Two Related Proteins, Edc1p and Edc2p, Stimulate mRNA Decapping in *Saccharomyces cerevisiae*

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ABSTRACT

The major mRNA decay pathway in *Saccharomyces cerevisiae* occurs through deadenylation, decapping, and 5′ to 3′ degradation of the mRNA. Decapping is a critical control point in this decay pathway. Two proteins, Dcp1p and Dcp2p, are required for mRNA decapping *in vivo* and for the production of active decapping enzyme. To understand the relationship between Dcp1p and Dcp2p, a combination of both genetic and biochemical approaches were used. First, we demonstrated that when Dcp1p is biochemically separated from Dcp2p, Dcp1p was active for decapping. This observation confirmed that Dcp1p is the decapping enzyme and indicated that Dcp2p functions to allow the production of active Dcp1p. We also identified two related proteins that stimulate decapping, Edc1p and Edc2p (*E*nhancer of mRNA *De*Capping). Overexpression of the *EDC1* and *EDC2* genes suppressed conditional alleles of *dcp1* and *dcp2*, respectively. Moreover, when mRNA decapping was compromised, deletion of the *EDC1* and/or *EDC2* genes caused significant mRNA decay defects. The Edc1p also co-immunoprecipitated with Dcp1p and Dcp2p. These results indicated that Edc1p and Edc2p interact with the decapping proteins and function to enhance the decapping rate.

An important control point in gene expression occurs at the level of mRNA stability (for reviews, see Ross 1995; Caponigro and Parker 1996; Jacobson and Peltz 1996). Work in the yeast *Saccharomyces cerevisiae* has demonstrated that mRNAs are degraded through two general pathways. The main decay pathway in yeast occurs through deadenylation followed by Dcp1p-catalyzed removal of the 5′ cap structure (Beelman *et al.* 1996), which exposes the body of the mRNA to Xrn1p-dependent 5′ to 3′ exonucleolytic degradation (Decker and Parker 1993; Hsu and Stevens 1993; Muhlrad *et al.* 1994, 1995). The second general mRNA decay pathway occurs via deadenylation of the polyadenylated mRNA, followed by 3′ to 5′ exonucleolytic digestion of the transcript body by the exosome complex (Muhlrad *et al.* 1995; Anderson and Parker 1998).

Multiple lines of evidence indicate that these general mRNA decay pathways are likely conserved in other eukaryotes. For example, deadenylated, decapped, full-length mRNA has been detected from murine liver cells (Couttet *et al.* 1997). Messenger RNA decay intermediates shortened from their 5′ ends have also been identified in both plant and animal cells (Lim and Maquat 1992; Higgs and Colbert 1994; Gera and Baker 1998). Decay of mRNA in a 3′ to 5′ direction has also been shown to occur in plants (Higgs and Colbert 1994). Last, the yeast proteins that are involved in the decay of mRNA, such as Dcp1p, Dcp2p, Xrn1p, Lsm1p-Lsm7p, Ski2p, and components of the exosome, are conserved in higher eukaryotes (Dangel *et al.* 1995; Lee *et al.* 1995; Bashkirov *et al.* 1997; Mitchel *et al.* 1997; Salgado-Garrido *et al.* 1999; Dunckley and Parker 1999).

Decapping is a critical step in the 5′ to 3′ mRNA decay pathway since it both precedes and permits the degradation of the mRNA body. Also, individual mRNAs are decapped at different rates, indicating that mRNA decapping is a controlled process that contributes to differential mRNA decay rates (Muhlrad *et al.* 1994, 1995). Decapping is also a key step in the degradation of aberrant mRNAs through the mRNA surveillance pathway (Muhlrad and Parker 1994; Hagan *et al.* 1995; Beelman *et al.* 1996; Dunckley and Parker 1999). The product of the *DCP1* gene has been shown to be required for mRNA decapping *in vivo* and sufficient for decapping activity *in vitro*, suggesting that *DCP1* encodes a mRNA decapping enzyme (Beelman *et al.* 1996; LaGrandeur and Parker 1998). An additional protein, Dcp2p, is also required for mRNA decapping (Dunckley and Parker 1999). Dcp2p purified from wild-type yeast copurifies with Dcp1p as well as with mRNA decapping activity (Dunckley and Parker 1999). If purified from a *dcp1*Δ mutant, Dcp2p no longer copurifies with decapping activity, indicating that the presence of functional Dcp1p is required for decapping activity. Additionally, Dcp1p purified from a *dcp2*Δ mutant is enzymatically inactive for mRNA decapping *in vitro* (Dunckley and Parker 1999). These results sug-

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TABLE 1

Strains used in this study

Strain	Genotype	Source
yRP840	*MAT***a** *his4-539 leu2-3112 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	Hatfield *et al.* (1996)
yRP841	*MAT*α *leu2-3112 lys2-201 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	Hatfield *et al.* (1996)
yRP1070	*MAT***a** *his4-539 leu2-3112 trp1 ura3-52 DCP1::URA3 cup1::LEU2/PGK1pG/MFA2pG*	Beelman *et al.* (1996)
yRP1340	*MAT***a** *his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp1-2::TRP1*	Dunckley and Parker (1999)
yRP1341	*MAT***a** *his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp1-2::TRP1* [MFA2pG/LYS2]	Dunckley and Parker (1999)
yRP1345	*MAT***a** *his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 ski8::URA3*	Dunckley and Parker (1999)
yRP1356	*MAT***a** *his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp1::URA3 cup1::URA3* [MFA2pG/LYS2]	Dunckley and Parker (1999)
yRP1500	*MAT***a** *his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp1::URA3 dcp2::TRP1 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1501	*MAT*α *leu2-3112 lys2-201 trp1 ura3-52 dcp2-7::URA3*	This study
yRP1502	*MAT***a** *his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp2-7::URA3 ski3::TRP1*	This study
yRP1503	*MAT***a** *his3-Δ200 ade2-101 leu2-3112 lys2-201 trp1 ura3-52 edc1::HIS3 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1504	*MAT*α *his4-539 leu2-3112 trp1 ura3-52 edc2::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1506	*MAT***a** *ade2 leu2-3112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 edc1::HIS3 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1507	*MAT*α *ade2 leu2-3112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 edc2::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1508	*MAT***a** *ade2 leu2-3112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 edc1::HIS3 edc2::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1509	*MAT***a** *leu2-3112 lys2-201 trp1 ura3-52 dcp2-7::URA3 edc1::HIS3 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1510	*MAT*α *leu2-3112 lys2-201 trp1 ura3-52 his3-Δ200 dcp2-7::URA3 edc2::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1511	*MAT*α *ade2 leu2-3112 lys2-201 trp1 ura3-52 dcp2-7::URA3 edc1::HIS3 edc2::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1512	*MAT*α *leu2-3112 lys2-201 trp1 ura3-52 his3-Δ200 dcp2::TRP1 edc1::HIS3 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1513	*MAT***a** *his4-539 leu2-3112 lys2-201 trp1 ura3-52 dcp2::TRP1 edc2::NEO cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP1514	*MAT***a** *his3-Δ200 ade2-101 leu2-3112 lys2-201 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	This study

gest two possibilities. First, Dcp2p could be required for the production of active Dcp1p decapping enzyme. Second, Dcp1p and Dcp2p could be essential members of a multi-subunit decapping enzyme.

Determining the relationship between Dcp1p and Dcp2p, as well as identifying additional factors that control the activity of Dcp1p and Dcp2p, will be important for understanding mRNA decapping. For this reason, we have performed a combination of biochemical and genetic experiments addressing the relevance of the interaction between Dcp1p and Dcp2p to the production of mRNA decapping activity. In addition, we identify two *E*nhancer of mRNA *De*Capping proteins, Edc1p and Edc2p, that stimulate mRNA decapping *in vivo*.

MATERIALS AND METHODS

Plasmids and strains: All strains used in this study are listed in Table 1. Strain yRP1501 was constructed in three steps. First, a portion of genomic DNA 3′ of the *DCP2* gene was PCR amplified from plasmid pRP930 and cloned into the *Sma*I/*Cla*I sites of a pBluescriptII KS+ vector that also contained the *URA3* gene in the *Bam*HI site, generating plasmid pRP988. Next, the *dcp2-7* allele was excised from plasmid pRP994 using *Sac*I/*Spe*I and cloned into the corresponding sites of pRP988 to generate plasmid pRP989. pRP989 was then cut with *Apa*I/*Sac*I to release the *dcp2-7* allele, *URA3* gene, and 3′ region of *DCP2*. This DNA fragment was transformed into yRP1358 and all fast-growing URA+ colonies were then screened for loss of the TRP1 marker. Integration of the *dcp2-7* allele at the *dcp2*Δ locus was confirmed by Southern analyses.

Strain yRP1503 was constructed by homologous recombination in strain yRP1514. The *HIS3* gene was PCR amplified from genomic DNA using primers oRP827 and oRP828, containing regions of DNA complementary to the sequence immediately 5′ of the *EDC1* initiation codon and 3′ of the stop codon. This PCR product was then transformed into yRP1514. Deletion of the *EDC1* gene was confirmed by Southern analysis. Deletion of *EDC2* was accomplished by PCR of a *NEO* deletion cassette from the commercially available *edc2*Δ mutant (Research Genetics, Birmingham, AL) using oRP990 and oRP991, followed by transformation of the PCR product into yRP841. Deletion of *EDC2* was confirmed by Southern analysis. Strain yRP1504 was then later obtained from dissection of a diploid heterozygous for the *edc1*Δ and *edc2*Δ mutations.

To make plasmid pRP984, the *EDC1* gene was amplified

from genomic DNA using oRP987 and oRP988. This PCR product was then cloned into yEP351, a 2μ plasmid, using the *Bam*HI and *Sac*I sites introduced on the oligonucleotides. Plasmid pRP985 was made similarly through amplification of *EDC2* using oRP931 and oRP932. Plasmid pRP982 was constructed by amplification of the *GPD* promoter, HIS-*DCP1* gene, and *PGK1* terminator from plasmid pRP785 using oligonucleotides oRP910 and oRP916. This PCR product was then cotransformed into a dcp1Δ strain (yRP1070) with *Nco*I/*Nde*I-digested pRP66. Plasmids generated from *in vivo* recombination that complemented the *dcp1*Δ growth defect were rescued to *Escherichia coli* using a yeast DNA isolation system (Stratagene, La Jolla, CA) and sequenced. The pRP983 plasmid was constructed by amplification of the *GPD* promoter, FLAG-*DCP2* gene, and *PGK1* terminator from plasmid pRP936 using oligonucleotides oRP899 and oRP915. The resulting PCR product was then cotransformed into a *dcp2*Δ strain (yRP1346) with *Eco*RI-digested pRS317. Plasmids that complemented the *dcp2*Δ growth defect were rescued to *E. coli* and sequenced. To make the FLAG-*EDC1* plasmid (pRP986), the 5′ untranslated region (UTR) and promoter region of *EDC1* was first PCR amplified using oligonucleotides oRP959 and oRP960, which contained the reverse complement of the FLAG nucleotide sequence. Next, the *EDC1* coding region and 3′ UTR were PCR amplified using oRP962 and oRP961, which contains the FLAG nucleotide sequence at its 5′ end. These two PCR products were cotransformed into the *edc1*Δ strain (yRP1503) with *Eco*RI-digested pRS317. Plasmids generated from *in vivo* recombination were rescued from the resulting transformants and sequenced. The V5-*DCP2* plasmid (pRP999) was constructed similarly to the FLAG-*EDC1* plasmid. The 5′ UTR of *DCP2* was PCR amplified using oRP985 and oRP968, which contained the reverse complement of the V5 nucleotide sequence. The *DCP2* coding region and 3′ UTR were amplified using oRP984 and oRP967, which contained the V5 nucleotide sequence. These PCR products were cotransformed into yRP1346 with *Eco*RI-digested pRS317 and complementing plasmids were rescued to *E. coli* and sequenced. The insert from pRP999 was then subcloned into pRS416 using *Apa*I/*Sac*I, generating pRP987.

Genetic screening procedures: *EDC1* was identified using a screening procedure for suppressors of the *dcp1-2 ski8*Δ mutant as previously described (Dunckley and Parker 1999). To screen for high-copy suppressors of the *dcp2-7 ski3*Δ mutant, strain yRP1502 was transformed with a yeast 2μ genomic DNA library (ATCC 37323). Transformants were selected at 24°. Positive transformants were then replica plated and screened for growth at 24° and 37°. Plasmids were isolated from clones that supported growth at 37°, retransformed into yRP1502, and retested for growth at the restrictive temperature. The inserts from plasmids that conferred growth at high temperature were then sequenced.

RNA procedures: RNA samples were prepared and isolated as previously described (Caponigro *et al.* 1993). Half-lives were determined by quantitation of blots using a Molecular Dynamics (Sunnyvale, CA) PhosphorImager. Loading corrections for quantitation were determined by hybridization to the 7S RNA, a stable RNA polymerase III transcript.

Determination of *dcp1-2* and *dcp2-7* suppression: To determine suppression of *dcp1-2* and *dcp2-7*, strains yRP840, yRP1341, yRP1341 [pRP984], yRP1356, yRP1356 [pRP984], yRP1501 [*MFA2*pG/LYS2], yRP1501 [pRP985] [*MFA2*pG/LYS2], yRP1358 [*MFA2*pG/LYS2], and yRP1358 [pRP985] [*MFA2*pG/LYS2] were grown at 24° to midlog phase in selective media containing 2% galactose. For *dcp1-2* strains, cultures were then shifted to 33° for 1 hr. For *dcp2-7* strains, cultures were shifted to 37° for 1.5 hr. Following the temperature shift, cells were harvested and RNA was prepared as previously described (Caponigro *et al.* 1993).

***In vitro* separation of Dcp1p and Dcp2p:** Four liters of strain yRP1500 containing pRP982 and pRP983 were grown at 30° in selective media to an OD_{600} of 1.0. Cells were lysed and the FLAG-Dcp2p was purified as previously described (Dunckley and Parker 1999), with one exception. The immunopellet was washed with Tris magnesium sodium (TMN) buffer containing 2.5 M NaCl, rather than with TMN containing 750 mM NaCl. All protein samples were dialyzed against 4 liters of TMN-150 (10 mM Tris-HCl, pH 7.7, 150 mM NaCl, 5 mM $MgCl_2$).

The FLAG-Dcp2p and HIS-Dcp2p were analyzed by standard SDS-PAGE on a 10% gel (Laemmli 1970). FLAG-Dcp2p and HIS-Dcp1p were detected by Western analyses using α-FLAG antibody (Kodak, Rochester, NY) and polyclonal antibodies to full-length Dcp1p, respectively. Protein size markers were purchased from GIBCO BRL (Gaithersburg, MD). The *in vitro* decapping assays were performed as previously described (Beelman *et al.* 1996; LaGrandeur and Parker 1998).

Immunoprecipitation of FLAG-Edc1p: Two liters of strain yRP1512 containing pRP986 and pRP987 was grown in selective media at 24° to an OD_{600} of 0.8. Cells were harvested and lysed as previously described (Dunckley and Parker 1999). Immunoprecipitation of FLAG-Edc1p was performed as previously described for FLAG-Dcp2p (Dunckley and Parker 1999) with one exception. Binding of the FLAG-Dsp1p was performed in TMN-25 buffer containing 25 mM NaCl. The immunopellet was then washed either with TMN-50, containing 50 mM NaCl, or with TMN-150, containing 150 mM NaCl, prior to elution of FLAG-Dsp1p. The RNase treatment was performed as previously described (Tharun *et al.* 2000). The V5-Dcp2p fusion protein was detected using commercially available α-V5 antibody (Invitrogen, San Diego) following the manufacturer's protocol.

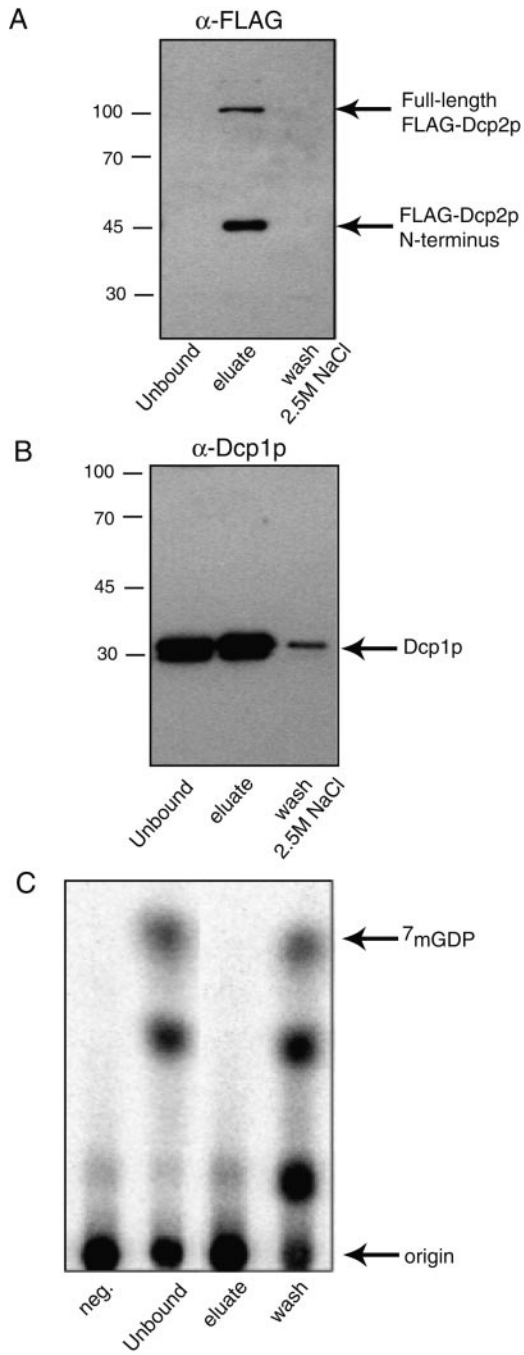
RESULTS

Separation of Dcp1p from Dcp2p yields active decapping enzyme: On the basis of the available data, there are two possible functions for Dcp2p in mRNA decapping. First, Dcp2p could be required for the activation of the Dcp1p decapping enzyme and would be dispensable for decapping once Dcp1p was activated. Alternatively, Dcp1p and Dcp2p could be essential members of a multi-subunit decapping enzyme, in which case Dcp2p would always be required for decapping activity. To distinguish between these two possibilities, we biochemically separated a fraction of Dcp1p from Dcp2p and assayed for mRNA decapping activity of the different protein fractions *in vitro* to determine which protein, or proteins, are required for decapping.

To do this experiment, we purified a FLAG-tagged Dcp2 protein from a yeast strain that overexpressed both HIS-tagged Dcp1p and FLAG-Dcp2p (see materials and methods). As has been observed previously, purification of FLAG-Dcp2p yields both the full-length protein and a shorter form of the protein (Figure 1A; Dunckley and Parker 1999). Importantly, FLAG-Dcp2p is detectable by Western analysis only in the eluate (Figure 1A), even after very long exposures (data not shown). In contrast, Dcp1p is readily detectable in the unbound, high-salt wash, and eluate fractions (Figure 1B). By this paradigm, we have separated the

Dcp1p present in the cell into three pools: a pool that is not bound by Dcp2p and is present in the unbound fraction, a pool that is bound to Dcp2p but is released in the high-salt wash, and a pool that is tightly bound to Dcp2p and elutes only when Dcp2p is released from the column by peptide elution.

Assaying the protein fractions for mRNA decapping activity revealed several important observations. First, *in vitro* decapping activity is present in the unbound and wash fractions, which contain Dcp1p but no FLAG-Dcp2p (Figure 1C). Further, these two fractions contain comparable amounts of decapping activity relative to the amount of Dcp1p that was used in the reactions. In the figure shown, approximately 30 ng of Dcp1p was included in each decapping reaction based on comparisons of the amount of Dcp1p in each fraction to purified Dcp1p. This observation demonstrated that, once produced in an active form, Dcp1p no longer required Dcp2p for decapping activity. This result strongly argued that the essential function of Dcp2p in decapping is to activate the Dcp1p decapping enzyme. Furthermore, this result confirms previous observations suggesting that Dcp1p alone is sufficient for mRNA decapping (LaGrandeur and Parker 1998).



A second important observation was that, following a 2.5 M NaCl wash, Dcp2p remains associated with a large fraction of Dcp1p, indicating that Dcp2p interacts tightly with a significant amount of Dcp1p *in vivo*. Moreover, the Dcp1p that is present in this fraction is inactive for decapping *in vitro* (Figure 1). This is a surprising result and suggests several intriguing possibilities. First, Dcp1p may exist *in vivo* in an active and inactive form. In this model, Dcp2p would associate more tightly with the inactive form than with the active form of Dcp1p. The affinity of Dcp2p for Dcp1p would decrease following Dcp2p-dependent activation of Dcp1p. An alternative, but related, possibility is that Dcp1p is inactive if bound by Dcp2p. This model requires that Dcp2p function both as an activator and as an inhibitor of Dcp1p (see DISCUSSION).

Identification of *EDC1* and *EDC2*: To further characterize the relationship between Dcp1p and Dcp2p we used a genetic screen to identify proteins that affect their function. In this case, we took advantage of the observation that loss-of-function mutations in either *dcp1* or *dcp2* are synthetically lethal with *ski2*Δ, *ski3*Δ, *ski4-1*, *ski7*Δ, or *ski8*Δ mutations, which prevent efficient 3′ to 5′ mRNA decay (Anderson and Parker 1998; van Hoof *et al.* 2000). Two observations suggest this synthetic lethality results from lack of sufficient mRNA degradation. First, mRNAs are extremely stable in a *dcp1-2 ski8*Δ strain (Anderson and Parker 1998). Sec-

Figure 1.—Dcp2p activates Dcp1p. (A) A Western blot of the FLAG-*DCP2* preparation using anti-FLAG antibody is shown. The lanes shown are: Unbound, supernatant remaining following binding to α-FLAG immunoaffinity matrix; eluate, fraction eluted from the α-FLAG matrix; wash, 2.5 M NaCl wash fraction performed prior to elution of FLAG-Dcp2p. Numbers on the left indicate molecular weight standards in kilodaltons. (B) Western blot using antibodies specific for the Dcp1 protein. Lanes shown are the same as those for A. (C) Decapping assays using the same fractions as in A and B are shown, with the addition of a negative control consisting of substrate RNA with no added protein. The products produced at the end of a 20-min time course are shown. One-ninth of the unbound sample was used relative to the wash fraction so that comparable amounts of Dcp1p were present in the two reactions. The spots beneath the ^{7}mGDP decapping product result from the activity of other nucleases normally present in the unbound and wash fractions (T. Dunckley and R. Parker, unpublished observations).

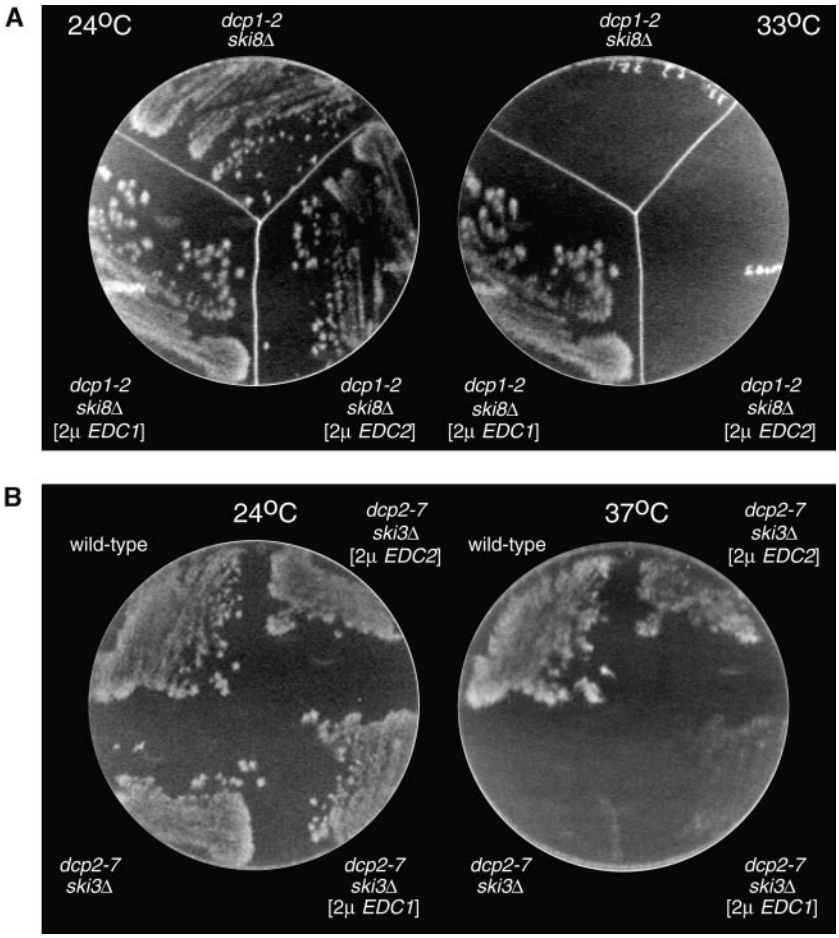


FIGURE 2.—*EDC1* and *EDC2* overexpression rescues the growth defect of *dcp1-2ski8*Δ mutants and of *dcp2-7ski3*Δ mutants, respectively. (A) Strain yRP1345, containing either a 2μ plasmid (yEP351), *EDC1* on a 2μ plasmid (pRP984), or *EDC2* on a 2μ plasmid (pRP985), was grown at 24° for 4 days on minimal media lacking leucine and then replica plated and grown for an additional 2 days at either 24° or 33°. (B) Strains yRP840 and yRP1502, containing either a 2μ plasmid (yEP351), *EDC1* on a 2μ plasmid (pRP984), or *EDC2* on a 2μ plasmid (pRP985), were grown at 24° for 4 days on minimal media lacking leucine and then replica plated and grown for an additional 2 days at either 24° or 37°.

ond, all suppressors of this lethality isolated to date restore mRNA decay to some extent (DUNCKLEY and PARKER 1999 and unpublished observations). We have used this synthetic lethality, in combination with a temperature-sensitive allele of the *DCP1* gene (*dcp1-2*), to isolate high-copy suppressors that restore growth at high temperature to a *dcp1-2 ski8*Δ strain (DUNCKLEY and PARKER 1999). This genetic screen identified *DCP2* and *EDC1* [open reading frame (ORF) no. YGL222C] as high-copy suppressors of the synthetic lethality of *dcp1-2 ski8*Δ mutants (Figure 2A; DUNCKLEY and PARKER 1999). Characterization of the role of Edc1p in mRNA decay is presented below.

To identify proteins that influence Dcp2p activity, we made the synthetic lethality of *dcp2*Δ *ski3*Δ double mutants conditional using a temperature-sensitive mutation in *dcp2*, termed *dcp2-7*. This allele contains three point mutations in the N terminus of the protein (N60D, I68V, and D142V) and allows nearly normal mRNA decay at 24° but is essentially a null at 37° (T. DUNCKLEY and R. PARKER, unpublished results). We then screened three genome equivalents of a yeast 2μ genomic DNA library for genes that, when overexpressed, would suppress the conditional growth defect of *dcp2-7 ski3*Δ mutants at 37° (see MATERIALS AND METHODS). The *EDC2* gene (ORF no. YER035W) was identified as an overexpression suppressor of the conditional synthetic lethality of *dcp2-7 ski3*Δ mutants (Figure 2B).

***EDC1* and *EDC2* encode related proteins with distinct functions:** Analysis of the *EDC1* and *EDC2* coding regions revealed several interesting features. First, both proteins are relatively small (Edc1p = 19.1 kD, Edc2p = 16.1 kD) and basic (Edc1p pI = 11.01, Edc2p pI = 10.05). Most strikingly, a BLAST search revealed that Edc1p and Edc2p are related (Figure 3). The most significant homology is clustered in two regions, one at the C termini of the two proteins and the other toward the N termini. At the present time, no other proteins in the databases show these conserved motifs.

The conservation of these two regions suggested that Edc1p and Edc2p might share a similar function. For this reason, we tested if overexpression of Edc1p would suppress the conditional lethality of *dcp2-7 ski3*Δ mutants and if overexpression of Edc2p would suppress the lethality of the *dcp1-2 ski8*Δ mutant. Interestingly, overexpression of Edc1p suppressed the growth defect of the *dcp1-2 ski8*Δ mutant as seen previously, but not that of the *dcp2-7 ski3*Δ mutant (Figure 2, compare A and B). In contrast, overexpression of Edc2p suppressed the growth defect of the *dcp2-7 ski3*Δ mutant but not that of the *dcp1-2 ski8*Δ mutant (Figure 2, compare A and B). However, a screen for genes that would suppress

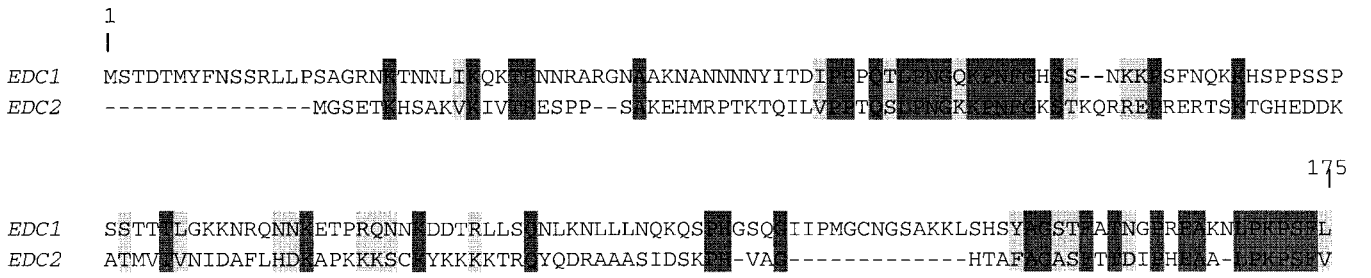


FIGURE 3.—Edc1p and Edc2p are related proteins. Shown is an alignment of Edc1p and Edc2p. Darker shaded boxes indicate identity and lighter shaded boxes indicate similarity. The two proteins are 26% identical and 42% similar to each other. The alignment was generated using the ClustalW alignment program with the default parameters. Accession numbers for the sequences are: *EDC1*, CAA96938.1; *EDC2*, AAB64570.1.

the *dcp1-2* mutation if highly overexpressed from the *GAL* promoter yielded the *EDC2* gene as a suppressor (data not shown). This suggests that high levels of Edc2p can suppress the *dcp1-2* mutation. Combined, these results suggest that, although Edc1p and Edc2p are homologous proteins, they might have subtly different functions with respect to their interactions with the decapping machinery (see below).

Edc1p and Edc2p overexpression suppresses mRNA decapping defects: In principle, the restoration of growth to the conditional *dcp ski*Δ strains could be accompanied by a partial restoration of either 5′ to 3′ mRNA decay or 3′ to 5′ mRNA decay. To determine whether *EDC1* and *EDC2* overexpression restored mRNA decay through either degradation pathway, we made use of a galactose-inducible *MFA2* mRNA that contains a poly(G) insertion in its 3′ UTR, termed *MFA2*pG (DECKER and PARKER 1993). The poly(G) tract forms a very stable RNA secondary structure that blocks both 5′ to 3′ and 3′ to 5′ RNA degrading exonucleases, thereby trapping intermediates in mRNA decay (VREKEN and RAUE 1992; DECKER and PARKER 1993; ANDERSON and PARKER 1998). Moreover, the specific degradation intermediates that are trapped can be used to infer the directionality of mRNA decay (for discussion see HE and PARKER 1999). Therefore, we introduced the *EDC1* gene on a high-copy-number plasmid into either *dcp1-2* or *ski8*Δ strains that also contained the *MFA2*pG-inducible reporter mRNA and determined the mRNA decay phenotype of the resulting strains. It should be noted that we examined the effects of Edc1p or Edc2p in strains deficient in only 5′ to 3′ decay or 3′ to 5′ decay to simplify the analysis. Similarly, the *EDC2* gene on a high-copy-number plasmid was introduced into either *dcp2-7* or *ski3*Δ strains with the *MFA2*pG plasmid, and the mRNA decay phenotypes of these strains were also determined (see MATERIALS AND METHODS).

As shown in Figure 4A, overexpression of Edc1p partially rescued the mRNA decay defect of the *dcp1-2* mutation at the restrictive temperature. This was observed in the *dcp1-2* mutant that was overexpressing Edc1p as an increased production of 5′ to 3′ mRNA degradation product relative to the *dcp1-2* mutant alone. In contrast, *EDC1* overexpression does not restore mRNA decay to a *dcp1*Δ mutant, indicating that *EDC1* overexpression is not a bypass suppressor of *dcp1* mutations (Figure 4A).

Overexpression of *EDC2* in the *dcp2-7* mutant at restrictive temperature revealed that *EDC2* partially restores decapping in the presence of the *dcp2-7* mutation (Figure 4B). Overexpression of *EDC2* does not bypass the *dcp2-7* mutation as there is no effect of *EDC2* overexpression on the decapping defect seen in the *dcp2*Δ mutant (Figure 4B). Finally, neither *EDC1* nor *EDC2* overexpression had a detectable effect on the 3′ to 5′ decay defect of the respective *ski*Δ mutation (data not shown), suggesting that neither *EDC1* nor *EDC2* overexpression significantly affects 3′ to 5′ mRNA decay. These results suggest that overexpression of *EDC1*p suppresses *dcp1-2* whereas overexpression of Edc2p is more specific for suppression of *dcp2-7*. However, there is some partial redundancy regarding the roles of Edc1p and Edc2p in decapping (see below).

Edc1p and Edc2p are not rate limiting for mRNA decay: The suppression of conditional decapping defects by Edc1p and Edc2p overexpression suggests that these proteins are involved in mRNA decapping. To determine the role of Edc1p and Edc2p in mRNA decay, we first analyzed the mRNA decay phenotypes of strains that are deleted for these two homologous genes (see MATERIALS AND METHODS). The *edc1*Δ, *edc2*Δ, and *edc1*Δ *edc2*Δ double deletion strains do not result in growth defects at any temperature tested from 18° to 37°. Furthermore, analysis of mRNA degradation in these strains revealed no defect in the decay of either the unstable *MFA2*pG reporter mRNA or the stable *PGK1*pG mRNA (Figure 5A and data not shown). This suggests that in otherwise wild-type cells Edc1p and Edc2p are not rate limiting for mRNA decapping on these substrates.

The *edc1*Δ and *edc2*Δ slow mRNA decapping in strains compromised for decapping activity: The observation that Edc1p and Edc2p overexpression can enhance decapping, in combination with the observation that the *edc1*Δ *edc2*Δ double mutant showed normal mRNA turnover, suggested that these proteins may be involved in

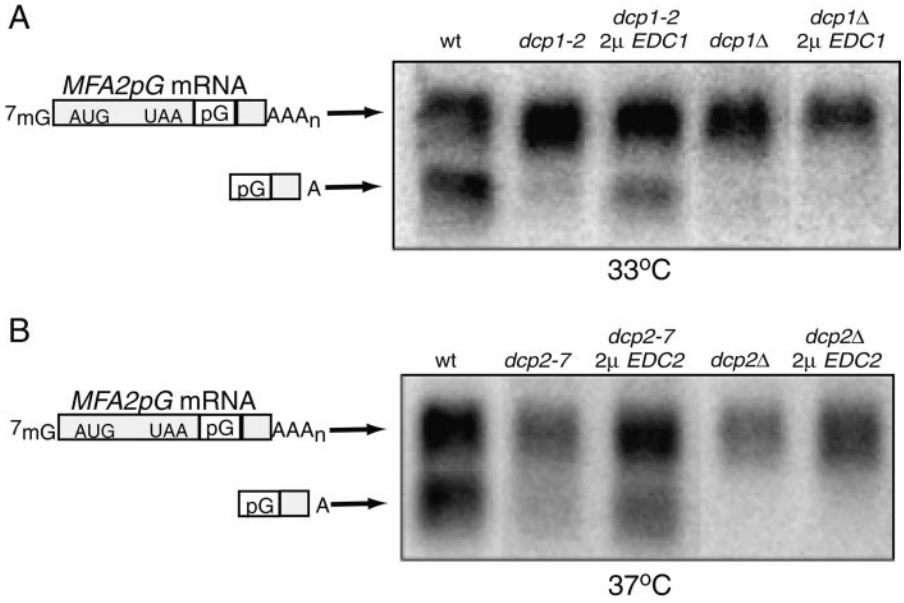


FIGURE 4.—Overexpression of *EDC1* and *EDC2* genes suppresses mRNA decapping defects. The distribution of full-length *MFA2*pG relative to 5′ to 3′ degradation product is shown. The amount of smaller 5′ to 3′ degradation product is a measure of decapping activity in the strains shown. The experiment was performed as described in MATERIALS AND METHODS. Hybridizations were performed with an oligonucleotide specific for the *MFA2*pG mRNA (oRP140, CAPONIGRO and PARKER 1996).

aspects of mRNA decapping that are not normally rate limiting. One possibility is that the *EDC1* and *EDC2* proteins are members of a larger protein complex involved in mRNA decapping (see below). In this case loss of the individual Edc proteins may not significantly affect the assembly or function of the larger complex. This type of situation, where individual members of a larger complex of proteins are dispensable for the normal function of the assembled complex, has been observed previously. For example, certain individual members of the pre-mRNA splicing machinery are dispensable for the splicing of pre-mRNA under otherwise wild-type conditions (RUTZ and SERAPHIN 2000). However, under conditions where splicing is inefficient, as is the case for specific mutations in the consensus splice site sequences (JACQUIER *et al.* 1985), loss of these individual proteins can be seen to be required for efficient splicing of the intron and, hence, for the efficient function of the splicing machinery. This illustrates a useful principle for identifying important functions of individual members of large complex assemblies.

Given this logic, we examined the effects of the *edc1*Δ and *edc2*Δ mutations in strains that were partially compromised for mRNA decapping. Both the conditional *dcp1-2* and *dcp2-7* alleles are very slightly defective for mRNA decapping *in vivo* at the permissive temperature (THARUN and PARKER 1999; T. DUNCKLEY and R. PARKER, unpublished results). Therefore, a reasonable expectation is that a mutation that further compromises the decapping rate will be evidenced more clearly in these strain backgrounds than in an otherwise wild-type cell. Therefore, we introduced the *edc1*Δ and *edc2*Δ into either the *dcp1-2* or *dcp2-7* mutant and assayed the mRNA decay defects of the resulting strains at 24°, which corresponds to the permissive temperature for both *dcp1-2* and *dcp2-7* alleles in an otherwise wild-type background.

An analysis of the mRNA decay phenotypes of the *edc1*Δ and *edc2*Δ in the *dcp1-2* background revealed four important observations. First, the *edc1*Δ in the *dcp1-2* background slowed the decay rate of the *MFA2*pG mRNA greater than twofold (Figure 5B). Similar results

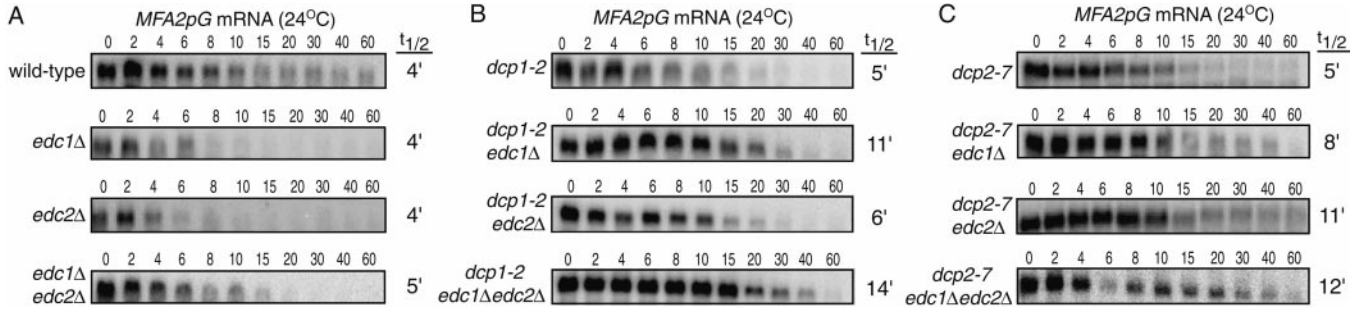


FIGURE 5.—*EDC1* and *EDC2* are required for efficient mRNA decapping. The decay of the *MFA2*pG mRNA in the indicated strains is shown. Strains were grown at 24° to midlog phase in YEP medium containing 2% galactose. Cells were harvested and resuspended in YEP medium. Glucose was added to a final concentration of 4% to repress transcription. Time points represent minutes after transcriptional repression. Hybridizations were performed as for Figure 4.

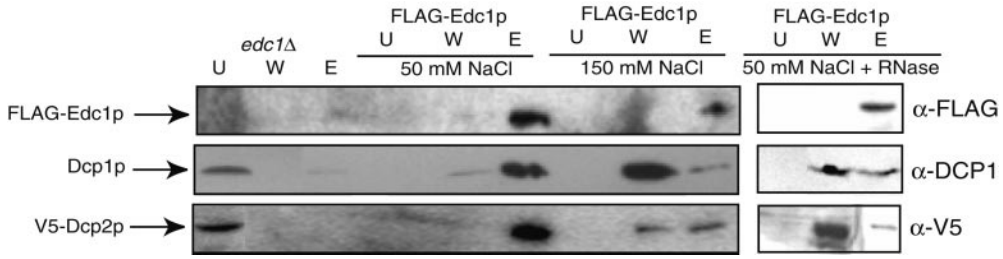


FIGURE 6.—Edc1p interacts with Dcp1p and Dcp2p. Shown is an α-FLAG immunoprecipitation from an *edc1*Δ strain (yRP1512 containing the V5-*DCP2* plasmid, pRP987) and from a FLAG-Edc1p-expressing strain (yRP1512 containing pRP987 and the FLAG-*EDC1* plasmid, pRP986). The experiment was performed as described in MATERIALS AND METHODS. For the last panel, RNase treatment was performed on the cell extract prior to binding to the α-FLAG matrix. Specific protein bands are indicated on the left and the antibodies used to detect them are indicated on the right of the Western blots. U, unbound fraction; W, wash fraction at the indicated salt concentrations; E, eluate.

were seen with the stable *PGK1*pG mRNA (data not shown). This result indicates that, when Dcp1p is partially defective, Edc1p functions to stimulate the mRNA decapping rate. Second, the *edc2*Δ did not significantly affect the decay of the *MFA2*pG mRNA in the *dcp1-2* mutant. This result is consistent with the suppression data described above where Edc1p on a 2μ plasmid would suppress the conditional lethality of the *dcp1-2ski8*Δ strain, but not the conditional lethality of the *dcp2-7 ski3*Δ strain (Figure 2). A third important observation was that, in the *dcp1-2* background, the combined *edc1*Δ *edc2*Δ double deletion slowed the decay rate of the *MFA2*pG mRNA greater than did the *edc1*Δ mutant alone. In fact, loss of both Edc1p and Edc2p in the *dcp1-2* mutant results in mRNA half-lives comparable to those observed in a *dcp1*Δ (BEELMAN *et al.* 1996), suggesting that mRNA decapping is essentially completely eliminated in this strain. Consistent with a specific defect in decapping, none of these strains exhibited altered deadenylation rates compared to the wild-type strain (data not shown). Last, a comparison of the *MFA2*pG half-life in the *dcp1-2 edc1*Δ *edc2*Δ strains (14′) to that in the *dcp1-2 edc1*Δ (11′) strain also suggests that, if Edc1p is absent, Edc2p is able to partially substitute for Edc1p (Figure 5B). Consistent with this interpretation, the Edc2p can partially suppress both the growth defect of *dcp1-2 ski2*Δ mutants and the mRNA decay defect of the *dcp1-2* mutation if very highly overexpressed from the *GAL* promoter (data not shown). Combined, the above results suggest distinct but partially redundant functions for Edc1p and Edc2p in the stimulation of decapping.

Analyses of the mRNA decay phenotypes of the *edc1*Δ and *edc2*Δ in the *dcp2-7* background also yielded several interesting observations. First, the *dcp2-7 edc2*Δ mutant showed a longer half-life for both the *MFA2*pG (11′, Figure 5C) and *PGK1*pG mRNA (data not shown) than did the *dcp2-7 edc1*Δ (*MFA2*pG $t_{1/2}$ = 8′). This result was in contrast to the case with the *dcp1-2* allele where the *edc1*Δ had the larger effect (Figure 5B). The *edc1*Δ*edc2*Δ double deletion in combination with *dcp2-7* had an increase in half-life similar to the value obtained from the *dcp2-7 edc2*Δ strain. These results are consistent with the view that the *dcp2-7* allele is more sensitive to the function of the Edc2p than to the function of the Edc1p. These results indicated that, while not normally rate limiting under conditions where mRNA decapping is maximally efficient on the *MFA2* and *PGK1* mRNAs, the *EDC1* and *EDC2* proteins can function as enhancers of the mRNA decapping machinery (see DISCUSSION).

Edc1p associates with the mRNA decapping machinery *in vivo*: The proteins encoded by the *EDC1* and *EDC2* genes could affect mRNA decapping in multiple ways. One simple interpretation of the above genetic data is that these proteins interact with Dcp1p and Dcp2p and form a larger protein complex that carries out mRNA decapping. To test this possibility, we utilized co-immunoprecipitation experiments to determine if the Edc1p interacts with either Dcp1p or Dcp2p *in vivo*. For this experiment, we constructed a version of Edc1p with a flag epitope at its N terminus and confirmed that this tagged version of the protein was functional (data not shown). We were unable to construct a functional epitope-tagged version of Edc2p so we have restricted our analysis to the Edc1p to date.

Results of the co-immunoprecipitation demonstrated that both Dcp1p and a functional epitope-tagged version of Dcp2p associate with Edc1p (Figure 6). In this experiment we purified the Flag-Edc1p over a flag affinity column. On the basis of gel analysis and silver staining of the various fractions, we determined that the vast majority of the cellular protein is in the unbound and first wash fractions, with only a small percentage of the total protein present in the eluted fraction (data not shown). Interestingly, essentially all of the Dcp1p and Dcp2p in the cell interacted with Edc1p under low-salt conditions. The interactions between Edc1p and Dcp1p or Dcp2p were reduced, but not eliminated, by washing the immunopellet with 150 mM NaCl prior to elution of the FLAG-Edc1p (Figure 6). These results argue that Edc1p physically interacts with Dcp1p and Dcp2p.

Because the observed interaction was reduced under relatively low-salt conditions, we hypothesized that the interaction may involve RNA. To test this possibility, we

performed the immunoprecipitation experiment using cell extracts that were pretreated with RNase prior to binding to the α-FLAG immunoaffinity matrix. The RNase treatment reduced, but did not eliminate, the observed interaction. Following treatment with RNase, there was no detectable Dcp1p or Dcp2p in the unbound fraction, indicating that the bulk of cellular Dcp1p and Dcp2p remain associated with FLAG-Edc1p in the absence of RNA (Figure 6). Interestingly, after RNase treatment, the interaction between Edc1p, Dcp1p, and Dcp2p became more labile. A large amount of the Dcp1p and most of the V5-Dcp2p fusion protein no longer interact with Edc1p at 50 mM NaCl instead of at 150 mM NaCl, as was seen in the presence of RNA. This result suggests that the observed interaction between Edc1p and Dcp1p/Dcp2p was not RNA dependent, but rather was strengthened by the presence of RNA. The identity of the RNA involved is currently unknown, but the mRNA itself represents a reasonable possibility. These results also imply that Edc1p, and likely Edc2p as well, may be members of a larger protein complex that assembles either prior to or during decapping (see DISCUSSION).

DISCUSSION

Dcp2p functions to activate Dcp1p: Two important observations indicate that Dcp2p's critical function in mRNA decapping is to activate the Dcp1p decapping enzyme. First, previous findings show that Dcp1p is inactive for mRNA decapping *in vivo* and *in vitro* if produced in a *dcp2*Δ strain (DUNCKLEY and PARKER 1999). Second, Dcp1p is a functional mRNA decapping enzyme if produced in the presence of Dcp2p and subsequently separated from Dcp2p (Figure 1). These results suggest that, once produced, Dcp1p must undergo a Dcp2p-dependent activation event that makes the protein competent for mRNA decapping. The specific mechanism of Dcp1p activation is unknown. However, the Dcp2 protein contains a functional MutT motif that is required for its mRNA decapping functions (DUNCKLEY and PARKER 1999). The MutT motif is found in a large family of pyrophosphatases (for review of MutT proteins see KOONIN 1993; BESSMAN *et al.* 1996). This suggests that Dcp2p likely functions in some manner as a pyrophosphatase to activate Dcp1p.

Dcp2p associates with active and inactive Dcp1p with differing affinity: Dcp2p associates with a large fraction of Dcp1p that is not removed in a 2.5 M salt wash, suggesting a very tight interaction between these two proteins (Figure 1, A and B). Furthermore, this fraction of Dcp1p is catalytically inactive *in vitro* (Figure 1C). These observations suggest two possible interpretations. In one model, Dcp2p associates with Dcp1p, inhibiting its activity. Hydrolysis of Dcp2p's substrate, mediated by the protein's MutT motif, would release Dcp1p, thereby allowing the Dcp1p enzyme to function. This makes the prediction that Dcp1p will be functional for decapping provided that the protein is not bound by Dcp2p. The fact that Dcp1p in the dcp2Δ mutant is completely inactive *in vivo* and *in vitro* (DUNCKLEY and PARKER 1999) makes this model unlikely. A more likely possibility is that cleavage of Dcp2p's substrate results in either a specific covalent or noncovalent modification of Dcp1p that converts inactive Dcp1p to active Dcp1p. This model could also explain why the Dcp1p that remains bound to Dcp2p is inactive (Figure 1). The simplest explanation is that Dcp2p associates tightly with an inactive form of Dcp1p and that cleavage of Dcp2p's substrate converts the inactive Dcp1p to active Dcp1p, which may have a decreased affinity for Dcp2p. As an extension of this model, it is possible that Dcp2p may function to inactivate Dcp1p through essentially the reverse of the activation reaction. Following cleavage of the cap structure, this would provide a simple mechanism for inactivating Dcp1p and readying the protein for another round of decapping.

Edc1p and Edc2p stimulate the mRNA decapping machinery: Several observations show that Edc1p and Edc2p enhance the function of the mRNA decapping machinery. First, *EDC1* and *EDC2* overexpression suppresses loss-of-function mutations in the Dcp1p decapping enzyme and Dcp2p, respectively (Figures 2, A and B, and 4, A and B). Second, the *edc1*Δ and *edc2*Δ deletions greatly exaggerate the partial mRNA decapping defects seen at the permissive temperature of conditional *dcp1* and *dcp2* mutants (Figure 5, B and C). Third, Edc1p co-immunoprecipitated from cell extracts with the Dcp1p and Dcp2p (Figure 6). To date, we have been unable to construct a functional epitope-tagged version of the Edc2p, but the prediction is that this protein will also associate with the Dcp1p and Dcp2p. Taken together, we interpret these observations to argue that Edc1p and Edc2p interact with the Dcp1p/Dcp2p decapping machinery to stimulate the mRNA decapping rate.

An alternative formal possibility is that Edc1p and Edc2p function to stabilize aberrant proteins much like chaperones and that their interaction with the decapping proteins is revealing a broader role in protein metabolism. However, two observations make this possibility unlikely. First, Edc1p interacts with wild-type Dcp1p. Second, high levels of Edc2 (from the Gal promoter) can increase decapping in wild-type strains as assessed by the ratio of full-length mRNA to the 5′ to 3′ decay product. These observations demonstrate that the Edc proteins can interact with wild-type Dcp1p and Dcp2p and therefore their function is not likely to be limited to stabilizing mutant proteins.

One of the intriguing properties of Edc1p and Edc2p is their apparent specificity for Dcp1p and Dcp2p, respectively. For example, the *EDC1* gene is a high-copy suppressor of *dcp1-2* but not *dcp2-7*, whereas the *EDC2* gene is a high-copy suppressor of *dcp2-7* but not *dcp1-2*

(Figure 2). Similarly, the *edc1*Δ reveals a stronger decapping defect when combined with the *dcp1-2* allele than when combined with the *dcp2-7* allele, whereas the *edc2*Δ reveals a decapping defect when combined with the *dcp2-7* allele but not with the *dcp1-2* allele (Figure 5). One possibility is that the specificity of Edc1p for Dcp1p, and of Edc2p for Dcp2p, results from the Edc proteins best stabilizing the decapping protein they interact with directly. Alternatively, the specificity could result from the *dcp1-2* and *dcp2-7* lesions affecting distinct substeps in the overall process of decapping. This possibility is based on the finding that mRNA decapping requires multiple steps and interactions between multiple proteins (Dunckley and Parker 1999; Bouveret *et al.* 2000; Schwartz and Parker 2000; Tharun *et al.* 2000). The *dcp1-2* mutation affects the catalytic activity of the enzyme (Tharun and Parker 1999). The specific step in decapping that is affected in the *dcp2-7* lesion is unknown, but it is likely to be prior to Dcp1p cleavage. In this instance, Edc1p and Edc2p would show differential effects because each protein would stimulate a different substep of decapping. Different substeps that could be affected would include the assembly of a decapping complex on the mRNA, or potentially the activation of Dcp1p by Dcp2p (see below).

How might Edc1p and Edc2p function in decapping? On the basis of our current understanding of the mechanism of mRNA decapping, Edc1p and Edc2p could function to enhance decapping in one, or more, of three possible manners. First, these proteins could function to promote the disassembly of the cytoplasmic cap binding complex. This possibility is based on the observations that the cytoplasmic cap binding complex is an inhibitor of decapping and needs to dissociate before decapping can occur (Schwartz and Parker 1999, 2000). Currently, there is no evidence to favor this mechanism of function for the Edc proteins. Two more likely models are that the Edc proteins either facilitate the assembly of a decapping complex or function to enhance the Dcp2p-dependent activation of Dcp1p.

Evidence that the EDC proteins are part of a larger assembly of proteins that performs the decapping reaction comes from the observation that Edc1p co-immunoprecipitates with Dcp1p and Dcp2p (Figure 6). Additional evidence for the assembly of a large protein complex to promote decapping is based on the analyses of other proteins functioning in decapping. In addition to Edc1p and Edc2p, decapping of mRNA is now known to involve the function of a set of interacting proteins, including Dcp1p, Dcp2p, Pat1p/Mrt1p, and the cytoplasmic *L*ike-*SM* complex of proteins consisting of Lsm1p-Lsm7p (Beelman *et al.* 1996; Hatfield *et al.* 1996; Boeck *et al.* 1998; Dunckley and Parker 1999; Bouveret *et al.* 2000; Tharun *et al.* 2000). Four observations suggest that these proteins form two distinct complexes that associate together with the mRNA before decapping. First, Dcp1p and Dcp2p copurify (Dunckley and Parker 1999; Figure 1). Second, the Lsm complex, consisting of Lsm1p-Lsm7p, copurifies with Pat1/Mrt1p and with the Xrn1 exoribonuclease (Bouveret *et al.* 2000). Third, both Dcp1p and Lsm proteins can be shown to co-immunoprecipitate mRNA (Tharun *et al.* 2000). Finally, Dcp1p co-immunoprecipitates with the Lsm complex, but in an RNase-sensitive manner. Our data that Edc1p co-immunoprecipitated with the majority of the Dcp1p and Dcp2p in the cell suggest that Edc1p and Edc2p may be part of this larger assembly.

The interpretation that Edc1p and Edc2p are part of a larger assembly of proteins promoting decapping provides an explanation for their genetic phenotypes. Prior observations have shown that some individual members of large complexes are dispensable for the function of the complex under optimal conditions. However, the function of these individual components can be revealed when the process in which the complex functions is inefficient. The interpretation that Edc1p and Edc2p are modulatory components of a larger decapping complex is consistent with this precedent. Additional evidence that decapping involves a number of redundant protein interactions has come from a mutational analysis of the Dcp1p where several mutations have been identified that individually do not affect decapping but, when combined, give rise to a strong defect in decapping (Tharun and Parker 1999). Since these mutations do not affect the enzymatic function of purified Dcp1p, this suggests that the Dcp1p makes multiple redundant physical interactions that promote decapping. An interesting possibility for future work is to determine if these dcp1 point mutations disrupt the interaction of the Dcp1p with Edc1p or, potentially, Edc2p.

An important goal will be to determine the timing and location of the interaction between Edc1p and Dcp1p/Dcp2p, as well as other proteins involved in decapping. We have shown that the interaction between Edc1p and Dcp1p/Dcp2p is largely eliminated at low-salt concentrations following RNase treatment (Figure 6). This suggests that the presence of RNA stabilizes the interaction between Edc1p and the decapping machinery. Although the identity of the RNA is unknown, a likely possibility is the mRNA itself. The Edc1p and likely Edc2p, because they interact with Dcp1p and Dcp2p, would be in a complex known to associate in some manner with the mRNA. Because Edc1p interacts with most Dcp1p and Dcp2p in the cell, Edc1p would likely interact with Dcp1p and Dcp2p both prior to and after their recruitment to the mRNA. Edc1p and Edc2p may function either to promote the assembly of the decapping complex on the mRNA or, once on the mRNA, Edc1p and Edc2p may stimulate a transition that results in the Dcp2p-mediated activation of Dcp1p. Using the mRNA to stimulate the activation of Dcp1p would provide an elegant mechanism for the localization of decap-

ping activity to mRNAs that have been targeted for degradation.

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