

The *Saccharomyces cerevisiae* Hyperrecombination Mutant *hpr1Δ* Is Synthetically Lethal with Two Conditional Alleles of the Acetyl Coenzyme A Carboxylase Gene and Causes a Defect in Nuclear Export of Polyadenylated RNA

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In a screen for mutants that display synthetic lethal interaction with *hpr1Δ*, a hyperrecombination mutant of *Saccharomyces cerevisiae*, we have isolated a novel cold-sensitive allele of the acetyl coenzyme A (CoA) carboxylase gene, *acc1^{cs}*, encoding the rate-limiting enzyme of fatty acid synthesis. The synthetic lethal phenotype of the *acc1^{cs} hpr1Δ* double mutant was only partially complemented by exogenous fatty acids. *hpr1Δ* was also synthetically lethal with a previously isolated, temperature-sensitive allele of *ACCI*, *mtr7* (mRNA transport), indicating that the lethality of the *acc1^{cs} hpr1Δ* double mutant was not allele specific. The basis for the interaction between conditional *acc1* alleles and *hpr1Δ* was investigated in more detail. In the *hpr1Δ* mutant background, acetyl-CoA carboxylase enzyme activity was reduced about 15-fold and steady-state levels of biotinylated Acc1p and *ACCI* mRNA were reduced 2-fold. The reduced Acc1p activity in *hpr1Δ* cells, however, did not result in an altered lipid or fatty acid composition of the mutant membranes but rendered cells hypersensitive to soraphen A, an inhibitor of Acc1p. Similar to *mtr7*, *hpr1Δ* and *acc1^{cs}* mutant cells displayed a defect in nuclear export of polyadenylated RNA. Oversized transcripts were detected in *hpr1Δ*, and rRNA processing was disturbed, but pre-mRNA splicing appeared wild type. Surprisingly, the transport defect of *hpr1Δ* and *acc1^{cs}* mutant cells was accompanied by an altered ring-shaped structure of the nucleolus. These observations suggest that the basis for the synthetic lethal interaction between *hpr1Δ* and *acc1* may lie in a functional overlap of the two mutations in nuclear poly(A)⁺ RNA production and export that results in an altered structure of the nucleolus.

The *hpr1Δ* mutant of *Saccharomyces cerevisiae* was isolated in a screen for mutations that confer an increased mitotic recombination (1, 2). The *hpr1Δ* null mutant is temperature sensitive for growth at 37°C and displays a 700-fold-elevated rate of mitotic intrachromatid recombination. Hpr1p has two regions of homology to topoisomerase I, Top1p (3, 40), and *hpr1Δ* mutants display synthetic lethality with mutations in all three DNA topoisomerase genes, *TOP1*, *TOP2*, and *TOP3* (3). A fourth synthetic lethal interaction has been found between *hpr1Δ* and a mutant carrying a deletion of one copy of the histone H3-H4 genes (10).

In *hpr1Δ* null mutants, transcription of many physiologically unrelated genes is affected (44) and the temperature-sensitive growth phenotype of *hpr1Δ* mutants is suppressed by mutations in components of the general transcription machinery (12, 27, 41). The Hpr1 protein has been found to be in a distinct RNA polymerase II complex (8) and has been suggested to have a functional role in transcription elongation (9).

To better understand the in vivo function of Hpr1p, a screen was initiated to isolate additional mutants that exhibit synthetic lethal interaction with *hpr1Δ*. This screen yielded a novel cold-

sensitive allele of the acetyl coenzyme A (CoA) carboxylase gene, *acc1-200^{cs}*, hereafter referred to as *acc1^{cs}* (14).

The acetyl-CoA carboxylase gene, *ACCI*, encodes a biotin-containing enzyme that synthesizes malonyl-CoA from acetyl-CoA and bicarbonate, with the hydrolysis of ATP (4). Acc1p is the rate-limiting enzyme of the de novo fatty acid biosynthetic pathway. Expression of the *ACCI* gene is under coordinate transcriptional regulation by the phospholipid precursors inositol and choline (15). A temperature-sensitive allele of *ACCI*, *mtr7*, has been isolated in a screen for mutants that affect nuclear export of polyadenylated RNA (18; for a review, see reference 39). The nuclear transport defect of this *acc1^{cs}* allele has been proposed to be due to a special lipid requirement of the nuclear membrane-nuclear pore complex (31, 33).

The basis for the synthetic lethality between *hpr1Δ* and the cold-sensitive *acc1^{cs}* allele was investigated in more detail. We find that *hpr1Δ* mutant cells have a very strong defect in export of nuclear poly(A)⁺ RNA, a phenotype previously observed in a temperature-sensitive allele of *ACCI* (31), and propose that the lethal interaction between *hpr1Δ* and *acc1^{cs}* is due to a combined effect of the two mutations on nuclear export of polyadenylated RNA.

MATERIALS AND METHODS

Yeast strains and culture conditions. The *S. cerevisiae* strains used for these experiments are listed in Table 1. Strains were grown in YEPD medium (1% yeast extract, 2% Bacto Peptone, 2% glucose) or synthetic minimal medium (35) supplemented with appropriate amino acids and glucose. Medium supplemented with fatty acids contained 1% Tween 40, 0.015% palmitic acid, and 0.015% stearic acid (28). Soraphen A, a kind gift from A. Hinnen, was added to medium

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TABLE 1. Yeast strain genotypes and construction

Strain	Relevant genotype	Source or reference
479-2A	<i>MATα acc1^{cs} leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 can1-100</i>	H. Klein
485-13A	<i>MATα acc1^{cs} leu2-3,112 ura3-1 ade2-1 his3-11,15 can1-100</i>	H. Klein
U768-1C	<i>MATα hpr1Δ3::HIS3 leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 can1-100</i>	R. Rothstein
U768-4C	<i>MATα hpr1Δ3::HIS3 leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 can1-100</i>	R. Rothstein
W303d	<i>MATα/MATα leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 ade2-1/ade2-1 his3-11,15/his3-11,15 can1-100/can1-100</i>	R. Rothstein
YRXS12	<i>MATα acc1^{ts} leu2-Δ1 ura3-52 ade2-101 lys2-801 his3-Δ200</i>	Schneiter et al. (31)
YRXS300C-1	<i>MATα hpr1Δ3::HIS3 leu2 TRP1 ura3 ade2 his3 lys2-801</i>	This work; spore from cross between U768-4C and YRXS12
YRXS300C-2	<i>MATα hpr1Δ3::HIS3 acc1^{ts} leu2 trp1 ura3 ade2 his3 lys2-801</i>	This work; spore from cross between U768-4C and YRXS12
YRXS300C-3	<i>MATα leu2 TRP1 ura3 ade2 his3</i>	This work; spore from cross between U768-4C and YRXS12
YRXS300C-4	<i>MATα acc1^{ts} leu2 trp1 ura3 ade2 his3</i>	This work; spore from cross between U768-4C and YRXS12

from a 10-mg/ml stock solution in methanol. Optical density at 600 nm was monitored every hour for growth rate determinations. The exponential growth rate in the presence of soraphen A was established after a 3-h lag period and was expressed as a percentage of the growth rate in the absence of the inhibitor.

Isolation and analysis of RNA. Total yeast RNA was extracted from exponentially growing cultures as described previously (29). Ten milligrams of RNA per well was fractionated by electrophoresis through a 0.8% agarose-3.7% formaldehyde gel and prepared for Northern blot analysis as described previously (29). For quantification of *ACC1* transcription, ³²P-labeled DNA probes were prepared according to the method in reference 13. Blots were serially hybridized to *ACC1*-specific and *ACT1*-specific DNA probes, in that order and without an intermediate probe-stripping step. After autoradiography of the blots, each lane in the exposed film was scanned in one dimension with the UltraScan XL system (LKB), and the *ACC1/ACT1* ratios of peak areas were calculated relative to the ratio in the *MAT α* wild-type strain. For detection of *CRY1* message, filters were probed with digoxigenin-labeled probe and signal was detected by enhanced chemiluminescence with CDP-Star substrate (Tropix, Bedford, Mass.). For the analysis of rRNA processing, mutant cells were transformed with pRS316 (36) to make them uracil prototrophic. Cells were incubated at nonpermissive temperatures for 1 h and then labeled with 0.05 mCi of [³H]uridine per ml for 10 min.

Immunofluorescence and in situ hybridization. Early-logarithmic-phase cells were prepared for immunofluorescence microscopy as previously described (31). Mouse monoclonal antibody against the yeast fibrillar homologue, Nop1p, was applied at a 1:5,000 dilution (6). Secondary fluorescein isothiocyanate-conjugated antibody (Oncogene Science, Cambridge, Mass.) was used at a 1:200 dilution. Detection of accumulated poly(A)⁺ RNA was performed essentially as described elsewhere (31), with the modification that digoxigenin-labeled oligo(dT)₂₅₋₃₀ was used for hybridization. Secondary fluorescein isothiocyanate-conjugated anti-sheep antibody was used at a 1:200 dilution. Laser scanning microscopic analysis was performed with a Leica TCS 4d confocal microscope equipped with a PL APO 100 \times /1.40 objective, and photographs were recorded on Ektachrome 100 film (Eastman Kodak Co., Rochester, N.Y.). Corresponding pictures were recorded with identical pinhole openings and amplification settings and were printed with the same exposure time.

Western blot analysis. Whole-cell protein extracts were prepared as described elsewhere (43), and protein concentration was determined according to the method of Lowry et al. (22), with bovine serum albumin as a standard. Proteins were separated by one-dimensional sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (20), with 4% stacking and 10% separating gels. Proteins were transferred to nitrocellulose sheets (Hybond C; Amersham) and stained with Ponceau S to assess transfer efficiency. Membranes were incubated overnight in blocking solution containing 5% fat-free dry milk powder in Tris-buffered saline (TBS) (50 mM Tris-HCl, 150 mM NaCl, pH 8.0). After two washes with TTBS (0.1% Tween 20 in TBS), membranes were incubated with 10 μ g of avidin-peroxidase conjugate (ExtrAvidin peroxidase; Sigma, St. Louis, Mo.), rabbit anti-Acc1p (1:700 [17]), or rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5,000) per ml, diluted in TBS. Peroxidase-conjugated anti-rabbit immunoglobulin G (Sigma) was used as secondary antibody. Signal detection was carried out according to the instructions provided by the manufacturer with the ECL system (Amersham) and the SuperSignal CL-HRP substrate system (Pierce, Rockford, Ill.). Densitometric scanning of X-ray films and quantification of signals were carried out with NIH Image 1.54 software.

Acc1p activity determination. The cytosolic fraction used for Acc1p enzyme activity assays was prepared as follows. Cells were harvested at 1,200 \times g for 10 min, washed with 0.1 M K₂HPO₄-KH₂PO₄ buffer (pH 6.5), mixed with breaking buffer (50 mM Tris-HCl, 100 mM NaF, 1 mM EDTA, 10 mM β -mercaptoethanol, 0.25 M sucrose, 1 mM phenylmethylsulfonyl fluoride, pH 7.5) and glass beads (0.30-mm diameter) in a ratio of 1:1:1 (wt/vol/wt), and disrupted by four

1-min bursts in a Braun-Melsungen homogenizer under CO₂ cooling. Glass beads were collected by centrifugation at 3,000 \times g for 5 min, and the supernatant was centrifuged at 20,000 \times g for 20 min and centrifuged again at 195,000 \times g for 80 min. Saturated (NH₄)₂SO₄ was then added in three portions within 20 min to 50% saturation, and samples were stirred for an additional 30 min. The precipitate was collected by centrifugation at 15,000 \times g, dissolved in HEPES buffer (50 mM HEPES, 1 mM EDTA, 0.02% Na-azide, 50% glycerol, pH 7.0), and stored frozen at -20°C. Enzymatic activities of Acc1p were found to be stable over a period of 3 weeks after freezing of samples. All steps were carried out at 4°C. Activity of acetyl-CoA carboxylase was determined by a photometric assay in a coupled enzymatic reaction as described elsewhere (23). All enzyme measurements were carried out at 24°C.

RESULTS

Synthetic lethality between *acc1^{cs}* and *hpr1 Δ* . The *acc1^{cs} acc1-200^{cs}* allele was recovered in a screen for mutants that display a synthetic lethal interaction with a null allele of *HPRI* (14). The synthetic lethality of the *acc1^{cs} hpr1 Δ* double mutant is indicated by the failure to recover spore colonies of this genotype from a cross between an *acc1^{cs}* strain and an *hpr1 Δ* strain. When the tetrads from this cross were dissected on a YEPD plate supplemented with fatty acids, the *acc1^{cs} hpr1 Δ* double mutant was viable but still showed reduced growth compared to the single mutant strain or the wild-type strain (Fig. 1).

Soraphen A sensitivity of *acc1^{cs}* and *hpr1 Δ* mutants. Soraphen A is a potent inhibitor of Acc1p activity in yeast (42). The *acc1^{cs}* allele was found to confer hypersensitivity to soraphen A at the permissive temperature (Fig. 2A). Since the *acc1^{cs}* allele is synthetically lethal with the *hpr1 Δ* mutation, we examined the *hpr1 Δ* mutant for soraphen A sensitivity. The *hpr1 Δ* null mutant was found to be as soraphen A sensitive as the *acc1^{cs}* mutant on solid medium (Fig. 2A) and in liquid medium (Fig. 2B).

The synthetic lethal interaction between *hpr1 Δ* and *acc1* is not allele specific. A temperature-sensitive allele of *ACC1*, *mtr7*, herein referred to as *acc1^{ts}*, had previously been isolated in a screen for conditional mRNA transport mutants (31). To determine whether the synthetic lethal interaction between *acc1^{cs}* and *hpr1 Δ* is an allele-specific phenomenon, a cross between *acc1^{ts}* and *hpr1 Δ* was performed. Diploids were sporulated, and tetrads were dissected on YEPD and YEPD plates supplemented with 1 M sorbitol. Sorbitol supplementation has been found to rescue the temperature-dependent growth phenotype of both conditional *acc1* alleles (data not shown). While *acc1^{ts} hpr1 Δ* double mutants were viable on sorbitol-supplemented medium, no *acc1^{ts} hpr1 Δ* double mutants were recov-

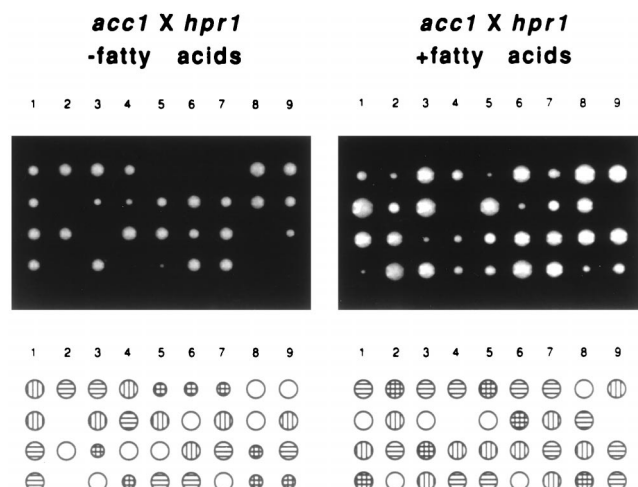


FIG. 1. *acc1^{cs}* is synthetically lethal with *hpr1Δ*. Growth of spore segregants from an *acc1^{cs} × hpr1Δ* cross. Asci were dissected on YEPD medium alone or YEPD medium supplemented with fatty acids and incubated for 3 days at 30°C. Germinated spores from each tetrad are arranged in vertical columns (1 to 9), and the corresponding genotypes are indicated by circles below as follows: wild-type spores, open circles; *acc1^{cs}* spores, vertically striped circles; *hpr1Δ* spores, horizontally striped circles; and double mutant spores, crosshatched circles.

ered from YEPD or fatty acid-supplemented plates, indicating that *acc1^{cs}* is synthetically lethal with *hpr1Δ* (Fig. 3).

***hpr1Δ* affects Acc1p activity at the level of transcription.** The observation that *hpr1Δ* is synthetically lethal with two different conditional *acc1* alleles and that *hpr1Δ* itself is hypersensitive to soraphen A suggested that Acc1p activity is affected in the *hpr1Δ* mutant. Acc1p enzymatic activity was determined in cytosolic fractions from the wild type and the *hpr1Δ* mutant strain. Cells were grown in YEPD medium at 30°C, cytosol was prepared, and Acc1p activity was determined as described previously (23). Acc1p activity in the *hpr1Δ* mutant was reduced approximately 15-fold compared to that in the wild type, as shown by the following data (means ± standard deviations of three determinations): enzyme activity of wild type, $38.3 \times 10^{-5} \pm 3.7 \times 10^{-5} \Delta E/\text{min}/\mu\text{g}$; enzyme activity of the *hpr1Δ* mutant, $2.6 \times 10^{-5} \pm 1.7 \times 10^{-5} \Delta E/\text{min}/\mu\text{g}$. This greatly reduced Acc1p activity in the *hpr1Δ* mutant background, however, did not result in an altered lipid or fatty acid composition of *hpr1Δ* mutant cells compared to the wild type (data not shown).

To determine at which level the lack of Hpr1p function affects the activity of Acc1p, we analyzed steady-state levels of biotinylated Acc1p. Protein was isolated from whole cells and blotted with peroxidase-labeled avidin to simultaneously detect biotinylated Acc1p and the two pyruvate carboxylase isoenzymes Pyc1p and Pyc2p (Fig. 4). To control for internal loading, GAPDH was detected by an anti-GAPDH antibody. Signal intensities of these blots were quantified, and ratios of biotinylated Acc1p to biotinylated Pyc are listed in Table 2. This analysis revealed that the percentage of biotinylated Acc1p relative to biotinylated Pyc in the *hpr1Δ* mutant was 50% of that of the wild type.

Preliminary results with a plasmid-borne *ACC1-lacZ* fusion as a reporter gene indicated that *ACC1* expression was reduced twofold in *hpr1Δ* strains compared to the wild type (data not shown). To confirm that expression of the chromosomal *ACC1* gene was affected in *hpr1Δ* strains, Northern blot analysis was carried out with total RNA. The relative levels of steady-state

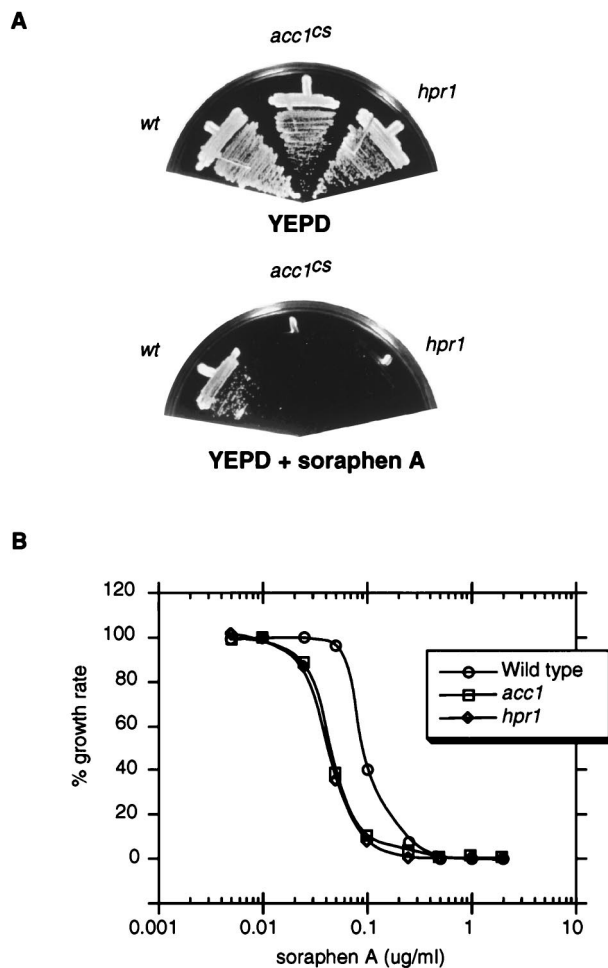


FIG. 2. Soraphen A sensitivity of the *hpr1Δ* mutant. (A) Strains with indicated genotypes were streaked onto YEPD medium (top) and YEPD medium containing 0.25 mg of soraphen A per ml (bottom) and incubated for 2 days at 30°C. wt, wild type. (B) Dose-response curves for soraphen A. The exponential growth rate was established after a 3-h lag period for various concentrations of soraphen A and is expressed as a percentage of the growth rate in the absence of soraphen A. The results for two strains of each genotype were averaged and plotted against the concentration of soraphen A. Curves are the best fit calculated with respect to Hill's equation (Hill coefficients, 3 to 4). Calculated 50% infective doses are 0.09 mg/ml for the wild type and 0.04 mg/ml for both the *acc1^{cs}* and the *hpr1Δ* strains.

ACC1 mRNA were determined by normalization to *ACT1* mRNA. For each genotype, the results from two strains of opposite mating type and from two separate experiments were combined. The average *ACC1* mRNA level ± standard deviation ($n = 4$) is shown as a percentage of the wild-type value. The percentages were as follows: wild type, 100.0 ± 24.0 ; *hpr1Δ* mutant, 45.4 ± 3.8 ; *acc1^{cs}* mutant, 90.7 ± 18.5 . No significant difference was found in the levels of *ACC1* message between the *acc1^{cs}* mutant and the wild type. In contrast, *ACC1* expression was reduced twofold in the *hpr1Δ* mutant compared to the wild type. These data indicate that the reduced Acc1p activity in the *hpr1Δ* mutant is partially due to a diminished level of steady-state *ACC1* message made in the absence of Hpr1p and accounts for about half of the reduction in Acc1p activity. The fact that the biotinylated levels of Acc1p in the *hpr1Δ* mutant are also only 50% of the wild-type level indicates that transport and stability of the *ACC1* message are not further reduced. Most likely, there is some posttransla-

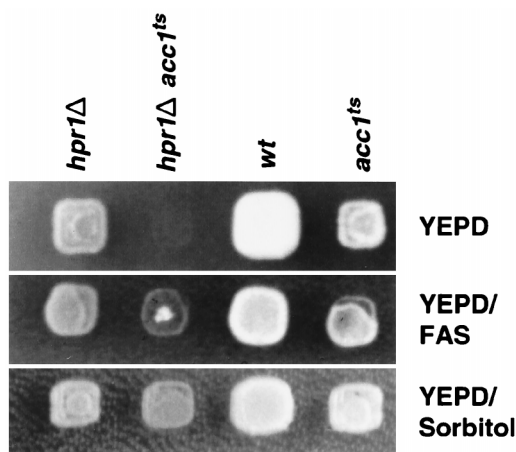


FIG. 3. *acc1^{ts}* is synthetically lethal with *hpr1Δ*. An *acc1^{ts}* strain (YRXS12) was crossed with an *hpr1Δ* strain (U768-4C), diploids were sporulated, and tetrads were dissected. Four spores of a complete tetrad were replica plated on YEPD plates (top), YEPD supplemented with fatty acids (middle), and YEPD plates supplemented with 1 M sorbitol (bottom) and incubated at 30°C for 3 days. The relevant genotype of the spores is indicated on top. wt, wild type.

tional process that is altered in the *hpr1Δ* mutant that further reduced that Acc1p activity.

Cells lacking Hpr1p have recently been shown to be defective in transcriptional elongation, but transcription initiation or activation appears not to be affected (9). *ACC1* is an unusually large yeast gene of approximately 7 kb. We thus reasoned that the combination of a defect in transcriptional elongation and a mutation in a large gene might cause the synthetic lethality between *hpr1Δ* and *acc1*. Conditional *acc1* alleles were therefore tested for hypersensitivity towards the transcriptional elongation inhibitor 6-azauracil (5), to see if this would mimic the *hpr1Δ acc1* synthetic lethality. Conditional *acc1* mutants were found to be as sensitive towards 6-azauracil as were wild-type cells (data not shown). Thus, the synthetic lethal interaction between *hpr1Δ* and *acc1* was not due to the reduced transcriptional elongation in the *hpr1Δ* mutant background.

***hpr1Δ* and *acc1^{ts}* affect nuclear export of polyadenylated RNA.** The temperature-sensitive allele of *acc1*, *mtr7*, has been isolated in a screen for mutants that affect mRNA transport (*mtr* [18]; for a review, see reference 39). This function of Acc1p in nuclear export of RNA is not yet fully understood, but it has been suggested that acyl chain elongation and hence the synthesis of the C_{26:0} very-long-chain fatty acid are re-

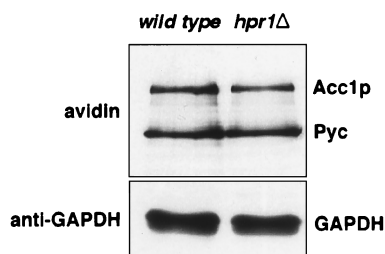


FIG. 4. Western blot analysis of biotinylated Acc1p levels in wild-type and *hpr1Δ* cells. Equal amounts of protein (10 μg) isolated from a whole-cell lysate were separated by SDS-gel electrophoresis, blotted, and probed with peroxidase-labeled avidin to detect biotinylated Acc1p and pyruvate carboxylase (Pyc) and anti-GAPDH or anti-Acc1p antibodies. Signal intensities were quantified and are listed in Table 2.

TABLE 2. Biotinylated levels of Acc1 protein in wild-type and *hpr1Δ* cells^a

Strain	Ratio (%)		
	Pyc/Acc	GAPDH/Acc	GAPDH/Pyc
Wild type	<1.4 (100)	<2.7 (100)	<1.9 (100)
<i>hpr1Δ</i> mutant	2.2 (50)	3.5 (77)	1.6 (119)

^a Cells were grown in YEPD medium at 30°C; whole-cell protein was prepared; and equal amounts of protein were separated by SDS-gel electrophoresis, blotted, and probed with peroxidase-labeled avidin to detect biotinylated Acc1p and pyruvate carboxylase (Pyc1/2p) and an anti-GAPDH antibody to control for loading. Signal intensities were quantified densitometrically, and ratios are given. Percentages are relative to the ratio seen in wild-type cells.

quired for a functional nuclear membrane-nuclear pore complex (31, 33). We thus investigated whether the synthetic lethal interaction between *hpr1Δ* and the conditional *acc1* alleles could be due to a defect of *hpr1Δ* in nuclear export of polyadenylated RNA. In situ hybridization with a digoxigenin-labeled oligo(dT) probe revealed a very strong defect of *hpr1Δ* cells in exporting nuclear poly(A)⁺ RNA at the nonpermissive growth temperature of 37°C in approximately 30% of all cells. A similar albeit much weaker (comparable to that of *acc1^{ts}*) defect in mRNA export was also observed in *acc1^{ts}* cells (Fig. 5).

To characterize the transport defect in more detail, we analyzed pre-mRNA splicing and mRNA processing (transcription initiation and 3'-end formation) in *hpr1Δ* and *acc1^{ts}* mutant cells. Equal amounts of total RNA isolated from cells incubated at permissive and nonpermissive temperatures were subjected to Northern blot analysis with *CRY1*, which codes for a ribosomal protein (21), as a probe (Fig. 6). As has previously been described for a number of temperature-sensitive mutants that block nuclear export of polyadenylated RNA (e.g., *mtr1*, *mtr3*, *mtr4*, and *mtr17* [18]), the synthesis of oversized *CRY1* transcripts was observed for *hpr1Δ* cells. Oversized transcripts were not detected in any of the other lanes shown in Fig. 6 even on overexposure of the blot (data not shown). Detection of aberrant transcripts confirms the previously proposed function of Hpr1p in some steps of transcription. Similar to *acc1^{ts}* (*mtr7* [18]), *acc1^{ts}* did not affect *CRY1* processing.

To determine whether the observed block in mRNA export also affects processing of rRNA, mutant strains were incubated at either permissive or nonpermissive temperatures and then pulse-labeled with [³H]uridine for 10 min at the same temperature. The efficiency of labeling in *hpr1Δ* was greatly reduced at 37°C (2.6%) compared to 23°C, suggesting that in the *hpr1Δ* mutant either the synthesis of RNA, [³H]uridine uptake, or [³H]UTP synthesis is decreased at nonpermissive temperatures. To normalize this effect, the labeled RNA samples analyzed on agarose gels each were loaded with equal amounts of radioactivity. As shown in Fig. 7, rRNA processing appeared to be disturbed in the *hpr1Δ*, but not the *acc1^{ts}*, mutant. The labeling of all rRNA species was greatly reduced in the *hpr1Δ* mutant.

***hpr1Δ* and *acc1^{ts}* cells display an aberrant ring-shaped nucleolus.** The yeast nucleolus is a crescent-shaped structure that makes extensive contact with the nuclear envelope. A defect in nuclear export of polyadenylated RNA is frequently accompanied by an altered structure of the nucleolus, i.e., fragmentation and/or enlargement (18, 32). We thus analyzed the structure of the nucleolus in *hpr1Δ* and *acc1^{ts}* mutant cells by immunofluorescence microscopy with an antibody against an abundant nucleolar protein, Nop1p (6). As shown in Fig. 8, at permissive temperatures the nucleolus in *hpr1Δ* and *acc1^{ts}* cells displayed a ring-shaped structure rather than the typical crescent-shaped structure seen in wild-type cells. This ring-like structure collapses into a normal-looking, crescent-shaped nu-

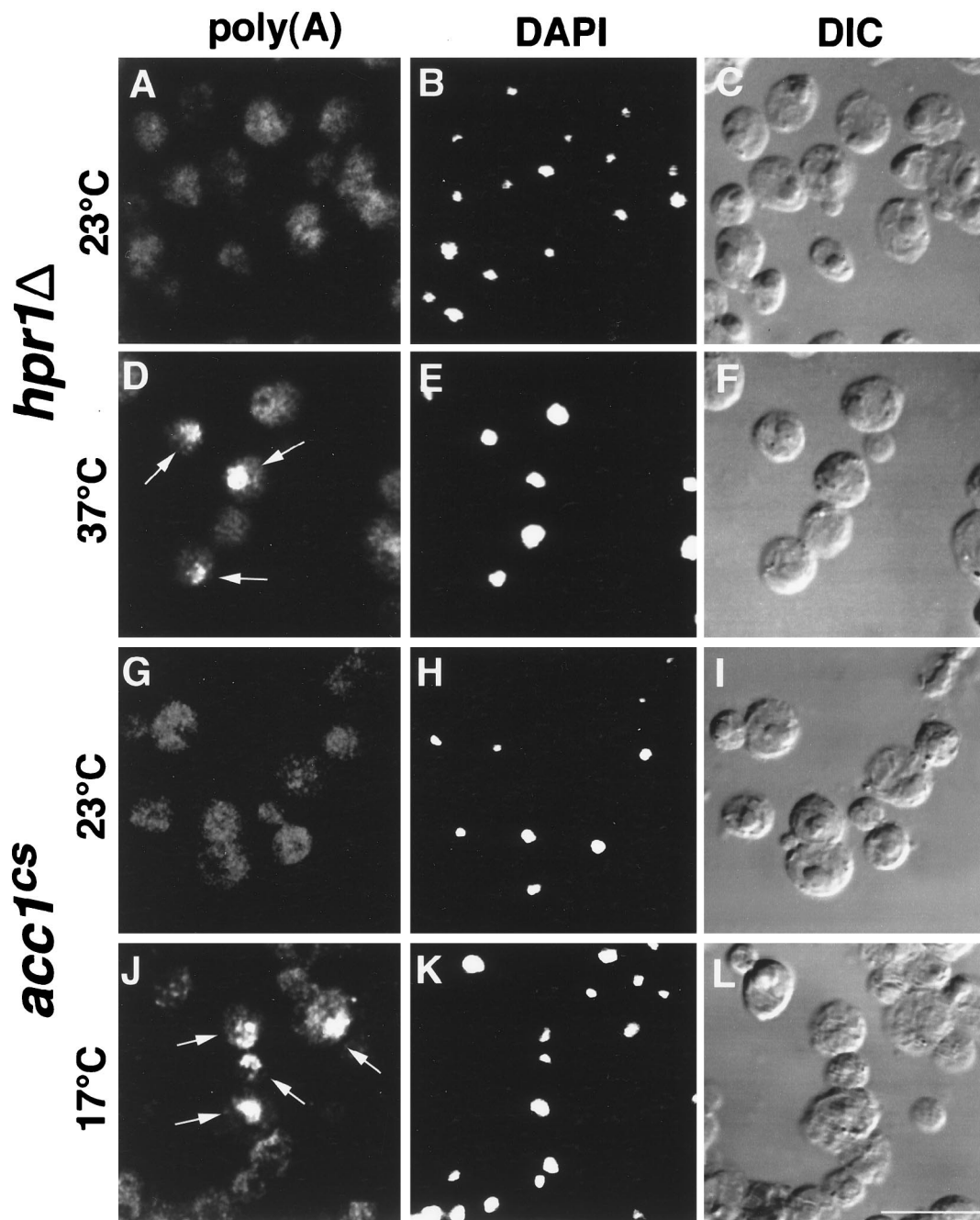


FIG. 5. *hpr1Δ* and *acc1^{cs}* cells are defective in nuclear export of polyadenylated RNA. Nuclear accumulation of polyadenylated RNA in *hpr1Δ* and *acc1^{cs}* cells at the nonpermissive temperature is shown. Cells were grown to early logarithmic phase at 23°C and shifted to 37 and 17°C, respectively, for 4 h, and fixed and processed for in situ hybridization with a digoxigenin-labeled oligo(dT)₂₅₋₃₀ probe. Strong accumulation of nuclear poly(A)⁺ RNA was visible in *hpr1Δ* and *acc1^{cs}* cells shifted to nonpermissive temperatures (arrows in panels D and J) but not in cells grown at permissive temperatures (A and G). DAPI (4',6-diamidino-2-phenylindole) staining and differential interference contrast (DIC) pictures of the same visual field are shown to the right. Bar, 10 μm.

cleolus upon shifting of *hpr1Δ* and *acc1^{cs}* cells to nonpermissive temperatures (data not shown).

DISCUSSION

We have described a synthetic lethal interaction between a loss-of-function allele of *HPRI* and two conditional alleles of *ACCI*, encoding yeast acetyl-CoA carboxylase. Acc1p is an essential cytoplasmic enzyme that catalyzes the rate-limiting step of de novo synthesis of fatty acids. In the *hpr1Δ* mutant

background, Acc1p activity was reduced approximately 15-fold. This reduced activity did not result in gross alterations of the lipid or fatty acid composition of the *hpr1Δ* mutant but rendered the mutant hypersensitive to sorafen A, an inhibitor of Acc1p activity. Analysis of steady-state levels of biotinylated Acc1p and *ACCI* mRNA revealed that levels of biotinylated Acc1p and *ACCI* transcripts were reduced twofold in the absence of Hpr1p, indicating that the lack of Hpr1p affected cellular Acc1p activity already at the level of transcription.

Several lines of evidence suggested that the synthetic lethal

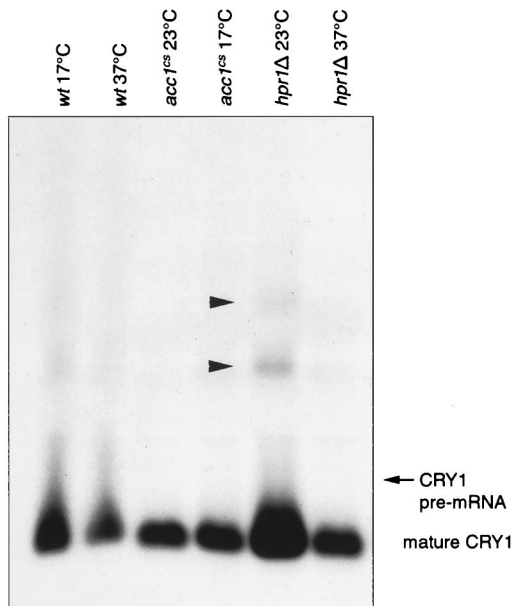


FIG. 6. Northern analysis of *CRY1* mRNA processing in wild-type, *hpr1Δ*, and *acc1^{cs}* cells. Ten micrograms of total RNA isolated from cells grown at the permissive temperature (23°C) or shifted for 4 h to a nonpermissive temperature (37 or 17°C) was analyzed by Northern hybridization with *CRY1* as a probe. The position of mature *CRY1* mRNA (540 bases) is shown. The position of *CRY1* pre-mRNA (847 bases) is indicated by the arrow. Oversized transcripts detected in *hpr1Δ* cells are indicated by the arrowheads pointing to the right. wt, wild type.

interaction of *hpr1Δ* with conditional *acc1* alleles was not simply due to the combination of a twofold reduction of *ACC1* transcription with an enzymatically challenged mutant *Acc1p* allele. First, like other genes encoding lipid biosynthetic enzymes, *ACC1* transcription is under the regulatory control of the transcriptional activators Ino2p and Ino4p. In the absence of Ino2p or Ino4p, *ACC1* transcription is reduced two- to threefold (15). *acc1^{cs} ino2* or *acc1^{cs} ino4* double mutants, grown in the presence of 11 μM inositol to rescue the inositol auxotrophy, however, did not show any reduced growth compared to the *acc1^{cs}* mutant alone (data not shown). Moreover, the *hpr1Δ* mutation did not result in an inositol auxotrophic phenotype or a reduction in *INO1* mRNA levels (10), indicating that *HPR1* does not function like Ino2p and Ino4p in coordinate regulation by inositol (26). Second, *ACC1* transcription was reduced twofold in a *top1Δ* mutant background (data not shown). Unlike *hpr1Δ*, however, *top1Δ* was not synthetically lethal with *acc1^{cs}* or *acc1^{ts}*. As is the case for the *ino2* and *ino4* mutants, a twofold reduction of *acc1^{cs}* levels in a *top1Δ* mutant is thus not sufficient to cause synthetic lethality. These data argue that the interaction between *hpr1Δ* and conditional *acc1* alleles is characteristic for the way that *hpr1Δ* affects *ACC1* expression and that the synthetic lethality is not solely due to a twofold reduction of *ACC1* transcription in the *hpr1Δ* mutant background.

The phenotypes that have been studied for *hpr1Δ* strains are all related to nuclear events: hyperrecombination of direct repeats in chromosomal DNA (3), synthetic lethality with a mutant allele of DNA topoisomerase genes (3), or a mutation in one of the histone genes (10). The temperature-sensitive growth of *hpr1Δ* is suppressed by mutations in *SOH* genes that play a role in transcription (10). Some of the *soh* mutants suppress the soraphen A sensitivity of *hpr1Δ* and the synthetic phenotype of the *acc1^{cs} hpr1Δ* double mutant (11). The pres-

ence of a nuclear localization sequence in Hpr1p suggests that this protein is most likely nuclear, and recent studies using a FLAG-tagged Hpr1p have confirmed this localization (11). Whether Hpr1p is confined to the chromatin-rich nucleoplasm or to the nucleolus is currently being investigated.

We now report that *hpr1Δ* cells are conditionally defective in nuclear export of polyadenylated RNA, synthesize oversized *CRY1* transcripts, are impaired in rRNA processing, and display an aberrant structure of the nucleolus. These are phenotypic changes that previously have been analyzed to characterize a set of conditional mutants that block mRNA transport (*mtr* [18]). A defect in nuclear export of polyadenylated RNA has previously also been observed in a temperature-sensitive *acc1^{ts} (mtr7)* mutant (31), and we now report that a second, cold-sensitive *acc1^{cs}* mutant also has an Mtr phenotype. We thus propose that the basis for the synthetic phenotype between *hpr1Δ* and conditional *acc1* alleles lies in their common defect in mRNA export and that the combination of mutations that affect nuclear mRNA export is lethal. This would explain the absence of a synthetic phenotype in the *acc1^{cs} top1Δ* and *acc1^{cs} ino2/4* double mutants. The synthetic phenotype would indicate either that each mutant slightly decreases mRNA export and that the additive effect of the two mutations is lethal or that each mutant blocks transport through a different pathway (for a review, see reference 39). Whether Hpr1p acts directly or indirectly to affect transport is not known, but it is likely that Hpr1p affects expression of a gene(s) that is involved in transport and that *Acc1p* affects nuclear membrane synthesis and hence nuclear pore complex function. M. Chang et al. have suggested that the RNA polymerase II complex in which Hpr1p is found is involved in the expression of cell wall genes (7). The cell wall defects of mutants encoding components of the RNA polymerase II complex may be analogous to the nuclear transport phenotype that we see in *hpr1Δ* mutants, which may be caused by a reduced expression of nuclear transport factors.

Interestingly, *hpr1Δ* and *acc1^{cs}* mutant cells display an aberrant circle-shaped nucleolus. The nucleolus is the site of ribo-

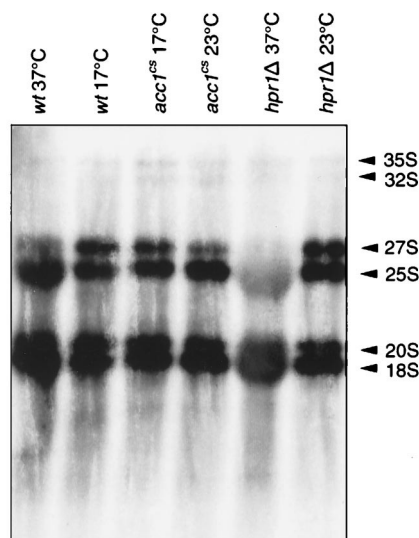


FIG. 7. rRNA processing in wild-type, *hpr1Δ*, and *acc1^{cs}* strains. Strains were preincubated for 1 h at permissive or nonpermissive temperatures and pulse-labeled for 10 min with [³H]uridine. RNA was isolated, and equal amounts of incorporated radioactivity were loaded on a denaturing agarose gel. The positions of 35, 32, 27, 25, 20, and 18S rRNA are shown by arrowheads. wt, wild type.

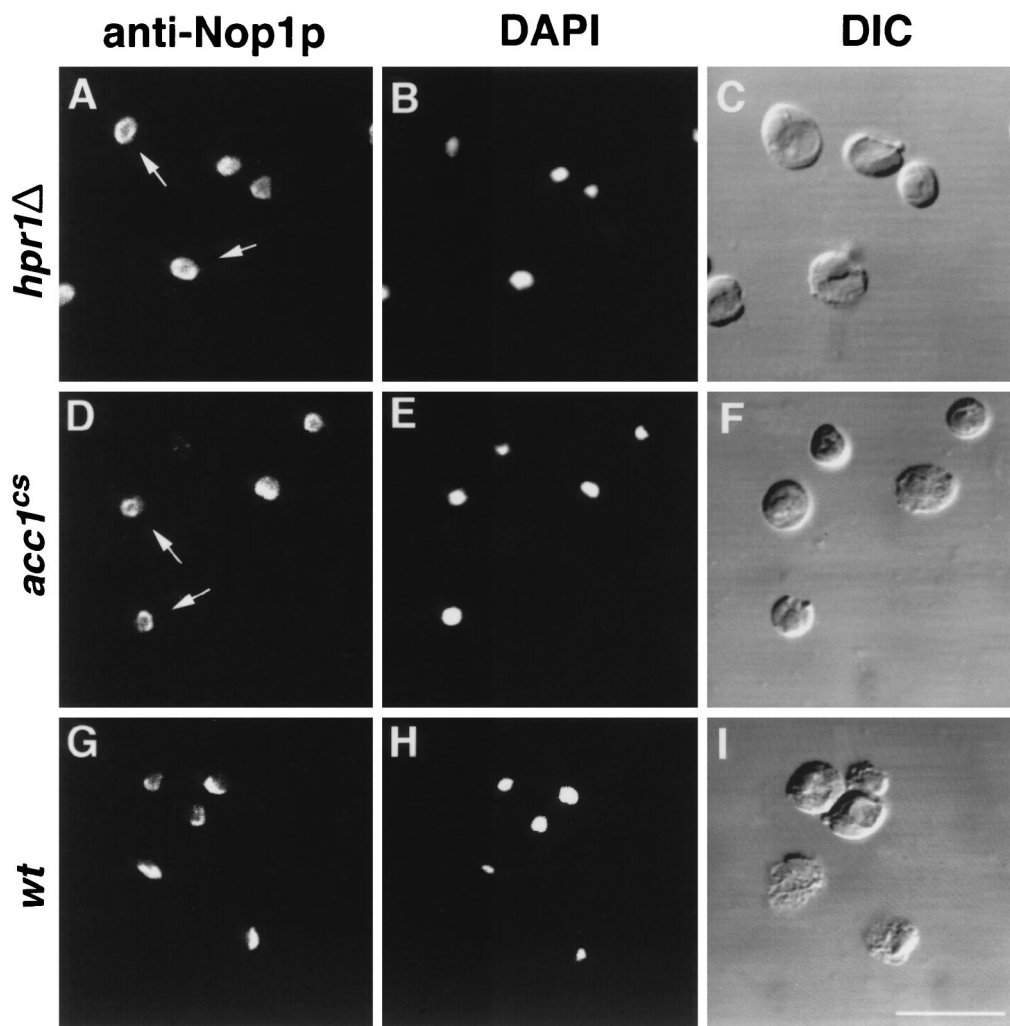


FIG. 8. Ring-shaped structure of the nucleolus in *hpr1Δ* and *acc1^{cs}* cells. Shown is immunofluorescence localization of the nucleolar antigen Nop1p in *hpr1Δ*, *acc1^{cs}*, and wild-type cells. Strains were cultivated in YEPD to early logarithmic growth phase and fixed and processed for immunofluorescence detection of Nop1p as described in Materials and Methods (A, D, and G). Arrows in panels A and D point to ring-shaped nucleoli. DAPI (4',6-diamidino-2-phenylindole) staining (B, E, and H) and differential interference contrast (DIC) pictures (C, F, and I) of the same visual field are shown to the right. Bar, 10 μ m. wt, wild type.

somal DNA (rDNA) transcription by RNA polymerase I, processing of rDNA transcripts, and assembly of ribosomes (for reviews, see references 24, 30, and 34). In wild-type yeast cells, the nucleolus is a crescent-shaped region of the nucleus that stands in close contact with the nuclear envelope (16). A rounded nucleolar structure that often lacked extensive contact with the nuclear envelope has recently been observed for strains that express polymerase II-transcribed 35S rRNA (25). Furthermore, many of the previously characterized mRNA transport mutants display a fragmented or enlarged nucleolus (18), an observation that led us to propose an involvement of the nucleolus or nucleolar proteins in the mRNA export pathway (32). More recently, a correlation between structural alterations of the nucleolus and aging of yeast cells has also been observed (19). In this case, nucleolar changes appear to be due to the accumulation of extrachromosomal rDNA circles in old cells (37, 38). The significance of the observed morphological alterations of the nucleolus in *hpr1Δ* and *acc1^{cs}* mutant cells is not clear at present. Hpr1p affects nuclear events that may be connected to rDNA transcription and/or recombination, but the *hpr1Δ* mutant has not been found to have any altered rate

of rDNA recombination (3). Acc1p, on the other hand, affects the lipid composition of all cellular membranes, including the nuclear envelope that contacts the nucleolus. How this contact between nucleolar structures and the nuclear envelope is maintained is not known. The two proteins thus clearly have distinct functions, and there is no obvious overlap between them. Nevertheless, both mutants affect nuclear export of polyadenylated RNA and display an altered nucleolar morphology. We propose that the two mutations affect two different, but overlapping, functions required for efficient nucleocytoplasmic transport: the lipid composition of the nuclear envelope in the case of the *acc1^{cs}* mutation and transcription-packaging of nascent transcripts into a transport-competent state in the case of the *hpr1Δ* mutation. Reducing the efficiency of both processes at the same time is lethal.

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