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Degradation of Normal mRNA in the Nucleus of *Saccharomyces cerevisiae*

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A nuclear mRNA degradation (DRN) system was identified from analysis of mRNA turnover rates in *nup116*-Δ strains of *Saccharomyces cerevisiae* lacking the ability to export all RNAs, including poly(A) mRNAs, at the restrictive temperature. Northern blotting, in situ hybridization, and blocking transcription with thiolutin in *nup116*-Δ strains revealed a rapid degradation of mRNAs in the nucleus that was suppressed by the *rrp6*-Δ, *rai1*-Δ, and *cbc1*-Δ deletions, but not by the *upf1*-Δ deletion, suggesting that DRN requires Rrp6p, a 3′-to-5′ nuclear exonuclease, the Rat1p, a 5′-to-3′ nuclear exonuclease, and Cbc1p, a component of CBC, the nuclear cap binding complex, which may direct the mRNAs to the site of degradation. We propose that certain normal mRNAs retained in the nucleus are degraded by the DRN system, similar to degradation of transcripts with 3′ end formation defects in certain mutants.

The rate of synthesis of a protein is determined primarily by the steady-state level of the corresponding mRNA, which, in turn, is determined by the rate of synthesis and degradation of the mRNA. Thus, mRNA stability is an important parameter in the regulation of gene expression, affecting both the steady-state level of the protein and the transient time of translation of the formed transcript.

Normal mature mRNAs of *Saccharomyces cerevisiae* are degraded through a major 5′-to-3′ (5′→3′) pathway and a minor 3′→5′ pathway, both of which take place in the cytoplasm. Both degradation pathways begin with the shortening of the poly(A) tail to a track of A_{10} or less, caused by a Pop2p-Ccr4p-Caf1p poly(A) nuclease complex (16, 73). In the major pathway, deadenylation causes disassociation of the Pab1p [poly(A) binding protein] from the cap binding protein eIF4G, followed by the removal of the 5′ cap by the decapping enzyme Dcp1p. Subsequently, the decapped mRNA is rapidly degraded by the 5′→3′ exonuclease Xrn1p and by assistance of Sbp8p and other protein components (4, 5, 17, 32, 47, 57, 58, 71).

In the minor pathway, deadenylated mRNAs are subjected to 3′→5′ degradation by the action of the exosome, a complex of 10 3′→5′ riboexonucleases that also plays a central role in the precise formation of the 3′ ends of several types of RNAs (9), including processing precursors for rRNAs in the nucleus. The exosome may also degrade fragments of mRNA released by endonucleolytic cleavage. Yeast mutants lacking either exonucleolytic pathway degrade their mRNAs more slowly, but the loss of both pathways is lethal (54).

While most mRNAs are slowly deadenylated before rapid degradation, certain normal and mutant mRNAs are rapidly degraded by a third specialized pathway, known as nonsense-mediated mRNA decay (NMD) pathway, or mRNA surveillance, which triggers decapping before deadenylation (26, 29, 55). Substrates of the NMD pathway include not only mRNAs containing nonsense mutations but also wild-type mRNAs that contain the following: inefficiently spliced pre-mRNAs that enter the cytoplasm upstream open reading frames (14, 77) and certain codons subject to leaky scanning (80). In fact, Lelivelt and Culbertson (48) showed that mutation of protein components of NMD could actually lead to the increase in the steady-state levels of a wide spectrum of normal mRNAs. The NMD pathway discriminates between nonsense codons on the basis of downstream sequence elements located 3′ to susceptible nonsense codons (60, 87).

In addition, yeast has the capacity to recognize and degrade mRNAs lacking all termination codons, a process that occurs by a mechanism distinct from NMD and from the major mRNA turnover pathway that requires deadenylation, decapping, and 5′→3′ exonucleolytic decay (20, 76).

Previously, Das et al. (15) presented preliminary evidence, based on the analysis of *cyc1-512* suppressors, that Cbc1p, the large subunit of nuclear cap binding complex, is involved in a novel mRNA degradation system. The *cyc1-512* mutation causes a 90% reduction in the level of iso-1-cytochrome *c* because of the lack of a proper 3′ end-forming signal, resulting in low levels of eight aberrantly long *cyc1-512* mRNAs, which differ in length at their 3′ termini (15, 85). Suppression analysis of *cyc1-512* showed that it can be suppressed by deletion of either of the nonessential genes *CBC1* or *CBC2*, which encode, respectively, the CBP80 or CBP20 subunits of the nuclear cap binding complex, or by deletion of the nonessential gene *UPF1*, which encodes a major component of the mRNA surveillance complex responsible for NMD. Suppression of *cyc1-512* by *cbc1*-Δ occurred by two different mechanisms. The levels of the shorter *cyc1-512* transcripts were enhanced in the *cbc1*-Δ mutants by promoting 3′-end formation at otherwise weak sites; whereas the levels of the longer *cyc1-512* transcripts, as well as all mRNAs, were slightly enhanced by diminishing degradation. Furthermore, *cbc1*-Δ greatly suppressed the degradation of mRNAs and other phenotypes of a *rat7-1* strain that is defective in mRNA export. These findings led Das et al. (15) to

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TABLE 1. List of yeast strains used in this study

Strain designation	Genotype	Abbreviated genotype	Reference
B-11598	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100*	*NUP116* or *HPR1*	95
B-11592	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 hpr1*::*HIS3*	*hpr1*-Δ	This study
B-11599	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 nup116*::*HIS3*	*nup116*-Δ	95
B-13398	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 nup116*::*HIS3 cbc1*::*URA3*	*nup116*-Δ *cbc1*-Δ	This study
B-13755	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 nup116*::*HIS3 rrp6*::*URA3*	*nup116*-Δ *rrp6*-Δ	This study
B-14236	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 nup116*::*HIS3 upf1*::*URA3*	*nup116*-Δ *upf1*-Δ	This study
B-14238	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 nup116*::*HIS3 rail*::*LEU2*	*nup116*-Δ *rail*-Δ	This study
B-14366	*MAT*α *leu2-3,112 his3-11,15 ade2-1 ura3-1 trp1-1 can1-100 nup116*::*HIS3 cbc1*::*URA3 rrp6*::*KAN*	*nup116*-Δ *cbc1*-Δ *rrp6*-Δ	This study
B-10603	*MAT***a** *his3*-Δ*200 ura3-52 leu2*-Δ*1* (also denoted FY86)	*RAT7*	96
B-10095	*MAT***a** *his3*-Δ*200 ura3-52 leu2*-Δ*1 rat7-1*	*rat7-1*	25
B-10096	*MAT***a** *his3*-Δ*200 ura3-52 leu2*-Δ*1 rat7-1 cbc1*::*URA3*	*rat7-1 cbc1*-Δ	18
B-10097	*MAT***a** *his3*-Δ*200 ura3-52 leu2*-Δ*1 rat7-1 upf1*::*URA3*	*rat7-1 upf1*-Δ	18

suggest that Cbc1p possibly defines a novel degradation pathway that acts on mRNAs partially retained in nuclei. However, the interpretation of the results obtained with *rat7-1* was complicated by the suppression of the mRNA export defect by *cbc1*-Δ, allowing growth at the restrictive temperature, and thus preventing meaningful studies with mRNA half-lives and in situ mRNA localization using fluorescence in situ hybridization (FISH). Thus, it remained to be definitively established if the Cbc1p-dependent mRNA decay system was located in the nucleus.

In this study, we definitely established the existence of this novel mRNA degradation pathway which we named the DRN (for decay of RNA in the nucleus) pathway, and we conclusively confirmed the involvement of Cbc1p in this pathway. We have investigated the nature of this degradation pathway, primarily by using a mutation (*nup116*-Δ) in *NUP116* which encodes a nucleoporin that plays a central role in nuclear mRNA export (3, 31). *nup116*-Δ strains grow slowly at 25°C and are inviable at 37°C (81). The lethal phenotype correlates with defects in mRNA export and perturbations of structures of the nuclear envelope and nuclear pore complexes, resulting in the complete nuclear accumulation of mRNA (82). We show that retention of mRNAs in the nucleus causes accelerated degradation of representative transcripts. Deletions of either *CBC1* or *RRP6*, which encodes a nuclear 3′→5′ exoribonuclease associated with the exosome, suppressed the rapid mRNA degradation phenotype. Deletion of *RAI1*, which encodes a nuclear protein required for the activity of the nuclear 5′→3′ exoribonuclease Rat1p, also suppressed the rapid degradation, but to a lesser extent. We conclude that DRN involves the Rrp6p and Rat1p nuclear exonucleases, as well as the CBC, the nuclear cap binding complex, which may direct the mRNAs to the site of degradation.

MATERIALS AND METHODS

Strains, media, and yeast genetics. Standard genetic nomenclature was used to designate wild-type alleles (for example, *NUP116*, *RAT7*, *CYC1*, *CYH2*, and *ACT1*), recessive mutant alleles (for example, *cyc1-512* and *rat7-1*, etc.), and disruptants or deletion mutants (for example, *cbc1*-Δ and *cbc1*::*URA3*, etc). The genotypes of *S. cerevisiae* strains used in this study and the abbreviated genotypes are listed in Table 1. Standard YPD, YPG, SC-Ura (uracil omission), SC-Leu (leucine omission), and other omission media were used for testing and growth of yeast propagation and testing (70). Yeast genetic analysis was carried out by standard procedures described by Sherman (70).

Transformation, nucleic acid isolation, and manipulation. *S. cerevisiae* cultures were transformed with linear DNA for gene disruption (66), using the lithium acetate method (33), followed by selection on SC-Ura (uracil omission) or SC-Leu (leucine omission) media. The yeast chromosomal gene *CBC1* was disrupted by transforming the appropriate yeast strains with DNA fragments that were prepared by digesting the plasmid pAB1100 with *Sal*I and *Bam*HI. Similarly, the *UPF1* gene on the chromosome was disrupted by transforming the suitable yeast strains with DNA fragments that was prepared by digesting the plasmid YCpPL51 (47) with *Bam*HI and *Eco*RI. The *RRP6*, *RAI1*, and *XRN1* chromosomal genes were similarly disrupted by digesting plasmids pAB2755 with *Bam*HI and *Pvu*II (7), pAB2806 with *Sst*I and *Hin*dIII (84), and pAB2809 with *Xho*I and *Sal*I (46), respectively, and transforming yeast strains with the appropriate DNA fragments. *Escherichia coli* strains DH5α and XL1-Blue were transformed by the protocol of Hanahan (22). Standard techniques of DNA manipulation such as cloning, subcloning, and sequencing, etc., used in this study are described by Sambrook et al. (68).

Analysis of mRNA steady-state levels and stability. The stability of the various mRNAs and pre-mRNAs were determined by the inhibition of transcription with thiolutin (4 μg/ml) at 37°C unless mentioned otherwise, as described previously (15). Total RNA was isolated as described by Russo et al. (67) from approximately 10^8 cells. Northern blot analysis of different mRNAs was conducted as outlined by Russo et al. (67). mRNA levels were quantified by storage phosphorimager analysis (model 425E; Molecular Dynamics) and normalized against the 18S rRNA signals.

The decay rates and half-lives were estimated with the SigmaPlot (version 4.0) regression analysis program, using either a single exponential decay formula, $y = 100\ e^{-bx}$, or a four-parameter double-exponential decay formula, $y = ae^{-bx} + ce^{-dx}$ (where $a + c = 100$).

FISH analysis. Cells for FISH analysis were grown in YPD medium to early log phase at 23°C. Half of the culture was then mixed with an equal volume of prewarmed medium and shifted to 37°C. Aliquots of 10^8 cells were removed both from the mock-shifted as well as from shifted culture at different time intervals after temperature shift and mixed with fresh 4% formaldehyde. The cultures were immediately centrifuged at 3,500 × *g* for 5 min and fixed in 1/10 volume of freshly prepared solution of 0.1 M potassium phosphate buffer (pH 6.5), 3.7% formaldehyde, and 10% methanol for 1 h at room temperature. The cells were centrifuged, and the cell pellets were washed three times with 0.1 M potassium phosphate, pH 6.5, and once with SCP buffer (which contains 0.1 M dipotassium hydrogen phosphate, 0.033 M citric acid, and 1.2 M sorbitol) and were subsequently resuspended in 100 μl of SCP. Spheroplasts were generated by incubating 10^8 cells in 100 μl SCP containing 1/40 volume of glusulase (NEN) and 100 μg of zymolase T-20 (U.S. Biologicals) for 1 h at 30°C. Spheroplasts were washed three time with SCP and adhered to coverslips precoated with 0.01% poly-lysine and plunged into ice-cold methanol for 5 min followed by rinsing in acetone for 30 s at room temperature and dry at same temperature for 20 s.

Each coverslip for in situ hybridization was rehydrated in 5 ml of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 5 min and prehybridized in a solution containing 2× SSC, 50% formamide, 1% bovine serum albumin, 10 mM VRC (Gibco-BRL), 10% dextran sulfate, salmon sperm DNA (500 μg/ml), and *E. coli* tRNA (125 μg/ml) for 1 h at 37°C. Coverslips were inverted on 24 μl of this solution containing10 ng of a Cy3-labeled 43-mer oligo(dT), and hybridizations were performed overnight at 37°C. Following hybridization, each cov-

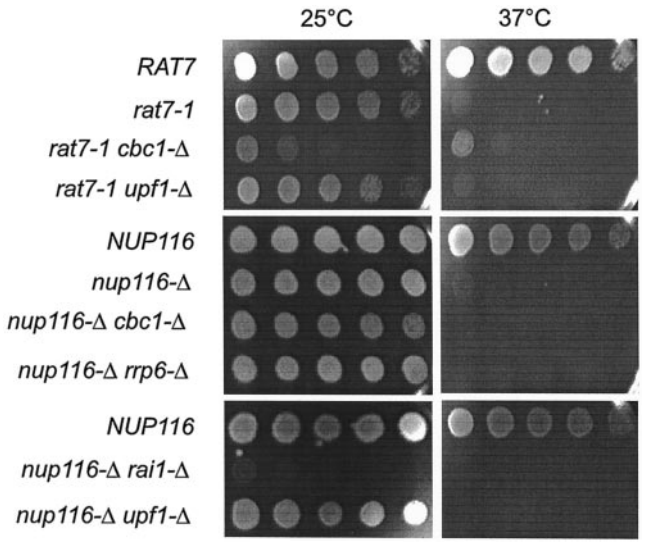

FIG. 1. The growth of 1/10 serial dilutions of suspensions of various strains (Table 1) at 25°C for 4 days or 37°C for 2 days on YPD medium, demonstrating that the growth defect at 37°C of *nup116*-Δ strains is not suppressed by *cbc1*-Δ, *rrp6*-Δ, or other mutations, whereas the growth defect at 37°C of *rat7-1* strains are partially suppressed by *cbc1*-Δ.

erslip was washed twice at 37°C for 15 min in a solution of 10% formamide and 2× SSC, once in a solution of 2× SSC and 0.1% Triton X-100 for 15 min, twice in 1× SSC for 15 min, and once in 1× phosphate-buffered saline (1 mM KH_2PO_4, 10 mM Na_2HPO_4, 140 mM NaCl, 3 mM KCl [pH 7.4]) for 15 min. Coverslips were mounted in phenylenediamine containing glycerol and DAPI (4′,6-diamino-2-phenylindole).

Cells were examined with a Nikon Diaphot inverted epifluorescence microscope, using a 100× objective. Digital images were captured using a Princeton Instruments (Princeton, N.J.) Micromax camera and analyzed with MetaFluor software from Universal Imaging (Downingtown, Pa.). The images were processed with Adobe Photoshop 5.5 software.

Poly(A) tail lengths. Poly(A) tail lengths were analyzed as described by Butler et al. (10).

RESULTS

Experimental approach. In this investigation, we have demonstrated the existence of DRN, a novel nuclear mRNA decay pathway, primarily by using strains containing the *nup116*-Δ mutation that was previously reported to prevent the export of RNA at the restrictive condition of 37°C (3, 31, 81). We first demonstrated that lethal effect of *nup116*-Δ was not suppressed by *cbc1*-Δ or *rrp6*-Δ, etc., which are mutations in putative components of DRN (Fig. 1), thus suggesting that mRNA is retained in the nucleus in these mutant strains. Subsequently, we used the FISH procedure to directly verify that total poly(A) RNA is retained in the nucleus at the restrictive condition of 37°C in the *nup116*-Δ, as well as in the *cbc1*-Δ *nup116*-Δ strain (Fig. 2). Furthermore, Northern blot analysis was used to verify the cytological results by determining the steady-state levels (Fig. 3) and half-lives (Fig. 4) of the representative *CYC1*, *CYH2*, and *ACT1* mRNAs in *nup116*-Δ strains under the restrictive condition. Finally the effect of the *cbc1*-Δ, *rrp6*-Δ, and other mutations on the degradation of the representative mRNAs in a set of isogenic *nup116*-Δ strains were tested by examining steady-state levels (Fig. 3) and half-lives (Fig. 4 and 5). Graphical representation of some of the half-lives are presented in Fig. 6.

***cbc1*-Δ and other mutations do not suppress the growth defect of *nup116*-Δ.** We previously demonstrated that *cbc1*-Δ suppressed both the growth defect as well as rapid decay of specific mRNAs in the *rat7-1* strain at 37°C (15). The suppression of *rat7-1* by *cbc1*-Δ complicated our efforts to study the fate of mRNAs retained in the nucleus, so we have extended our studies of the Cbc1p-dependent decay of mRNAs by investigating a number of mutants defective in mRNA export, including *rat7-1*, *nup116*-Δ, and *hpr1*-Δ (Table 1). These mRNA export defective mutants were tested for suppression of their growth defect by *cbc1*-Δ, *rrp6*-Δ, and several other mutants. As shown in Fig. 1, *cbc1*-Δ and all other tested mutations did not suppress the growth defects of *nup116*-Δ at 37°C, a finding that is critical for the studies described below. The lack of growth of *nup116*-Δ *cbc1*-Δ and other double mutant strains (Table 1) at the restrictive temperature implies that total poly(A) RNA in these strains is not exported to the cytoplasm and still remains in the nucleus. This allowed us to directly investigate the effect of *cbc1*-Δ and other mutations on mRNAs retained in the nucleus.

Existence of DRN, a Cbc1p-dependent nuclear mRNA degradation pathway: cytological evidence. The inability of the *cbc1*-Δ deletion mutant and the several other mutants to suppress the temperature sensitive mRNA export defective *nup116*-Δ deletion mutant (82) prompted us to test the nuclear retention of total poly(A) RNA in a *nup116*-Δ *cbc1*-Δ strain. FISH analysis was used to verify if total poly(A) RNA accumulates in the nucleus under our experimental conditions and to demonstrate the existence of the DRN system. This technique distinguishes between the nuclear and cytoplasmic distribution of poly(A) RNA. A steady-state FISH analysis of poly(A) RNA, using Cy3-labeled 43-mer oligo(dT) probe, revealed that after 1 h of a shift from the permissive condition of 25°C to restrictive condition of 37°C, the fluorescent signal is predominantly nuclear in both the *nup116*-Δ and *nup116*-Δ *cbc1*-Δ strains when transcription is blocked, thus confirming that under this condition the vast majority of the cellular total poly(A) RNA is nuclear (data not shown; compare Fig. 2E and G and with Fig. 2F and H, which represents the position of the nucleus counterstained with DAPI). However, we sometimes observed a weak cytoplasmic background signal in a fraction of cells of both *nup116*-Δ and *nup116*-Δ *cbc1*-Δ strains. These observations justify the method employed and the condition used to investigate the degradation of total poly(A) RNA, as well as specific mRNAs by determining their half-lives, as described below.

The rates of degradation in situ of poly(A) RNA in the nucleus of both *nup116*-Δ and *nup116*-Δ *cbc1*-Δ strains were similarly investigated by using the Cy3-labeled 43-mer oligo(dT) probe under the following conditions: (i) the control culture at 25°C (Fig. 2A to D; designated 25°C) before the temperature shift, when both the export and transcription are still progressing; (ii) 1 h after the shift to 37°C and just before transcription is blocked (Fig. 2E to H; designated 37°C, 0 min); and (iii) 30 min (Fig. 2I to L; designated 37°C, 30 min) and (iv) 60 min (Fig. 2M to P; designated 37°C, 60 min) after the transcription is blocked. The left half of each pair of panels for each time point shows the poly(A) RNA localization and the

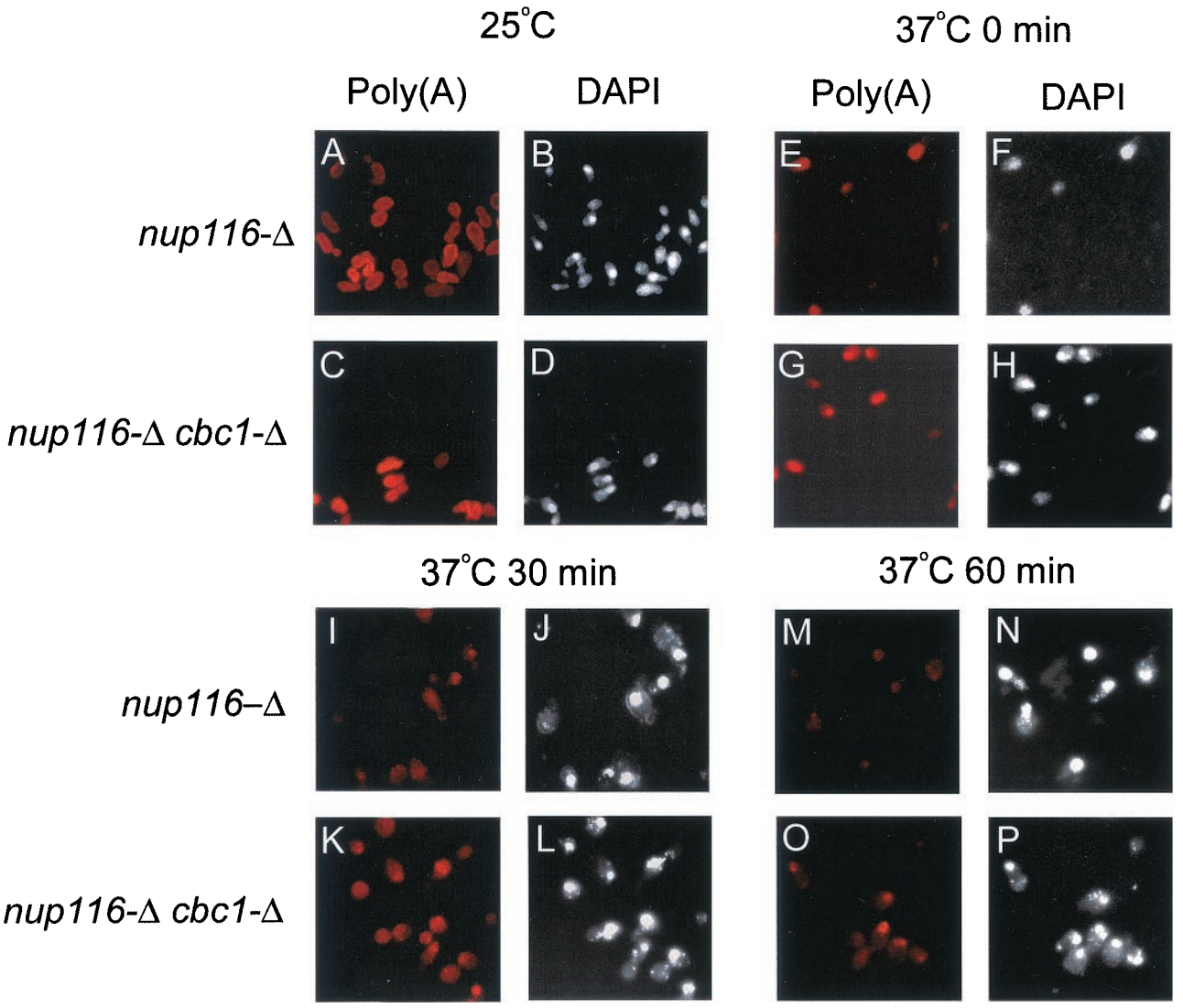

FIG. 2. FISH analyses revealing that total poly(A)$^+$ RNA in *nup116*-Δ and *nup116*-Δ *cbc1*-Δ strains are retained in the nucleus and that there is less degradation in the *nup116*-Δ *cbc1*-Δ strain. The isogenic pairs of strains were grown at 25°C to the mid-logarithmic phase of growth. Subsequently, one-half of each culture was transferred to the restrictive temperature of 37°C; the cultures were further incubated for one additional hour at both temperatures. Transcription of the cells in the culture shifted to 37°C was inhibited by the addition of thiolutin (4 μg/ml); the cells were harvested at various times after transcription block as indicated on top of each panel in the figure and subsequently fixed and processed for FISH and DAPI analysis as described in Materials and Methods. Left panels of mock shifted as well as each time point after transcription block show the localization and decay of the total poly(A)$^+$ RNA as visualized using Cy3-labeled oligo(dT) are denoted as Poly(A), whereas right panels of respective time points show the nuclear DNA as visualized using DAPI staining denoted as DAPI. The time indicated at the top of each panel represents the time after transcription block to after shift to 37°C. See the Results ("Existence of DRN, a Cbc1p-dependent nuclear mRNA degradation pathway: cytological evidence") for details of each panel.

right half shows the counter staining of the nucleus by DAPI. As shown in Fig. 2, the poly(A) RNA signal was diffuse over both the nucleus and cytoplasm of both *nup116*-Δ and *nup116*-Δ *cbc1*-Δ strains at the permissive condition of 25°C (Fig. 2A to D). After 1 h at 37°C and before blocking transcription (37°C, 0 min), the signal was predominantly nuclear in both strains, as revealed by the colocalization of the DAPI and poly(A) signals (Fig. 2E to H). On continued incubation at 37°C after blocking transcription, the nuclear fluorescence of the poly(A) RNA decreases more rapidly in the *nup116*-Δ strain compared to the *nup116*-Δ *cbc1*-Δ strain (Fig. 2I to L for 30 min and Fig. 2M to P for 60 min after transcription arrest; compare Fig. 2M with 2O). After 60 min of transcription block, the majority of the poly(A) signal disappeared in the nuclei of *nup116*-Δ strain (Fig. 2M). On the other hand under the same condition, the poly(A) signal still remained much stronger in the nuclei of *nup116*-Δ *cbc1*-Δ strain (Fig. 2O). Thus, this result clearly suggests that the decay of poly(A) RNA occurs in the nucleus of *nup116*-Δ strains, and this degradation requires Cbc1p. Importantly, these findings indicate that this method will allow us to determine the fate of mRNAs retained in the nucleus and thereby establish details of the DRN system.

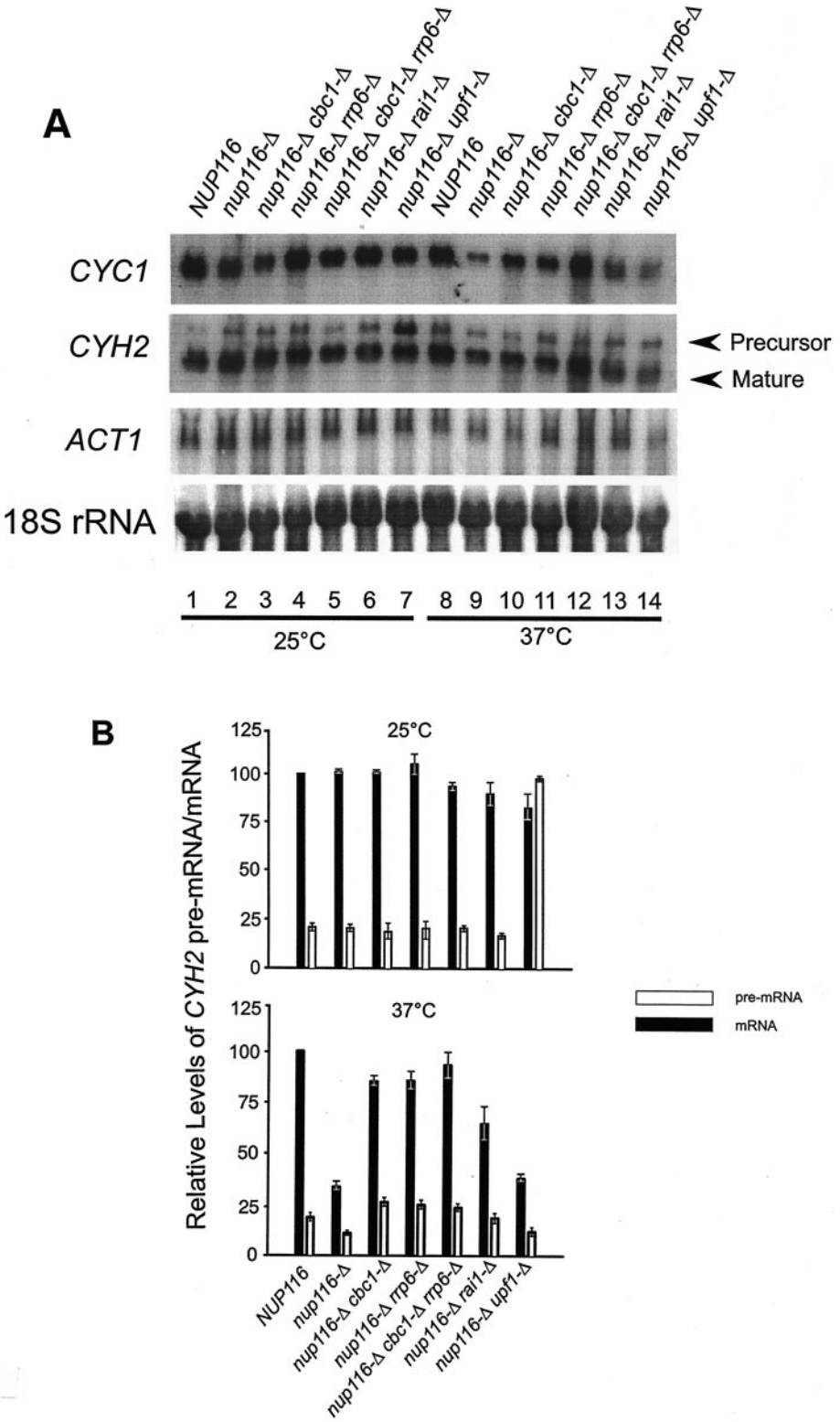

FIG. 3. Comparison of the steady-state levels of *CYC1*, *ACT1*, and *CYH2* precursor and matured mRNAs at 25°C (mock-shifted) and 37°C (shifted) in *NUP116* (normal), *nup116*-Δ, *nup116*-Δ *cbc1*-Δ, *nup116*-Δ *rrp6*-Δ, *nup116*-Δ *cbc1*-Δ *rrp6*-Δ, *nup116*-Δ *rai1*-Δ, and *nup116*-Δ *upf1*-Δ deletion strains. (A) Northern blots of steady-state levels of *CYC1*, *ACT1*, and *CYH2* mRNAs and pre-mRNA in different strains are indicated at the top of each lane. All the strains were grown at 25°C until mid-log phase, half of the culture of each strain was then shifted to 37°C. Both cultures of each strain at 25 and 37°C were incubated for 1 h at the respective temperature and harvested. Subsequently, the steady-state levels of the each mRNA and pre-mRNA were determined by Northern blot analysis with the total RNA isolated from each of these strains and probing for respective pre-mRNA and mRNAs isolated from strains grown at 25°C (lanes 1 to 7) and shifted to 37°C (lanes 8 to 14). The signal for each mRNA

Existence of DRN. (i) Representative mRNAs retained in the nucleus are degraded more rapidly. In order to substantiate the cytological observation described above, we first tested if intranuclear retention of specific mRNAs would affect their steady-state levels. Total RNAs from the normal *NUP116* strain and the isogenic *nup116*-Δ mutant (Table 1) were subjected to Northern blot analysis after the strains were shifted from 25°C to 37°C for 1 h, a condition that results in the complete nuclear retention of mRNAs in *nup116*-Δ (82) (see above). After a 1-h temperature shift to 37°C, the steady-state levels of mRNAs of the three representative transcripts, *CYC1*, *CYH2*, and *ACT1*, were found to be lower in the *nup116*-Δ mutant compared to the normal *NUP116* strain (Table 2; Fig. 3), indicating that the normal mRNAs retained in the nucleus of *nup116*-Δ strain might be unstable. Subsequent half-life measurements established that the lower abundance of all the three mRNAs in the *nup116*-Δ mutant was due to faster degradation (Tables 3 and 4; Fig. 4 and 6). Mock shifted control conditions, on the other hand, did not reveal any significant difference between the *NUP116* and *nup116*-Δ strains in either the abundance (Table 2; Fig. 3) or half-lives of these transcripts (Table 3; Fig. 4 and 6) when the export of mRNAs in *nup116*-Δ strain is not blocked. The lower abundance in steady-state level and the enhanced instability of these representative mRNAs observed only with the *nup116*-Δ strain under the condition of complete retention of poly(A) RNA, i.e., after 1 h at 37°C, provides strong evidence for the existence of the DRN pathway, a conclusion that is also substantiated by the FISH analysis described above.

(ii) Instability of mRNAs retained in the nucleus is dependent on Cbc1p. In subsequent experiments, we used Northern blot analysis of mRNA levels and decay rates to confirm the cytological observation that Cbc1p is responsible for decay of total poly(A) RNA in the nucleus (Fig. 2). Total RNA from a *nup116*-Δ strain and *nup116*-Δ *cbc1*-Δ strains shifted to 37°C for 1 h was isolated and subjected to Northern blot analysis, which revealed that the steady-state levels of the three representative transcripts, *CYC1*, *CYH2*, and *ACT1*, increased by approximately 1.5- to 3-fold compared to the levels in the *nup116*-Δ strain under the same condition (Table 2; Fig. 3). Also no significant difference in the levels of the mRNAs was observed in the control experiment where the *nup116*-Δ *cbc1*-Δ strain was maintained at 25°C for 1 h (Table 2; Fig. 3). Subsequent measurement of mRNA half-lives after inhibition of transcription of *NUP116*, *nup116*-Δ, and *nup116*-Δ *cbc1*-Δ strains (Table 1) by thiolutin revealed that the *CYC1*, *CYH2*, and *ACT1* mRNAs are degraded more rapidly in *nup116*-Δ strain when the strains are shifted to 37°C, a condition that prevents export of RNAs from the nucleus (Table 3; Fig. 4 and 6). Furthermore, this rapid degradation is suppressed by *cbc1*-Δ, resulting in half-lives that were more-than-threefold longer than those obtained with *nup116*-Δ (Table 3; Fig. 4 and 6). In fact these half-lives are even higher than those of *NUP116* mRNAs. It appears from the combined results of the FISH and Northern analyses that mRNAs are rapidly degraded if retained in the nucleus and that Cbc1p is a required component of this degradation system.

DRN is distinct from NMD. We have investigated potential relationships between DRN and NMD by testing possible effects of *upf* and other mutations on the degradation of representative mRNAs in *nup116*-Δ strains. This issue was addressed by investigating the degradation of *CYC1*, *CYH2*, and *ACT1* mRNAs in the *nup116*-Δ and *nup116*-Δ *upf1*-Δ strains and revealed that the *upf1*-Δ deletion did not stabilize any of the transcripts (Fig. 5; Table 4). These results indicate that the decay of transcripts in the nucleus of *nup116*-Δ strain is independent and distinct from the cytoplasmic NMD pathway. Furthermore, the steady-state levels of the intron-containing *CYH2* pre-mRNA, one of the well characterized natural substrate of NMD pathway (23), was determined in the *NUP116*, *nup116*-Δ, *nup116*-Δ *cbc1*-Δ, and *nup116*-Δ *upf1*-Δ strains. As expected, the level of the *CYH2* pre-mRNA remained the same in *NUP116*, *nup116*-Δ, and *nup116*-Δ *cbc1*-Δ strains at 25°C but accumulated by approximately 10-fold in the *nup116*-Δ *upf1*-Δ strain under the same condition (Fig. 3). Because the export of poly(A) RNA can occur in the *nup116*-Δ strain at 25°C, the majority of the *CYH2* pre-mRNA is cytoplasmic and thus accumulates in the *nup116*-Δ *upf1*-Δ strain due to the lack of NMD. In contrast, *CYH2* pre-mRNA did not accumulate when the *nup116*-Δ *upf1*-Δ strain was incubated at 37°C for 1 h, a condition that prevents the export of pre-mRNA and allows degradation in the nucleus. These findings confirm that transcripts in the *nup116*-Δ and *nup116*-Δ *upf1*-Δ strains were degraded in the nucleus and not in the cytoplasm. Thus, the stability analysis of different transcripts and the in situ localization study described above clearly identifies, DRN, a novel, Cbc1p-dependent nuclear pathway of mRNA decay that is distinct from the previously known cytoplasmic pathways.

We were unable to determine if the transcripts were stabilized in *nup116*-Δ *xrn1*-Δ strains, because the double deletion mutant was not recovered, suggesting that *xrn1*-Δ may be synthetically lethal with *nup116*-Δ.

The Rrp6p and possibly Rat1p exoribonucleases are components of DRN. We investigated the major question of which exoribonuclease is associated with DRN by determining if mutation of any of the known nuclear exoribonucleases could suppress *cyc1-512*, similar to suppression by *cbc1*-Δ. We considered the well-characterized 5′→3′ exoribonuclease, Rat1p (41), which is involved in 5′-end processing of snoRNAs and rRNAs and degradation of spacer fragments of pre-snRNA and pre-rRNAs (25, 61). The nuclear Rat1p is homologous to the cytosolic Xrn1p, which is engaged in 5′→3′ degradation of mRNAs. In spite of being homologous, Xrn1p is nonessential,

in each lane was quantified as described in Materials and Methods and normalized against the 18S rRNA signals (shown at the lowest panel of A) for loading errors and the relative steady-state levels of each mRNA are presented in Table 2. (B) Quantification of the *CYH2* pre-mRNA and mRNA signals. The intensity of each band of mature and precursor mRNA were determined by scanning the blots with a PhosphorImager and by normalizing for loading differences with respect to 18S rRNA signals. The relative levels of pre-mRNA and mRNA in each strain were expressed with respect to that in the *NUP116* (normal) strain at each temperature, which was considered to be 100%. The error bar represents the range of three independent experiments.

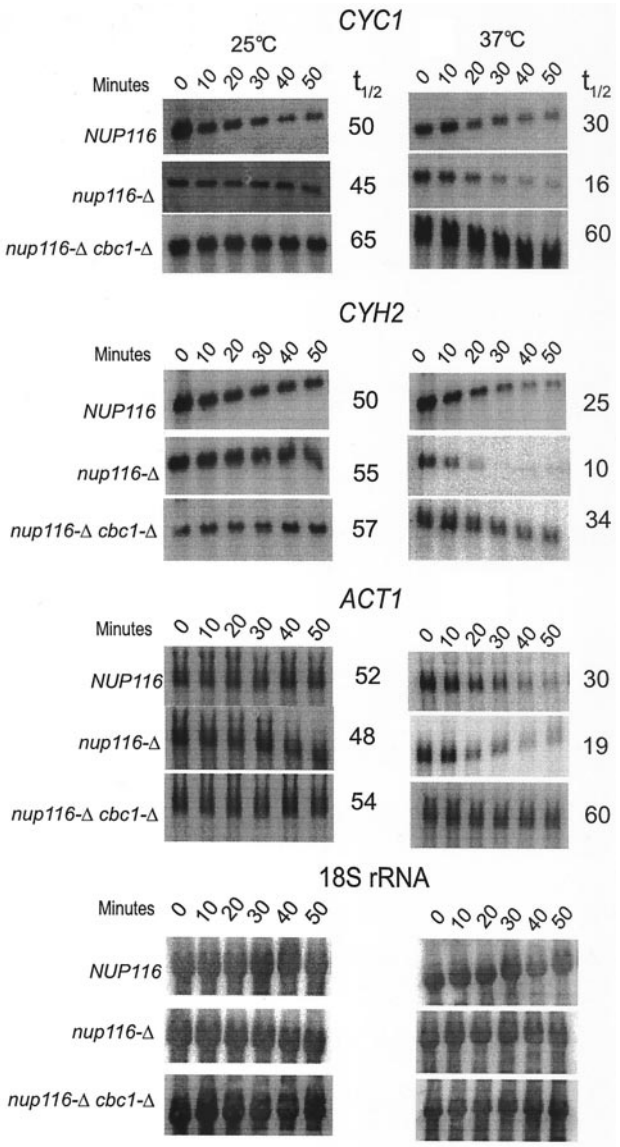

FIG. 4. Northern blot analysis revealing an increased degradation of *CYC1*, *CYH2*, and *ACT1* mRNAs that are retained in the nucleus because of the export deficiency caused by *nup116*-Δ mutation. Furthermore, the Northern blot analysis also revealed that the degradation is suppressed by *cbc1*-Δ at 37°C. The *NUP116* (normal), *nup116*-Δ, and *nup116*-Δ *cbc1*-Δ strains were grown at 25°C to the mid-logarithmic phase of growth. Subsequently, one-half of each culture was transferred to the restrictive temperature of 37°C. Both the cultures of each strain at 25 and 37°C were further incubated for one additional hour at both temperatures, and transcription was inhibited by the addition of thiolutin (4 μg/ml), as described in Materials and Methods. Cells of each strain from both the temperatures, mock shifted and shifted, were harvested after various times of thiolutin addition; Northern blots were prepared with total RNA; the half-lives of *CYC1*, *CYH2*, and *ACT1* mRNA were determined as described in Materials and Methods and normalized against 18S rRNA shown at the bottom of the figure. The half-lives are presented beside each panel as well as in Table 3.

whereas Rat1p is essential (39). Examination of the iso-1-cytochrome *c* level in a *cyc1-512 rat1-1* strain, constructed with a conditional allele of *RAT1*, revealed that *rat1-1* did not suppress *cyc1-512*, suggesting that Rat1p may not be involved in DRN. However, the results with conditional mutants can be ambiguous, as the lack of growth at the restrictive temperature may prevent manifestation of suppression.

The role of Rat1p was addressed further by examining the degradation rates of representative *CYC1*, *CYH2*, and *ACT1* mRNAs in a *nup116*-Δ *rai1*-Δ strain. *RAI1* is an unessential gene that binds to and modulates the activity of Rat1p both in vivo and in vitro. Furthermore, *rai1*-Δ strains are viable and lack Rat1p activity in vivo (84). However, *rai1*-Δ strains, including the *nup116*-Δ *rai1*-Δ strain, grow poorly (Fig. 1). Northern blots of total RNA from the *nup116*-Δ *rai1*-Δ strain were analyzed at 37°C and at different times after the temperature shift and transcription inhibition, according to the procedure used for the other strains. The results of the steady-state levels and half-life measurements at 37°C of the three representative *CYC1*, *CYH2*, and *ACT1* mRNA transcripts in a *nup116*-Δ *rai1*-Δ strain (Fig. 3 and 5; Tables 2 and 4) indicated a twofold increase in both steady-state levels and stability compared to that of *nup116*-Δ under the same condition, suggesting that Rat1p possibly plays a minor role in DRN.

In addition, we have also considered the possibility of involvement of nuclear exoribonuclease Rrp6p, a protein involved in the 3′ processing of the 5.8S rRNA (7) and part of nuclear exosome (2). Burkard and Butler (8) provided direct evidence for the 3′→5′exonuclease activity of this protein in vitro (8). *RRP6* is not essential for viability (7), and a strain carrying a precise deletion of *RRP6* is impaired in growth at all temperatures and is nonviable at 37°C. We have established its role in DRN by demonstrating suppression of *cyc1-512* by *rrp6*-Δ; the level of cytochrome *c* was increased from 10% of the normal level in the *cyc1-512* strain to 30% in the *cyc1-512* *rrp6*-Δ strain (data not shown). Consistent with this genetic evidence, the abundance of the *CYC1*, *ACT1*, and *CYH2* mRNAs and *CYH2* pre-mRNA (Fig. 3; Table 2) was found to increase by approximately threefold. Furthermore, degradation of the representative transcripts, *CYC1*, *CYH2*, and *ACT1* mRNAs, was diminished approximately threefold in a *nup116*-Δ *rrp6*-Δ strain at 37°C under the condition of complete nuclear retention of poly(A) RNA, compared to the control *nup116*-Δ strain. This finding is similar to the effect of *cbc1*-Δ (Fig. 5 and 6; Table 4). These results indicate that Rrp6p is a component of DRN and that the degradation takes place at least in part in a 3′→5′ direction. Furthermore, because the rates of *ACT1*, *CYH2*, and *CYC1* mRNA degradation in the *nup116*-Δ *cbc1*-Δ *rrp6*-Δ strain were similar to the rates of degradation in *nup116*-Δ *cbc1*-Δ and *nup116*-Δ *rrp6*-Δ strains (Fig. 5; Table 4), both Cbc1p and Rrp6p appear to be involved in the same pathway, not in separate parallel pathways. Taken together these results revealed a major role of Rrp6p in DRN.

Another block in mRNA export enhances mRNA degradation. We also tested the degradation of *ACT1* mRNA in an additional conditionally lethal mutant, *hpr1*-Δ, defective in mRNA export. Similar to the results with the *nup116*-Δ (see above) and *rat7-1* mutants (15), the steady-state levels (data not shown) and half-lives of the *ACT1* mRNA were diminished in the *hpr1*-Δ mutant (Table 4; Fig. 7). Therefore it appears that this accelerated nuclear mRNA decay in *nup116*-Δ strains is not specific for this export defect. Rather, accelerated mRNA decay occurs as a consequence of nuclear retention of

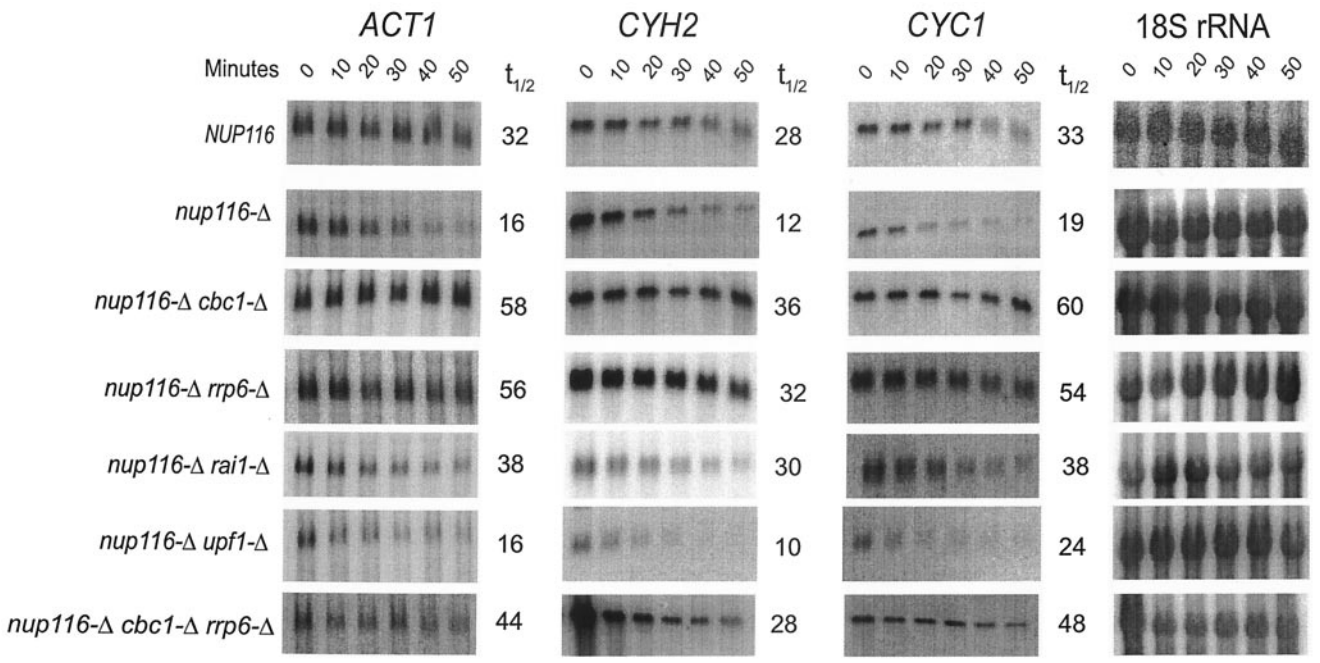

FIG. 5. A comparison of the decay of *ACT1*, *CYH2*, and *CYC1* mRNAs in *NUP116* and various *nup116*-Δ strains by Northern blot analysis. The analysis was performed as described in the legend of Fig. 4 after normalizing each signal against that from 18S rRNA internal control (as shown on the rightmost panels) for each strain and the half-lives are presented beside each panel as well as in Table 4.

mRNAs, whether the deficiency is due to defective nucleoporins, such as that with *rat7-1* and *nup116*-Δ, or due to a defect in another pathway, such as that with *hpr1*-Δ, which causes defects in elongation and metabolism of nascent mRNA and mRNA export proteins (38) and which results in RNA export defects at 37°C (69). In addition, increased degradation can also be due to the intrinsic defect in the sequence or the structure of mRNA that causes partial retention of a specific defective mRNA in the nucleus without any export or processing defect, such as in the *cyc1-512* mutant (15).

The *nup116*-Δ mutation does not cause hyperadenylation. Mutations that block the export of mRNA from the nucleus often result in hyperadenylation of transcripts and are associated with mRNA retention in nuclear foci that may represent the site of transcription (37). We tested whether nuclear retention of mRNAs caused by the *nup116*-Δ mutation resulted in hyperadenylation of mRNAs by labeling their poly(A) tails and comparing the amounts and lengths in *NUP116* and *nup116*-Δ strains. The results for the normal and mutant strains show an increase in the amount of the longest poly(A) tails, which is typical for yeast strains grown at 37°C (37, 63). Cells with the *nup116*-Δ mutation show a slight increase of ~15 to 20 nucleotides (nt) in the poly(A) lengths at 25°C and after a shift to 37°C for 60 min (Fig. 8, lanes 1 to 4). This small increase in poly(A) tail length is not affected by the *cbc1*-Δ mutation (Fig. 8, lanes 5 to 6). These findings indicate that while the *nup116*-D mutation results in a small increase in the longest poly(A) tails, it does not result in the 50- to100-nt increase observed for other nuclear export mutations (37).

DISCUSSION

DRN is a general consequence of retention of mRNAs and pre-mRNAs in the nucleus. This investigation clearly revealed that normal mRNAs are degraded when retained in the nucleus, and that this so-called DRN degradation is suppressed by the *rrp6*-Δ, *rai1*-Δ, and *cbc1*-Δ deletions. Thus, DRN involves the following: the 3′→5′ nuclear exonuclease, Rrp6p; the 5′→3′ nuclear exonuclease, Rat1p; and CBC, the nuclear cap binding complex, which may direct the mRNAs to the site of degradation. Degradation was investigated primarily by examining the stability of representative *ACT1*, *CYH2*, and *CYC1* mRNAs after inhibition of transcription with thiolutin in *nup116*-Δ strains, which retain mRNAs in the nucleus under restrictive conditions.

The decay rates of specific eukaryotic mRNAs can vary by more than 100-fold (11, 64, 65) and can range from approximately 1 min to more than 90 min in *S. cerevisiae* (27, 51, 79). The diversity in turnover can be attributed in part to a wide variety of mechanisms that have been suggested to take place by-and-large in the cytosol (12, 56). Here we demonstrate that normal mRNAs are degraded also in the nucleus. These studies extend the findings of Das et al. (15), who reported that *ACT1* and *CYH2* mRNAs were only marginally stabilized, 20 to 25%, whereas the longer *cyc1-512* transcripts were stabilized by approximately 200% by *cbc1*-Δ. Because the turnover of *CYC1*, as well as *ACT1* and *CYH2* mRNAs were not significantly affected by *cbc1*-Δ, Das et al. (15) suggested that long *cyc1-512* mRNAs are partially retained in the nucleus, and the net destruction of a particular mRNA is determined in part by the length of time spent in the nucleus. It is reasonable to

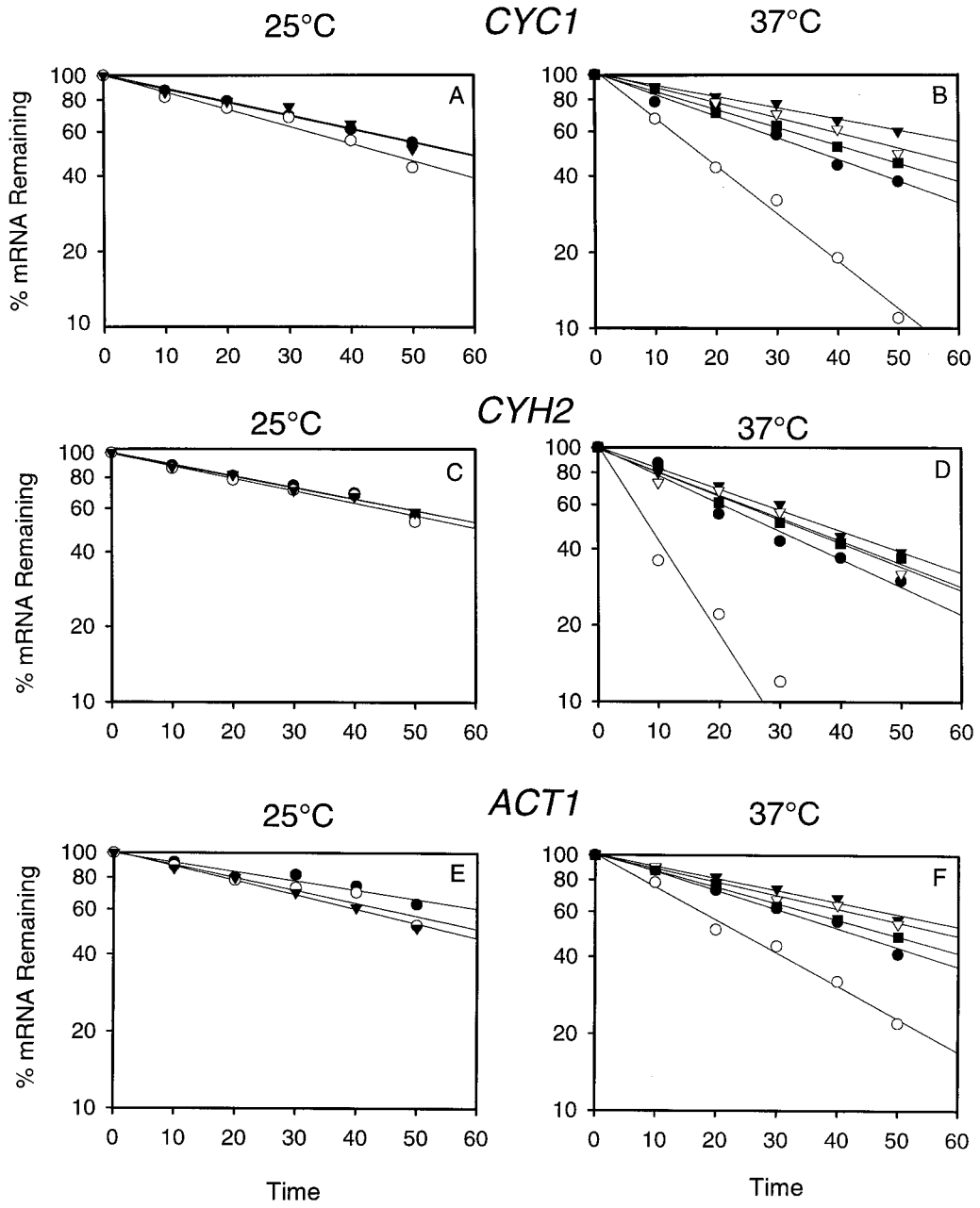

FIG. 6. Graphical representation of decay of *CYC1*, *CYH2* and *ACT1* mRNAs at 25°C (A, C, and E) and at 37°C (B, D, and F) from thiolutin treated cells of *NUP116* (●), *nup116*-Δ (○), *nup116*-Δ *cbc1*-Δ (▼), *nup116*-Δ *rrp6*-Δ (▽), and *nup116*-Δ *cbc1*-Δ *rrp6*-Δ (■). The decay was determined by Northern blot analysis of the RNA extracted from the strains mentioned above treated with thiolutin from 0 to 50 min. The result from one typical experiment from each strain at different temperatures are presented as the percentage of mRNA remaining versus time of incubation of thiolutin.

suggest that the turnover of certain mRNAs is also controlled in part by intrinsic properties of restricting their export from the nucleus. Therefore, it would be of considerable interest to determine if any wild-type mRNAs have a property similar to the mutant *cyc1-512* mRNAs, a study that is in progress.

Our results showed that increased rates of *ACT1* mRNA degradation were observed when they were retained in the nucleus by any of the mutations—*nup116*-Δ, *rat7-1*, and *hpr1*-Δ—which act by a variety of mechanisms. Furthermore, these results are consistent with the results of the FISH analysis, which indicate that total poly(A) RNA was similarly affected. However, most of the studies in this investigation were carried out with *nup116*-Δ strains, because the mRNA export defect in this strain is essentially complete at the restrictive condition and because the *nup116*-Δ defect was not apparently suppressed by any of the *rrp6*-Δ, *rai1*-Δ, and *cbc1*-Δ deletions, which were used to characterize DRN. In contrast, *cbc1*-Δ suppressed the temperature-sensitive growth of *rat7-1* (15) and

TABLE 2. Relative steady-state levels of *CYC1*, *ACT1*, and *CYH2* pre-mRNA and mRNA in various strains[a]

Pertinent genotype	Steady-state level							
	CYC1		*CYH2*		*CYH2* pre-mRNA		*ACT1*	
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
NUP116	100	100	100	100	20 ± 0.5	23 ± 1.5	100	100
nup116-Δ	94 ± 2	27 ± 2.5	102 ± 2	38 ± 2.5	21 ± 1.5	10 ± 0.25	101 ± 3	53 ± 2.5
nup116-Δ *cbc1*-Δ	91 ± 4	76 ± 3	101 ± 2	84 ± 3	19 ± 2	29 ± 3	102 ± 1	81 ± 3
nup116-Δ *rrp6*-Δ	86 ± 3.5	66 ± 2	109 ± 8	85 ± 5	24 ± 3	26 ± 3	101 ± 3.5	75 ± 4
nup116-Δ *rail*-Δ	94 ± 4	55 ± 1.5	87 ± 8	71 ± 6	18 ± 0.5	20 ± 2	83 ± 4	70 ± 2
nup116-Δ *upf1*-Δ	92 ± 6	37 ± 2	83 ± 7	45 ± 2	102 ± 4	13 ± 1.5	96 ± 7	43 ± 2.5
nup116-Δ *cbc1*-Δ *rrp6*-Δ	105 ± 6	66 ± 2.5	92 ± 2	90 ± 6	23 ± 1	22 ± 2.5	93 ± 1	79 ± 6

[a] The steady-state levels were determined with total RNA isolated from the indicated strains either from 25°C or after 1 h of temperature shift at 37°C as described in Materials and Methods. The level of each mRNA at each temperature in the *NUP116* strain was arbitrarily chosen as 100%, and the relative levels at which mRNA in different strains were expressed as a percentage of that of strain *NUP116* at respective temperatures. The level of *CYH2* pre-mRNA in each strain was expressed as a percentage of the *CYH2* mRNA level in strain *NUP116* at each temperature. Where applicable, the mean values are presented (determined from three independent experiments) to nearest whole number. The number after the mean value represents the range of three independent experiments.

hpr1-Δ; consequently, these mutants were avoided for examining mRNA retention and decay in the nucleus. Uemura et al. (75) also reported that a *cbc1* mutation suppressed the temperature-sensitive growth of *hpr1* mutants, which are conditionally defective in the nuclear export of poly(A) RNA (69).

DRN involves both 3′→5′ and 5′→3′ degradation pathways. The suppression of degradation of *ACT1*, *CYH2*, and *CYC1* mRNAs in thiolutin treated *nup116*-Δ *rrp6*-Δ strains established that the 3′→5′ nuclear exonuclease, Rrp6p, is the major nuclease acting in DRN, adding to its other major function, nuclear pre-rRNA processing (8, 9). Rrp6p specifically associates with the nuclear form of the exosome (2, 8). Although *RRP6* is not essential for viability (7) all other components of the exosome were found to be essential (1, 2, 53, 54). Like the other exosome mutants the *rrp6*-Δ strain is defective in the 3′ processing of the 5.8S rRNA, but differed from the others insofar as it accumulated a discrete species, 5.8S + 30, which was 3′ extended by ~30 nt (7). These differences in phenotypes of *rrp6*$^-$ and other exosome mutants led Burkard and Butler (8) to speculate that Rrp6p may act independently of the exosome as a monomeric exonuclease or in conjunction with another set of proteins. Our results show a threefold increase in both the steady-state levels and in the stability of all the representative normal mRNAs in *nup116*-Δ *rrp6*-Δ strains thereby demonstrating that Rrp6p plays a major role in DRN. It is notable, however that although a 5- to 10-fold stabilization of various intron-containing pre-mRNAs in absence of Rrp6p was previously reported (6), we did not observe such a high degree of stabilization. This difference in the degree of stabilization may reflect the differences in the experimental systems employed in the previous work. Finally, we would like to speculate that DRN might be taking place in the nucleolus as Rrp6p is localized more densely in that region although it is present throughout the nucleoplasm (9).

Rat1p, the major nuclear 5′→3′exoribonuclease participates in a variety of functions such as the 5′-end processing of snoRNAs and rRNAs and degradation of spacer fragments of pre-snRNA and pre-rRNAs (25, 61) and requires Rai1p for both of its in vitro and in vivo activity (84). Because *rat1*-Δ deletion mutants are lethal, we examined *rai1*-Δ strains, which lack Rat1p activity in vivo (84). A relatively modest degree of suppression of degradation was observed in *nup116*-Δ *rai1*-Δ strains which, although are viable, exhibit greatly reduced growth, suggesting the elimination of certain critical functions described above. This observation suggests that Rat1p, might also act in DRN, but its effect is relatively modest; thus, we suggest that both the 5′→3′ and 3′→5′ pathways participate in DRN with 3′→5′ pathway being the major pathway. However, we have not determined the relative contribution of each.

DRN requires CBC, the nuclear cap binding complex. CBC, the nuclear cap binding complex, is a critical component of DRN and defines this pathway (15). A major question is the mechanism by which CBC is required for DRN. CBC consists of a heterodimer of two proteins, denoted CBP80 and CBP20 in higher eukaryotes and Cbc1p and Cbc2p, respectively, in yeast (19, 34, 35, 36, 40, 41, 59). Mutant forms of the CBC components were recovered in numerous genetic screens with yeast, and *CBC1* has been previously designated *SUT1* (15), *GCR3* (75), and *STO1* (13), whereas *CBC2* has also been designated *MUD13* (13). Similar to higher eukaryotic CBC,

TABLE 3. Half-lives of *ACT1*, *CYH2*, and *CYC1* mRNAs in various strains[a]

Pertinent genotype	Half-life (min) of:					
	ACT1		*CYH2*		*CYC1*	
	25°C	37°C	25°C	37°C	25°C	37°C
NUP116	52 ± 4.5	32 ± 1.5	50 ± 3.5	26 ± 2.0	50 ± 3.5	31 ± 2.5
nup116-Δ	48 ± 3.25	18 ± 1.5	55 ± 4.0	11 ± 1.0	45 ± 2.0	18 ± 1.5
nup116-Δ *cbc1*-Δ	54 ± 4.25	60 ± 2.0	57 ± 3.75	37 ± 3.0	65 ± 3.0	61 ± 1.0

[a] The half-lives were determined with total RNA from thiolutin-treated cells as described in Materials and Methods. The values of multiple determinations fell within 15% of the mean. Where applicable, the mean values are presented (determined from three independent experiments) to nearest whole number. The number after the mean value represents the range of three independent experiments.

TABLE 4. Comparison of half-lives of *ACT1*, *CYH2*, and *CYC1* mRNAs in different mutant strains under the restrictive condition of 37°C after temperature shift[a]

Pertinent genotype	Half-life (min) of:		
	ACT1	*CYH2*	*CYC1*
NUP116	32 ± 1.5	26 ± 2.0	31 ± 2.5
nup116-Δ	18 ± 1.5	11 ± 1.0	18 ± 1.5
nup116-Δ *cbc1*-Δ	60 ± 2.0	37 ± 3.0	61 ± 1.0
nup116-Δ *rrp6*-Δ	56 ± 6.5	28 ± 3.0	52 ± 5.5
nup116-Δ *rail*-Δ	38 ± 2.5	30 ± 2.5	38 ± 3.0
nup116-Δ *upf1*-Δ	16 ± 1.5	10 ± 1.0	24 ± 1.0
nup116-Δ *cbc1*-Δ *rrp6*-Δ	44 ± 3.0	26 ± 2.0	48 ± 3.5
HPR1	34 ± 3.5	ND[b]	ND
hpr1-Δ	22 ± 1.5	ND	ND

[a] The half-lives were determined with total RNA from thiolutin-treated cells as described in Materials and Methods. The values of multiple determinations fell within 15% of the mean. Where applicable, the mean values are presented (determined from three independent experiments) to nearest whole number. The number after the mean value represents the range of three independent experiments.

[b] ND, not determined.

yeast CBC is primarily located in the nucleus. Experiments performed in vivo and in vitro indicate that CBC plays a role in both pre-mRNA splicing and U snRNA export (34, 35, 49). CBC associates with the cap structures of pre-mRNA and nuclear mRNA in vivo and accompanies mRNA through nuclear pore complexes to the cytoplasm (78).

CBC is not essential for growth in yeast, although the growth of *cbc1*-Δ strains is severely retarded on glucose medium but only mildly diminished on glycerol (74) and raffinose (15) media. Fortes et al. (19) relied on the nonessentiality of CBC to

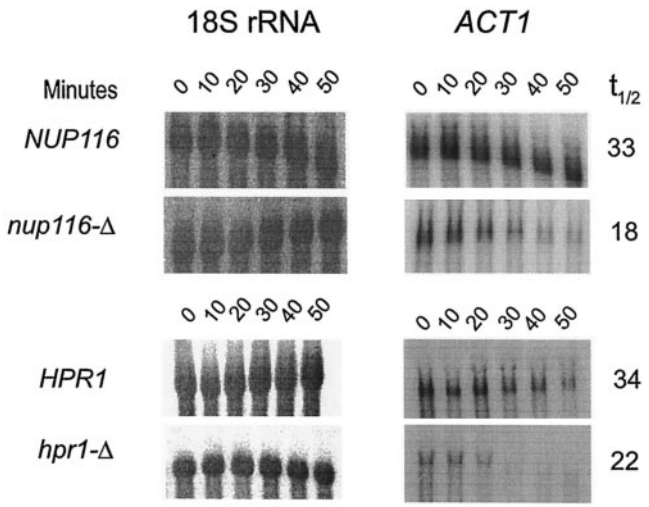

FIG. 7. Northern blot analysis revealing an increased degradation of *ACT1* mRNA (right panels), which is retained in the nucleus because of the export deficiency caused by *nup116*-Δ or *hpr1*-Δ mutations at the restrictive temperature of 37°C when compared to the corresponding isogenic normal strain. The *NUP116* (normal), *nup116*-Δ, and *HPR1* and *hpr1*-Δ strains were grown at 25°C to the mid-logarithmic phase of growth. Subsequently, one-half of each culture was transferred to the restrictive temperature of 37°C; the cultures were further incubated for one additional hour at that temperature; and transcription was inhibited by the addition of thiolutin (4 μg/ml), as described in Materials and Methods. Northern blots were prepared using total RNA extracted from cells after various times, 0 to 50 min, of thiolutin addition. The half-lives, presented in Table 4, were determined from these blots after normalization to the 18S rRNA signals shown at the left panels. The numbers beside each panel represents the half-lives in minutes.

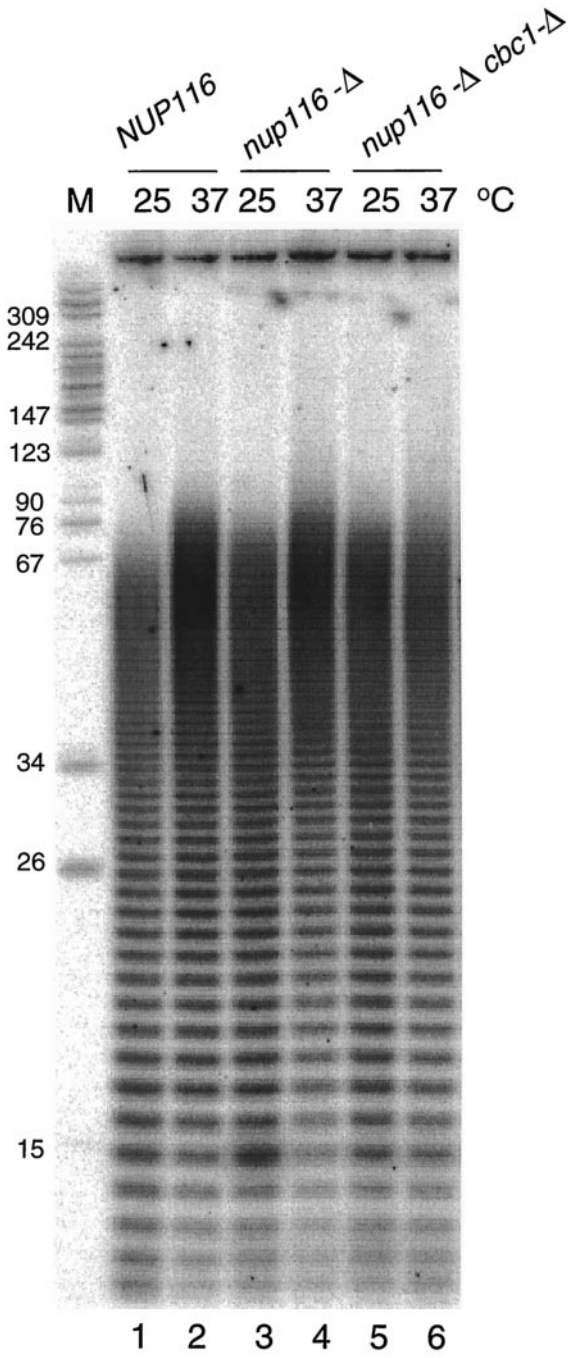

FIG. 8. Poly(A) tail analysis of mRNA from strains carrying the *nup116*-Δ mutation. Poly(A) tails were analyzed by 3′ end labeling of 1 μg of total RNA with [^{32}P]CP and RNA ligase, followed by hydrolysis with RNase A and RNase T_1, electrophoretic separation on a 16% acrylamide–8 M urea gel, and storage phosphorimager analysis. Lane M indicates pBR322 *Msp*I-cut length markers.

carry out a genetic screen for components that show synthetic lethality with a *cbc1*-Δ *cbc2*-Δ double deletion mutant strain. One group of synthetically lethal mutations was due to alterations that were complemented by components of U1 snRNP and the yeast splicing commitment complex. These interactions confirmed the role of CBC in commitment complex formation in yeast. Fortes et al. (19) also demonstrated the physical interaction of Cbc1p and Cbc2p with the commitment complex components Mud10p and Mud2p, which may directly mediate function. Most interestingly, Fortes et al. (19) identified five synthetically lethal mutations that were complemented by *CBF5* and *NOP58*, which encode components of the two major classes of yeast snoRNPs functioning in the maturation of rRNA precursors. Cbf5p and Nop58p are essential nucleolar proteins that are core components of the box C+D and box H+ACA families of snoRNPs, respectively (42, 43). Both Nop58p and Cbf5p are required for the early pre-rRNA processing steps at sites A0, A1, and A2 in the pathway of 18S rRNA synthesis, and the synthetically lethal strains had defects in pre-rRNA processing at these steps (19). Most importantly, the *cbc1*-Δ *cbc2*-Δ strain by itself was defective in the cleaving at sites A0, A1, and A2, possibly explaining synergism and the synthetic lethality with *CBF5* and *NOP58* mutations. Although the role of CBC in nucleolar pre-rRNA processing has not been explained, it is tempting to speculate that the diminished cleavage of pre-rRNA and the diminished degradation of nuclear mRNAs have a common mode of action that involves the enhanced localization of capped RNAs, such as mRNAs and snoRNAs, to nucleoli. However, the cap may not be absolutely required for nucleolar localization of snoRNAs (45).

CBC and Rrp6p appear to act in the same pathway. mRNA degradation in *nup116*-Δ revealed that CBC and Rrp6p both participate in the degradation of normal mRNAs in the nucleus. In order to determine whether the CBC and Rrp6p participate in the same or parallel pathways, the rates of *ACT1*, *CYH2*, and *CYC1* mRNA degradation in the *nup116*-Δ *cbc1*-Δ *rrp6*-Δ strain were compared to the rates of degradation in *nup116*-Δ *cbc1*-Δ and *nup116*-Δ *rrp6*-Δ strains. The similar values and the lack of increased stability in the *nup116*-Δ *cbc1*-Δ *rrp6*-Δ strain (Table 4; Fig. 5) suggest that both CBC and Rrp6p are components of the same pathway and are in the same epistasis group.

NMD does not occur in the nucleus. The extent of NMD of mRNA in the nucleus was assessed by determining the levels of *CYH2* pre-mRNA in the *nup116*-Δ *upf1*-Δ strain at 25 and 37°C. As expected, the degradation of *CYH2* pre-mRNA was clearly suppressed by *upf1*-Δ at the nonrestricted temperature of 25°C (Fig. 3; Table 2). In contrast, the *CYH2* pre-mRNA levels at 37°C, a condition in which mRNA is retained in the nucleus, was approximately the same in both the *nup116*-Δ and *nup116*-Δ *upf1*-Δ strains. This finding clearly suggests that NMD does not act in the nucleus of *S. cerevisiae*. Consistent with this observation, Maderazo et al. (52) showed that NMD in *S. cerevisiae* most likely takes place in the cytoplasm.

Pathways for degrading abnormal mRNAs in the nucleus. One major question is whether DRN, which acts on normal mRNAs and which is dependent on CBC, corresponds to any of the degradation pathways that act on abnormal and defective mRNAs. Recent studies have revealed nuclear surveillance systems that selectively degrade abnormal and defective RNA species, including the following: (i) pre-mRNAs that accumulate due to inefficient splicing, such as in splicing defective *prp2-1* mutants (6); (ii) mRNAs produced after cessation of polyadenylation in a *pap1-1* strain (8); (iii) aberrantly 3′-extended mRNAs due to lack of functional Rna14p and Rna15p, which are components of cleavage and polyadenylation factor CF1A (50, 72); (iv) mRNA that accumulates in the nucleus due to inefficient loading of essential mRNA export factors to the assembling mRNP (86); (v) hyperadenylated mRNAs, which appear as a consequence of nuclear retention, such as in export defective *rat7-1* or *rip1*-Δ strains (37); and (vi) hypoadenylated mRNAs, which occurs in *pap1-1* strains that lack functional poly(A) polymerase (28).

It should be emphasized that mRNAs in *nup116*-Δ strains are not hyperadenylated, as they are in other mutants defective in mRNA export, including *rat7-1*, *rip1*-Δ, *gle1-4*, *mex67-5*, *rat8*-2 (30, 37), and *nab2*-Δ (24). The marginal increases in poly(A) tail lengths of approximately 15 to 20 nt that is associated with the *nup116*-Δ mutation (Fig. 8) are similar to those seen in *rna1-1* and *prp20-1* mutants (18, 62), but contrast significantly with other nuclear export mutations, which resulted in poly(A) tail lengths up to 100 nt longer than normal (37). The reason for these differences remains unclear, but the results do indicate that retention of mRNAs in the nucleus does not necessarily lead to hyperadenylation. While the hyperadenylated mRNAs are clearly abnormal, the mRNAs in the *nup116*-Δ strains are considered to be normal.

Similar to the DRN pathway, Rrp6p is a component of the certain nuclear pathways acting on abnormal mRNAs. In the *prp2-1* strain, Rrp6p acts as a 3′→5′ exonuclease degrading unspliced pre-mRNAs (6). In addition, Rrp6p appears to degrade unadenylated mRNAs in a *pap1-1* strain (8). Furthermore, in the *rna14-1* and *rna15-2* strains, Rrp6p functions further to degrade the processively degraded pre-mRNA intermediates already acted on by the exosome and Dob1p (72). In this regard, *pap1-1* is suppressed by *rrp6*-Δ (8). While DRN acts on *CYH2* pre-RNA (Fig. 6), the results with *nup116*-Δ strains indicate that DRN action on pre-mRNA and mRNA may be similar.

However, the role of Rrp6p in degradation of unadenylated and hyperadenylated mRNAs in the intranuclear foci of the *rat7-1*, *rip1*-Δ, or *pap1-1* strains is unclear (28). In fact, Hilleren et al. (28) demonstrated that *PGK1* mRNA from *pap1-1* strain becomes destabilized in absence of Rrp6p; they further demonstrated that unadenylated *SSA4* mRNAs in *pap1-1* strain accumulated at the specific intranuclear foci as a consequence of nuclear retention and that in *pap1-1 rrp6*-Δ strain they were released from these foci and exported. Thus, the degradation of unadenylated and hyperadenylated mRNAs at intranuclear foci differs significantly from the other nuclear pathways.

The relationship of DRN to the RNA surveillance pathways acting on defective forms of pre-mRNA as described by Bousquet-Antonelli et al. (6) and Torchet et al. (72) has yet to be defined. While all of these degradation pathways require Rrp6p and presumably the nuclear exosome, it is unknown whether the RNA surveillance pathways require CBC. Experiments with strains containing *cbc1*-Δ and other appropriate mutations should reveal the role of CBC in the RNA surveillance pathways.

DRN may play physiological roles in degrading aberrant mRNAs and in regulating the abundance of specific normal mRNAs. Another major question is whether DRN is restricted to mRNAs that are retained in the nucleus by abnormal physiological conditions caused by the mutations affecting mRNA export. As stressed above, the degradation of certain *cyc1-512* transcripts are suppressed by *cbc1*-Δ, *cbc2*-Δ and *rrp6*-Δ in strains having the normal mRNA export apparatus. In fact, the initial proposal of DRN was based on the on the assumption that long *cyc1-512* transcripts are partially retained in the nucleus due to an intrinsic property of these mRNAs (15). We believe that DRN plays a positive role in identifying and eliminating defective mRNAs in the nucleus in wild type cells where export proceeds normally. Finding certain wild-type mRNAs that are particularly protected from degradation by *cbc1*-Δ and *rrp6*-Δ will provide evidence consistent with the view that DRN is a normal pathway acting at a high rate on a special class of normal mRNAs.

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