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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 (C_{26} 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, 43a, 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 Opi1p; i.e., it is under the general control of phospholipid synthesis (14, 31).

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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 enzyme which is necessary for producing active Acc1p, biotin: 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 C_{16} to C_{18} 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% KPO_4, 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% K-PO_4, 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 constructs. 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 *Sph*I fragment of pRXS5 containing the entire *ACC1* gene

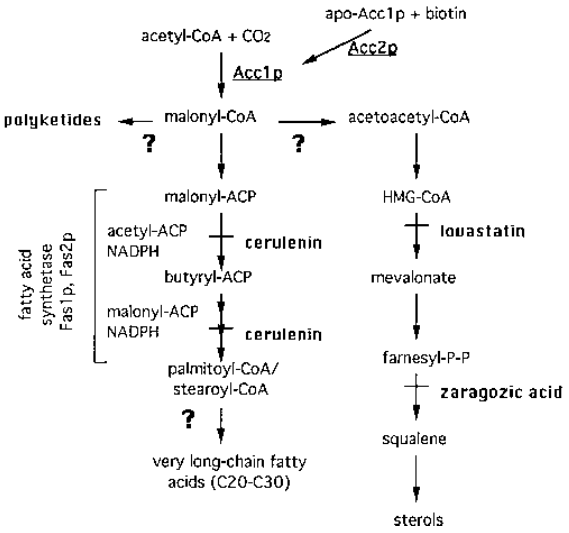

FIG. 1. Metabolic pathways of fatty acid synthesis and malonate metabolism. The question marks indicate steps in which malonate may be involved (see text). HMG-CoA, hydroxymethylglutaryl-CoA.

was cloned into a centromeric plasmid (pFL38 [10]) to yield pRXS53, which does complement the temperature-sensitive and mRNA accumulation phenotype of *acc1-7-1* at 37°C.

Partial DNA sequence was determined by the dideoxy chain termination method (70) with [α-^{35}S]dATP (1,000 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) and Sequenase version 2.0 (United States Biochemicals Corp., Cleveland, Ohio). Unique oligonucleotide primers employed in this study were synthesized by Integrated DNA Technologies (Coralville, Iowa). DNA and predicted protein sequences were compared with those in the GenBank and EMBL databases by the BLAST and FASTA algorithms (4, 64).

Disruption of *ACC1* was performed by integrative DNA transformation (68) as follows. The *Bgl*II fragment containing the *LEU2* gene from PS118 (79) was ligated into *Bgl*II-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 *Sst*I 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 *Bgl*II- and *Ecl*136II-digested genomic DNA, probed with the *Ecl*136II 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 *Bam*HI-*Sal*I fragment of pRXS16 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 TTG CAT TCT CAT AAC TTC 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 *Bgl*II-*Cla*I fragment of pRXS76 was subsequently cloned into *Bgl*II-*Cla*I-cut pRXS16, yielding pRXS77, from which the *Bam*HI-*Sal*I fragment was cloned into *Bam*HI-*Sal*I-cut pRXS74 to yield pRXS85. Moving the *Bam*HI-*Eag*I fragment from pRXS85 to *Bam*HI-*Eag*I-cut pRXS73 produced pRXS89, which was used in the red/white sectoring assay to determine whether the K735R allele can complement the null allele.

Expression of Acc1p was put under transcriptional control of the *GAL1* promoter by cloning a *Sal*I-digested PCR-generated fragment which covers the 5′ part of *ACC1* (sequence positions −47 to 710) into *Sma*I-*Sal*I-digested pGP316, yielding pRXS71. The 3′ part of *ACC1* was then moved as an *Sal*I-*Ecl*136II fragment into *Sal*I-*Ecl*136II-cut pRXS71, generating pRXS81. This plasmid was transformed into an *acc1-7-1*::*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.

TABLE 1. Yeast strain genotype and construction[a]

Strain	Relevant genotype	Source or reference
acc1-2150	*MAT*α *acc1-2150*	55
acc2-3826	*MAT***a** *acc2-3826*	55
YRXS9	*MAT*α *mtr2-1 acc1-7-1 ura3-52 leu2-*Δ*1 lys2-801*	Segregant of cross between *acc1-7-1* (T229 [41]) and *mtr2-1* (T5 [42])
YRXS11	*MAT***a** *acc1-7-1 ade2-101 ura3-52 leu2-*Δ*1 lys2-801 acc1-7-1*	Segregant of backcross between T229 (41) and YPH259 (78)
YRXS12	*MAT*α *acc1-7-1 ura3-52 his3-*Δ*200 leu2-*Δ*1 lys2-801*	Segregant of backcross between *acc1-7-1* (T229 [41]) and YPH259 (78)
YRXS22	*MAT*α *ade2-101 ura3-52 his3-*Δ*200 leu2-*Δ*1 lys2-801 ACC1*::*URA3*	Integrative transformation of YPH259 (78) with *Nhe*I-linearized pRXS49
YRXS23	*MAT***a**/*MAT*α *ade2-101*/*ade2-101 ura3-52*/*ura3-52 his3-*Δ*200*/*his3-*Δ*200 trp1-*Δ*63*/*trp1-63 leu2-*Δ*1 lys2-801*/*lys2-801 ACC1*/*acc1-7-1*::*LEU2*	Integrative transformation of YPH501 (78) with *Sst*I-digested pRXS57
YRXS64	*MAT***a** *ade2-101 ura3-52 his3-*Δ*200 trp1-*Δ*63 leu2-*Δ*1 lys2-801 acc1-7-1*::*LEU2* pRXS73(*HIS*)	Transformation of YRXS23 with pRXS73, sporulation, resulting viable segregant
YRXS65	*MAT***a**/*MAT*α *ade2-101*/*ade2-101 ura3-52*/*ura3-52 his3-*Δ*200*/*his3-*Δ*200 trp1-*Δ*63*/*trp1-63 leu2-*Δ*1*/*leu2-*Δ*1 lys2-801*/*lys2-801 acc1-7-1*::*LEU2*/*acc1-7-1*::*LEU2* pRXS72(*HIS*)	Transformation of YRXS23 with pRXS72, sporulation, two resulting viable segregants crossed to produce diploid
YRXS66	*MAT***a**/*MAT*α *ade2-101*/*ade2-101 ura3-52*/*ura3-52 his3-*Δ*200*/*his3-*Δ*200 trp1-*Δ*63*/*trp1-63 leu2-*Δ*1*/*leu2-*Δ*1 lys2-801*/*lys2-801 acc1-7-1*::*LEU2*/*acc1-7-1*::*LEU2* pRXS73(*HIS*)	Transformation of YRXS23 with pRXS73, sporulation, two resulting viable segregants crossed to produce diploid
YRXS67	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 acc1*::*URA3* pRXS72(*HIS*)	Transformation of W303D-Acc1p$^{\Delta U}$ (31) with pRXS72, sporulation, resulting viable segregant
YRXS69	*MAT***a**/*MAT*α *acc1-7-1*/*acc1-7-1 ade2-101*/+ *ura3-52*/*ura3-52 leu2-*Δ*1*/*leu2-*Δ*1 his3-*Δ*200*/+ *lys2-801*/*lys2-801*	*acc1-7-1* diploid resulting from the cross of YRXS11 and YRXS12
YRXS73	*MAT***a** *ade2-101 ura3-52 his3-*Δ*200 trp1-*Δ*63 leu2-*Δ*1 lys2-801 acc1-7-1*::*LEU2* pRXS81(*URA*)	Transformation of YRXS23 with pRXS81, sporulation, resulting viable segregant
YRXS75	*MAT*α *ade2 ade3 ura3 his3 trp1 leu2 acc1-7-1*::*LEU2* pRXS80(*URA*)	Cross of YRXS64 and pRXS80 transformed CH1462 (46), selected for loss of pRXS73
YRXS80	*MAT*α *ade2 ade3 ura3 his3 trp1 leu2 acc1-7-1*::*LEU2* pRXS80(*URA*) pRXS73(*HIS*)	YRXS75 transformed with pRXS73
YRXS81	*MAT*α *ade2 ade3 ura3 his3 trp1 leu2 acc1-7-1*::*LEU2* pRXS80(*URA*) pRXS89(*HIS*)	YRXS75 transformed with pRXS89

[a] 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-7Zf was obtained from Promega Biotec.

TABLE 2. Plasmid construction[a]

Designation	Marker(s)	Comments	Source
YCp50-Acc1p	CEN *URA3 ACC1*	*ACC1*-containing plasmid	Hasslacher et al. (31)
pRXS5	2μm *URA3 ACC1*	*ACC1* isolated from YEp24-based genomic library	This work
pRXS16	CEN *URA3*	3.7-kb *Bam*HI-*Sal*I fragment from pRXS5 into *Bam*HI-*Sal*I site of pRS316	This work
pRXS49	*URA3*	*Sph*I fragment of pRXS5 into *Sph*I-cut pRS306	This work
pRXS51	*ACC1*	*Sph*I fragment of pRXS5 into *Sph*I site of pGEM-7Zf(+)	This work
pRXS53	CEN *URA3 ACC1*	*Sph*I fragment of pRXS5 into *Sph*I site of pFL38	This work
pRXS57	*acc1-7-1*::*LEU2*	*Bgl*II fragment containing *LEU2* from pS118 into *Bgl*II-digested pRXS51	This work
pRXS60	*ACC1*^{myc}	myc-tagged derivative of pRXS51 (see "Experimental procedures")	This work
pRXS72	CEN *HIS3 ACC1*^{myc}	*Ecl*136II fragment of pRXS60 into *Sma*I site of pRS313	This work
pRXS73	CEN *HIS3 ACC1*	*Ecl*136II fragment of pRXS51 into *Sma*I site of pRS313	This work
pRXS74	CEN *URA3 ACC1*	*Ecl*136II fragment of pRXS51 into *Xho*I-fill in-*Ecl*136II-cut pRS316	This work
pRXS80	CEN *URA3 ADE3 ACC1*	*Ecl*136II fragment of pRXS51 into *Sma*I site of pCH1122	This work
pRXS81	CEN *URA3 GAL1-ACC1*	Expression of *ACC1* under control of *GAL1* promoter (see Materials and Methods)	This work
pRXS85	CEN *URA3*	K735R mutant of *ACC1* in pRXS74 (see Materials and Methods), *acc1*^{K735R}	This work
pRXS87	CEN *URA3 GAL1-ACC1*^{myc}	myc-tagged derivative of pRXS81 (see Materials and Methods)	This work
pRXS89	CEN HIS *acc1*^{K735R}	*Bam*HI-*Eag*I fragment of pRXS85 into *Bam*HI-*Eag*I-cut pRXS73	This work

[a] 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-7Zf 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 *Sal*I 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 the insertion was confirmed by DNA sequencing and PCR analysis. The overhangs destroy the original *Sal*I site, and a new *Hin*dIII site is generated (underlined):

Sal-myc1, 5′-TC GAA CAA AAG CTT ATT TCT GAA GAA GAC TTG G-3′
Sal-myc2, 3′-T GTT TTC GAA TAA AGA CTT CTT CTG AAC CAG CT-5′

Cell fractionation and Western blot (immunoblot) analysis. Subcellular fractionation was performed essentially as described previously (54). Cells harboring myc-tagged (YRXS65) and untagged (YRXS66) versions of *ACC1* were grown in synthetic minimal medium-Leu-His at 23°C and harvested during the exponential growth phase. Spheroplasts were prepared from 800-ml cultures with Zymolyase 100T (ICN Biomedicals, Inc., Irvine, Calif.) at 0.5 mg/ml, resuspended in 1.2 M sorbitol–50 mM KPO_4 (pH 6.5), and centrifuged through a cushion of 0.6 M sorbitol–0.6 M sucrose–2% Ficoll 400 in 20 mM KPO_4 (pH 6.5). Pellets were resuspended in homogenization buffer (20% Ficoll 400, 20 mM KPO_4 [pH 6.5], 1 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, 2 mg of chymostatin per ml, 10 mg of aprotinin per ml, and 2 mg of leupeptin per ml). Homogenization was performed at 4°C with a glass-on-glass homogenizer until the majority of spheroplasts were broken as visualized by 4′,6-diamidino-2-phenylindole (DAPI) staining. Unlysed cells and cell debris were removed by centrifugation at 4,000 × *g* (Beckman type 70Ti rotor [7,300 rpm]) for 10 min at 4°C. Nuclei were then pelleted at 16,500 × *g* (Beckman type 70Ti rotor [15,000 rpm]) for 30 min and resuspended in homogenization buffer.

Total yeast cell extracts were made as described previously (92) and electrophoresed on sodium dodecyl sulfate–6% polyacrylamide gels (47) before electrophoretic transfer to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, Mass.). Blots were probed with a 1:1,000 dilution of anti-myc (9E10 [22]; Cambridge Research Biochemicals, Wilmington, Del.) or anti-NOP1 (A66.1 [5]) monoclonal antibodies (MAbs) in TBST (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 0.05% Tween 20) plus 1% bovine serum albumin (BSA). After TBST washes, antibody binding was detected with an alkaline phosphatase-conjugated anti-mouse secondary antibody (Promega Corp., Madison, Wis.).

Immunofluorescence and in situ hybridization. Early-logarithmic-phase yeast cells were prepared for immunofluorescence microscopy exactly as described in reference 88. Binding of primary antibody was visualized with fluorescein isothiocyanate-, rhodamine-, or Cy3-conjugated anti-mouse or anti-rabbit immunoglobulin G (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) at a 1:200 dilution. Detection of polyadenylated RNA by in situ hybridization was performed as described previously (42). Photographs were taken with identical exposure times.

Electron microscopy. Cells were collected by centrifugation, washed, and fixed in 2% glutaraldehyde in buffer A (40 mM K_2HPO_4-KH_2PO_4 [pH 6.5], 0.5 mM $MgCl_2$) for either 30 min at room temperature or overnight at 4°C. Cell walls were then removed with Zymolyase 100T and postfixed with 2% osmium tetroxide for 30 min at room temperature. Cells were washed, transferred to 1% uranyl acetate in water at 4°C overnight, ethanol dehydrated, and embedded in LX112 resin (Polysciences, Inc.). Silver sections were cut and stained with 2% uranyl acetate at 37°C for 40 min, 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 MAb 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. Cerulenin (Sigma Chemical Co., St. Louis, Mo.) was prepared as a 1,000× stock (25 mM 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.). Zaragozic acid was prepared as a 200× stock solution in dimethyl sulfoxide and used at 62 mg/ml (6). 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 methanolysis and separated by gas-liquid chromatography on a Hewlett-Packard Ultra 2 capillary column (5% Ph Me silicone) 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

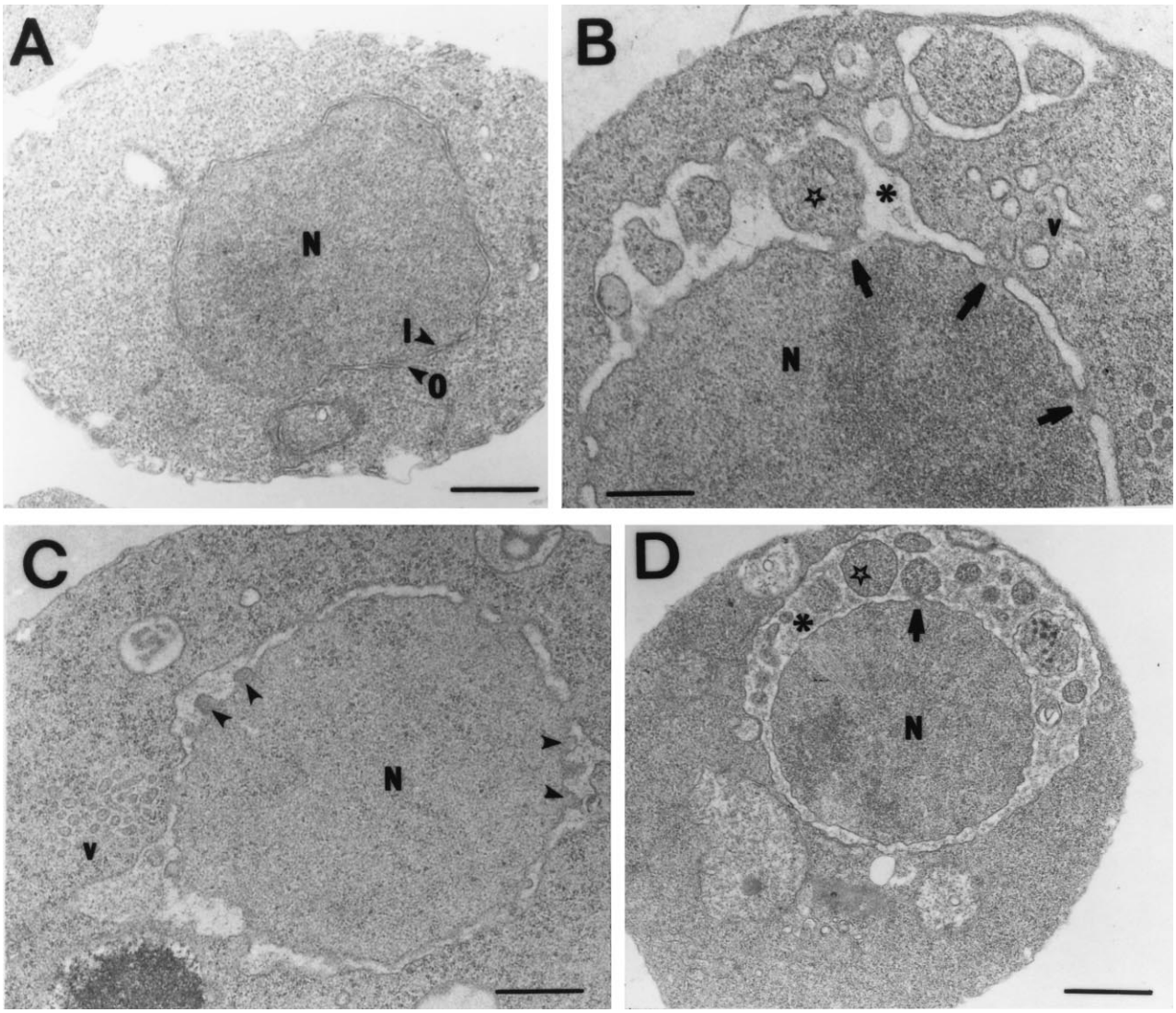

FIG. 2. Structural alterations of the nuclear envelope in *acc1-7-1*. Transmission electron micrographs of *acc1-7-1* cells incubated at the permissive or the restrictive temperature are shown. *acc1-7-1* (YRXS12) was incubated at either 23°C (A) or 37°C (B to D) for 5 h and processed for transmission 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.

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.

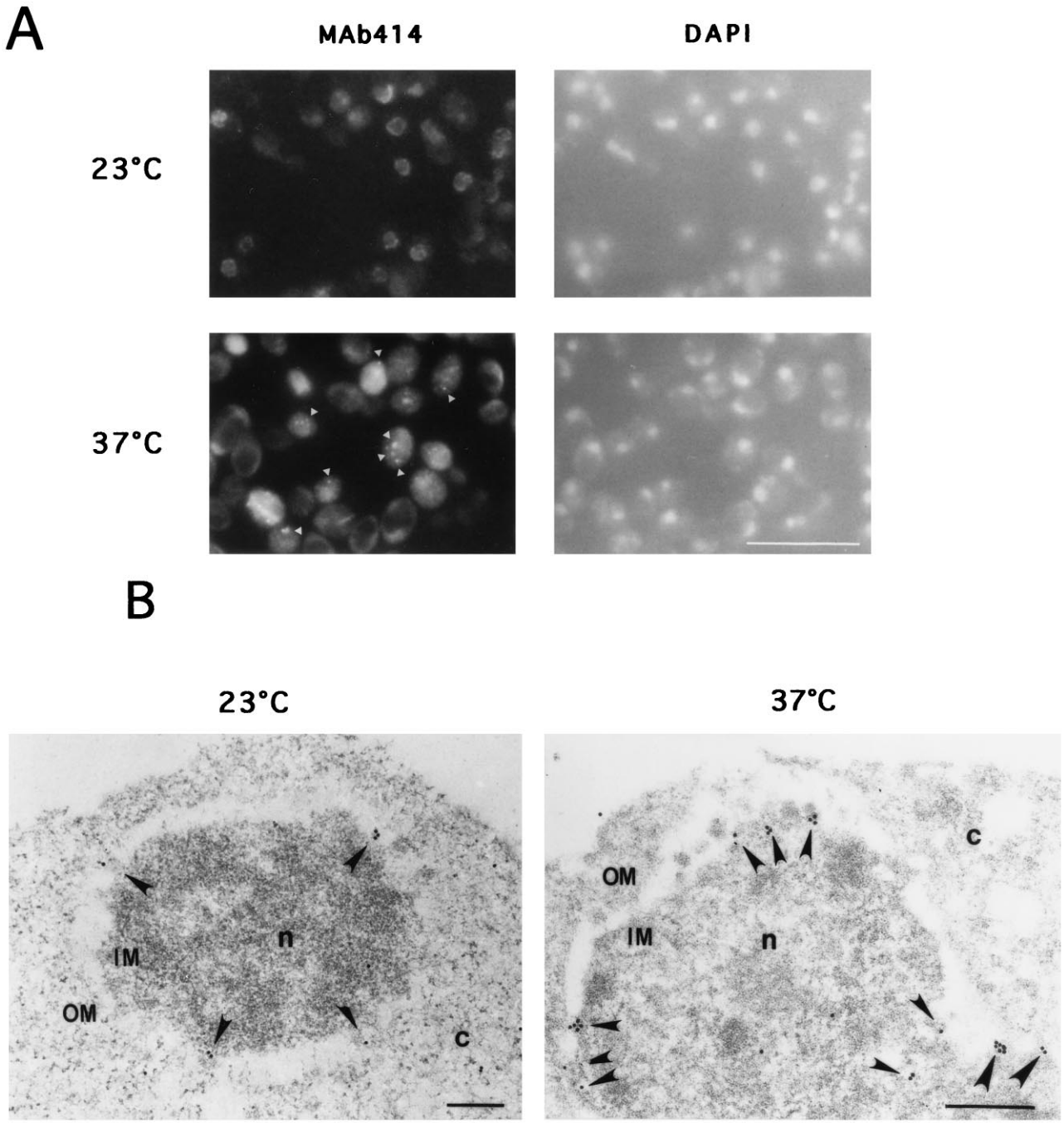

FIG. 3. Localization of nuclear pores in *acc1-7-1*. (A) Indirect immunofluorescence localization of the nuclear pores in *acc1-7-1* with MAb 414. *acc1-7-1* diploid cells (YRXS69) were incubated at either 23°C (top row) or 37°C (bottom row) for 4 h and processed for immunofluorescence. The wild-type punctate nuclear rim staining seen in cells incubated at the permissive temperature changes when cells are shifted to the restrictive temperature. The signal appears to concentrate in spots (white arrowheads), and the level of cytoplasmic staining is increased. DAPI staining of the same field of cells is shown in the right photo of each panel. In a number of cells which were incubated at the restrictive temperature (bottom panel), the MAb 414 antigen does not encircle the DAPI-stained chromatin. This noncoincidence may be caused by the disruption of pores which may be targeted for degradation. Wild-type cells prepared in parallel display punctate nuclear rim staining as observed at 23 and 37°C. Bar, 8 mm. (B) Immunogold localization of the NPCs by electron microscopy. Thin sections of *acc1-7-1* diploid cells (YRXS69), incubated at 23°C (left panel) or 37°C (right panel) for 4 h, were sequentially incubated with MAb 414 and 15-nm-diameter colloidal gold particle-coated goat anti-mouse immunoglobulin antibody. The positions of the gold particles are indicated by arrowheads. The outer nuclear membrane (OM), inner nuclear membrane (IM), nucleoplasm (n), and cytoplasm (c) are marked. Bar, 0.35 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

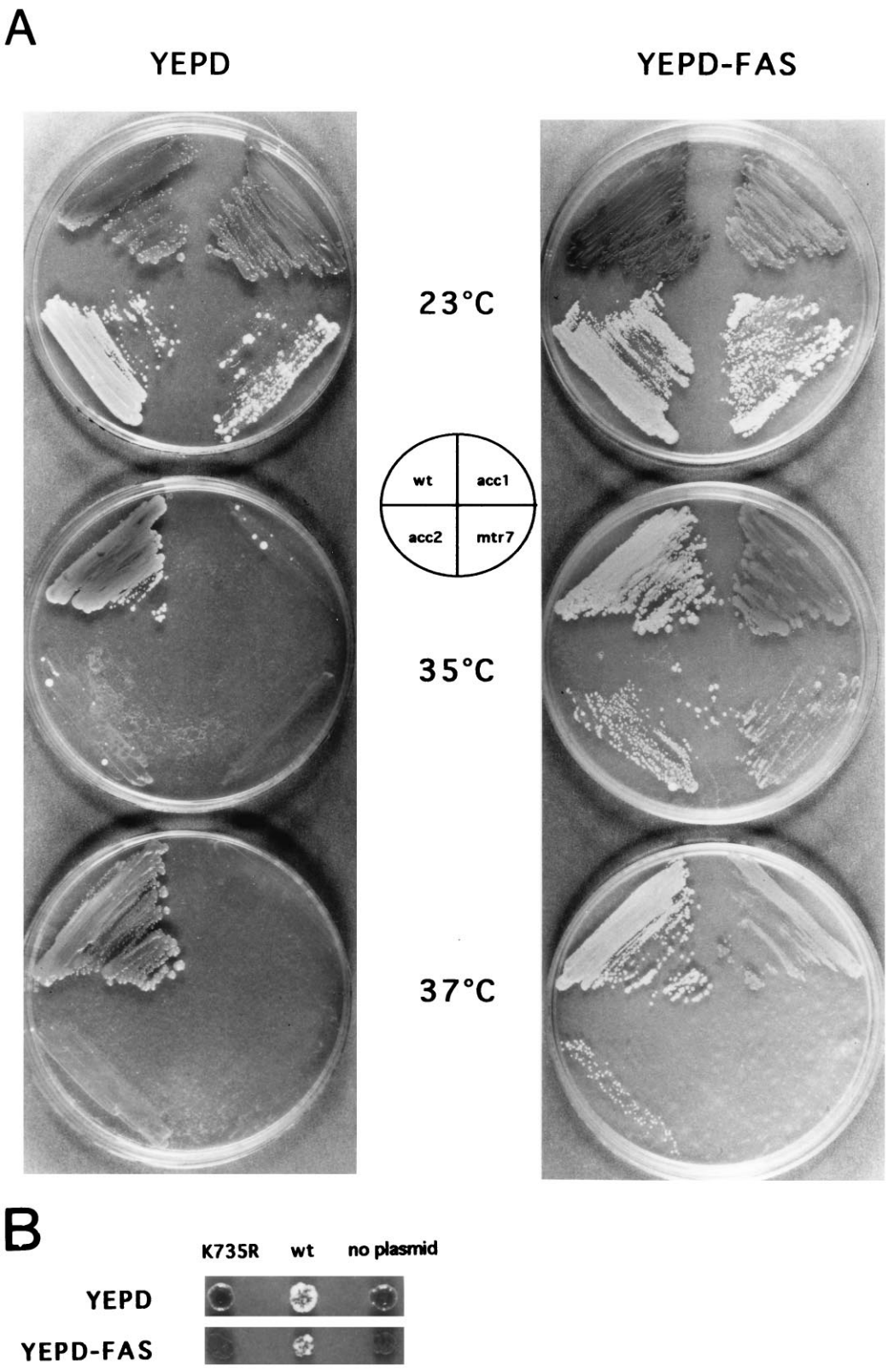

FIG. 4. *acc1-7-1* and a loss-of-function allele of *ACC1* are not rescued by fatty acid supplementation or by an enzymatically inactive mutant allele (K735R) of acetyl-CoA carboxylase. (A) The temperature-sensitive growth phenotype of *acc1-7-1* is not rescued by addition of fatty acids to the medium. 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]).

from which the insert of pRXS5 is derived (yielding the *acc1-7-1*::*URA3* allele of strain YRXS22 [see Materials and Methods]). Analysis of 16 tetrads from a cross between this strain and *acc1-7-1* (YRXS11) revealed only spores of the parental ditype, indicating that the insert of pRXS5 is tightly linked to the genomic position of the temperature-sensitive mutation. Sequence analysis of an essential part of pRXS5 revealed that the gene in question is identical to the gene encoding yeast acetyl-CoA carboxylase, *ACC1*/*FAS3* (2, 31). The gene is therefore referred to as *ACC1*, and the original mutant is referred to as *acc1-7-1* rather than *mtr7-1*. By probing a set of filters which contain clones covering most the genome of *S. cerevisiae* (66), the gene was physically mapped to the right arm of chromosome XIV, close to the centromere. These data are in good agreement with recent genetic mapping data (27). Several observations provide further proof that the gene that is defective in *acc1-7-1* is identical to *ACC1*: (i) the restriction map of pRXS5 is similar to that of *ACC1*, (ii) transformation of *acc1-7-1* with an *ACC1*-containing plasmid (YCp50-*ACC1* [31]) complements the temperature-sensitive growth phenotype of *acc1-7-1*, (iii) pRXS5 complements the lethality of an *acc1* disruption allele (*acc1*::*URA3*; YRXS67), and (iv) a heterozygous diploid generated from *acc1-7-1* and *acc1-2150* (55) is still a temperature-sensitive fatty acid auxotroph.

Using the temperature-sensitive fatty acid auxotrophic alleles of *acc1* (*acc1-2150*) and *acc2* (*acc2-3826*) as controls, we tested whether growth of *acc1-7-1* is rescued by palmitic acid supplementation. Strikingly, with *acc1-7-1*, only microcolonies are seen at 35°C, while *acc1-2150* and *acc2-3826* are fully rescued at their restrictive temperature of 35°C (Fig. 4A) (55).

ACC1 disruptants cannot live even if the medium is supplemented with fatty acids (31). Moreover, sporulation followed by tetrad dissection of an *ACC1*/*acc1-7-1*::*LEU2* diploid strain (YRXS23) on YEPD with or without palmitate supplementation did not yield any viable leucine prototrophic spores (not shown). This observation and the fact that the temperature-sensitive phenotype of *acc1-7-1* is not rescued by fatty acid supplementation indicate that Acc1p harbors a function which is independent of de novo fatty acid synthesis.

We therefore investigated whether the essential function of *ACC1* can be separated from its carboxylase activity by generating an enzymatically inactive mutant form of the protein. Lysine 735, which is conserved in all known carboxylases and undergoes biotinylation (83), was substituted by an arginine residue, generating the *acc1*^{K735R} plasmid pRXS85 (see Materials and Methods). This nonbiotinylatable mutant form of the enzyme did not complement an *acc1*::*LEU2* null allele in the presence or absence of palmitate (Fig. 4B) or rescue the temperature sensitivity of *acc1-7-1* (not shown). Even though it is formally possible that the K735R point mutation also affects a putative nonenzymatic function, e.g., the structure of the enzyme, we favor the suggestion that the essential function of Acc1p depends on its carboxylase activity and that its enzymatic product, malonyl-CoA, is required for this essential function.

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 acetoacetyl-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 zaragozic 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 nucleoplasm (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-

(B) An enzymatically deficient allele of *acc1* (K735R) does not provide the essential function of acetyl-CoA carboxylase. A red/white sectoring readout of *ade2 ade3* cells which harbor a chromosomal disruption of *ACC1* and an *ADE3*-containing extrachromosomal copy of *ACC1* (YRXS75) is shown. Cells were transformed with a plasmid containing the K735R allele of *acc1* (pRXS89; YRXS81), a wild-type copy of *ACC1* (pRXS73; YRXS80), or no additional copy of *ACC1* (no plasmid; YRXS75) and then were replica stamped on YEPD (top) or YEPD-FAS (bottom) plates. Only the colony in the middle, which harbors a second wild-type copy of *ACC1*, is capable of forming white sectors, indicating that it can lose the *ADE3*-linked copy of *ACC1*. Cells carrying the K735R allele or no additional copy of *ACC1* do not form white sectors. The absence of white sectors in the other two cases indicates that the K735R mutant does not complement the chromosomal null allele and that *ACC1* is essential even in the presence of fatty acids.

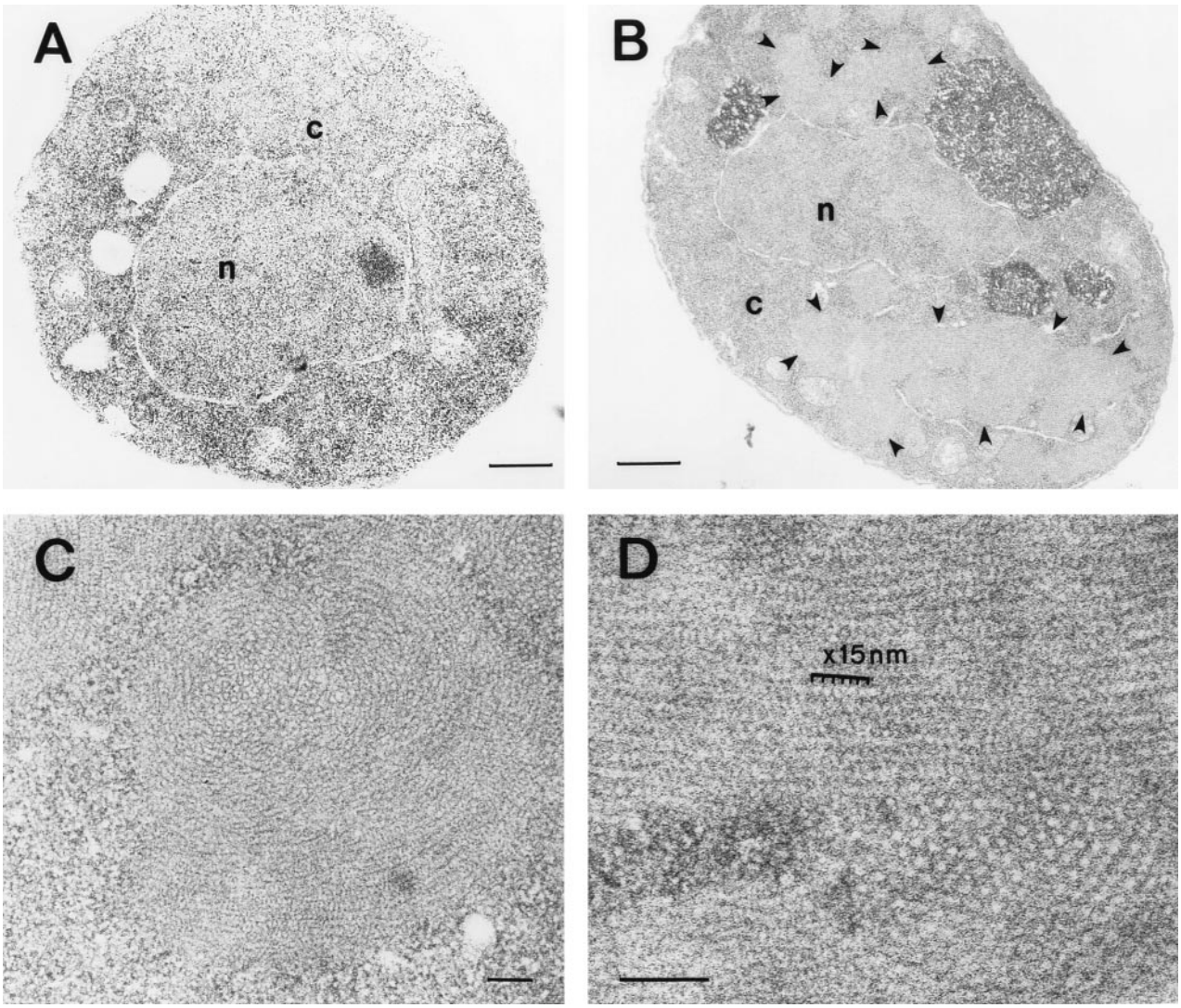

FIG. 5. Cytoplasmic filamentous structures accumulate upon overexpression of Acc1p. Transmission electron micrographs of cells which express *ACC1* under control of the *GAL1* promoter are shown. YRXS73 was grown in raffinose-containing medium at 23°C and then was transferred to YEPD-glucose (A) or YEPD-galactose (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.

tion of Mtr7p, nuclear and cytoplasmic fractions of YRXS65 were prepared and analyzed by Western blotting. Unexpectedly, 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 (C_{13} to C_{18}), 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 (C_{26} and OH C_{26}) 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 chromatography 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 (C_{14} atoms), and palmitoleic acid ($C_{16:1}$) is the major unsaturated fatty acid, at the expense of oleic acid ($C_{18:1}$), even at 23°C. The ratios of C_{14}:C_{16}:C_{18} fatty acids are 0.04:1:0.7 in the wild type and 0.14:1:0.3 in *acc1-7-1*. At 37°C, most strikingly, C_{26} 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 detrimental, we have analyzed the growth and fatty acid composition of wild-type cells and *acc1-7-1* and *acc1* mutant cells on plates supplemented with myristic acid (Table 4). Growth of

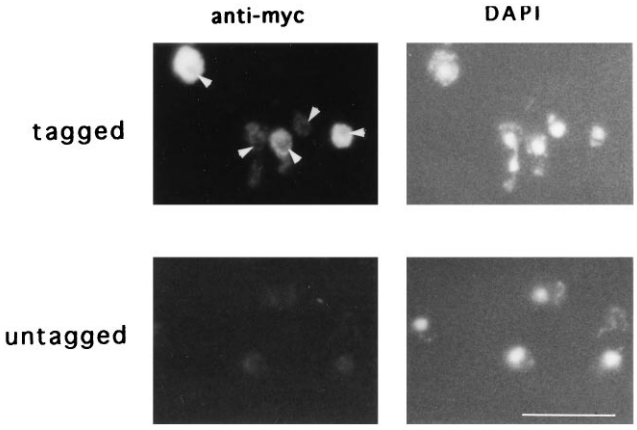

FIG. 6. Localization of epitope-tagged Acc1p. Results from immunolocalization of epitope-tagged Acc1p are shown. Immunofluorescence staining of cells carrying either a myc-tagged copy of Acc1p (YRXS65) driven by a *ACC1* promoter or an untagged copy (YRXS66) of Acc1p was performed with DAPI staining of the same field is shown in the right photo of each panel. The cytoplasmic localization of the myc-tagged Acc1p and lack of a nuclear signal (arrowheads) are indicated. Corresponding negatives were taken with identical exposure settings. To visualize background staining in YRXS65, the print had to be overexposed. Bar, 8 mm.

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% $C_{14:1}$ in *acc1-2150* versus 14% $C_{14:1}$ in *acc1-7-1*), excluding the possible toxicity of accumulated C_{14} and $C_{14:1}$ fatty acids. Both *acc1-7-1* and *acc1-2150* contain a higher proportion of C_{14} and C_{16} 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 C_{26} fatty acid remained constant in *acc1* 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 C_{26} fatty acids. This residual activity is strikingly reduced in *acc1-7-1* at 37°C.

TABLE 3. Fatty acid composition[a]

Fatty acid	Fatty acid composition (%)				Relative fatty acid composition[b]			
	Wild type		*acc1-7-1*		Wild type		*acc1-7-1*	
	24°C	37°C	24°C	37°C	24°C	37°C	24°C	37°C
$C_{14:0}$	1.5	0.7	4.2	2.9				
$C_{14:1}$	0.6	0.2	4.6	5.0				
$C_{14:0} + C_{14:1}$					0.04	0.02	0.14	0.14
$C_{16:0}$	13.5	13.0	12.4	7.2				
$C_{16:1}$	41.5	30.4	52.0	51.0				
$C_{16:0} + C_{16:1}$					1.00	1.00	1.00	1.00
$C_{18:0}$	3.8	6.1	2.3	9.6				
$C_{18:1}$	33.5	42.9	18.8	19.2				
$C_{18:0} + C_{18:1}$					0.68	1.10	0.32	0.50
$C_{26:0}$	2.1	2.5	1.4	0.9				
Others	3.4	3.3	3.3	4.1				
Ratio[c]					4.02	3.55	4.04	3.80

[a] 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.

[b] Sum of percent fatty acid composition for indicated carbon chain length relative to the sum for $C_{16:0}$ plus $C_{16:1}$.

[c] Ratio of composition for double-bond-containing chains to that for chains without double bonds.

TABLE 4. Fatty acid composition after culture with myristate[a]

Fatty acid	Fatty acid composition (%)					
	Wild type		*acc1-2150*		*acc1-7-1*	
	24°C	37°C	24°C	37°C	24°C	37°C
$C_{14:0}$						
$C_{14:1}$	8.2	5.1	18.8	17.3	14.3	13.7
$C_{16:0}$	14.4	19.8	10.3	14.2	21.4	23.0
$C_{16:1}$	45.9	42.1	48.6	47.9	41.1	42.5
$C_{18:0}$	2.5	3.3	1.1	1.6	2.3	2.5
$C_{18:1}$	25.1	25.7	13.2	14.4	12.5	13.3
$C_{26:0}$	1.3	1.4	1.2	1.3	1.3	0.6
Others	2.5	2.7	6.8	3.4	7.2	4.4

[a] 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 (Kohlwein 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

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 nuclear envelope 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_{14} 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 glycerophospholipids, 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).

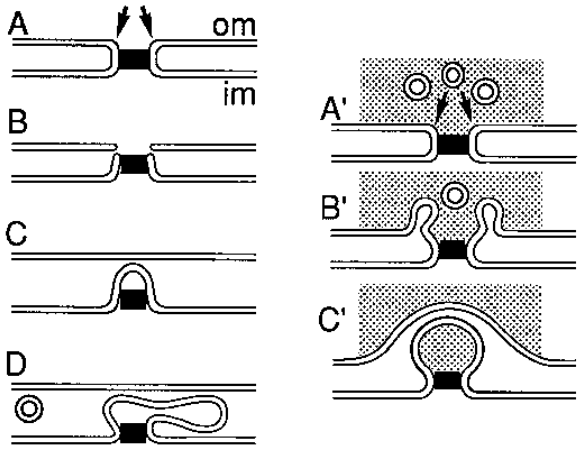

FIG. 7. Hypothetical intermediates which result in the separation of the two nuclear membranes. A wild-type pore complex anchored to the outer (om) and inner (im) nuclear membrane is outlined at the top left (A). As indicated, if very-long-chain fatty acids become limiting, the bend which connects the two membranes breaks (B). The outer membrane then seals again to generate protuberances (C). Continued export at this stage could result in the accumulation of exported material in vesicles within the intermembraneous space (D). Alternatively (A′ to C′), the formation of vesicles within the intermembrane space may result from local fusion of cytosolic vesicles. Cytosol is shaded in these images. The weakened sites of the outer membrane are indicated by arrowheads.

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 lumenal 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, Lα 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 ($C_{20:4}$ 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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REFERENCES

1. **Alberts, A. W., J. Cheng, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Alberts-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch, and J. Springer.** 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proc. Natl. Acad. Sci. USA **77:**3957–3961.
2. **Al-Feel, W., S. Chirala, and S. Wakil.** 1992. Cloning of the yeast FAS3 gene and primary structure of yeast acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. USA **89:**4534–4538.
3. **Allan, D., and P. J. Raval.** 1987. A sphingomyelinase-resistant pool of sphingomyelin in the nuclear membrane of hen erythrocytes. Biochim. Biophys. Acta **897:**355–363.
4. **Altschul, S. F., W. Gish, W. Miller, E. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
5. **Aris, J. P., and G. Blobel.** 1988. Identification and characterization of a yeast nucleolar protein that is similar to a rat liver nucleolar protein. J. Cell Biol. **107:**17–31.
6. **Bergstrom, J. D., M. Kurtz, D. Rew, A. Amend, J. Karkas, R. Bostedor, V. Bansal, C. Dufresne, F. van Middlesworth, O. Hensens, J. Liesch, D. Zink, K. Wilson, J. Onishi, J. Milligan, G. Bills, L. Kaplan, M. Omstead, R. Jenkins, L. Huang, M. Meinz, L. Quinn, R. Burg, Y. Kong, S. Mochales, M. Mojena, I. Martin, F. Pelaez, M. Diez, and A. W. Alberts.** 1993. Zaragozic acids: a family of fungal metabolites that are picomolar competitive inhibitors of squalene synthase. Proc. Natl. Acad. Sci. USA **90:**80–84.
7. **Bishop, R. W., and R. M. Bell.** 1988. Assembly of phospholipids into cellular membranes: biosynthesis, transmembrane movement and intracellular translocation. Annu. Rev. Cell Biol. **4:**579–610.
8. **Bloom, M., E. Evans, and O. G. Mouritsen.** 1991. Physical properties of the fluid lipid-bilayer component of cell membranes: a perspective. Q. Rev. Biophys. **24:**293–397.
9. **Bogerd, A. M., J. Hoffman, D. Amberg, G. Fink, and L. Davis.** 1994. *nup1* mutants exhibit pleiotropic defects in nuclear pore complex function. J. Cell Biol. **127:**319–332.
10. **Bonneaud, N., O. Ozier-Kalogeropoulos, G. Li, M. Labouesse, L. Minvielle-Sebastia, and F. Lacroute.** 1991. A family of low and high copy replicative, integrative and single-stranded *S. cerevisiae/E. coli* shuttle vectors. Yeast **7:**609–615.
11. **Brown, M. S., J. Faust, J. Goldstein, I. Kaneko, and A. Endo.** 1978. Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts incubated with compactin (ML-236B), a competitive inhibitor of the reductase. J. Biol. Chem. **253:**1121–1128.
12. **Cane, D. E.** 1994. Polyketide biosynthesis: molecular recognition or genetic programming? Science **263:**338–340.
13. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNA with different 5′ end encode secreted and intracellular forms of yeast invertase. Cell **28:**145–154.
14. **Chirala, S. S., Q. Zhong, W. Huang, and W. Al-Feel.** 1994. Analysis of *FAS3/ACC* regulatory region of *Saccharomyces cerevisiae*: identification of a functional UASINO and sequences responsible for fatty acid mediated repression. Nucleic Acids Res. **22:**412–418.
15. **Cinti, D. L., L. Cook, M. Nagi, and S. K. Suneja.** 1992. The fatty acid chain elongation system of mammalian endoplasmic reticulum. Prog. Lipid Res. **31:**1–51.
16. **Conzelmann, A., A. Puoti, R. Lester, and C. Desponds.** 1992. Two different types of lipid moieties are present in glycophosphoinositol-anchored membrane proteins of Saccharomyces cerevisiae. EMBO J. **11:**457–466.
17. **Davis, L. I.** 1995. The nuclear pore complex. Annu. Rev. Biochem. **64:**365–396.
18. **Dekker, C., G. Van Kessel, J. Klomp, J. Pieters, and B. De Kruijff.** 1983. Synthesis and polymorphic phase behavior of polyunsaturated phosphatidylcholines and phosphatidylethanolamines. Chem. Phys. Lipids **33:**93–106.
19. **Dietlein, G., and E. Schweizer.** 1975. Control of fatty acid-synthetase biosynthesis in *Saccharomyces cerevisiae*. Eur. J. Biochem. **58:**177–184.
20. **Dingwall, C., and R. Laskey.** 1992. The nuclear membrane. Science **258:**942–947.
21. **Doye, V., R. Wepf, and E. Hurt.** 1994. A novel nuclear pore protein Nup133p with distinct roles in poly(A)+ RNA transport and nuclear pore distribution. EMBO J. **13:**6062–6075.
22. **Evan, G. I., G. K. Lewis, G. Ramsay, and J. M. Bishop.** 1985. Isolation of monoclonal antibodies specific for human c-*myc* proto-oncogene product. Mol. Cell. Biol. **5:**3610–3616.
23. **Fulco, A. J.** 1967. Chain elongation, 2-hydroxylation, and decarboxylation of long chain fatty acids by yeast. J. Biol. Chem. **242:**3608–3613.
24. **Gorsch, L. C., T. Dockendorff, and C. N. Cole.** 1995. A conditional allele of the novel repeat-containing yeast nucleoporin RAT7/NUP159 causes both rapid cessation of mRNA export and reversible clustering of nuclear pore complexes. J. Cell Biol. **129:**939–955.
25. **Greber, U. F., and L. Gerace.** 1992. Nuclear protein import is inhibited by an antibody to a lumenal epitope of a nuclear pore complex glycoprotein. J. Cell Biol. **116:**15–30.
26. **Guchhait, R. B., G. Putz, and J. W. Porter.** 1966. Synthesis of long-chain fatty acids by microsomes of pigeon liver. Arch. Biochem. Biophys. **117:**541–549.
27. **Guerra, C. E., and H. L. Klein.** 1995. Mapping of the ACC1/FAS3 gene to the right arm of chromosome XIV of *Saccharomyces cerevisiae*. Yeast **11:**697–700.
28. **Hampton, R. Y., and J. Rine.** 1994. Regulated degradation of HMG-CoA reductase, an integral membrane protein of the endoplasmic reticulum, in yeast. J. Cell Biol. **125:**299–312.
29. **Hardie, D. G.** 1989. Regulation of fatty acid synthesis via phosphorylation of acetyl-CoA carboxylase. Prog. Lipid Res. **28:**117–146.
30. **Harrington, A., C. Herbert, and P. Slonimski.** 1992. A beta-ketoacyl synthase is essential for normal mitochondrial function. Yeast **8:**S414.
31. **Hasslacher, M., A. Ivessa, F. Paltauf, and S. D. Kohlwein.** 1993. Acetyl-CoA carboxylase from yeast is an essential enzyme and is regulated by factors that control phospholipid metabolism. J. Biol. Chem. **268:**10946–10952.
32. **Hechtberger, P., and G. Daum.** 1995. Intracellular transport of inositol-containing sphingolipids in the yeast, *Saccharomyces cerevisiae*. FEBS Lett. **367:**201–204.
33. **Higgins, M. J. P., and R. Kekwick.** 1973. An investigation into the role of malonyl-coenzyme A in isoprenoid biosynthesis. Biochem. J. **134:**295–310.
34. **Hopwood, D. A., and D. Sherman.** 1990. Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. Annu. Rev. Genet. **24:**37–66.
35. **Hori, T., N. Nakamura, and H. Okuyama.** 1987. Possible involvement of acetyl coenzyme A carboxylase as well as fatty acid synthetase in the temperature-controlled synthesis of fatty acids in *Saccharomyces cerevisiae*. J. Biochem. **101:**949–956.
36. **Hui, S., T. Mason, and C.-H. Huang.** 1984. Acyl chain interdigitation in saturated mixed-chain phosphatidylcholine bilayer dispersions. Biochemistry **23:**5570–5577.
37. **Israelachvili, J. N., S. Marcelja, and R. G. Horn.** 1980. Physical principles of membrane organization. Q. Rev. Biophys. **13:**121–200.
38. **Ito, H., Y. Fukada, K. Murata, and A. Kimura.** 1983. Transformation of intact cells with alkali cations. J. Bacteriol. **153:**163–168.
39. **Jandrositz, A., F. Turnowsky, and G. Hegenauer.** 1991. The gene encoding squalene epoxidase from *Saccharomyces cerevisiae*: cloning and characterization. Gene **107:**155–160.
40. **Johnson, D. R., L. Knoll, D. Levin, and J. I. Gordon.** 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:**751–762.
41. **Kadowaki, T., S. Chen, M. Hitomi, E. Jacobs, C. Kumagai, S. Liang, R. Schneiter, D. Singleton, J. Wisniewska, and A. M. Tartakoff.** 1994. Isolation and characterization of *Saccharomyces cerevisiae* mRNA transport-defective (*mtr*) mutants. J. Cell Biol. **126:**649–659.
42. **Kadowaki, T., Y. Zhao, and A. M. Tartakoff.** 1992. A conditional yeast mutant deficient in mRNA transport from nucleus to cytoplasm. Proc. Natl. Acad. Sci. USA **89:**2312–2316.
43. **Klimov, A. N., L. Vasil′eva, T. Klimova, and E. Dizhe.** 1990. Does malonate

participate in sterol synthesis? Biokhimiya **55:**549–553.
43a. **Kohlwein, S. D.** Unpublished observations.
44. **Kohlwein, S. D., and F. Paltauf.** 1983. Uptake of fatty acids by the yeast, *Saccharomyces cerevisiae* and *Yarrowia lipolytica*. Biochim. Biophys. Acta **792:**310–317.
45. **Kottig, H., G. Rottner, K.-F. Beck, M. Schweizer, and E. Schweizer.** 1991. The pentafunctional *FAS1* genes of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* are co-linear and considerably longer than previously estimated. Mol. Gen. Genet. **226:**310–314.
46. **Kranz, J., and C. Holm.** 1990. Cloning by function: an alternative approach for identifying yeast homologs of genes from other organisms. Proc. Natl. Acad. Sci. USA **87:**6629–6633.
47. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
48. **Lane, M. D., J. Moss, and S. E. Polakis.** 1975. Acetyl coenzyme A carboxylase, p. 181–221. *In* K. E. Ebner (ed.), Subunit enzymes. Biochemistry and function. Marcel Dekker, Inc., New York.
49. **Lester, R. L., and R. C. Dickson.** 1993. Sphingolipids with inositolphosphate-containing head groups. Adv. Lipid Res. **26:**253–274.
50. **Lester, R. L., G. Wells, G. Oxford, and R. C. Dickson.** 1993. Mutant strains of Saccharomyces cerevisiae lacking sphingolipids synthesize novel inositol glycerolipids that mimic sphingolipid structure. J. Biol. Chem. **268:**845–856.
51. **Li, O., C. Heath, D. Amberg, T. Dockendorff, C. Copeland, M. Snyder, and C. N. Cole.** 1995. Mutation or deletion of the *Saccharomyces cerevisiae RAT3/NUP133* gene causes temperature-dependent nuclear accumulation of poly(A)+ RNA and constitutive clustering of nuclear pore complexes. Mol. Biol. Cell **6:**401–417.
52. **Lohner, K.** 1991. Effects of small organic molecules on phospholipid phase transitions. Chem. Phys. Lipids **57:**341–362.
53. **Mackall, J. C., and M. D. Lane.** 1978. Subunit size and paracrystal structure of avian liver acetyl-CoA carboxylase. J. Mol. Biol. **123:**595–606.
54. **Mirzayan, C., C. Copeland, and M. Snyder.** 1992. The *NUF1* gene encodes an essential coiled-coil related protein that is a potential component of the yeast nucleoskeleton. J. Cell Biol. **116:**1319–1332.
55. **Mishina, M., R. Roggenkamp, and E. Schweizer.** 1980. Yeast mutants defective in acetyl-coenzyme A carboxylase and biotin: apocarboxylase ligase. Eur. J. Biochem. **111:**79–87.
56. **Mohamed, A. H., S. Chirala, N. Mody, W.-Y. Huang, and S. Wakil.** 1988. Primary structure of the multifunctional a subunit protein of yeast fatty acid synthase derived from *FAS2* gene sequence. J. Biol. Chem. **263:**12315–12325.
57. **Mouritsen, O. G., and M. Bloom.** 1984. Mattress model of lipid-protein interactions in membranes. Biophys. J. **46:**141–153.
58. **Mutvei, A., S. Dihlmann, W. Herth, and E. Hurt.** 1992. *NSP1* depletion in yeast affects nuclear pore formation and nuclear accumulation. Eur. J. Cell Biol. **59:**280–295.
59. **Nurminen, T., and H. Suomalainen.** 1971. Occurrence of long-chain fatty acids and glycolipids in the cell envelope fraction of baker's yeast. Biochem. J. **125:**963–969.
60. **Obermayer, M., and F. Lynen.** 1976. Structure of biotin enzymes. Trends Biochem. Sci. **1:**169–171.
61. **Omura, S.** 1981. Cerulenin. Methods Enzymol. **72:**520–532.
62. **Pagano, R. E.** 1990. Lipid traffic in eukaryotic cells: mechanisms for intracellular transport and organelle-specific enrichment of lipids. Curr. Opin. Cell Biol. **2:**652–663.
63. **Patton, J. L., and R. L. Lester.** 1991. The phosphoinositol sphingolipids of *Saccharomyces cerevisiae* are highly localized in the plasma membrane. J. Bacteriol. **173:**3101–3108.
64. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
65. **Pinto, W. J., B. Srinivasan, S. Shepherd, A. Schmidt, R. C. Dickson, and R. L. Lester.** 1992. Sphingolipid long-chain-base auxotrophs of *Saccharomyces cerevisiae*: genetics, physiology, and a method for their selection. J. Bacteriol. **174:**2565–2574.
66. **Riles, L., J. Dutchik, A. Baktha, B. McCauley, E. Thayer, M. Leckie, V. Braden, J. Depke, and M. Olson.** 1993. Physical maps of the six smallest chromosomes of *Saccharomyces cerevisiae* at a resolution of 2.6 kilobase pairs. Genetics **134:**81–150.
67. **Roggenkamp, R., S. Numa, and E. Schweizer.** 1980. Fatty acid-requiring mutant of *Saccharomyces cerevisiae* defective in acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. USA **77:**1814–1817.
68. **Rothstein, R.** 1991. Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. **194:**281–301.
69. **Sambrook, J., E. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
70. **Sanger, F., S. Nicklen, and A. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
71. **Schneider, P., M. Ferguson, M. McConville, A. Mehlert, S. Homans, and C. Bordier.** 1990. Structure of the glycosylphosphatidylinositol membrane anchor of the *Leishmania major* promastigote surface protease. J. Biol. Chem. **265:**16955–16964.
72. **Schweizer, E.** 1989. Biosynthesis of fatty acids and related compounds, p. 3–50. *In* C. Ratledge and S. G. Wilkinson (ed.), Microbial lipids, vol. 2. Academic Press, New York.
73. **Schweizer, E., and H. Bolling.** 1970. A *Saccharomyces cerevisiae* mutant defective in saturated fatty acid biosynthesis. Proc. Natl. Acad. Sci. USA **67:**660–666.
74. **Schweizer, E., B. Kniep, H. Castorph, and U. Holzner.** 1973. Pantetheine-free mutants of the yeast fatty-acid-synthetase complex. Eur. J. Biochem. **39:**353–362.
75. **Severs, N. J., E. Jordan, and D. H. Williamson.** 1976. Nuclear pore absence from areas of close association between nucleus and vacuole in synchronous yeast cultures. J. Ultrastruct. Res. **54:**374–387.
76. **Shen, B., and C. R. Hutchinson.** 1993. Enzymatic synthesis of a bacterial polyketide from acetyl and malonyl coenzyme A. Science **262:**1535–1540.
77. **Sherman, F., G. Fink, and J. Hicks.** 1986. Methods in yeast genetics: a laboratory course manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
78. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
79. **Silver, P. A., A. Chiang, and I. Sadler.** 1988. Mutations that alter both localization and production of a yeast nuclear protein. Genes Dev. **2:**707–717.
80. **Stadler, J., T. Keenan, G. Bauer, and G. Gerisch.** 1989. The contact site A glycoprotein of *Dictyostelium discoideum* carries a phospholipid anchor of a novel type. EMBO J. **8:**371–377.
81. **Stewart, L. C., and M. Y. Yaffe.** 1991. A role for unsaturated fatty acids in mitochondrial movement and inheritance. J. Cell Biol. **115:**1249–1257.
82. **Sumper, M., D. Oesterhelt, C. Riepertinger, and F. Lynen.** 1969. Die Synthese verschiedener Carbonsauren durch den Multienzymkomplex der Fettsauresynthese aus Hefe und die Erklarung ihrer Bildung. Eur. J. Biochem. **10:**377–387.
83. **Toh, H., H. Kondo, and T. Tanabe.** 1993. Molecular evolution of biotin-dependent carboxylases. Eur. J. Biochem. **215:**687–696.
84. **Wagner, S., and F. Paltauf.** 1994. Generation of glycerophospholipid molecular species in the yeast *Saccharomyces cerevisiae*. Fatty acid pattern of phospholipid classes and selective acyl turnover at *sn-1* and *sn-2* positions. Yeast **10:**1429–1437.
85. **Wells, G. B., and R. L. Lester.** 1983. The isolation and characterization of a mutant strain of *Saccharomyces cerevisiae* that requires a long chain base for growth and for synthesis of phosphosphingolipids. J. Biol. Chem. **258:**10200–10203.
86. **Wente, S. R., and G. Blobel.** 1993. A temperature-sensitive *NUP116* null mutant forms a nuclear envelope seal over the yeast nuclear pore complex thereby blocking nucleocytoplasmic traffic. J. Cell Biol. **123:**275–284.
87. **Wente, S. R., and G. Blobel.** 1994. *NUP145* encodes a novel yeast glycine-leucine-phenylalanine-glycine (GLFG) nucleoporin required for nuclear envelope structure. J. Cell Biol. **125:**955–969.
88. **Wente, S. R., M. Rout, and G. Blobel.** 1992. A new family of yeast nuclear pore complex proteins. J. Cell Biol. **119:**705–723.
89. **Willison, J. H. M., and G. C. Johnston.** 1978. Altered nuclear pore diameters in G1-arrested cells of the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **136:** 318–323.
90. **Witters, L. A., and T. D. Watts.** 1990. Yeast acetyl-CoA carboxylase: in vitro phosphorylation by mammalian and yeast protein kinases. Biochem. Biophys. Res. Commun. **169:**369–376.
91. **Wozniak, R. W., G. Blobel, and M. P. Rout.** 1994. POM152 is an integral protein of the pore membrane domain of the yeast nuclear envelope. J. Cell Biol. **125:**31–42.
92. **Yaffe, M. P., and G. Schatz.** 1984. Two nuclear mutations that block mitochondrial protein import. Proc. Natl. Acad. Sci. USA **81:**4819–4823.