthesis mutants and acc1-7-1 indicate that the continued synthesis of malonyl-CoA, the enzymatic product of acetyl-CoA carboxylase, is required for an essential pathway which is independent from de novo synthesis of fatty acids. We provide evidence that synthesis of very-long-chain fatty acids (C25 atoms) is inhibited in acc1-7-1, suggesting that very-long-chain fatty acid synthesis is required to maintain a functional nuclear envelope.

The regulation of synthesis and transport of phospholipids has been characterized at length, but the signals which determine the phospholipid and fatty acid composition of particular membranes and membrane-specific lipid requirements are not well understood (7, 62, 81, 83). The present investigation, which is the outgrowth of analysis of a yeast (Saccharomyces cerevisiae) mutant which accumulates poly(A)* RNA in the nucleus at the restrictive temperature (41), documents a critical relationship between fatty acid chain length and the integrity of the nuclear membrane and nuclear pore complex (NPC).

The biosynthesis of very-long-chain fatty acids requires four enzyme systems: acetyl coenzyme A (CoA) carboxylase, fatty acid synthase, fatty acid desaturase, and the fatty acyl chain elongation system (Fig. 1). The rate-limiting step of the de novo synthesis of fatty acids is under control of the first of these, acetyl-CoA carboxylase (Acc1p; EC 6.4.1.2). This biotinylated enzyme catalyzes the ATP-dependent carboxylation of acetyl-CoA to yield malonyl-CoA, which serves as the two-carbon-unit donor for the subsequent synthesis of long-chain fatty acids by the fatty acid synthase complex. The chain length of newly synthesized fatty acids appears to depend on the concentration of malonyl-CoA rather than on the activity of the fatty acid synthase complex (35, 45a, 82). Acc1p thus regulates both the overall rate of de novo synthesis and chain-length distribution of long-chain fatty acids. It is perhaps for this reason that its activity is subject to complex regulation (48). Yeast Acc1p/Fas3p has a subunit molecular mass of 250 kDa, is active as a tetramer, and is subject to short-term regulation by phosphorylation (2, 60, 90). Its transcription is positively regulated by Ino2p and Ino4p and negatively regulated by Op1p; i.e., it is under the general control of phospholipid synthesis (14, 31).

Many yeast long-chain fatty acid auxotrophs have been isolated (72). Sixty-one are alleles of acc1 (55, 67), while others bear mutations in fatty acid synthase (19, 73, 74) or in the apo-Acc1p ligase (Acc2p [55]).

Since acc1 fatty acid auxotrophs can be obtained, it is most surprising that cells carrying a null allele of ACC1 are not rescued by fatty acid supplementation (31). This observation suggests that Acc1p performs some function other than the synthesis of C16 to C18 fatty acids. Confirming this hypothesis, we find that synthesis of very-long-chain fatty acids is inhibited in a conditional mutant of ACC1 (acc1-7-1), which cannot be rescued by fatty acid supplementation. Genetic and biochemical analyses suggest that lipids substituted with very-long-chain fatty acids stabilize the interface between the nuclear membrane and the NPC, which includes a sharp bend of the membrane (17, 20).

MATERIALS AND METHODS

Yeast strains, plasmids, media, and microbiological techniques. Tables 1 and 2 describe the yeast strains and plasmids referred to in this study. Yeast strains were grown in either YEPD (1% yeast extract, 2% Bacto Peptone, 2% glucose) or synthetic minimal medium (77) supplemented with the appropriate amino acids and glucose. Medium supplemented with fatty acid was prepared as described previously (0.4% yeast extract, 0.4% peptone, 2% sucrose, 1% KPO4, 0.01% aspartic acid, 1% Brij 58, 0.03% palmitic acid) (55). For fatty acid analyses of cells grown in the presence of myristic acid, the medium contained 0.4% yeast extract, 0.4% peptone, 2% sucrose, 1% KPO4, 0.5% Tween 40, and 0.04% myristic acid. Sterol-supplemented medium contained 20 mg of ergosterol per ml and 0.05% Tween 80 in YEPD. Transformation was performed with lithium acetate (38). Selection against uracil prototrophic strains was done by culturing on solid synthetic media containing 1 mg of 5′-fluoroorotic acid (PCR, Inc., Gainesville, Fla.) per ml. Bacterial cells were cultured in Luria broth and transformed or infected with phage by standard methods (69). (Construction of plasmids is described in the next section and in Table 2.)

Gene cloning, gene disruption, and plasmid constructions. The temperature-sensitive mutation in acc1-7-1 was complemented by transformation with a yeast genomic library inserted in YEp24 (13). Despite some variability in colony size at 23°C, there was no difficulty in recognizing temperature-sensitive transformants. The essential region of the complementing plasmid, pRXS5, was further defined by subcloning of individual fragments. To determine whether the high copy number of pRXS5 is essential to rescue the temperature-sensitive phenotype of acc1-7-1, the SphI fragment of pRXS5 containing the entire ACC1 gene
Disruption of ACC1 was performed by integrative DNA transformation (68) as follows. A BglII fragment containing the LEU2 gene from PS118 (79) was ligated into BglII-digested pRXS51 (generating a 5.9-kb deletion [87% of the open reading frame] within the coding sequence of ACC1) to yield pRXS57. pRXS57 was then cut with SrfI to release the insert. The gel-purified fragment was subsequently used for the transformation of the diploid strain YPH501 (78). Leucine prototrophic transformants were selected, and integration was confirmed by genomic Southern hybridization of BglII- and EclI-digested genomic DNA, probed with the EclI-digested fragment of pRXS51. The strain carrying the desired acc1::LEU2 allele was named YRXS23.

The biotin-deficient allele was generated by replacing the lysine residue which normally is biotinylated (position 735) with an arginine residue (K735R). To this end, the BamHI-SstI fragment of pRXS56 was cloned into M13mp19, generating pRXS75, which was subjected to site-directed mutagenesis with a commercial kit (Amersham Corp.) and the primer 5'-AGG CAT TTC CAT TCT CAT AAT TTC. The single base change which results in the K735R substitution is underlined. A clone harboring the desired mutation was identified by sequencing and named pRXS76. The BglII-ClaI fragment of pRXS76 was subsequently cloned into BglII-ClaI-cut pRXS76, yielding pRXS77, from which the BamHI-SstI fragment was cloned into BamHI-SstI-cut pRXS77 to yield pRXS85. Moving the BamHI-EagI fragment from pRXS55 to BamHI-EagI-cut pRXS73 produced pRXS89, which was used in the red/white sectoring assay to determine whether the K735R allele could complement the null allele.

Expression of Acc1p was put under transcriptional control of the GAL1 promoter by cloning a SacII-digested PCR-generated fragment which covers the 5' part of ACC1 (sequence positions 47 to 710) into Smal-SstI-digested pRXS76, yielding pRXS77. The 3' part of ACC1 was then moved as an SacII-EclI fragment into SacII-EclI-digested pRXS77, generating pRXS81. This plasmid was transformed into an acc1::LEU2/ACC1 heterozygous diploid (YRXS23), which was sporulated to yield the leucine and uracil prototrophic haploid YRXS73. This strain can be maintained on galactose plates at 23°C.

![FIG. 1. Metabolic pathways of fatty acid synthesis and malonate metabolism.](image)

The question marks indicate steps in which malonate may be involved (see text). HMG-CoA, hydroxymethylglutaryl-CoA.
**TABLE 2. Plasmid construction**

<table>
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<th>Designation</th>
<th>Marker(s)</th>
<th>Comments</th>
<th>Source</th>
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<tr>
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<td>CEN URA3 ACC1</td>
<td>ACC1-containing plasmid</td>
<td>Hassellacher et al. (31)</td>
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<td>pRXS5</td>
<td>2μm URA3 ACC1</td>
<td>ACC1 isolated from Yepl24-based genomic library</td>
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</tr>
<tr>
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<td>URA3</td>
<td>SalI fragment of pRXS5 into SalI-cut pRS306</td>
<td>This work</td>
</tr>
<tr>
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<td>ACC1</td>
<td>SalI fragment of pRXS5 into SalI site of pGEM-7ZI(+)</td>
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<tr>
<td>pRXS53</td>
<td>CEN ACC1 URA3</td>
<td>SalI fragment of pRXS5 into SalI site of pFL38</td>
<td>This work</td>
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<tr>
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<td>acc1-1/-1:LEU2</td>
<td>BglII fragment containing LEU2 from pS118 into BglII-digested pRXS51</td>
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<td>myc-tagged derivative of pRXS51 (see “Experimental procedures”)</td>
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<td>CEN URA3 GALI-ACC1</td>
<td>Expression of ACC1 under control of GAL1 promoter (see Materials and Methods)</td>
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<td>myc-tagged derivative of pRXS81 (see Materials and Methods)</td>
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<td>CEN HIS acc1K735R</td>
<td>BamHI-EagI fragment of pRXS55 into BamHI-EagI-cut pRX373</td>
<td>This work</td>
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*All pRS vectors were obtained from P. Hieter (78), pFL44L and pFL38 were obtained from F. Lacroute (10), pS118 was obtained from P. Silver (79), pCH1122 was obtained from C. Holm (46), pGP316 was obtained from D. Templeton, and pGEM-7ZI was obtained from Promega Biotec.*

For subcellular localization of Mtr7p, a myc epitope-tagged allele was constructed by ligating an excess of the myc linker shown below into the unique SalI site of pRXS51, resulting in pRXS60. In-frame insertion of this linker results in a 10-residue insertion between amino acids 223 (Asp) and 224 (Asp) (within the putative biotin carboxylase domain of Acc1p) in pRXS60. The orientation of this insertion was confirmed by DNA sequencing and PCR analysis. The overhangs of this linker were resuspended in homogenization buffer (20% Ficoll 400, 20 mM KPO4 [pH 6.5], and centrifuged through a cushion of 0.6 M sorbitol–50 mM KPO4 (pH 6.5), and centrifuged at 4 °C for 10 min at 15000 g. The supernatant was then removed by centrifugation at 4 °C for 10 min at 15000 g, followed by fresh Reynolds lead citrate at room temperature for 5 min.

**Immunoelectron microscopic localization of the NPCs.** Diploid acc1-7-1 mutant cells (YRXS69) were grown at 23°C to the early logarithmic phase and then shifted to 37°C for 4 h or kept at 23°C. Fixation, cell wall removal, dehydration, and embedding in LR white were performed exactly as described previously (88). Thin sections were collected on nickel grids coated with Formvar, stabilized with carbon, blocked with 1% BSA-0.2% fish gelatin in phosphate-buffered saline, and reacted with a 1:20 dilution of Ab 414 (Berkeley Antibody Co., Richmond, Calif.) in blocking buffer. After extensive washes with blocking buffer, the grids were incubated for 1 h in a suspension of 15-nm-diameter colloidal gold particles coated with goat anti-mouse antibody (Amersham Corp.) diluted 1:20 in Tris-buffered saline. After the final washes, the grids were contrasted by staining in 2% uranyl acetate. Samples were examined in a JEM-100CXII electron microscope (JEOL USA Inc., Peabody, Mass.) at 80 kV, and photographs were recorded with Kodak EM film (Eastman Kodak Co., Rochester, N.Y.).

**Drug treatment of wild-type cells.** Cereulcin (Sigma Chemical Co., St, Louis, Mo.) was prepared as a 1,000× stock (25 mg/ml in ethyl acetate; used at 5.5 mg/ml [61]). Lovastatin and zaragozic acid were the kind gifts of A. Alberts (Merck & Co., Inc., Rahway, N.J.), and zaragozic acid was prepared as a 200× stock solution in dimethyl sulfoxide and used at 62 mg/ml (60). Lovastatin was converted from the lactone prodrug to the active dihydroxy open acid as described previously (28) and used at 50 mg/ml (1, 11). The activity of drugs was assessed by monitoring their inhibitory effect on cell growth and by measuring changes in cell density and the increase in volume of the vacuole in drug-treated cells, as observed by differential interference contrast microscopy.

**Fatty acid analyses.** Glycerophospholipids and sphingolipids were extracted as described previously (32). After alkaline hydrolysis of lipids, fatty acids were converted to methyl esters by BF3-catalyzed methanalysis and separated by gas-liquid chromatography on a Hewlett-Packard Ultra 2 capillary column (5% Ph Me silcone) with a temperature gradient (20 min at 200°C, 10°C/min to 280°C, 15 min at 280°C). Fatty acids were identified by commercial methyl ester standards (NuCheck, Inc., Elysian).

**RESULTS**

The phenotype of acc1-7-1 mutant cells. acc1-7-1 (identical to mtr7-1 [see below]) was isolated as a temperature-sensitive strain of S. cerevisiae defective in mRNA transport. The mutation is recessive, and only one allele was recovered. Previous
characterization of all mtr mutants, after backcross, indicated that acc1-7-1 affects processing of rRNA but does not obviously affect pre-mRNA splicing or tRNA processing. The length of the poly(A) tail of RNA polymerase II transcripts, however, increases at the restrictive temperature. Unlike some other mtr strains, no fragmentation or enlargement of the nucleolus is detectable, and there is no evidence of accumulation of nuclear proteins in the cytoplasm (41).

acc1-7-1 affects the structure of the nuclear envelope. Examination of acc1-7-1 in the electron microscope shows that the nuclear envelope is frequently perturbed, although some seemingly normal NPCs persist (Fig. 2). Cells incubated at the restrictive temperature display a characteristic separation of the inner and outer nuclear membranes which may run along the entire perimeter of the envelope. The inner membrane often is distorted by protuberances which face the intermembrane space, which contains membrane profiles, some of which are attached to the inner membrane. Debris is also present in the intermembrane space. Clusters of small, smooth vesicles are often seen in the cytoplasm adjacent to NPCs. No obvious changes of the nucleoplasm are seen, but the endoplasmic reticulum often is more elaborate and dilated than it is in controls. In favorable sections one can detect continuity between the endoplasmic reticulum and dilated nuclear envelope (41). The changes seen in acc1-7-1 are distinct from those of nucleoporin mutants (see Discussion). We do not know whether the separation of the nuclear envelope is reversible; however, the original selection of mtr mutants involved incubation for 3 h at 37°C followed by recovery at 23°C.

Figure 3A illustrates the localization of the XFXFG family of nucleoporins as detected with MAb 414 (17). At the permissive temperature, acc1-7-1 cells display punctate nuclear rim staining, i.e., characteristic wild-type distribution of NPCs. At the restrictive temperature, staining is restricted to a few intensely stained spots at the nuclear perimeter, and there is an elevated level of cytoplasmic staining.

FIG. 2. Structural alterations of the nuclear envelope in acc1-7-1. Transmission electron micrographs of acc1-7-1 cells incubated at the permissive or the restrictive temperature are shown. acc1-7-1 (YRXS12) was incubated at either 23°C (A) or 37°C (B to D) for 5 h and processed for transmission electron microscopy. At 37°C, the inner (I) and outer (O) nuclear membranes often separate (asterisks), “islands” accumulate between the two membranes (open stars in panels B and D), and protuberances are seen extending from the inner membrane into the intermembrane space (arrowheads in panel C). Small, smooth vesicles near the nuclear pores are indicated (V) in panel B. The arrows indicate nuclear pores. N, nucleus. Bar in panels A and D, 0.6 mm; bar in panel B, 0.3 mm; bar in panel C, 0.4 mm.
At the electronmicroscopic level, immunogold labeling is seen both at intact NPCs and at the base of the protuberances of the inner nuclear membrane (Fig. 3B).

**MTR7** encodes acetyl-CoA carboxylase, is allelic to **ACC1/FAS3**, and is essential for growth. The temperature-sensitive growth phenotype of **acc1-7-1** was complemented by transformation with a yeast genomic library. To determine whether the insert of the complementing plasmid, pRXS5, maps to the region responsible for temperature sensitivity, a linkage analysis was performed. **URA3** was targeted to the genomic site.
FIG. 4. acc1-7-1 and a loss-of-function allele of ACC1 are not rescued by fatty acid supplementation or by an enzymatically inactive mutant allele (K735R) of acetyl-CoA carboxylase. (A) The temperature-sensitive growth phenotype of acc1-7-1 is not rescued by addition of fatty acids to the medium. Wild-type (wt) and acc1-2150, acc1-7-1 (YRXS12), and acc2-3826 mutant cells were streaked on YEPD or fatty acid-supplemented YEPD plates (YEPD-FAS) and incubated at 23, 35, or 37°C. None of the mutants grows at 35 or 37°C on YEPD. At 35°C, some degree of rescue of acc1-7-1 on YEPD-FAS plates is observed, but at 37°C, no growth of acc1-7-1 on either YEPD or YEPD-FAS plates is visible. acc1-2150 does grow at 37°C on YEPD-FAS. Growth of acc2-3826 at 37°C is severely reduced, even when the cells are grown on YEPD-FAS plates. The two fatty acid auxotrophic strains were isolated as temperature sensitive for growth at 35°C (Mishina et al. [55]).
Inhibition of fatty acid or ergosterol synthesis does not affect the structure of the nuclear envelope. Malonyl-CoA is a two-carbon-unit donor for de novo fatty acid synthesis (Fig. 1). It may also be a substrate for acetocarboxyl-CoA synthesis and thus may participate in sterol biosynthesis (33, 43). We therefore asked whether inhibition of de novo fatty acid or sterol synthesis results in an ultrastructural phenotype similar to that of acc1-7-1 or accumulation of poly(A)\(^+\) RNA. Wild-type cells were treated for 4 h with cerulenin to inhibit fatty acid synthesis (61), lovastatin to inhibit hydroxymethylglutaryl-CoA reductase (1, 11), or zaragolic acid to inhibit squalene synthase (6). These drug-treated cells do not display perturbation of the nuclear envelope comparable to what is observed in acc1-7-1, nor do they exhibit nuclear accumulation of poly(A)\(^+\) RNA (not shown). Furthermore, acc1-7-1 did not grow at 37°C on media supplemented with sterols under conditions which rescue sterol synthesis mutants (e.g., erg1 [39]). Thus, the acc1-7-1 phenotype is not simply the result of inhibition of fatty acid or sterol synthesis.

The fatty acid auxotrophic acc1-2150 strain also does not show ultrastructural changes during 4 h at 37°C or nuclear accumulation of poly(A)\(^+\) RNA, while a low percentage of acc2-3826 mutant cells does accumulate poly(A)\(^+\) RNA in the nucleus (not shown).

Overexpression of ACC1. In mammalian cells and possibly also in yeast cells, the activity of acetyl-CoA carboxylase is elaborately regulated (29, 90). To study the effects of overexpression of ACC1, we constructed a haploid strain in which a CEN plasmid carries a copy of ACC1 under GAL1 control (YRXS73 [see Materials and Methods]). Galactose induction of this strain does increase Acc1p levels, as judged by Western blotting (not shown). This strain cannot be maintained in galactose-containing liquid medium at 37°C; however, during the early period of induction, a striking accumulation of filamentous material nearly fills the cytoplasm without altering the nuclear envelope (Fig. 5). These filaments are likely to be Acc1p, since they are absent in glucose medium and since purified avian and bovine acetyl-CoA carboxylase form polymeric filamentous helical ribbons and paracrystals composed of staggered laterally packed filaments (48, 53). Higher magnification of the filaments reveals a similar association into paracrystals. The subunit spacing along the filament axis is comparable to that found for the avian enzyme, ~15 nm.

Subcellular localization of Acc1p. A myc epitope-tagged version of the enzyme was generated (pRXS72) and used to determine its subcellular localization. The tagged version of Acc1p complements the null allele (YRXS65), indicating that it is functional and hence is likely to localize as the wild-type enzyme. Staining of diploid cells which carry the tagged allele (in a null background) with an anti-myc antibody reveals cytoplasmic staining (Fig. 6). Not all of the cells in a given field, however, are stained with the same intensity. There may be significant differences in the cytoplasmic concentration of the protein; however, fixation may be critical. A rabbit antihistone antibody stained all cell nuclei uniformly (not shown).

To obtain additional evidence for the cytoplasmic localiza-
tion of Mtr7p, nuclear and cytoplasmic fractions of YRXS65 were prepared and analyzed by Western blotting. Unexpect-
edly, the majority of the tagged protein was recovered in the “nuclear” pellet, possibly because of its filamentous nature (not shown).

Analysis of fatty acid composition of acc1-7-1. Since the fatty acid auxotrophic acc1-167 mutant does elongate fatty acid chains (C₁₃ to C₁₈), it was proposed that malonyl-CoA is not critical in yeasts (67), unlike in other organisms (15). It is now evident that the fatty acid auxotrophic alleles of acc1 do synthesize limited amounts of malonyl-CoA (data not shown). We therefore have investigated the fatty acid composition of the wild type, acc1-7-1, and conditional fatty acid auxotrophic acc1 mutants. Very-long-chain fatty acids (C₁₆₅ and OH C₁₈₃) are mainly components of sphingolipids, which are essential in yeast cells (4₉, 6₅, 8₅).

A mixture of glycerophospholipids and sphingolipids (3₂, 5₀) was extracted and analyzed (Table 3). Gas-liquid chroma-
tography analysis demonstrates major changes in the fatty acid composition of acc1-7-1. In comparison with the wild type, acc1-7-1 accumulates a significant amount of myristic acid (C₁₄ atoms), and palmitoleic acid (C₁₆₁) is the major unsaturated fatty acid, at the expense of oleic acid (C₁₈₁), even at 2₃°C. The ratios of C₁₄:C₁₆:C₁₈ fatty acids are 0.0₄:1.0₇ in the wild type and 0.1₄:1:0.₃ in acc1-7-1. At 3₇°C, most strikingly, C₂₆ fatty acid is reduced by 4₀%, i.e., to about 3₀% of the wild-type level under these cultivation conditions. Thus, fatty acid chain length is dramatically impaired, and the fatty acids are shifted towards a shorter chain length. The degree of unsaturation, on the other hand, is unaffected by the mutation.

To test whether accumulation of myristic acid might be det-
imental, we have analyzed the growth and fatty acid compo-
sition of wild-type cells and acc1-7-1 and acc1 mutant cells on plates supplemented with myristic acid (Table 4). Growth of

FIG. 5. Cytoplasmic filamentous structures accumulate upon overexpression of Acc1p. Transmission electron micrographs of cells which express ACC1 under control of the GAL1 promoter are shown. YRXS73 was grown in raffinose-containing medium at 2₃°C and then was transferred to YEPD-glucose (A) or YEPD-galactose (B to D) for 1₂ h at 3₇°C. Accumulation of filamentous material is observed in the cytoplasm of cells which were grown in galactose-containing medium (arrowheads in panel B) but not in cells grown in glucose-containing medium (A). The staggered lateral packing of filaments into higher-ordered paracrystals is visible at a higher magnification (C and D). Subunits spaced at regular intervals of about 1₅ nm along the axis of the filaments are indicated. n, nucleoplasm; c, cytoplasm. Bars in panels A and B, 0.6 mm; bars in panels C and D, 0.₁₄ mm.
Fatty acid composition after culture with myristate

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*Cells were grown in medium supplemented with myristic acid for 21 h at 24°C. The cultures were split, a fraction was maintained at 24°C, and the second fraction was shifted to 37°C for 1 h. Total lipids were extracted and subjected to alkaline hydrolysis, and fatty acids were converted to methyl esters by BF3-catalyzed methanolysis. Fatty acid methyl esters were separated by gas-liquid chromatography as described in Materials and Methods. Myristic acid was excluded from the calculation since absorption to the surface of the cells cannot be excluded (Kohlewein and Paltauf [44]).

**DISCUSSION**

**acc1-7-1** is characterized by severe structural alterations of the nuclear envelope. One way to rationalize these changes is to postulate that destabilization of the junction between the NPCs and the outer membrane occurs, which generates NPC-derived protuberances and membrane vesicles within the intermembrane space. These changes are distinct from other alterations of NPCs and the nuclear envelope which have been previously reported. For example, the distribution and size of nuclear pores change during the cell cycle (75, 89). Moreover, mutation of individual nuclear pore proteins can cause clustering of NPCs (Nsp1p [58] and nup133/rat3 and nup159/rat7 [21, 24, 51]), intranuclear annulate lamellae and “herniated NPCs” (nup116 [86]), grape-like clusters of NPC herniations (nup145 [87]), and impressive elaborations of the undilated nuclear membrane into the cytoplasm (nup1 [9]).

**ACC1** is essential, and the temperature-sensitive phenotype of **acc1-7-1**, like the **ACC1** disruption, is not rescued by fatty acid supplementation. This indicates that acetyl-CoA carboxylase harbors two functions, only one of which is required for de novo fatty acid synthesis. To determine whether the enzymatic activity of acetyl-CoA carboxylase rather than some other, possibly structural, function of **ACC1** is essential (e.g., as a component of filaments associated with NPCs), an enzymatically inactive point mutant version of the enzyme was constructed and tested for complementation of the null allele and **acc1-7-1**. The failure of this mutant protein to complement, even in the presence of fatty acid supplement, strongly suggests that its known enzymatic activity—the synthesis of malonyl-CoA—is an essential function of **ACC1**. The apparent paradox, in comparison with the fatty acid auxotrophic phenotype of the wild-type and mutant cells at 23°C was not affected by myristic acid (data not shown), and **acc1-2150** mutants tolerated even larger amounts of shorter-chain fatty acids (18% C14:1 in **acc1-2150** versus 14% C14:1 in **acc1-7-1**), excluding the possible toxicity of accumulated C14 and C16 fatty acids. Both **acc1-7-1** and **acc1-2150** contain a higher proportion of C14 and C16 fatty acids than the wild type does, consistent with the notion that fatty acid chain length distribution depends on Acc1p activity. In contrast, the content of C26 fatty acid remained constant in **acc1-7-1** at 37°C, but 50% reduction was observed with **acc1-7-1** after a shift to the restrictive temperature for 1 h. Since **acc1-2150** is a temperature-sensitive fatty acid auxotroph that remains viable when supplemented with myristic acid, we propose that sufficient malonyl-CoA is synthesized in **acc1-2150** under restrictive conditions to support elongation and produce C26 fatty acids. This residual activity is strikingly reduced in **acc1-7-1** at 37°C.

**TABLE 3. Fatty acid composition**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Wild type</th>
<th>acc1-7-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24°C</td>
<td>37°C</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>C14:0 + C14:1</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>C16:0</td>
<td>13.5</td>
<td>13.0</td>
</tr>
<tr>
<td>C16:1</td>
<td>41.5</td>
<td>30.4</td>
</tr>
<tr>
<td>C16:0 + C16:1</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.8</td>
<td>6.1</td>
</tr>
<tr>
<td>C18:1</td>
<td>33.5</td>
<td>42.9</td>
</tr>
<tr>
<td>C18:0 + C18:1</td>
<td>0.68</td>
<td>1.10</td>
</tr>
<tr>
<td>C26:0</td>
<td>2.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Others</td>
<td>3.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Ratio*</td>
<td>4.02</td>
<td>3.55</td>
</tr>
</tbody>
</table>

*Cells were grown on YPD medium for 21 h at 24°C. The cultures were split, and a fraction was maintained at 24°C, and the second fraction was shifted to 37°C for 4 h. Total lipids were extracted and subjected to alkaline hydrolysis, and fatty acids were converted to methyl esters by BF3-catalyzed methanolysis. Fatty acid methyl esters were separated by gas-liquid chromatography as described in Materials and Methods.

*Sum of percent fatty acid composition for indicated carbon chain length relative to the sum for C16:0 plus C16:1.

*Ratio of composition for double-bond-containing chains to that for chains without double bonds.
previously available *acc1* alleles, appears to be due to the leakiness of available *acc1* mutants, which thus provide enough malonyl-CoA to maintain the essential pathway (31). This notion is supported by the low frequency with which fatty acid auxotrophic *acc1* mutants are recovered, which in many cases make full-length enzyme with residual overall acetyl-CoA carboxylase activity (55). It will be of interest to learn whether the phenotype of *acc1-7-1* reflects a structural alteration or absence of Acc1p at the restrictive temperature.

The *acc1-7-1* phenotype does not simply result from the absence of long-chain (*C*16 and *C*18) fatty acid synthesis, judging from the morphological analysis of two fatty acid auxotrophic strains (*acc1-2150* and *acc2-3826*) and a wild-type strain treated with cerulenin. The observation that a limited fraction of *acc2-3826* cells (defective in apoenzyme-biotin ligase) accumulates nuclear poly(A)*+* RNA supports the hypothesis that the phenotype of *acc1-7-1* depends on the activity of acetyl-CoA carboxylase. The fact that no stronger nuclear phenotype is seen in *acc2-3826* is best explained by (i) the presumed leakiness of the allele and (ii) the possibility that the mutants die or stop RNA synthesis because of reduced activity of other biotin-dependent enzymes (e.g., pyruvate carboxylase) before they can accumulate RNA (55).

Why is malonyl-CoA required? It may be a substrate for polyketide synthesis (12, 76) or for sterol biosynthesis (33, 43), or it may serve as a two-carbon unit in acyl-chain elongation (26). We investigated a possible requirement for malonyl-CoA in these pathways by determining whether their inhibition results in an *acc1-7-1* phenotype.

Polyketides are secondary metabolites which are preferentially synthesized during cell differentiation (for a review, see reference 34). To our knowledge, there is no evidence of the existence of this class of compounds in yeasts. The yeast mitochondrial β-ketoacyl synthase shows significant homology to enzymes involved in polyketide synthesis in other organisms; however, it is not essential and is therefore unlikely to be important in the present context (30). The possibility that malonyl-CoA is required only when spores switch to vegetative growth has been previously tested by transformation of haploid cells with an integrative loss-of-function construct. No viable fatty acid-requiring *acc1* disruptants were obtained (31).

Malonyl-CoA has repeatedly been proposed to be a substrate for the synthesis of acetoacetyl-CoA and hence to participate in sterol synthesis (33, 43). Nevertheless, inhibition of sterol synthesis in wild-type cells did not alter the structure of the inner (im) nuclear membrane or cause poly(A)*+* RNA accumulation, nor did supplementation of *acc1-7-1* with ergosterol complement temperature sensitivity under aerobic or anaerobic conditions (not shown).

A possible lethal accumulation of acetyl-CoA in *acc1-7-1* has also been tested, since de novo fatty acid biosynthesis normally provides a major sink for this compound. Intracellular levels of acetyl-CoA, however, appeared not to be higher in *acc1-7-1* than in wild-type cells (not shown).

Analysis of fatty acid chain length distribution in *acc1-7-1* indicates that Acc1p and/or its products are needed for fatty acid chain elongation. For example, unlike the wild type, *acc1-7-1* and *acc1-2150* cells contain more *C*16 than *C*18 fatty acids and contain impressive quantities of *C*20 fatty acids. In striking contrast to *acc1-2150*, however, *C*26 fatty acids are significantly reduced in *acc1-7-1* at the restrictive temperature. Such very-long-chain fatty acids are found in the ceramide moiety of sphingolipids, which are essential (49, 65, 85). Although the majority of sphingolipids localize to the yeast plasma membrane (32, 59, 63), some are also present in the nuclear membrane (3). The functional significance of the very long chain length of fatty acids of sphingolipids is underlined by the observation that strains which survive without sphingolipids produce novel *C*26 fatty acid-containing glycosphingolipids, which structurally mimic sphingolipids (50). We have found considerable amounts of *C*26 fatty acid in lipids extracted from nuclear fractions of wild-type yeast cells; however, the nature of the lipids harboring this fatty acid has not been identified (43a).

Our observations thus argue that malonyl-CoA-dependent very-long-chain fatty acid synthesis is inhibited in *acc1-7-1* and that the reduction of these very-long-chain fatty acids results in a striking alteration of the nuclear envelope. Unfortunately, however, all attempts to rescue the essential function of *ACC1* by supplementation of mutant cells with malonyl-CoA or very-long-chain fatty acids were unsuccessful, most likely because of poor uptake and/or activation of such compounds (23, 31, 40, 44).

Why is the nuclear envelope affected upon depletion of very-long-chain fatty acids in *acc1-7-1*? There is little reason to implicate ceramide-based lipid glycans, which act as membrane anchors for proteins, since such units have been described only at the plasma membrane (16, 71, 80). It is possible that integral membrane proteins of the NPC, at least one of which can regulate transport via its endoplasmic reticulum luminal domain (25, 91), have stringent lipid requirements, as do other integral membrane proteins (8, 57); i.e., the lipid environment of NPC proteins may be critical for their function.

We nevertheless propose that there may be a direct requirement for membrane lipids substituted with very-long-chain fatty acids in stabilizing the NPC at the pore-membrane interface. This suggestion is based on observations of the relationship between the polar head group and acyl chain length of lipids and the type of membrane structure (bilayer, La phase or hexagonal phase, HII), membrane curvature, and thermodynamic stability. Head group size and surface exposed to the aqueous phase relative to the volume and length of the hydrocarbon portion determine the molecular shape of membrane lipids (37). Moreover, very-long-chain fatty acids in mem-
branes perturb bilayer structure and promote curvature of the membrane, since the extended hydrophobic tail requires interdigitation with the opposite membrane leaflet (36) and occupies the free volume in the hydrophobic core of the membrane bilayer (52). Moreover, arachidonic acid (C20:4 fatty acid) esterified in phosphatidylethanolamine, which has a small head group area, promotes highly negative membrane curvature (18). If the supply of very-long-chain fatty acids becomes limiting, as is proposed to occur in acc1-7-1, one might therefore predict that the membrane bend would become more and more unstable (illustrated in Fig. 7), eventually break apart and possibly separate the nuclear membrane from the NPC.

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