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, *acc1ts*, encoding the rate-limiting enzyme of fatty acid synthesis. The synthetic lethal phenotype of the *acc1ts hpr1Δ* double mutant was only partially complemented by exogenous fatty acids. *hpr1Δ* was also synthetically lethal with a previously isolated, temperature-sensitive allele of *ACC1*, *mtr7* (mRNA transport), indicating that the lethality of the *acc1ts 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 *ACC1* 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 *acc1ts* 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 *acc1ts* 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 *hpr1* gene, hereafter referred to as *hpr1Δ* (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, *acc1-200cs*, hereafter referred to as *acc1ts* (14).

The acetyl-CoA carboxylase gene, *ACC1*, 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 *ACC1* gene is under coordinate transcriptional regulation by the phospholipid precursors inositol and choline (15). A temperature-sensitive allele of *ACC1*, *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 *acc1ts* 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 *acc1ts* 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 *ACC1* (31), and propose that the lethal interaction between *hpr1Δ* and *acc1ts* is due to a combined effect of the two mutations on nuclear export of polyadenylated RNA.

The *hpr1Δ* mutant is due to a special lipid requirement of the nuclear membrane-nuclear pore complex (31, 33). The nuclear transport defect of this *acc1ts* allele has been proposed to be due to a special lipid requirement of the nuclear membrane-nuclear pore complex (31, 33).

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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.

Table 1. Yeast strain genotypes and construction

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<th>Strain</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
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<td>479-2A</td>
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<td>H. Klein</td>
</tr>
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<td>485-13A</td>
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<td>H. Klein</td>
</tr>
<tr>
<td>U706-1C</td>
<td>MATα hpr1Δ::HIS3 leu2-3,112 trp1-1 ura3-1 ade2-1 his3-1,115 can1-100</td>
<td>R. Rothstein</td>
</tr>
<tr>
<td>U706-4C</td>
<td>MATα hpr1Δ::HIS3 leu2-3,112 trp1-1 ura3-1 ade2-1 his3-1,115 can1-100</td>
<td>R. Rothstein</td>
</tr>
<tr>
<td>W303A</td>
<td>MATα hpr1Δ::HIS3 leu2-3,112 trp1-1 ura3-1 ade2-1 his3-1,115 can1-100</td>
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</tr>
<tr>
<td>YRXS12</td>
<td>MATα acc1ts leu2-3 trp1-1 ura3-1 ade2-1 his3-1,115 can1-100</td>
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<td>This work; spore from cross between U706-4C and YRXS12</td>
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<tr>
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Synthetic lethality between acc1ts and hpr1Δ. The acc1ts acc1-200 allele was recovered in a screen for mutants that display a synthetic lethal interaction with a null allele of HPR1 (14). The synthetic lethality of the acc1ts hpr1Δ double mutant is indicated by the failure to recover spore colonies of this genotype from a cross between an acc1ts strain and an hpr1Δ strain. When the tetrads from this cross were dissected on a YEPD plate supplemented with fatty acids, the acc1ts hpr1Δ 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 acc1ts and hpr1Δ mutants. Soraphen A is a potent inhibitor of Acc1p activity in yeast (42). The acc1ts allele was found to confer hypersensitivity to soraphen A at the permissive temperature (Fig. 2A). Since the acc1ts allele is synthetically lethal with the hpr1Δ mutation, we examined the hpr1Δ mutant for soraphen A sensitivity. The hpr1Δ null mutant was found to be as soraphen A sensitive as the acc1ts mutant on solid medium (Fig. 2A) and in liquid medium (Fig. 2B).

The synthetic lethal interaction between hpr1Δ and acc1 is not allele specific. A temperature-sensitive allele of ACC1, mit7, herein referred to as acc1ts, had previously been isolated in a screen for conditional mRNA transport mutants (31). To determine whether the synthetic lethal interaction between acc1ts and hpr1Δ is an allele-specific phenomenon, a cross between acc1ts and hpr1Δ 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 acc1ts hpr1Δ double mutants were viable on sorbitol-supplemented medium, no acc1ts hpr1Δ double mutants were recov-
Phpr1A affects Acc1p activity at the level of transcription. The observation that hpr1A is genetically lethal with two different conditional acc1 alleles and that hpr1A itself is hypersensitive to soraphen A suggested that Acc1p activity is affected in the hpr1A mutant. Acc1p enzymatic activity was determined in cytosolic fractions from the wild type and the hpr1A mutant strain. Cells were grown in YEPD medium at 30°C, cytosol was prepared, and Acc1p activity was determined as described previously (23). Acc1p enzyme activity in the hpr1A 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 × 10^{-5} ± 3.7 × 10^{-5} ΔE/min/μg; enzyme activity of the hpr1A mutant, 2.6 × 10^{-5} ± 1.7 × 10^{-5} ΔE/min/μg. This greatly reduced Acc1p activity in the hpr1A mutant background, however, did not result in an altered lipid or fatty acid composition of hpr1A 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 hpr1A 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 hpr1A strains compared to the wild type (data not shown). To confirm that expression of the chromosomal ACC1 gene was affected in hpr1A strains, Northern blot analysis was carried out with total RNA. The relative levels of steady-state 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; hpr1A mutant, 45.4 ± 3.8; acc1D mutant, 90.7 ± 18.5. No significant difference was found in the levels of ACC1 message between the acc1D mutant and the wild type. In contrast, ACC1 expression was reduced twofold in the hpr1A mutant compared to the wild type. These data indicate that the reduced Acc1p activity in the hpr1A 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 hpr1A 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-
required 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 acc1ts) defect in mRNA export was also observed in acc1cs mutants (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 acc1cs 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 acc1ts (mtr7 [18]), acc1cs 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 acc1ts, mutant. The labeling of all rRNA species was greatly reduced in the hpr1Δ mutant.

**hpr1Δ and acc1ts 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 acc1ts mutants 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 acc1ts 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-
cleolus upon shifting of hpr1Δ and acc1cs cells to nonpermissive temperatures (data not shown).

DISCUSSION

We have described a synthetic lethal interaction between a loss-of-function allele of HPR1 and two conditional alleles of ACC1, 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 soraphen A, an inhibitor of Acc1p activity. Analysis of steady-state levels of biotinylated Acc1p and ACC1 transcripts revealed that levels of biotinylated Acc1p and ACC1 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 interaction between a loss-of-function allele of HPR1 and two conditional alleles of ACC1:

1. Nuclear accumulation of polyadenylated RNA in hpr1Δ and acc1cs 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 olio(dT)25–30 probe. Strong accumulation of nuclear poly(A)⁺ RNA was visible in hpr1Δ and acc1cs 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.

FIG. 5. hpr1Δ and acc1cs cells are defective in nuclear export of polyadenylated RNA. Nuclear accumulation of polyadenylated RNA in hpr1Δ and acc1cs 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 olio(dT)25–30 probe. Strong accumulation of nuclear poly(A)⁺ RNA was visible in hpr1Δ and acc1cs 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.
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 to threefold (15). acc1Δ ino2 or acc1Δ 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 acc1Δ 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 acc1Δ or acc1Δ. As is the case for the ino2 and ino4 mutants, a twofold reduction of acc1Δ 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 of 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 acc1Δ hpr1Δ double mutant (11). The presence 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 nucleoleus 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 acc1Δ (mtr7) mutant (31), and we now report that a second, cold-sensitive acc1Δ 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 acc1Δ top1Δ and acc1Δ 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 acc1Δ mutant cells display an aberrant circle-shaped nucleolus. The nucleolus is the site of ribo-

FIG. 6. Northern analysis of CRY1 mRNA processing in wild-type, hpr1Δ, and acc1Δ 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.

FIG. 7. rRNA processing in wild-type, hpr1Δ, and acc1Δ cells. 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 35S, 32S, 27S, 25S, 20S, and 18S rRNA are shown by arrowheads. 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 acc1Δ 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 acc1Δ 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.

FIG. 8. Ring-shaped structure of the nucleolus in hpr1Δ and acc1Δ cells. Shown is immunofluorescence localization of the nucleolar antigen Nop1p in hpr1Δ, acc1Δ, 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 μm. wt, wild type.

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REFERENCES


