Inhibition of mRNA Turnover in Yeast by an *xrn1* Mutation Enhances the Requirement for eIF4E Binding to eIF4G and for Proper Capping of Transcripts by Ceg1p

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ABSTRACT

Null mutants of *XRN1*, encoding the major cytoplasmic exoribonuclease in yeast, are viable but accumulate decapped, deadenylated transcripts. A screen for mutations synthetic lethal with *xrn1*Δ identified a mutation in *CDC33*, encoding eIF4E. This mutation (glutamate to glycine at position 72) affected a highly conserved residue involved in interaction with eIF4G. Synthetic lethality between *xrn1* and *cdc33* was not relieved by high-copy expression of eIF4G or by disruption of the yeast eIF4E binding protein Caf20p. High-copy expression of a mutant eIF4G defective for eIF4E binding resulted in a dominant negative phenotype in an *xrn1* mutant, indicating the importance of this interaction in an *xrn1* mutant. Another allele of *CDC33*, *cdc33-1*, along with mutations in *CEG1*, encoding the nuclear guanylyltransferase, were also synthetic lethal with *xrn1*Δ, whereas mutations in *PRT1*, encoding a subunit of eIF3, were not. Mutations in *CDC33*, *CEG1*, *PRT1*, *PAB1*, and *TIF4631*, encoding eIF4G1, have been shown to lead to destabilization of mRNAs. Although such destabilization in *cdc33*, *ceg1*, and *pab1* mutants can be partially suppressed by an *xrn1* mutation, we observed synthetic lethality between *xrn1* and either *cdc33* or *ceg1* and no suppression of the inviability of a *pab1* null mutation by *xrn1*Δ. Thus, the inhibition of mRNA turnover by blocking Xrn1p function does not suppress the lethality of defects upstream in the turnover pathway but it does enhance the requirement for ^{7}mG caps and for proper formation of the eIF4E/eIF4G cap recognition complex.

TRANSLATION initiation, mRNA degradation, and the relationship between the two are the subject of much research (reviewed in Beelman and Parker 1995; Jacobson and Peltz 1996; Pain 1996). These processes are regulated by *trans*-acting factors and *cis*-acting elements of the mRNAs including the polyadenylate [poly(A)] tail and the 5′-7-methylguanosine (^{7}mG) cap. The synergistic stimulation of translation by ^{7}mG cap and poly(A) is mediated by the interaction of eIF4F with cap and poly(A) binding protein (Pab1p) with poly(A) (reviewed in Pain 1996; Sachs *et al.* 1997; Gallie 1998; McCarthy 1998). In yeast, the cap-binding complex (eIF4F) consists of eIF4E, the cap-binding protein that is required for recruitment of the translation initiation complex to the 5′-end of the mRNA (Altmann and Trachsel 1989; Lang *et al.* 1994; Vasilescu *et al.* 1996; Tarun and Sachs 1997), and eIF4G, which binds multiple factors including eIF4E and Pab1p (Tarun and Sachs 1996; reviewed in Morley *et al.* 1997). Pab1p is a multifunctional RNA binding protein that is necessary for poly(A)-dependent stimulation of translation (reviewed in Tarun and Sachs 1995; Gallie 1998). eIF4E, encoded by *CDC33*, Pab1p, encoded by *PAB1*, and either of the two different forms of eIF4G, encoded by *TIF4631* and *TIF4632* (Goyer *et al.* 1993), are all essential for viability.

Some translation factors also participate in mRNA degradation. For example, Pab1p has been reported to prevent degradation of mRNAs (Caponigro and Parker 1995; Coller *et al.* 1998). Transcript degradation is thought to be initiated by deadenylation (Vreken and Raue 1992; Decker and Parker 1993; Muhlrad *et al.* 1994) resulting in reduced Pab1p binding. The association of Pab1p with eIF4F suggested the model that reduced Pab1p binding to deadenylated mRNAs diminished eIF4E binding to the 5′-cap, allowing access to the cap for decapping enzyme Dcp1p (LaGrandeur and Parker 1998). However, the stabilizing function of Pab1p appears to be independent of eIF4G binding, suggesting an alternative and yet unknown mechanism of mRNA stabilization (Coller *et al.* 1998). Subsequent to decapping, the body of the transcript is degraded by the 5′-exoribonuclease Xrn1p (Stevens and Maupin 1987; Hsu and Stevens 1993; Beelman *et al.* 1996). The suppression of the lethality of a *PAB1* disruption by a *dcp1* partial loss-of-function mutation (Hatfield *et al.* 1996) suggests that transcript stabilization is a primary function of Pab1p. In addition to *PAB1*, mutations

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in *CDC33*, *TIF4631*, *PRT1*, encoding a subunit of eIF3, and the eIF4A gene *TIF1* have been shown to moderately destabilize certain mRNAs (Linz *et al.* 1997; Schwartz and Parker 1999). Furthermore this destabilization depends on the 5′-degradation pathway since it can be partially suppressed by mutations in *XRN1* (Schwartz and Parker 1999).

In a previous screen for mutations that are synthetic lethal with *xrn1*Δ, mutations in *SKI2* and *SKI3* were identified (Johnson and Kolodner 1995). *ski8* mutations are also synthetic lethal with *xrn1* (Jacobs Anderson and Parker 1998; J. T. Brown and A. W. Johnson, unpublished results). Since *SKI2*, *SKI3*, and *SKI8* are all required for normal 3′–5′ exonucleolytic mRNA degradation (Jacobs Anderson and Parker 1998), synthetic lethality with *xrn1*Δ is most easily explained as the result of completely blocking mRNA turnover by inhibiting two different degradation pathways. We now report that a separate class of synthetic lethal mutations affects cap-specific processes but does not act by blocking transcript degradation.

MATERIALS AND METHODS

Strains, media, and plasmids: The yeast strains used are described in Table 1. Construction of new strains is described below. Standard media including synthetic complete medium (SC) were described previously (Rose *et al.* 1990). Low Ade medium contained 6 mg/L adenine. Yeast transformations were performed as described elsewhere (Gietz and Schiestl 1995). Plasmids are listed in Table 2.

Isolation of *cdc33E72G*: To identify mutations synthetic lethal with *xrn1*Δ, UV mutagenesis was performed on yeast strain

TABLE 1

Yeast strains

Strain	Genotype or description	Source or reference
AJY201	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 cdc33E72G*	This study
AJY202	*MAT***a** *ade2 ade3 leu2 ura3-52*	This study
AJY208	*MAT*α *ade2 ade3 leu2 trp1*Δ*63 ura3-52 xrn1*Δ	This study
AJY210	*MAT*α *ade2 ade3 leu2 lys2-801 ura3-52 xrn1*Δ	This study
AJY215	AJY816 except *caf20::LEU2* / pRDK297	This study
AJY219	*MAT***a** *ade2 ade3 leu2 lys2-801 trp1*Δ*63 ura3-52 xrn1*Δ *cdc33E72G* / pRDK297	This study
AJY234	RKY1997 except *cdc33E72G*	
AJY517	*MAT*α *ade2 ade3 leu2 lys2-801 ura3-52*	This study
AJY559	*MAT***a** *his3*Δ*200 leu2 (lys2-801?) ura3 xrn1*Δ	This study
AJY816	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 xrn1*Δ *cdc33E72G* / pRDK297	This study
AJY837	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52*	This study
AJY838	*MAT*α *ade2 ade3 leu2 lys2-801 ura3-52*	This study
AJY840	*MAT*α *ade2 ade3 leu2 trp1*Δ*63 ura3-52*	This study
AJY846	*MAT*α *ade2 ade3 leu2 lys2-801 ura3-52 cdc33E72G*	This study
AJY847	*MAT***a** *ade2 ade3 leu2 trp1*Δ*63 ura3-52 cdc33E72G*	This study
AJY848	*MAT*α *ade2 ade3 leu2 ura3-52*	This study
AJY891	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 ceg1-34*	This study
AJY892	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 ceg1-63*	This study
CH1305	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52*	Kranz and Holm (1990)
FY23	*MAT***a** *ura3-52 leu2*Δ*1 trp1*Δ*63*	Amberg *et al.* (1992)
RKY1976	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 xrn1::URA3*	Johnson and Kolodner (1995)
RKY1977	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 xrn1*Δ	Johnson and Kolodner (1995)
RKY1978	*MAT*α *ade2 ade3 leu2 his3 ura3-52 xrn1::URA3*	Johnson and Kolodner (1995)
RKY1979	*MAT*α *ade2 ade3 leu2 his3 ura3-52 xrn1*Δ	Johnson and Kolodner (1995)
RKY1997	*MAT***a** *leu2 ura3 trp1* L-A-o	A. W. Johnson and R. D. Kolodner (unpublished results)
RKY1999	*MAT*α *leu2 ura3 xrn1::URA3* L-A-o	A. W. Johnson and R. D. Kolodner (unpublished results)
RKY2062	RKY1977 / pRDK297	Johnson and Kolodner (1995)
TP11B-2-2	*MAT*α *ade1 leu2-3,112 ura3-52 prt1-1*	C. A. Barnes
TC3-212-3	*MAT*α *leu2-3,112 ura3-52 prt1-63*	C. A. Barnes
YAS1668	*MAT*α *ade2 his3 trp1 leu2 ura3 can1-100 pab1::HIS3* / p*PAB-URA3-CEN*	A. B. Sachs
YAS1888	*MAT***a** *ade2-1 his3-11,15 leu2-3,111 trp1-1 ura3-1 pep4::HIS3 cdc33-1*	Tarun and Sachs (1997)

RKY2062 as described (Johnson and Kolodner 1995). The gene for one arbitrary mutation (AJY816) was cloned by complementation from a *LEU2* centromeric yeast library (ATCC no. 77162, P. Hieter). Sequence analysis [Institute for Cell and Molecular Biology Core Facility (ICMB CF), University of Texas at Austin] of a complementing clone (pAJ166) revealed that the genomic insert contained *CDC33*. A *Hin*dIII fragment containing *CDC33* as the only intact open reading frame subcloned into YEp352 (yielding pAJ173) complemented the synthetic lethality of AJY816. The *CDC33*-containing *Hin*dIII fragment from pAJ166 was also subcloned into a centromeric plasmid to give pAJ174. The genomic *CDC33* locus from AJY816 was amplified by PCR and sequenced (ICMB CF, University of Texas at Austin).

Gap rescue and integration of *cdc33E72G*: pAJ173 was digested with *Spe*I and religated to create a collapsed plasmid (pAJ178) lacking the *CDC33* open reading frame. This plasmid was then linearized with *Spe*I and transformed into AJY816 to gap rescue the *cdc33E72G* allele onto the plasmid (yielding pAJ182). A *cdc33E72G*-containing *Hin*dIII fragment was moved from pAJ182 to *Hin*dIII-digested pRS406, a *URA3*-containing plasmid that lacks yeast replication sequences (Sikorski and Hieter 1989). The resultant plasmid (pAJ185) was linearized with *Avr*II and transformed into RKY1997. Ura^+ transformants were streaked to YPD to allow homologous recombination to occur between the integrated *cdc33E72G* and *CDC33*, thus losing the *URA3* gene and one copy of *CDC33*. Isolates were then patched to 5-fluoroorotic acid (5-FOA) plates to select for Ura^- recombinants. To identify *cdc33E72G* integrants, 5-FOA-resistant isolates were scored for temperature sensitivity that could be complemented by a centromeric *CDC33* plasmid (pAJ174). One such isolate (AJY234) was used for further study.

Integration of *ceg1* alleles: The plasmids pBR306-*ceg1-34* and pBR306-*ceg1-63* (S. Buratowski) were linearized within the *CEG1* gene with *Bam*HI and transformed into CH1305. Southern blotting identified correct integrants. 5-FOA-resistant, temperature-sensitive integrants were then identified (AJY891 and AJY892, respectively).

Matings of yeast strains: Novel *xrn1*Δ and wild-type yeast strains used in this report were constructed as follows. The mating type of yeast strain CH1305 was switched to *MAT*α by the method of Herskowitz and Jensen (1991). The resultant strain (AJY517) was mated with FY23 and sporulated to give the haploid strains AJY837, AJY838, and AJY840. AJY840 was mated with RKY1977 and sporulated to give the *xrn1*Δ strains AJY208 and 210.

Tetrad dissection of RKY1979 mated to AJY816 demonstrated cosegregation of synthetic lethality and temperature sensitivity by the 2:2 segregation of white:red^{ts}. Tetrad dissection of RKY1999 mated to AJY234 demonstrated synthetic lethality between *xrn1::URA3* and *cdc33E72G* in an L-A virus-deficient, nonmutagenized background by the absence of Ura^+ temperature-sensitive spore clones at room temperature. Tetrad dissection of RKY1978 mated to YAS1888 demonstrated the synthetic lethality between *xrn1::URA3* and *cdc33-1* by the absence of Ura^+ temperature-sensitive spore clones. The original *xrn1*Δ *cdc33E72G* synthetic lethal strain (AJY816) was backcrossed twice either (i) to wild-type strains AJY840 and then AJY838 to make *cdc33E72G* strains AJY846 and AJY847 and *CDC33* strain AJY848 or (ii) to wild-type strains AJY840 and then AJY837 to make *cdc33E72G* strain AJY201 and *CDC33* strain AJY202. AJY219, the *cdc33E72G xrn1*Δ double mutant containing pRDK297, was obtained from tetrad dissection of AJY847 mated with AJY210 carrying pRDK297. The *cdc33E72G* allele was scored by temperature sensitivity and the *xrn1*Δ allele was scored by PCR. Tetrad dissection of RKY1976 mated with TP11B-2-2 and TC3-212-3 demonstrated the lack of synthetic lethality between *xrn1::URA3* and both *prt1-1* and *prt1-63*, respectively, by the presence of Ura^+ temperature-sensitive spore clones. Tetrad dissection of RKY1978 mated with AJY891 and AJY892 demonstrated the synthetic lethality between *xrn1::URA3* and both *ceg1-34* and *ceg1-63*, respectively, by the absence of Ura^+ temperature-sensitive spore clones at 30°. We did not observe synthetic lethality between *cdc33E72G* and either *ceg1-34* or *ceg1-63* after sporulation of diploids made by crossing AJY846 with AJY891 and AJY892, respectively. For the *xrn1*Δ *pab1::HIS3* cross, AJY559 (*xrn1*Δ) was mated with

TABLE 2

Plasmids

Plasmid designation	Description	Source or reference
pAJ166	*CEN LEU2 CDC33* library clone	This study
pAJ167	*CEN URA3 CAF20*	This study
pAJ173	2 μm *URA3 CDC33*	This study
pAJ174	*CEN LEU2 CDC33*	This study
pAJ176	*CEN URA3 caf20::LEU2*	This study
pAJ178	2 μm *URA3 cdc33*Δ collapse	This study
pAJ182	2 μm *URA3 cdc33E72G*	This study
pAJ185	*URA3 cdc33E72G*	This study
pAS548	2 μm *TRP1 TIF4631*	Tarun and Sachs (1997)
p*GAL-MAT*α*1*	2 μm *URA3 MAT*α*1*	Hennigan and Jacobson (1996)
p*PAB-URA3-CEN*	*CEN URA3 PAB*	A. B. Sachs
pRDK297	2 μm *URA3 ADE3 XRN1*	Johnson and Kolodner (1995)
pRS306-*ceg1-34*	*URA3 ceg1-34*	Fresco and Buratowski (1996)
pRS306-*ceg1-63*	*URA3 ceg1-63*	Fresco and Buratowski (1996)
pRS406	*URA3*	Sikorski and Hieter (1989)
pRS416	*CEN URA3*	Sikorski and Hieter (1989)
pRS424	2 μm *TRP1*	Sikorski and Hieter (1989)
p*tif4631-459*	2 μm *TRP1 tif4631-459*	Tarun and Sachs (1997)
YEp13	2 μm *LEU2*	Myers *et al.* (1986)
YEp352	2 μm *URA3*	Myers *et al.* (1986)

YAS1668 (*pab1::HIS3* carrying *PAB1* on a *URA3 CEN* vector). The resulting diploid was sporulated and tetrads were dissected. The *pab1::HIS3* allele was scored by histidine prototrophy and the *xrn1*Δ allele was scored by PCR. All *pab1::HIS3* and *xrn1*Δ *pab1::HIS3* isolates were sensitive to 5-FOA.

***CAF20* disruption:** The *CAF20* locus was amplified by PCR and ligated as a *Spe*I-*Eco*RI fragment into pRS416 (yielding pAJ167). A *LEU2*-containing *Bst*YI fragment of YEp13 was ligated into *Bgl*II-*Bcl*I-linearized pAJ167, deleting the *CAF20* open reading frame from nucleotide 7 to 168 (yielding pAJ176). The *caf20::LEU2*-containing *Xba*I-*Hin*dIII fragment of pAJ176 was transformed into AJY816. A Leu^+ transformant (AJY215) was confirmed as a disruption integrant by PCR.

Biochemical techniques: 7-methyl-GTP (^{7}mGTP) column chromatography was performed essentially as previously described (Tarun and Sachs 1996). Briefly, 2 liters of RKY2062 and AJY816 were grown to a density of 1.5×10^7 cells/ml in SC-Ura. The cells were washed with water and then resuspended in 12 ml buffer A (100 mm potassium acetate, 2 mm magnesium acetate, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin A, 0.5 mm PMSF, 7 mm β-mercaptoethanol, 30 mm HEPES pH 7.4) in a 50-ml tube. Glass beads (48-g) were added. The tubes were then placed in a multivortexer and vortexed seven times for 1 min each with 1-min periods on ice in between. The samples were centrifuged twice at 30,000 × *g* for 5 min each. The extracts (~10 ml) were then loaded onto separate 0.5-ml ^{7}mGTP Sepharose 4B columns (Pharmacia, Piscataway, NJ) preequilibrated in buffer A. The columns were washed with 15 ml buffer B [100 mm potassium chloride, 0.2 mm EDTA, 0.01% Triton X-100, 0.5 μg/ml leupeptin, 0.7 μg/ml pepstatin A, 0.5 mm phenylmethylsulfonyl fluoride (PMSF), 7 mm β-mercaptoethanol, 20 mm HEPES pH 7.4] and then with 10 ml buffer B + 0.1 mm GDP. Proteins were eluted with buffer B + 0.1 mm ^{7}mGTP.

Extracts for analysis of eIF4G degradation were prepared as follows. Actively growing cultures of AJY201 and AJY202 at 26° were split equally and placed at either 26° or 37° and grown for an additional 2 hr at which time the cells were harvested and broken with glass beads and vortexing in the presence of a buffer consisting of 20 mm Tris (pH 7.5), 150 mm NaCl, 0.5 mm EDTA, and one Complete Mini, EDTA-free protease inhibitor cocktail tablet (Boehringer-Mannheim, Indianapolis) per 7 ml of buffer. Anti-eIF4G1 antiserum was a generous gift of Alan Sachs. Western blot analysis was carried out as previously described (Johnson 1997).

Northern blot analysis: For transcriptional pulse chase experiments, 40-ml cultures of strains carrying p*GAL-MATα1* were grown to mid-log in SC-Ura liquid medium. Cultures were washed and concentrated to 15 ml and then induced for 20 min with a 2% final concentration of galactose. Aliquots (1.9-ml) were taken before and at various times after addition of glucose (final concentration of 2%) and flash frozen in a dry ice ethanol bath. For transcriptional inhibition experiments, 50-ml cultures were grown to mid-log in YPD liquid medium and then concentrated to 10 ml. Aliquots (1.9-ml) were taken before and at various times after addition of thiolutin to 10 μg/ml and flash frozen in a dry ice ethanol bath. RNA was prepared, fractionated, blotted, probed, and imaged as previously described (Johnson 1997).

RESULTS

Synthetic lethality between *xrn1*Δ and *cdc33*: A screen for mutations synthetic lethal with *xrn1*Δ (Johnson and Kolodner 1995) was repeated and identified five additional complementation groups. The gene for one arbitrary mutation from this screen was cloned by complementation. The mutant was found to contain a temperature-sensitive mutation in *CDC33*, which encodes translation initiation factor eIF4E (Figure 1). The temperature sensitivity and synthetic lethality cosegregated after multiple backcrosses. Amplification by PCR and subsequent sequencing of the mutant genomic *cdc33* locus identified the mutation as an A to G transition at nucleotide 215 resulting in a change of glutamate to glycine at amino acid 72. To confirm that the synthetic lethality was not strain-specific or due to the presence of the double-stranded RNA virus L-A, the *cdc33* mutation was introduced into an L-A virus-deficient strain of a different genetic background. The *cdc33E72G* mutation again conferred temperature sensitivity. This strain was mated with an L-A-deficient *xrn1::URA3* strain and the resulting diploid was sporulated. No viable temperature-sensitive Ura^+ spores were recovered (see materials and methods). Thus, synthetic lethality was independent of both strain background and of the L-A virus, whose capsid protein is known to decap mRNAs (Blanc *et al.* 1994; Masison *et al.* 1995). The *cdc33E72G* mutation was a recessive partial loss-of-function mutation and synthetic lethality could be overcome by high-copy expression of the *cdc33E72G* mutant allele (data not shown). To determine if other *CDC33* mutations were also synthetic lethal with *xrn1*, a strain bearing a *cdc33-1* (Altmann and Trachsel 1989) allele was crossed to an *xrn1::URA3* strain. No temperature-sensitive Ura^+ spore clones were recovered, indicating synthetic lethality between *cdc33-1* and *xrn1::URA3* (data not shown).

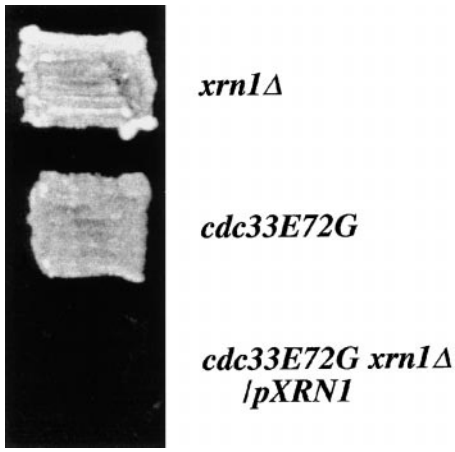

Figure 1.—Synthetic lethality between *xrn1* and *cdc33*. Strains RKY1977 (*xrn1*Δ), AJY201 (*cdc33E72G*), and AJY219 (*xrn1*Δ *cdc33E72G*/p*XRN1-URA3*) were patched onto plates containing 5-FOA and incubated at 30° for 3 days. The same results were observed at room temperature (data not shown).

***cdc33E72G* disrupts the eIF4E/eIF4G interaction:** The interaction of eIF4G with eIF4E is important for the stimulation of cap-dependent translation initiation (Mader *et al.* 1995; Tarun and Sachs 1997; Gallie 1998). Glutamate-72, which is altered in the *cdc33E72G*

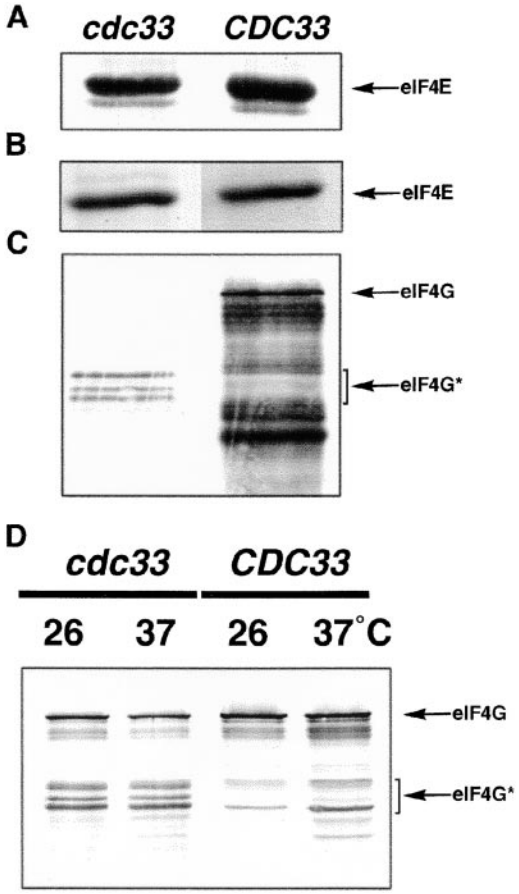

Figure 2.—Binding of eIF4G and cap analog by mutant eIF4E and novel degradation patterns for eIF4G in an eIF4E mutant. (A) Similar amounts of column input extracts from synthetic lethal strain AJY816 (*xrn1*Δ *cdc33E72G*/p*XRN1*) or parent strain RKY2062 (*xrn1*Δ *CDC33*/p*XRN1*) were analyzed by colorimetric Western blot using antibody raised against eIF4E. Equal amounts of protein eluted off of ^{7}mGTP Sepharose 4B columns were separated on 12% SDS-PAGE gels and visualized either (B) by Coomassie staining or (C) by colorimetric Western blot using antibody raised against eIF4G1. The identity of the band in B as eIF4E was confirmed by Western blot (data not shown). In D, equal amounts of protein from crude extracts from either AJY201 (*cdc33E72G*) or AJY202 (*CDC33*) grown at either 26° or 37° (2-hr shift) were separated on 12% SDS-PAGE gels and visualized by colorimetric Western blot using antibody raised against eIF4G1. The Western blot appeared unchanged when performed with cells that were grown at these temperatures for an additional 5 hr (data not shown).

mutant, is a highly conserved residue. Crystal structures of eIF4E (Marcotrigiano *et al.* 1997; Matsuo *et al.* 1997) show that this residue lies on the surface of eIF4E that is involved in interaction with eIF4G, suggesting that this mutation would affect eIF4G binding but not cap binding *per se.* These expectations were borne out experimentally. Protein extracts prepared from the wild-type and *cdc33E72G* strains had similar levels of eIF4E as measured by Western blotting (Figure 2A and data not shown). Equivalent amounts of each extract were applied to ^{7}mGTP Sepharose 4B columns (Pharmacia) and after extensive washing the bound proteins were eluted with free ^{7}mGTP. Although similar amounts of wild-type and mutant eIF4E were retained on these columns (Figure 2B), there was a striking reduction in the amount of eIF4G retained by the mutant eIF4E on the ^{7}mG column (Figure 2C). Since wild-type and mutant extracts contained similar levels of eIF4G (Figure 2D), the reduction in eIF4G retained on the column by mutant eIF4E was due to poor binding with mutant eIF4E. Thus the mutant eIF4E is severely impaired for eIF4G binding. The slight reduction in the amount of mutant eIF4E retained on the column compared to wild type may reflect the reduced *in vitro* binding of eIF4E to ^{7}mG in the absence of eIF4G (Ptushkina *et al.* 1998). Our results differ from those recently reported in which eIF4E mutations at glutamate-72 (E72A and E72D) were shown to have only a modest effect on the *in vitro* binding of the eIF4E binding domain of eIF4G to eIF4E at 4° (Ptushkina *et al.* 1998). This difference may arise from the use of different alleles or from the fact that the work of Ptushkina *et al.* was carried out with purified recombinant eIF4E protein and a recombinant eIF4G protein fragment *in vitro* in contrast to the work presented here using yeast extracts.

Interestingly, eIF4G degradation products were observed in the mutant eIF4E strain that were not apparent or were present at much lower levels in the wild-type strain (Figure 2, C and D). This enhanced proteolysis of eIF4G cosegregated with the *cdc33E72G* mutation but was not temperature dependent (Figure 2D) and thus did not appear to be the cause for the temperature sensitivity of *cdc33E72G*. eIF4G is highly susceptible to degradation *in vitro* and to proteolysis by various viral proteases *in vivo*. eIF4G proteolysis has also been noted in the absence of strong eIF4E/eIF4G interaction (Berset *et al.* 1998). To test if a lowered level of eIF4G due to proteolysis in the cdc33E72G mutant strain was responsible for synthetic lethality with *xrn1*Δ, we asked if high-copy eIF4G could suppress this lethality in a plasmid shuffle assay. A high-copy plasmid containing *TIF4631* encoding eIF4G1 (pAS548) was transformed into AJY219 (*xrn1*Δ *cdc33E72G*/p*XRN1-URA3*). Elevated levels of eIF4G1 protein were confirmed by Western blotting (data not shown). Transformants were scored for the ability to sector and for growth on 5-FOA. No complementation was observed (data not shown). Additionally, high-copy eIF4G1 was unable to reduce the temperature sensitivity of a *cdc33E72G* mutant (data not shown). Similarly, high-copy *PAB1*, which binds eIF4G, did not rescue the *xrn1*Δ *cdc33E72G* mutant, nor did it reduce the temperature sensitivity of a *cdc33E72G* single mutant (data not shown). Thus, synthetic lethality between *xrn1*Δ and *cdc33E72G* results from the disruption of eIF4E interaction with eIF4G and not simply from the loss of eIF4G due to heightened degradation.

We tested the idea that binding of eIF4E to eIF4G

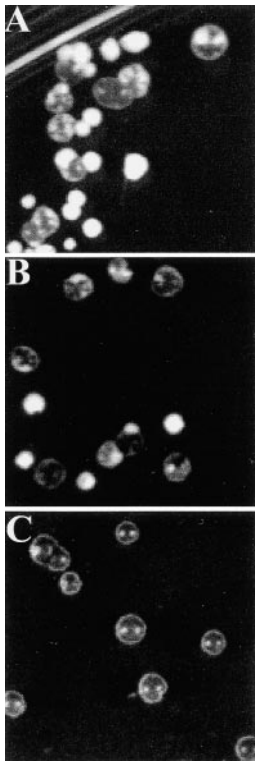

Figure 3.—Dominant negative phenotype of an eIF4E-binding mutant eIF4G in an *xrn1* mutant. Cotransformants of AJY208 (*xrn1*Δ) carrying pRDK297 (p*XRN1-URA3-ADE3*) and either (A) pRS424 (empty vector), (B) pAS548 (p*TIF4631*), or (C) p*tif4631-459* were streaked onto Trp^- low Ade plates and incubated at 30° for 5 days.

was critical in an *xrn1* mutant by asking if overexpression of an eIF4G mutant defective for eIF4E binding (*tif4631-459*; Tarun and Sachs 1997; Tarun *et al.* 1997) would confer a dominant negative phenotype in an *xrn1* mutant. High-copy vectors bearing mutant eIF4G1, wild-type eIF4G1, or empty vector were transformed into an *xrn1* mutant bearing an *XRN1-ADE3* plasmid. Transformants were scored for the ability to lose the *XRN1-ADE3* plasmid, indicated by sectoring on low Ade plates. Indeed, high-copy mutant eIF4G1 prevented the loss of the *XRN1-ADE3* plasmid, indicated by the solid red colonies in the mutant eIF4G1 transformant (Figure 3).

***CAF20* does not mediate synthetic lethality between *xrn1*Δ and *cdc33E72G*:** 4E-binding proteins (4E-BPs) in higher eukaryotes and Caf20p in yeast are negative regulators of eIF4E function that bind eIF4E competitively with eIF4G (Haghighat *et al.* 1995; Altmann *et al.* 1997; Ptushkina *et al.* 1998). They can be phosphorylated by mitogen-activated protein kinase via the FRAP/TOR signaling pathway in mammalian cells and by casein kinase II via the TOR pathway in yeast (Lin *et al.* 1994; Zanchin and McCarthy 1995; Barbet *et al.* 1996; Beretta *et al.* 1996). Such phosphorylation causes dissociation from eIF4E presumably by electrostatic repulsion. A cocrystal structure of mouse eIF4E bound to a functional homologue of Caf20p (mammalian 4E-BP2) identified residue E70 of mouse eIF4E (analogous to E72 of yeast eIF4E) as involved in 4E-BP binding (Matsuo *et al.* 1997). It could be argued that in the *cdc33E72G* mutant, the wild-type electrostatic repulsion between a negatively charged phosphate group and a negatively charged glutamate would be disrupted, allowing Caf20p to remain associated more tightly to eIF4E. It is also possible that normal interaction of Caf20p with eIF4E could more efficiently compete with weakened eIF4E/eIF4G interaction, thus blocking efficient translation initiation of capped transcripts. To examine whether the synthetic lethality was due to enhanced competition by eIF4E binding protein, *CAF20* was disrupted in the synthetic lethal strain (*xrn1*Δ *cdc33E72G*/p*XRN1-URA3*). This strain is unable to grow on 5-FOA plates because it requires *XRN1* on a *URA3*-containing plasmid for viability. Deletion of *CAF20* in this strain did not allow growth on 5-FOA. Thus, enhanced competition of binding by Caf20p is not responsible for synthetic lethality between *xrn1* and *cdc33E72G*.

The *cdc33E72G* mutation causes a modest mRNA destabilization effect: Although the mutant eIF4E binds ^{7}mG *in vitro*, disruption of its interaction with eIF4G may lead to reduced cap binding or translation initiation *in vivo* (Haghighat and Sonenberg 1997; Ptushkina *et al.* 1998). Such reduced cap binding by eIF4E could result in increased access of the decapping enzyme (Dcp1p) to the cap, leading to a general destabilization of mRNAs by exposing these transcripts to the processive 5′–3′ decay pathway (Sonenberg *et al.* 1979; LaGrandeur and Parker 1998). Indeed, different alleles of *cdc33* have recently been shown to have varying modest effects on mRNA stability (Linz *et al.* 1997; Schwartz and Parker 1999). The stability of *MAT*α*1* mRNA was examined in *cdc33E72G* and *CDC33* strains at permissive and nonpermissive temperatures using a transcriptional pulse chase analysis (Figure 4A). No change in stability was seen in the *cdc33E72G* mutant. The stabilities of *CYH2*, pre*CYH2*, and *PAB1* mRNAs were also examined at permissive temperature after inhibition of transcription with thiolutin. PreCYH2 is targeted for rapid degradation in the cytoplasm by the nonsense-mediated decay pathway (Leeds *et al.* 1992; He *et al.* 1993; Ruiz-Echevarria *et al.* 1996; Zhang and Maquat 1997; Hentze and Kulozik 1999; reviewed in Culbertson 1999). A modest destabilization was observed for *CYH2* mRNA ($t_{1/2}$ = 23 min in wild type and 16 min in the mutant) and for pre*CYH2* mRNA ($t_{1/2}$ = 10 min in wild type and 5 min in the mutant; Figure 4B) and no significant change in stability of *PAB1* mRNA was observed (data not shown). Thus, the *cdc33E72G* mutation leads to destabilization of some RNAs.

Reduced nuclear capping of mRNA is synthetic lethal with *xrn1*Δ: The eIF4E/eIF4G complex binds to the ^{7}mG cap *in vivo* to promote cap-dependent translation. *CEG1* is an essential gene that encodes the nuclear guanylyl-

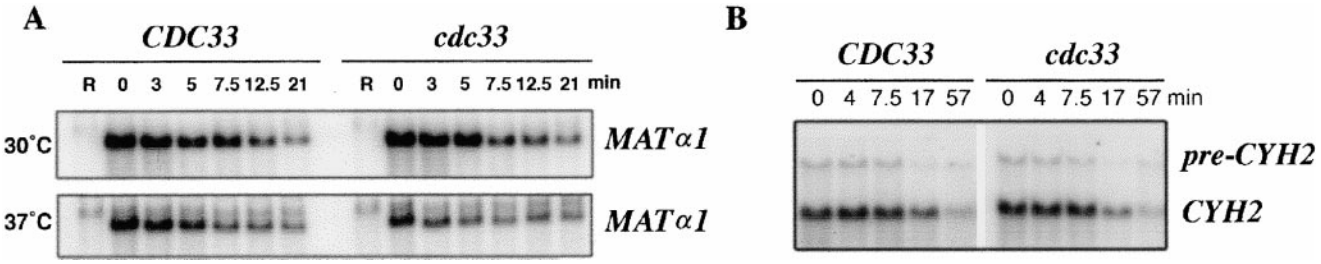

Figure 4.—Stability of *MATα1* and *CYH2* transcripts in an eIF4E mutant. (A) Cultures of AJY202 (*CDC33*) and AJY201 (*cdc33E72G*) carrying p*GAL-MATα1* were transiently induced with galactose to produce *MATα1* transcript. Temperature shift was for 2 hr. Northern blots were probed with a radiolabeled probe derived from random priming an *Eco*RV to *Hin*dIII digest of *MATα1*. "R" indicates cells growing in raffinose, before the addition of galactose for *GAL* promoter induction. The zero time point indicates cells growing in galactose, before the addition of glucose for repression of the *GAL* promoter. Other times indicate minutes after addition of glucose. (B) A thiolutin time course was performed on cultures of AJY848 (*CDC33*) and AJY846 (*cdc33E72G*) at 30°. Northern blots were probed with a radiolabeled probe derived from random priming a *Bam*HI to *Eco*RI digest of *CYH2*. The zero time point indicates cells growing in the absence of thiolutin. Other times indicate minutes after addition of thiolutin.

transferase that is responsible for capping RNA polymerase II transcripts in yeast (Fresco and Buratowski 1996). Mutants of *ceg1* generate unstable transcripts that can be stabilized by deleting *XRN1* (Schwer *et al.* 1998). In experiments to test the suppression of temperature-sensitive *ceg1* mutations by *xrn1*Δ, we found that *ceg1* mutations were synthetic lethal with *xrn1*Δ at 30°, a temperature at which the *ceg1* single mutants grew well (Figure 5). Thus even though deletion of *XRN1* can suppress the transcript instability of *ceg1* mutants, stabilization of the uncapped mRNAs resulting from the *ceg1* mutation is lethal. In separate experiments to test the suppression of temperature-sensitive *ceg1* mutations by temperature-sensitive mutations in *RAT1*, encoding the nuclear counterpart of Xrn1p (Kenna *et al.* 1993; Poole and Stevens 1995; Johnson 1997), no genetic interaction was observed (data not shown).

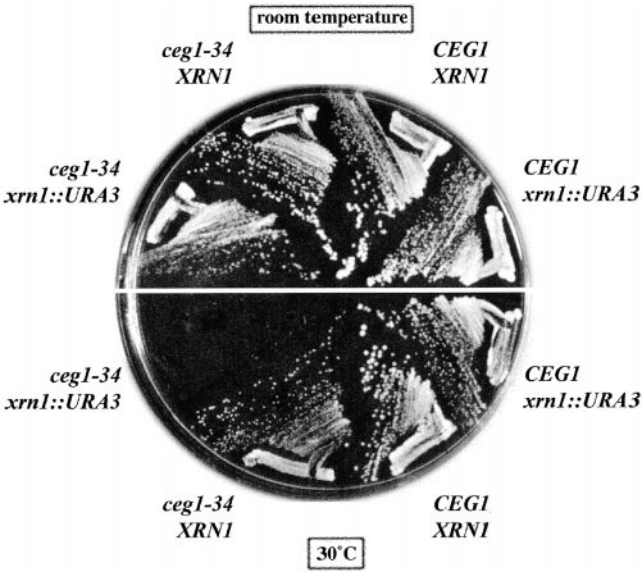

Figure 5.—Synthetic lethality between *xrn1* and *ceg1*. A diploid strain (*XRN1/xrn1::URA3 ceg1-34/CEG1*) was sporulated and dissected. Spore clones shown were grown on YPD at either room temperature for 3 days or at 30° for 2 days. The same results were obtained with the *ceg1-63* allele (data not shown).

Synthetic lethality with *xrn1*Δ is specific to cap-related processes: Because of the importance of ^{7}mG cap in translation initiation, mutations in the cap-binding complex (eIF4F) or in the capping enzyme (Ceg1p) reduce the overall translational efficiency in a cell (Altmann and Trachsel 1989; Tarun *et al.* 1997; Schwer *et al.* 1998; Schwartz and Parker 1999). Thus it seemed possible that reduced levels of translation in general, and not a cap-specific defect, were responsible for the lethality observed in *xrn1*Δ mutants. We tested this idea by asking if temperature-sensitive alleles of *PRT1* are synthetic lethal with *xrn1*Δ. *PRT1* encodes an essential subunit of the eIF3 complex that is involved in bringing the eIF2-GTP-Met-tRNA$_f$ ternary complex to the 40S ribosomal subunit (Feinberg *et al.* 1982; Hanic-Joyce *et al.* 1987b; Naranda *et al.* 1994; Chaudhuri *et al.* 1997). In addition, a *prt1-63* mutant shows destabilization of mRNAs (Schwartz and Parker 1999). Double mutants of *xrn1::URA3* and either *prt1-1* or *prt1-63* were constructed by mating and dissecting the appropriate strains. The presence of temperature-sensitive Ura^{+} spore clones that were viable at all temperatures at which the single *prt1* mutants were viable indicated no enhanced temperature sensitivity (Figure 6). Since the *prt1* single mutants display significantly lower translation rates as temperature increases (Hanic-Joyce *et al.* 1987a), the lack of enhanced temperature sensitivity indicated that reduced translation in general was not responsible for lethality between *xrn1*Δ and *cdc33*. Hence the genetic interaction is restricted to a subset of translation initiation factors. Additionally, even though lesions in *cdc33*, *ceg1*, and *prt1* accelerate the decay of some mRNAs, the genetic interaction with *xrn1* is only

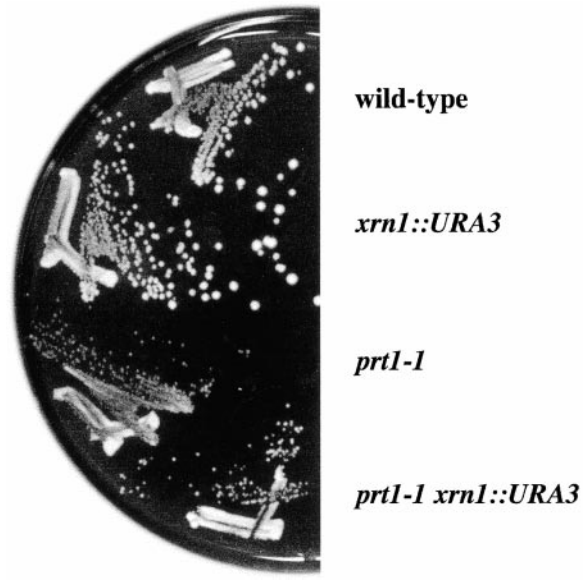

Figure 6.—The lack of synthetic lethality between *xrn1* and *prt1*. A diploid strain (*XRN1*/*xrn1::URA3 prt1-1*/*PRT1*) was sporulated and dissected. Spore clones shown were grown on YPD at 32° for 3 days. The same results were obtained with the *prt1-63* allele (data not shown).

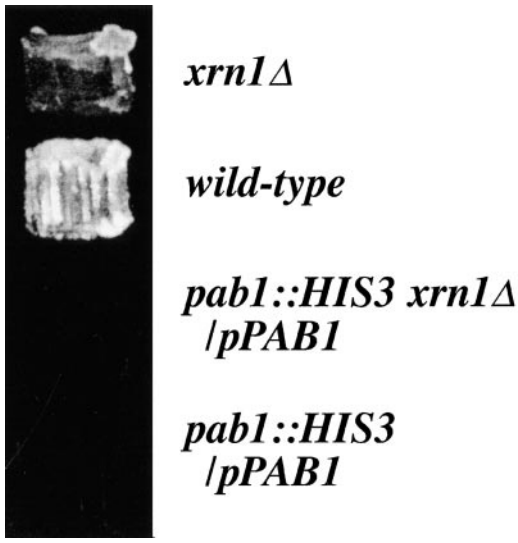

Figure 7.—The lack of suppression of *pab1* by *xrn1*. Diploid strain (*XRN1*/*xrn1*Δ *pab1::HIS3*/*PAB1*) was sporulated and dissected. Spore clones shown were grown on 5-FOA at room temperature for 4 days.

observed if distinctly cap-specific processes are perturbed.

Disruption of *PAB1* is not suppressed by *xrn1*Δ: Mutations in *CDC33*, *CEG1*, *PRT1*, and *PAB1* destabilize mRNAs (Caponigro and Parker 1995; Fresco and Buratowski 1996; Linz *et al.* 1997; Barnes 1998; Schwer *et al.* 1998; Schwartz and Parker 1999). This destabilization of RNA can be suppressed by mutations in *XRN1* that block the degradation of decapped transcripts (Caponigro and Parker 1995; Hatfield *et al.* 1996; Schwer *et al.* 1998; Schwartz and Parker 1999). Pab1p is a multifunctional protein required for efficient translation of poly(A) mRNA as well as stabilization of mRNAs (Caponigro and Parker 1995; Coller *et al.* 1998). Inactivation of Pab1p results in accelerated mRNA decapping and degradation. It has been suggested that stabilization of mRNAs in a *pab1* mutant by inactivation of downstream degradation steps can rescue the inviability of a *PAB1* deletion, indicating that stabilization of mRNA is an essential function of Pab1p (Caponigro and Parker 1995; Hatfield *et al.* 1996). Since Pab1p interacts with the eIF4G/eIF4E complex, we expected mutations in these genes to show similar genetic interactions with *xrn1*Δ. Because we observed synthetic lethality between *xrn1* and either *cdc33* or *ceg1* mutations (rather than suppression of inviability), we decided to reinvestigate the genetic interaction of *pab1*Δ with *xrn1*Δ. We found that an *xrn1* deletion did not rescue the inviability of a *PAB1* deletion mutant. In this experiment, an *xrn1*Δ mutant was crossed to a *pab1::HIS3* mutant that also contained *PAB1* on a *URA3*-containing centromeric plasmid. The diploid was sporulated and tetrads were dissected. All *pab1::HIS3 xrn1*Δ spore clones were 5-FOA-sensitive, indicating inviability in the absence of the *URA3*-plasmid-borne *PAB1* (Figure 7). A similar lack of suppression of *pab1*Δ by *xrn1*Δ has been observed by others (Morrissey *et al.* 1999). Thus, as with *cdc33* and *ceg1* mutants, stabilization of uncapped mRNAs by disruption of *xrn1* is not sufficient to suppress the inviability of a *PAB1* disruption.

DISCUSSION

Genetic interactions between *XRN1* and cap-related processes: In this report we have shown that a class of mutations affecting capping of mRNAs or the proper formation of the cap-binding complex eIF4F genetically interacts with *xrn1*. This class includes mutations in *CDC33*, *CEG1*, and *TIF4631*. *CEG1* encodes the nuclear guanylyltransferase responsible for adding the ^{7}mG cap to RNA polymerase II transcripts and *CDC33* and *TIF4631* encode the translation initiation factors eIF4E and eIF4G, respectively, which together form eIF4F and bind the ^{7}mG cap. The *cdc33E72G* mutation that we found from a synthetic lethal screen with *xrn1*Δ disrupts eIF4E/eIF4G interaction and renders cells temperature sensitive. This is consistent with the prior observation that mutations in the eIF4E binding site of eIF4G result in temperature sensitivity (Tarun *et al.* 1997). The *cdc33-1* mutant protein, which is unable to bind cap analog (Altmann and Trachsel 1989) and has reduced eIF4G binding (Tarun and Sachs 1997), was synthetic lethal with *xrn1*Δ as well. Synthetic lethality between *cdc33* and *xrn1* was also observed in a strain deficient for L-A virus, ruling out the possibility that increased binding of L-A

Gag protein to cap (Blanc *et al.* 1994; Masison *et al.* 1995) was responsible for lethality when eIF4E binding was reduced. Supporting the idea that deletion of *XRN1* enhances the requirement for eIF4E / eIF4G interaction, high-copy expression of a *tif4631* mutation that inhibits eIF4E binding gave a dominant negative phenotype in an *xrn1* mutant.

In addition to the genetic interaction with *cdc33* and *tif4631*, we found that *ceg1* mutations were synthetic lethal with *xrn1*. Ceg1p is a nuclear enzyme required for capping mRNAs. Since Xrn1p is a cytoplasmic protein, synthetic lethality with *ceg1* suggests that the *ceg1* defect giving rise to synthetic lethality is manifest in the cytoplasm. This was further supported by a lack of genetic interaction between *ceg1* alleles and a mutation in the nuclear exoribonuclease encoded by *RAT1* (A. W. Johnson, unpublished results). Previously it was reported that *ceg1 xrn1*Δ double mutants are viable but grow very slowly (Schwer *et al.* 1998). Our finding of synthetic lethality may be due to the use of different alleles, strains, or temperature.

These genetic interactions appeared to be specific to defects in nuclear capping of transcripts or assembly of the eIF4E / eIF4G cap-binding complex and not a result of reduced overall translational capacity since *prt1* mutations showed no synthetic interaction with *xrn1*Δ. *PRT1* encodes an essential subunit of the translation initiation factor 3 complex (Hanic-Joyce *et al.* 1987b; Naranda *et al.* 1994). eIF3 is required for recruitment of 40S ribosomes and formation of the preinitiation complex (Chaudhuri *et al.* 1997), a step distinct from cap recognition by eIF4F. We have found that several additional mutations that lead to substantially reduced 60S levels, including deletion of *SPB2* or *RAI1*, are also not synthetic lethal with *xrn1*Δ (Ho and Johnson 1999; Y. Xue and A. W. Johnson, unpublished results). Thus lowering the translational capacity of a cell in general is not lethal in an *xrn1* mutant. Because *cdc33*, *tif4631*, and *ceg1* mutants all affect cap-dependent reactions, we suggest that defects in assembling the eIF4F complex on the 5′-cap are lethal in combination with an *xrn1* mutation.

Suppression of RNA instability but not lethality by *xrn1*: Mutations in *CDC33*, *TIF4631*, *PRT1*, *PAB1*, and *CEG1* all lead to destabilization of mRNAs (Caponigro *et al.* 1993; Fresco and Buratowski 1996; Linz *et al.* 1997; Barnes 1998; Schwer *et al.* 1998; Schwartz and Parker 1999) with mutations in *PAB1* giving the most severe phenotype. Except for *CEG1*, mutations in these genes lead to premature decapping followed by degradation of the transcript by Xrn1p. Transcripts in *ceg1* mutants are unstable presumably because they are not protected by a cap structure. Thus, it is not surprising that the RNA stability phenotype of *cdc33*, *ceg1*, and *pab1* mutations can be partially suppressed by deletion of *XRN1* (Caponigro *et al.* 1993; Hatfield *et al.* 1996; Schwer *et al.* 1998; Schwartz and Parker 1999). However, we have shown that regardless of the suppression of the mRNA instability phenotype, *xrn1* mutations in combination with *ceg1* or *cdc33* mutations result in synthetic lethality. In addition, we observed genetic interaction between mutations in *XRN1* and *TIF4631* and no suppression of the inviability of a *pab1* deletion mutant. Indeed, synthetic lethality between *xrn1* and *pab1* mutations has recently been reported (Morrissey *et al.* 1999). These results are contrary to a model in which stabilization of mRNA turnover by deletion of *XRN1* suppresses the inviability of mutations in upstream factors in the turnover pathway (Caponigro and Parker 1995). We conclude that the accumulation of uncapped messages in an *xrn1* mutant is detrimental to a cell when coupled with particular defects in translation initiation.

Why are defects in cap-specific processes synthetic lethal with inhibition of mRNA turnover? Several models could explain the observed synthetic lethality. Deletion of *XRN1* is synthetic lethal with *SKI2* or *SKI3* (Johnson and Kolodner 1995) or *SKI8* (Jacobs Anderson and Parker 1998; J. T. Brown and A. W. Johnson, unpublished results). Ski2p, Ski3p, and Ski8p form a complex *in vivo* (Brown *et al.* 2000) and are required for a 3′–5′ mRNA degradation pathway (Jacobs Anderson and Parker 1998) and repression of translation of deadenylated mRNAs (reviewed in Wickner 1996; Benard *et al.* 1998). Hence, the mechanism for the synthetic lethality between *xrn1* and either *ski2*, *ski3*, or *ski8* has been proposed to be the complete inhibition of RNA decay by mutations in both the highly processive 5′ pathway and alternate 3′ pathway (Jacobs Anderson and Parker 1998). This is not the case for the synthetic lethality between *xrn1* and either *cdc33* or *ceg1* as these mutations lead to the destabilization and not stabilization of mRNAs (Schwer *et al.* 1998). Furthermore, mRNAs in an *xrn1 cdc33-42* double mutant are less stable than in an *xrn1* mutant alone, ruling out enhanced RNA stability as the cause of lethality (Schwartz and Parker 1999).

Inhibition of mRNA turnover in yeast by deletion of *XRN1* leads to a general stabilization of deadenylated decapped transcripts (Hsu and Stevens 1993). Although such degradation intermediates are not normally translated, in *xrn1*Δ cells these RNAs accumulate to high levels and they sediment in sucrose gradients in a position corresponding to polysomes, suggesting that they are translated (Hsu and Stevens 1993; Caponigro and Parker 1995). The translation of uncapped mRNAs is suggested from other work as well (Masison *et al.* 1995; Lo *et al.* 1998). It is possible that the accumulation of high levels of decapped deadenylated mRNAs titrates out RNA binding proteins and / or translation factors. This in turn would lead to reduced rates of translation of newly transcribed transcripts and relaxation of the gene regulation program of the cell. This situation in combination with mutations in factors required for cap-dependent translation may be lethal. However, it is important to note that whereas an *xrn1*

mutation does not suppress the lethality of a *pab1* mutation, a mutation in *DCP1*, encoding the decapping activity required prior to Xrn1p degradation, does suppress the lethality of a *pab1* mutant (Hatfield *et al.* 1996). Since a *dcp1* mutation stabilizes capped but deadenylated mRNAs, this suggests that there is a qualitative difference between stabilizing capped *vs.* decapped transcripts.

An alternate model that explains the genetic observations we have made with an *xrn1* mutation is that the decapped mRNAs that accumulate in *xrn1* mutants are translated aberrantly. Without cap-dependent recruitment of the translation machinery to the 5′-ends of messages and with the accumulation of decapped deadenylated mRNAs, translation may initiate at sites downstream of the normal initiation codon. This would lead to the production of truncated and novel proteins that could be lethal for the cell. General RNA binding proteins suppress cap-independent translation *in vitro*, apparently by masking alternative initiation codons (Svitkin *et al.* 1996). Thus, under *in vitro* conditions in which RNA binding factors are limiting, translation can initiate at internal and downstream sites. Similarly, the accumulation of decapped mRNA in an *xrn1* mutant may titrate cytoplasmic RNA binding proteins. When coupled with defects in recruitment of the ribosome to the 5′-end of an mRNA by eIF4F/cap interaction, this may lead to aberrant internal initiation at downstream AUG codons. The resulting translation products may then be responsible for the observed lethality. Because a *dcp1* mutant accumulates capped transcripts, higher levels of cap-dependent initiation would be maintained, resulting in greater fidelity of initiation. Recently, we have found a mutation in *GCD2* that is synthetic lethal with deletion of *XRN1* (J. T. Brown and A. W. Johnson, unpublished results). *GCD2* is a subunit of the eIF2B complex required for recycling eIF2, an essential translation initiation factor that delivers charged initiator tRNA to the 40S ribosomal subunit. Since mutations in *GCD2* can affect the position of translation reinitiation events (reviewed in Hinnebusch 1997), synthetic lethality between mutations in *XRN1* and *GCD2* supports a model in which aberrant translation initiation is lethal when mRNA turnover is inhibited by a mutation in *XRN1*.

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