Identification of Edc3p as an Enhancer of mRNA Decapping in *Saccharomyces cerevisiae*

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ABSTRACT

The major pathway of mRNA decay in yeast initiates with deadenylation, followed by mRNA decapping and 5′–3′ exonuclease digestion. An *in silico* approach was used to identify new proteins involved in the mRNA decay pathway. One such protein, Edc3p, was identified as a conserved protein of unknown function having extensive two-hybrid interactions with several proteins involved in mRNA decapping and 5′–3′ degradation including Dcp1p, Dcp2p, Dhh1p, Lsm1p, and the 5′–3′ exonuclease, Xrn1p. We show that Edc3p can stimulate mRNA decapping of both unstable and stable mRNAs in yeast when the decapping enzyme is compromised by temperature-sensitive alleles of either the *DCP1* or the *DCP2* genes. In these cases, deletion of *EDC3* caused a synergistic mRNA-decapping defect at the permissive temperatures. The *edc3*Δ had no effect when combined with the *lsm1*Δ, *dhh1*Δ, or *pat1*Δ mutations, which appear to affect an early step in the decapping pathway. This suggests that Edc3p specifically affects the function of the decapping enzyme *per se*. Consistent with a functional role in decapping, GFP-tagged Edc3p localizes to cytoplasmic foci involved in mRNA decapping referred to as P-bodies. These results identify Edc3p as a new protein involved in the decapping reaction.

IN eukaryotic cells, mRNA turnover and its regulation are essential determinants of gene expression. In *Saccharomyces cerevisiae*, a major pathway of mRNA turnover for both stable and unstable transcripts initiates with deadenylation of the 3′-polyadenosine [poly(A)] tail (Muhlrad and Parker 1992; Decker and Parker 1993). The deadenylation of transcripts is followed by the removal of the 5′ ^{7me}GTP cap and, subsequently, exonucleolytic digestion of the transcript occurs in the 5′–3′ direction (Decker and Parker 1993; Hsu and Stevens 1993; Muhlrad *et al.* 1994, 1995; Beelman *et al.* 1996). Several important proteins involved in different steps of the mRNA decay pathway have been characterized (Tucker and Parker 2000). The deadenylation step in the decay pathway requires Ccr4p and Pop2p, which are components of the major cytoplasmic deadenylase in *S. cerevisiae* (Daugeron *et al.* 2001; Tucker *et al.* 2001, 2002; Chen *et al.* 2002). After removal of the tail, the Dcp1p/Dcp2p complex decaps mRNAs at their 5′ end (Beelman *et al.* 1996; Dunckley and Parker 1999; Steiger and Parker 2002; Steiger *et al.* 2003), which then allows Xrn1p, the major yeast 5′–3′ exonuclease, to rapidly degrade the body of the transcript.

Decapping is an important step in the mRNA decay pathway as it allows the final degradation of the mRNA and is regulated by a number of proteins (Muhlrad *et al.* 1994, 1995; Tucker and Parker 2000). Apart from the decapping enzyme, which consists of Dcp1p and Dcp2p, several proteins including Pat1p, Dhh1p, the Sm-like (Lsm) complex (Lsm1p–Lsm7p), and Edc1p and Edc2p are enhancers of the decapping rate (Hatfield *et al.* 1996; Bonnerot *et al.* 2000; Bouveret *et al.* 2000; Tharun *et al.* 2000; Coller *et al.* 2001; Dunckley *et al.* 2001; He and Parker 2001; Tharun and Parker 2001; Fischer and Weis 2002). Lsm1p is part of the seven-member Lsm complex that binds to mRNA and is required for efficient decapping (Tharun *et al.* 2000). Pat1p is known to interact with the Lsm complex and is an enhancer of decapping (He and Parker 2001). Dhh1p is a member of the DEAD-box helicase proteins and is also required for efficient decapping (Coller *et al.* 2001). Edc1p and Edc2p are related proteins that are known to affect the decapping enzymes directly and are both enhancers of decapping (Dunckley *et al.* 2001; Schwartz *et al.* 2003; Steiger *et al.* 2003). Whether additional proteins are involved in decapping is not yet known.

One way to find new factors affecting mRNA decay is to utilize databases of protein-protein interactions on the basis of high-throughput two-hybrid screens, systematic mass spectrometry, and multidimensional protein identification technology screens to find proteins that show numerous interactions with known decapping factors. To do this, we examined the *S. cerevisiae* genomic-scale data and observed that the yeast open reading frame Yel015W showed numerous interactions with the Dcp1p/Dcp2p complex, Dhh1p, Xrn1p, Pat1p, and

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TABLE 1

Strains used in this study

Strains	Genotype	Source
yRP840	*MAT***a** *his4-539 leu 2-3,112 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	Hatfield *et al.* (1996)
yRP841	*MAT*α *leu 2-3,112 lys2-201 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	Hatfield *et al.* (1996)
yRP1745	*MAT***a** *his4-539 leu 2-3112 trp1 ura3-52 edc3::NEO cup1::LEU2/PGK1pG/ MFA2pG*	This study
yRP1746	*MAT*α *leu 2-3,112 lys2-201 trp1 ura3-52 edc3::NEO cup1::LEU2/PGK1pG/ MFA2pG*	This study
yRP1515	*MAT*α *leu 2-3,112 lys2-201 trp1 his4-539 ura3-52 dcp1-2::TRP1cup1::LEU2/ PGK1pG/MFA2pG*	Dunckley (2001)
yRP1747	*MAT***a** *his4 leu2-3,112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 edc3::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1516	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp2-7::URA3 cup1::LEU2/PGK1pG/MFA2pG*	Dunckley (2001)
yRP1748	*MAT***a** *his4-539 trp1 leu2-3,112 ura3-52 edc3::NEO dcp2-7::URA3 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1345	*MAT***a** *trp1 leu2-3,112 ura3-52 lys2-201 dcp1-2::TRP1 ski8::URA3*	Dunckley and Parker (1999)
yRP1749	*MAT*α *leu2-3112 ura3-52 lys2-201 edc3::NEO dcp1-2::TRP1 ski8::URA3 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1502	*MAT***a** *his4-539 trp1 leu2-3,112 ura3-52 lys2-201 dcp2-7::URA3 ski3::TRP1*	Dunckley and Parker (2001)
yRP1750	*MAT*α *his4-539 leu2-3,112 ura3-52 lys2-201 edc3::NEO dcp2-7::URA3 ski3::TRP1*	This study
yRP1365	*MAT*α *trp1 leu2-3,112 lys2-201 ura3-52 lsm1::TRP1 cup1::LEU2/PGKpG/ MGA2pG*	Tharun *et al.* (2000)
yRP1751	*MAT***a** *his4-539 trp1 leu2-3,112 lys2-201 ura3-52 lsm1::TRP1 edc3::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1561	*MAT*α *leu 2-3,112 lys2-201 trp1 his4-539 ura3-52 dhh1::URA3 cup1::LEU2/ PGK1pG/MFA2pG*	Coller *et al.* (2001)
yRP1753	*MAT***a** *his4-539 trp1 ura3-52 dhh1::URA3 edc3::NEO cup1::LEU2/ PGK1pG/MFA2pG*	This study
yRP1372	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 pat1::LEU2 cup1:LEU2/ PGK1pG/MFA2pG*	Tharun *et al.* (2000)
yRP1752	*MAT***a** *edc3::NEO trp1 ura3-52 leu 2-3,112 pat1::LEU2 cup1::LEU2/ PGK1pG/MFA2pG*	This study
yRP1070	*MAT*α *leu 2-3,112 lys2-201 trp1 ura3-52 dcp1::URA3 cup1::LEU2/PGK1pG/ MFA2pG*	Beelman *et al.* (1996)
yRP1331	*MAT***a** *his4-539 leu 2-3,112 trp1 ura3-52 xrnl::URA3 cup1::LEU2/ PGK1pG/MFA2pG*	Schwartz and Parker (1999)
yRP1373	*MAT***a** *his4-539 leu 2-3,112 lys2-201 trp1 ura3-52 upf1::URA3 cup1::LEU2/ PGK1pG/MFA2pG*	Schwartz and Parker (1999)
yRP1758	*MAT***a** *his4-539 trp1 leu2-3,112 ura3-52 EDC3-GFP-NEO cup1::LEU2/ PGK1pG/MFA2pG*	This study

members of the Lsm complex (Fromont-Racine *et al.* 1997, 2000; Schwikowski *et al.* 2000; Uetz *et al.* 2000; Hishigaki *et al.* 2001; Ito *et al.* 2001; Gavin *et al.* 2002; Giaever *et al.* 2002; Ho *et al.* 2002). This *in silico* analysis predicts Edc3p to be involved in mRNA turnover. In this study, we present experimental evidence that although Edc3p is not required for decapping, Edc3p can stimulate the rate of decapping *in vivo* and is found in sites of decapping in the cytoplasm.

MATERIALS AND METHODS

Sequence analysis: The Edc3p homolog in *S. pombe* was used as a query to identify homologs in other organisms using the BLAST algorithm (Altschul *et al.* 1990). Protein sequences of Edc3p and its homologs were aligned using the CLUSTAL W program (Thompson *et al.* 1994). The domains were identified using the BLOCKS program (Henikoff *et al.* 1995) and the position of the domains in the protein was used to draw a cartoon of the Edc3p and its homologs in Figure 1. The accession numbers for the proteins are listed in the legend to Figure 1.

Yeast strains and plasmids: All strains used in this study are listed in Table 1. The NEO deletion cassette from the genomic DNA of the commercially available *yel015w*Δ mutant strain (Research Genetics, Birmingham, AL) was PCR amplified using the oligonucleotides oRP 1202 (5′-GAA GCA TAT CGT AAG CAC AC-3′) and oRP 1203 (5′-GTG AGA CAC TGG CCT CGT CTG-3′). This amplified PCR fragment was used for transformation of the strains yRP840 and yRP841 to get yRP1745 and yRP1746 strains by homologous recombination.

Growth phenotype studies were done with the *dcp2-7ski3*Δ *edc3*Δ and *dcp1-2 ski8*Δ *edc3*Δ strains, which were obtained by

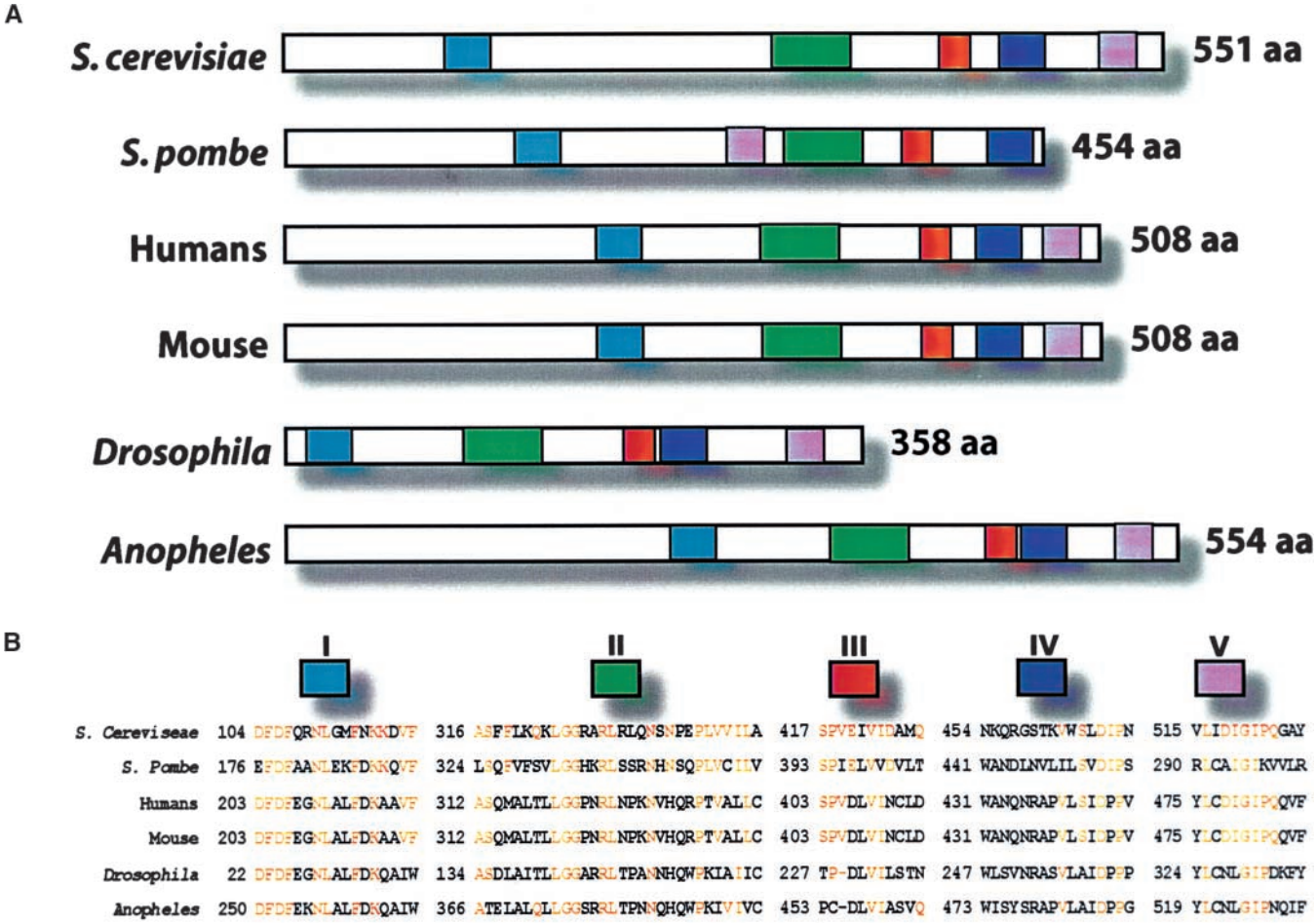

FIGURE 1.—The Edc3p family of proteins has five conserved domains. (A) Model of the relative arrangement of the five conserved putative domains in the respective protein sequences of Edc3p and its homologs. The total length of each of the proteins is indicated at the right. The color-coded legend shows the domain numbers. (B) The protein sequences corresponding to the five conserved domains are aligned in Edc3p and its homologs. The residues in red are present in *S. cerevisiae* Edc3p and its homologs and the black residues represent the residues that are not conserved in *S. cerevisiae* Edc3p but may be conserved in the homologs from other organisms. The alignment was generated using the BLOCKS program (HENIKOFF *et al.* 1995) that looks for regional conserved sequences in alignments. Accession numbers for the sequences are as follows: *S. cereviseae*, NP010901; *S. pombe*, NP595858; human, BAB15001; mouse, AAH31725; Drosophila, AAL25455; Anopheles, EAA08427.

crossing *edc3*Δ with *dcp2-7 ski3*Δ and *dcp1-2 ski8*Δ strains and dissection of the diploid strains. The triple mutants were then grown along with the wild type (WT) and single and double mutants at 24°, 30°, and 37° for 3 days.

The construction of the Lsm1p-RFP plasmid is described in SHETH and PARKER (2003). The EDC3-GFP strain construction was done as described in SHETH and PARKER (2003). The Edc3p-GFP strain was transformed with the Lsm1p-RFP plasmid for the colocalization studies.

RNA procedures: All RNA analyses were performed as described in MUHLRAD *et al.* (1994). For half-life measurements cells were grown to midlog phase containing 2% galactose. Cells were harvested and transcription was repressed by the addition of media containing 4% glucose. Aliquots were taken over a brief time course and frozen. Yeast total RNA extractions were performed as described in MUHLRAD *et al.* (1994) and CAPONIGRO *et al.* (1993). RNA was analyzed by running 10 μg of total RNA on either a 1.5% formaldehyde agarose gel as in Figures 2 and 3 or a 6% polyacrylamide/7.5 M urea gel as in Figure 4. All Northerns were performed using radiolabeled oligo probes directed against the MFA2pG reporter (oRP140) and the 7S RNA (oRP100). For the CYH2 Northern blot, a random-prime radiolabeled *CYH2* complementary DNA probe was used (THARUN *et al.* 2000). Half-lives were determined by quantitation of blots using a Molecular Dynamics (Sunnyvale, CA) Phosphorimager. Loading correction for quantitations was determined by hybridization with oRP100, an oligo directed to the 7S RNA, a stable RNA polymerase III transcript. Northerns shown in Figures 2, 3, and 5 were done at least three times.

A primer extension analysis was performed as described in HATFIELD *et al.* (1996). Oligo oRP131, which is complementary to the 5′ end of the PGK1 transcript, was radiolabeled and added to 20 μg of total RNA. Extension was performed using superscribe reverse transcriptase (GIBCO BRL, Gaithersburg, MD). The reactions were analyzed on 6% polyacrylamide gels and visualized using autoradiographs. The primer extension data shown in Figure 6 are representative of three independent experiments.

Confocal microscopy: The cells were grown to an OD of 0.4 at 30° in synthetic medium containing 2% dextrose, washed, resuspended in a smaller volume of the media, and observed. Observations were made as described in SHETH and PARKER (2003). The Edc3p-GFP strain with the LSM1-RFP plasmid

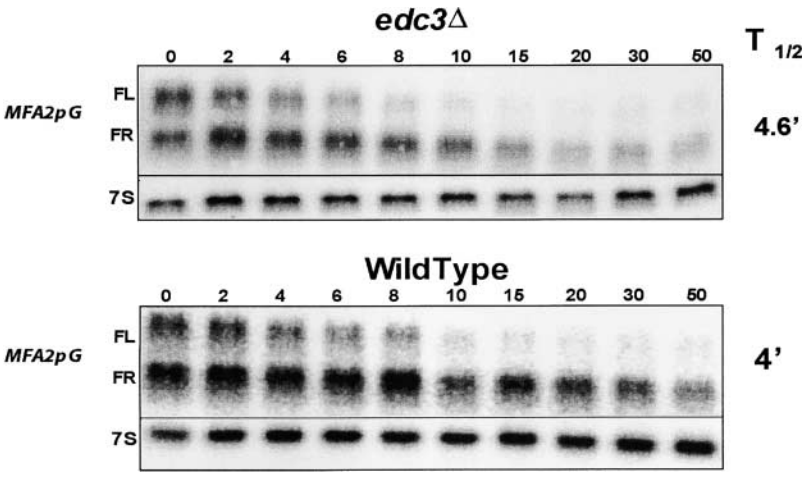

FIGURE 2.—Edc3p is not rate limiting for mRNA decay. Shown is the decay of the MFA2pG reporter following transcriptional repression in WT and *edc3*Δ strains. Time points indicated at the top represent minutes after transcriptional repression. The distribution of the full-length (FL) MFA2pG mRNA relative to the smaller 5′–3′ degradation intermediate (FR fragment) is shown. The amount of smaller 5′–3′ degradation product is a measure of decapping activity in the strains shown. The half-life of the MFA2pG reporter is shown on the right. Hybridizations were performed with an oligonucleotide specific for the MFA2pG reporter (oRP 140; CAPONIGRO and PARKER 1996). The oligo ORP 100 directed against the 7S RNA was used as a loading control. The experiment was performed as described in MATERIALS AND METHODS.

was grown to an OD of 0.4 at 30° on synthetic media containing 2% dextrose without uracil. The cells were collected and observed as described above. All images shown in Figure 7 are representative of at least three independent observations.

RESULTS

Edc3p belongs to a novel class of conserved proteins: To evaluate the possible significance of Edc3p, we first examined databases for homologs in other organisms. By a series of BLAST (ALTSCHUL *et al.* 1990) searches we were able to identify related proteins in several other organisms, including *Schizosaccharomyces pombe, S. castelli* (not shown), *S. kluyvri* (not shown), *Homo sapiens*, mouse, Drosophila, and Anopheles (Figure 1). Comparative sequence analysis showed that the *S. cerevisiae* Edc3p showed 21–28% identity with its homologs in these organisms. Interestingly, all the homologs of Edc3p are proteins of unknown function.

To determine which regions of Edc3p might be important for its function, we looked for conserved regions by several approaches. When Edc3p and its homologs were aligned using BLAST, a global alignment algorithm, no striking regions of similarity were observed. However, on using the alignment algorithms, CLUSTALW (THOMPSON *et al.* 1994) and BLOCKS (HENIKOFF *et al.* 1995), which look for regions of similarity over short stretches in the proteins, the *EDC3* class of proteins showed five conserved domains arranged in the same order in all the homologs except the *S. pombe* homolog, where domain V is rearranged and positioned after domain I (Figure 1). The conservation of these regions supports the identification of these proteins as being homologs and suggests that these are functionally significant regions within the Edc3p.

Edc3p is not rate limiting for mRNA decay: The extensive interactions/associations between Edc3p and different proteins involved in mRNA decay suggested that Edc3p might be involved in mRNA decay. To determine the role of Edc3p in mRNA decay, we created an edc3Δ strain, which grew normally at temperatures between 18° and 37°. We then analyzed the turnover of two reporter mRNAs in the *edc3*Δ strain. Analysis of mRNA degradation in these strains revealed no defect in the decay of either the unstable MFA2pG reporter mRNA (Figure 2) or the stable PGK1pG mRNA (data not shown). For example, the half-life of MFA2pG reporter mRNA was 4.6 min in the *edc3*Δ strain, which is not significantly different from the 4-min half-life of MFA2pG in the wild-type strain. This suggests that Edc3p either is not involved in mRNA decay or affects a step in decapping that is not rate limiting *in vivo*.

***edc3*Δ slows mRNA decay in strains compromised for the mRNA decay:** Given the extensive interactions with decapping factors, we hypothesized that Edc3p was involved in mRNA decapping, but affected a step that was not rate limiting. This interpretation was consistent with previous results identifying multiple substeps in the decapping reaction (SCHWARTZ and PARKER 2000) and revealing that defects in the decapping activator proteins, Edc1 and Edc2, show a decay defect only when cells are partially compromised for mRNA decapping activity (DUNCKLEY *et al.* 2001). Therefore, to test if Edc3p has some role in mRNA decapping, we created double mutants where the *edc3*Δ was combined with several mutations affecting decapping at different steps in the process. Specifically, we combined *edc3*Δ with conditional alleles in subunits of the decapping enzyme, *dcp1-2* and *dcp2-7*, which affect the actual activity of the decapping enzyme (DUNCKLEY *et al.* 2001; SCHWARTZ *et al.* 2003). In addition, we created double mutants where the *edc3*Δ was combined with *dhh1*Δ, *pat1*Δ, or *lsm1*Δ. These latter mutations affect an early step in the decapping pathway that is distinct from the actual catalytic cleavage (SCHWARTZ and PARKER 2000; SCHWARTZ *et al.* 2003; SHETH and PARKER 2003).

We first analyzed the mRNA decay defects of the *edc3*Δ *dcp1-2* and *edc3*Δ *dcp2-7* mutant strains. These experiments were done at 24°, which is the permissive tempera-

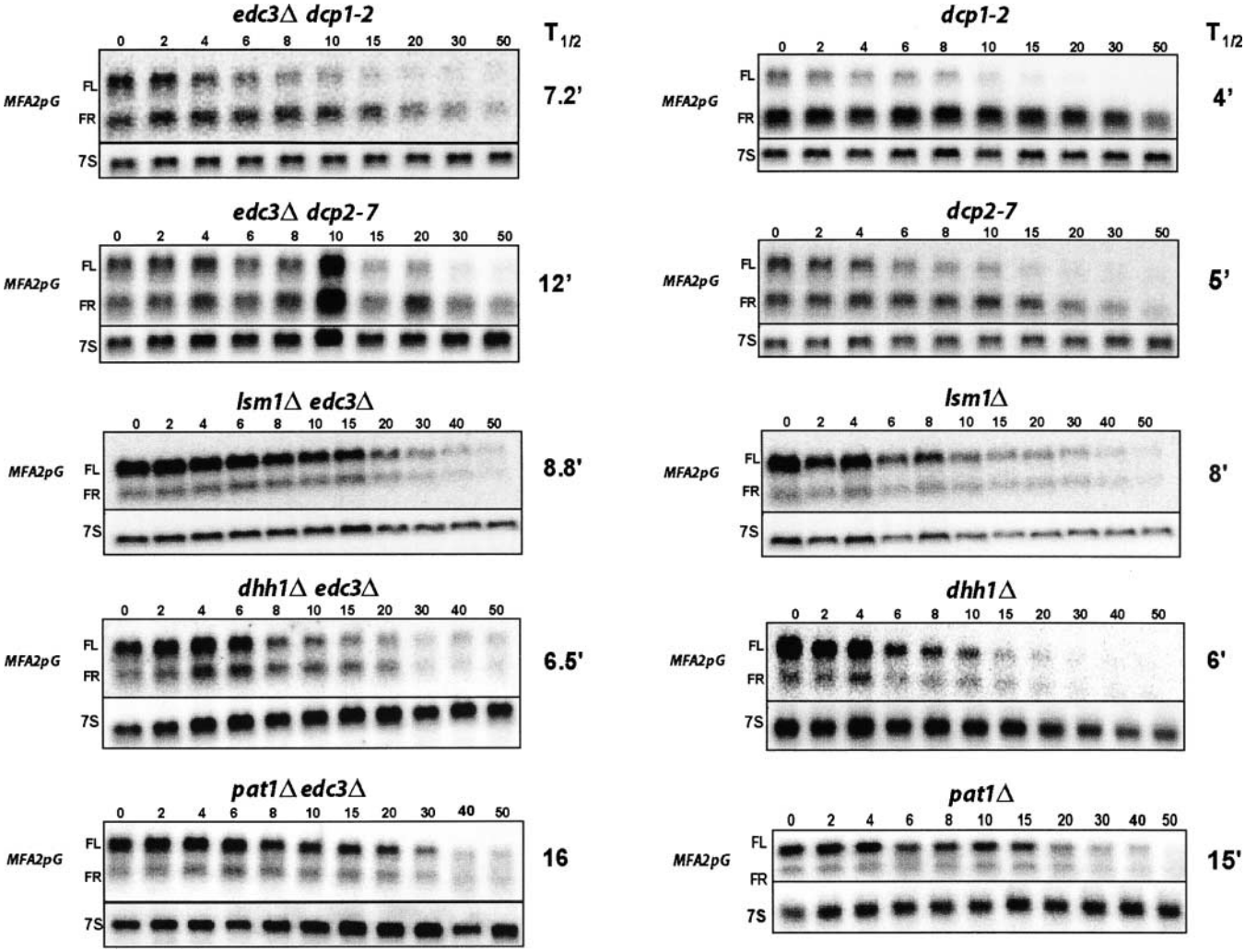

FIGURE 3.—Edc3p is required for efficient mRNA decay in strains compromised for mRNA decapping activity. The decay of the MFA2pG reporter mRNA after transcription repression by addition of glucose in *edc3*Δ combined with *dcp1-2*, *dcp2-7*, *lsm1*Δ, *dhh1*Δ, and *pat1*Δ strains is shown along with the control strains. Time points indicated at the top represent minutes after transcriptional repression. FL and FR represent the full length and fragment of the MFA2pG transcript. The bottom shows the loading control using the oligo for the 7S RNA, oRP100. The half-life of the MFA2pG reporter is shown on the right. The experiment was performed as described in MATERIALS AND METHODS.

ture for both *dcp1-2* and *dcp2-7* alleles. At this temperature, *dcp1-2* and *dcp2-7* alleles are very slightly defective in mRNA decay *in vivo* at the permissive temperature (DUNCKLEY *et al.* 2001; and Figure 3). When the *edc3*Δ was combined with either the *dcp1-2* or the *dcp2-7* background we observed a decrease in the rate of decay of the MFA2pG (Figure 3) and the PGK1pG mRNA (data not shown). In the *dcp2-7* background, the *edc3*Δ slowed the MFA2pG mRNA decay by at least twofold so that the half-life for the MFA2pG mRNA was increased to 12 min compared to the 5 min in strains with just the *dcp2-7* allele. The effect on MFA2pG half-life was not so dramatic (7.2 min) in the *dcp1-2* strains deleted for the *EDC3* gene compared to the *dcp1-2* strain alone (4 min). These results indicate that when Dcp2p, and possibly Dcp1p, are partially defective, a role for Edc3p in mRNA decay can be observed. In contrast, when the *edc3*Δ was combined with the *dhh1*Δ, *lsm1*Δ, or *pat1*Δ, no affect on decay rate was observed (Figure 3). We interpret these results to indicate that Edc3p functions as an enhancer of mRNA decapping and genetically interacts most strongly with the actual decapping enzyme.

Loss of *EDC3* slows growth in cells compromised for mRNA decapping: To obtain additional evidence that Edc3p affected decapping, we utilized a growth assay where the viability of the strains is dependent on efficient mRNA decapping. This assay is based on the observation that strains lacking both the major deadenylation-dependent decapping pathway and the alternative 3′–5′ degradation pathway are dead (JACOBS *et al.* 1998; VAN HOOF *et al.* 2000). This synthetic lethality can be made

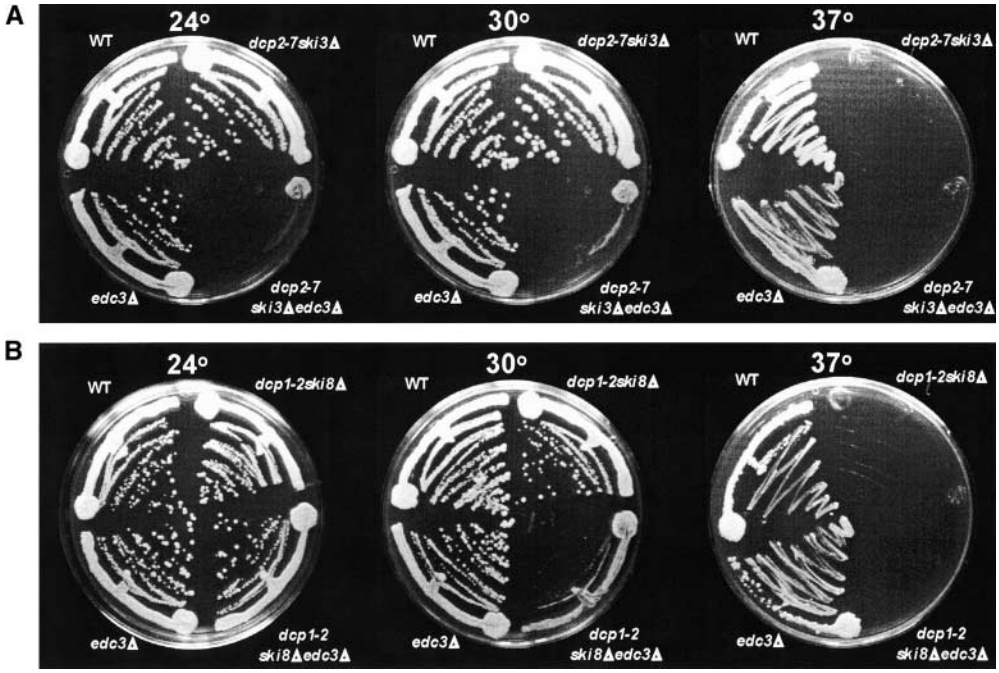

FIGURE 4.—Edc3p interacts with the decapping proteins Dcp1p and Dcp2p. *EDC3* was deleted in the *dcp1-2 ski3*Δ (A) and *dcp2-7 ski8*Δ (B) strain backgrounds and the growth of these strains at the indicated temperatures was monitored. The *edc3*Δ slows growth and causes synthetic lethality in strains compromised for mRNA decay at otherwise permissive temperatures for *dcp1-2 ski3*Δ and *dcp2-7 ski8*Δ strains.

conditional by combining temperature-sensitive alleles of the *DCP1* (*dcp1-2*) and *DCP2* (*dcp2-7*) genes with strains having defects in the exosome-mediated 3′–5′ decay pathway such as *ski3*Δ or *ski8*Δ. For example, a *dcp1-2 ski8*Δ strain grows at 24°, but is dead at 33° (JACOBS *et al.* 1998). Similarly, *dcp2-7 ski3*Δ grows at 24° and 30° but is dead at 37° (DUNCKLEY *et al.* 2001). These strains are conducive for studying effects of proteins that have a subtle effect on decapping. For example, if Edc3p reduces the function of the decapping enzyme, then *edc3*Δ should exacerbate the growth defect in the *dcp1-2 ski8*Δ and *dcp2-7 ski3*Δ strains and we would see a synthetic lethality at a temperature <33° or 37°.

We observed that combining the *edc3*Δ in strains with the *dcp1-2 ski8*Δ and *dcp2-7 ski3*Δ did lead to a clear exacerbation of the growth defects (Figure 4). At 24°, the *dcp2-7 ski3*Δ *edc3*Δ had an extremely slow growth phenotype compared to just the *dcp2-7 ski3*Δ. Moreover, the *dcp2-7 ski3*Δ *edc3*Δ failed to grow at 30° in comparison to the *dcp2-7 ski3*Δ that showed normal growth. Combination of *edc3*Δ with *dcp1-2 ski8*Δ also showed a growth defect phenotype at 30°, but almost normal growth at 24° in comparison to just the *dcp1-2 ski8*Δ strain that grows normally at both 30° and 24°. These growth defects correspond well with the mRNA decay phenotype of these mutants where Edc3p showed a greater defect when combined with the *dcp2-7* allele than when combined with the *dcp1-2* allele. These data provide a second line of genetic evidence for Edc3p affecting decapping.

Edc3p deletion affects the decapping step in mRNA decay: Mutations affecting the mRNA decay rate in yeast can be at any step during deadenylation, decapping, or 5′–3′ exonucleolytic degradation. The *edc3*Δ showed an mRNA decay defect phenotype only when combined in strains with either the *dcp1-2* or the *dcp2-7* mutation. Therefore, the most probable hypothesis is that Edc3p affects the decapping step of mRNA decay, which would be consistent with known Edc3p physical interactions. To determine what step in decapping Edc3p affects, we compared the decay of the MFA2pG mRNA in *dcp2-7* strains to *dcp2-7 edc3*Δ strains on polyacrylamide Northern gels, where the rates of deadenylation and subsequent decay can be observed. On analysis of the MFA2pG mRNA, we observed that there was no substantial difference in the deadenylation rate of the MFA2pG mRNA among the wild type, *edc3*Δ, *dcp2-7*, or *dcp2-7 edc3*Δ strains (Figure 5). This indicates that Edc3p does not affect the deadenylation rate. However, we did observe that the decay of the deadenylated species was slower in the *dcp2-7 edc3*Δ as compared to *dcp2-7* strains alone. This observation suggests that the Edc3p promotes either decapping or 5′–3′ exonucleolytic degradation in the *dcp2-7* strain.

A defect in mRNA decapping can be distinguished from a defect in the exonucleolytic digestion by a primer extension assay. This assay takes advantage of the observation that strains defective in 5′–3′ degradation accumulate a decapped PGK1 transcript that is two nucleotides shorter at the 5′ end compared to that of transcripts that still have the cap (HATFIELD *et al.* 1996; ZUK *et al.* 1999; COLLER *et al.* 2001). Given this, we examined the 5′ ends of the PGK1 mRNA in *edc3*Δ, *dcp2-7 edc3*Δ, and various control strains including the *xrn1*Δ strain (lacking the 5′–3′ exonuclease) and *dcp1*Δ (lacking the decapping enzyme) strains. We observed

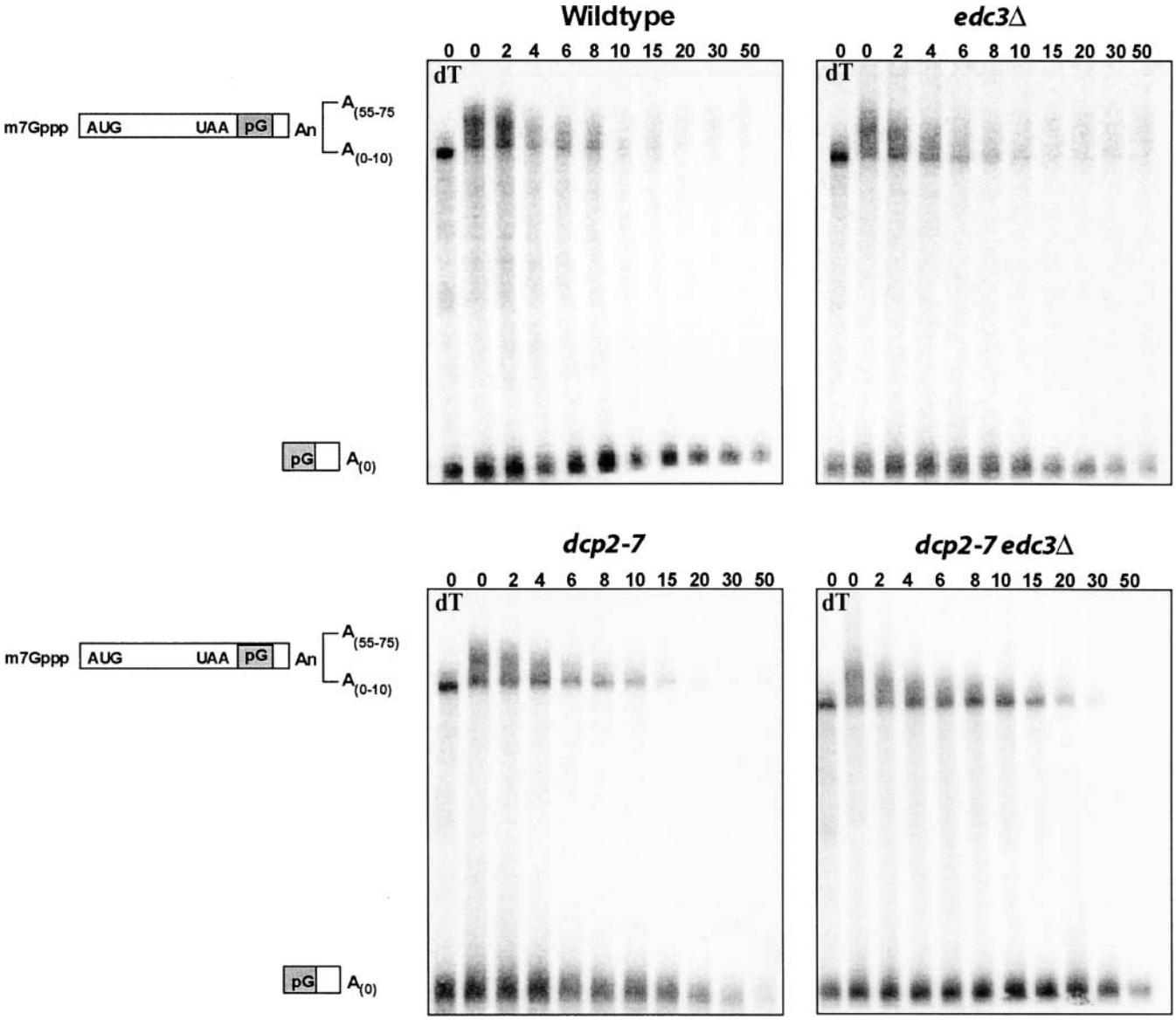

FIGURE 5.—*edc3*Δ affects the decapping step of mRNA decay. mRNA deadenylation of MFA2 transcripts after transcriptional repression from WT, *edc3*Δ, *dcp2-7*, and *edc3*Δ *dcp2-7* strains is shown on polyacrylamide Northern blots. Each first lane is a sample treated with RNaseH in the presence of oligo(dT) to remove the poly(A) tail. The positions of the full-length polyadenylated species, nonadenylated species, and the decay intermediate are shown. The time points after transcriptional repression are shown at the top.

that the *dcp2-7 edc3*Δ strain, where there is a defect in decay after deadenylation, contains a full-length transcript similar to only the wild-type and *dcp1*Δ strains. Further, the *edc3*Δ mutants did not have a defect exclusively in the 3′–5′ exonuclease activity. In contrast, the *xrn1*Δ strain shows the full-length transcript and a −2-nucleotide (nt) species (Figure 6). The above observations provide evidence that Edc3p affects the decapping step of mRNA degradation. However, it should be noted that we cannot rule out that Edc3p also affects the 5′–3′ degradation step.

Edc3p localizes to cytoplasmic foci similar to the P-bodies: It has been recently shown that the proteins involved specifically in mRNA decapping in yeast and mammals are concentrated in cytoplasmic foci called P-bodies (INGELFINGER *et al.* 2002; LYKKE-ANDERSEN 2002; VAN DIJK *et al.* 2002; SHETH and PARKER 2003). Given this observation, if Edc3p is involved in the mRNA decapping step, we would predict that Edc3p would be present in P-bodies. To assess this possibility, we constructed a C-terminal green fluorescent protein (GFP) fusion of Edc3p and localized the protein in the cell by confocal microscopy. We observed that Edc3p-GFP is present in discrete foci in the cytoplasm similar to P-bodies (Figure 7A). The identity of these foci as P-bodies is confirmed by the colocalization of Edc3p-

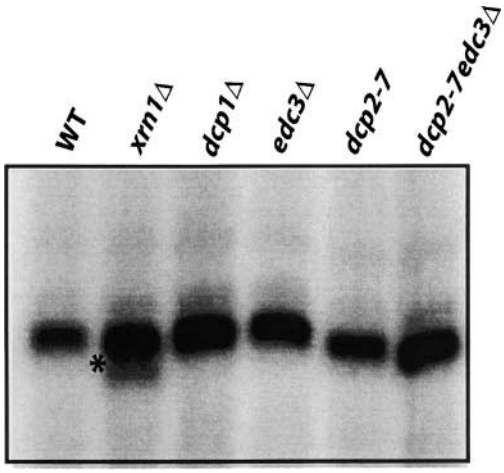

FIGURE 6.—*edc3*Δ mutants are not defective in 3′–5′ exonucleolytic decay. The primer oRP131, specific for the PGK1pG reporter, was used for the primer extension analysis in WT, *dcp1*Δ, *xrn1*Δ, *edc3*Δ, *dcp2-7*, and *edc3*Δ *dcp2-7* strains. After primer extension using reverse transcriptase, the samples were analyzed on a 6% polyacrylamide gel and visualized using autoradiographs. The asterisk indicates the expected −2 species below the full-length band in the *xrn1*Δ strain.

GFP with red fluorescent protein (RFP)-tagged Lsm1p (Figure 7B). Thus, like other proteins involved in mRNA decapping, Edc3p also localizes to the P-bodies. Interestingly, we observed a greater number of Edc3p-GFP foci as compared to the Lsm1p-RFP foci (Figure 7B). One possibility is that different forms of P-bodies exist, some of which are lacking Lsm1p, or another possibility is that we failed to detect Lsm1p in all P-bodies due to the lower signal from the Lsm1p-RFP fusion.

Additional supporting evidence that the Edc3p-GFP foci are P-bodies comes from the observation that trapping mRNAs in polysomes using cycloheximide inhibits decapping and leads to loss of P-bodies after treatment with the drug (SHETH and PARKER 2003). Just like P-bodies, the Edc3p-GFP foci also disappeared rapidly on treatment of the cells with cycloheximide (Figure 7A).

Edc3p does not affect nonsense-mediated decay: The decapping complex is also involved in nonsense-mediated decay (NMD), which is characterized by deadenylation-independent decapping; therefore we asked if Edc3p plays a role in NMD (WILUSZ *et al.* 2001; LYKKE-ANDERSEN 2002). To determine if Edc3p affects NMD, the levels of the CYH2 pre-mRNA in *edc3*Δ and *dcp2-7 edc3*Δ strains were analyzed. A known NMD substrate is the CYH2 pre-mRNA, which is inefficiently spliced and contains a pretermination codon (PTC; HE *et al.* 1993; HILLEREN and PARKER 1999). Analysis of CYH2 mRNA at steady state showed that the *edc3*Δ did not lead to increased levels of pre-CYH2 mRNA either in wild-type strains or in combination with *dcp1-2* and *dcp2-7* alleles (Figure 8). These results suggest that Edc3p does not play a significant role in NMD.

DISCUSSION

Several observations indicate that Edc3p has a role in mRNA decay and specifically enhances the function of decapping. First, Edc3p has been shown in numerous *in vivo* and *in vitro* genomic studies to physically interact with several mRNA decay factors (FROMONT-RACINE *et al.* 2000; UETZ *et al.* 2000; ITO *et al.* 2001; GAVIN *et al.*

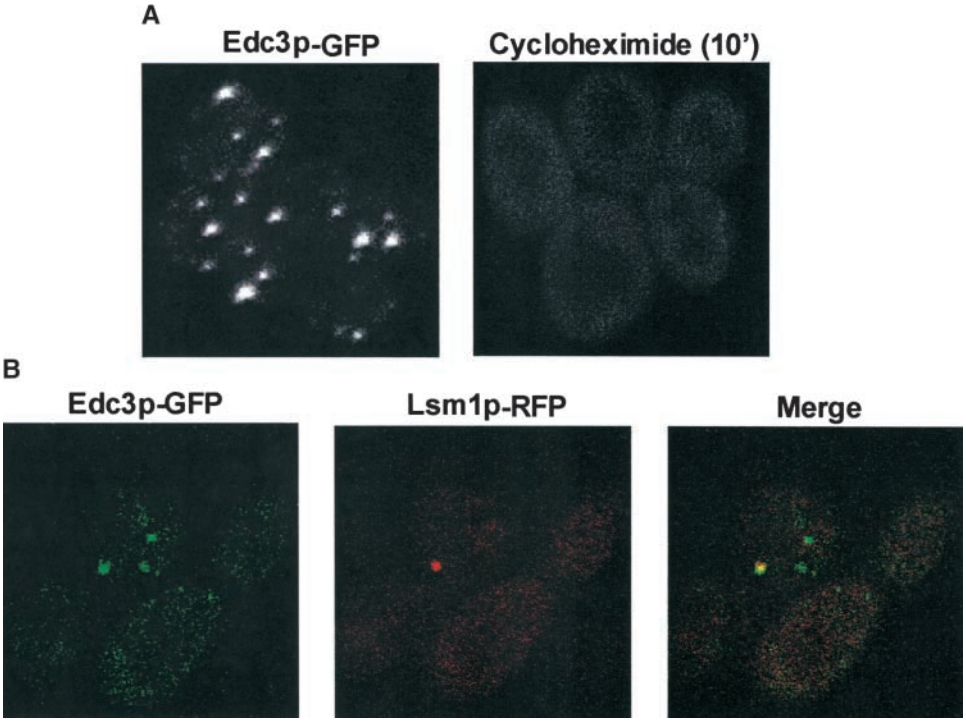

FIGURE 7.—Edc3p localizes to discrete cytoplasmic foci in the cell. (A) Edc3p was tagged with GFP as described in LONGTINE *et al.* (1998). Cells were grown to 0.3 OD in synthetic medium containing 2% dextrose at 30° and then visualized using the confocal microscope as described in MATERIALS AND METHODS. Cells were treated with 100 μg/ml cycloheximide for 10 min and then analyzed for the presence of the foci. (B) Strains with Edc3p-GFP were transformed with the Lsm1p-RFP plasmid and were examined for colocalization of the two proteins using Adobe Photoshop.

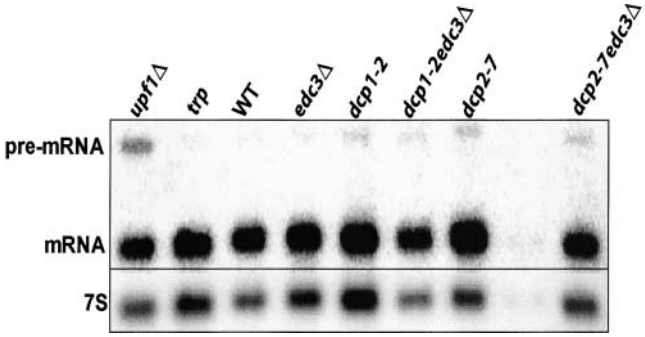

FIGURE 8.—Edc3p is not essential for NMD. The steady-state levels of *CYH2* pre-mRNA and mRNA in WT, *upf1*Δ, *trp*, *edc3*Δ, *dcp1-2*, *dcp2-7*, *edc3*Δ *dcp1-2*, and *edc3*Δ *dcp2-7* strains are shown. The lane second from the right is empty. Steady-state RNA from cells grown at 30° was isolated and analyzed on agarose Northern, using a random-primer-labeled probe that is specific for both pre-mRNA and mRNA of *CYH2*. The oligo oRP100 directed against the 7S RNA was used as a loading control. The ratio of the *CYH2* pre-mRNA and mRNA in all the strains was calculated (not shown).

2002; Ho *et al.* 2002). Second, deletion of the *EDC3* gene leads to a defect in mRNA degradation in strains carrying conditional alleles of Dcp1p and Dcp2p, even though the analysis is done at permissive temperatures where the *dcp1-2* and *dcp2-7* alleles have little or no effect on mRNA decay rates (Figure 3). Moreover, these *edc3*Δ *dcp2-7* strains accumulated deadenylated full-length MFA2pG reporter mRNA compared to the WT, *edc3*Δ, and *dcp2-7* allele alone (Figure 5). Third, *edc3*Δ exaggerated growth defects and caused synthetic lethality in strains containing the *dcp1-2* and *dcp2-7* alleles in combination with a block to the 3′–5′ decay pathway (Figure 4). Finally, an Edc3p-GFP fusion protein localizes in P-bodies (Figure 7), which are specialized cytoplasmic foci containing decapping proteins (SHETH and PARKER 2003). These observations together argue that Edc3p interacts with Dcp2p and Dcp1p to stimulate the mRNA decapping rate.

In principle, Edc3p could enhance decapping by acting at any of several distinct steps in the decapping process. Although poorly understood, previous experiments have suggested that decapping will require at least three distinct, but possibly related steps: (1) loss of the translation initiation complex bound to the 5′ end of the mRNA, (2) assembly of a decapping complex and localization within P-bodies, and (3) the actual catalytic step of decapping (SCHWARTZ and PARKER 2000; THARUN and PARKER 2001; SHETH and PARKER 2003).

Three observations are consistent with Edc3p primarily affecting the final step of enzymatic decapping, either by stabilizing Dcp1p/Dcp2p or by affecting their function in some manner. First, the *edc3*Δ shows only synergistic effects with other defects in the decapping activity and does not show any phenotypic interaction with deletions in the *LSM1*, *PAT1*, and *DHH1* genes, whose products are likely to affect the first two steps in the decapping process. Second, on the basis of copurification, Edc3p directly interacts with Dcp1p/Dcp2p (FROMONT-RACINE *et al.* 2000; UETZ *et al.* 2000; ITO *et al.* 2001; GAVIN *et al.* 2002; Ho *et al.* 2002). Third, the *edc3*Δ does not have a decay defect and previous results suggest that the actual decapping step in decay is not normally rate limiting. Evidence that the enzymatic decapping step is not rate limiting *in vivo* in our growth conditions is that the *dcp1-2*, *dcp2-7*, *edc1*Δ, and *edc2*Δ mutations all strongly affect mRNA decapping activity *in vitro*, yet have little effect on overall decay rate *in vivo* (DUNCKLEY *et al.* 2001; SCHWARTZ *et al.* 2003). However, it should be noted that decapping *per se* could be made the rate-limiting step in decapping *in vivo* (as in a strong *dcp1* or *dcp2* allele).

The Edc3p is part of an emerging set of proteins that perform the mRNA decapping step. Proteins involved in mRNA decay can be divided into three functional and phenotypic categories. The first class of proteins consists of Dcp1p and Dcp2p, which are required for the decapping enzyme to function, and strains lacking these proteins show a complete block to decapping (BEELMAN *et al.* 1996; DUNCKLEY and PARKER 1999; THARUN and PARKER 1999). The second group of decapping regulatory proteins (Lsm1-7p, Pat1p, and Dhh1p) appears to constitute a general activator of decapping complex. Strains lacking Lsm1-7p, Dhh1p, or Pat1p show a partial block to decapping for multiple mRNAs. Edc3p belongs to the third class of decapping factors, which, although they can affect the decapping process, are not normally rate limiting for decapping. The proteins Edc1p and Edc2p are also in this class.

Recent results suggest that the EDC family of proteins may also be important in the control of a subset of mRNAs or in the control of decapping under different conditions. For example, Edc1p has been shown to be important for growth during a shift from dextrose to glycerol, suggesting that it may be affecting specific mRNAs necessary for this carbon-source shift (SCHWARTZ *et al.* 2003). In addition, recent results show that Edc3p specifically affects the decapping of the RPS28B mRNA, possibly as part of an autoregulatory loop (G. BADIS, C. SAVEANU, M. FROMONT-RACINE and A. JACQUIER, unpublished results). Given these results, the emerging picture of the EDC family of proteins is that they will play a general role in assisting decapping and have been coopted for the control of specific mRNAs. An unresolved issue is how broad the specific role of these proteins will be in the cell.

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