Inhibition of mRNA Turnover in Yeast by an \textit{xrn1} Mutation Enhances the Requirement for elf4E Binding to elf4G and for Proper Capping of Transcripts by Ceg1p

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ABSTRACT

Null mutants of \textit{xrn1}, encoding the major cytoplasmic exonuclease in yeast, are viable but accumulate decapped, deadenylated transcripts. A screen for mutations synthetic lethal with \textit{xrn1} identified a mutation in \textit{cdc33}, encoding elf4E. This mutation (glutamate to glycine at position 72) affected a highly conserved residue involved in interaction with elf4G. Synthetic lethality between \textit{xrn1} and \textit{cdc33} was not relieved by high-copy expression of elf4G or by disruption of the yeast elf4E binding protein Caf20p. High-copy expression of a mutant elf4G defective for elf4E binding resulted in a dominant negative phenotype in an \textit{xrn1} mutant, indicating the importance of this interaction in an \textit{xrn1} mutant. Another allele of \textit{cdc33}, \textit{cdc33-1}, along with mutations in \textit{ceg1}, encoding the nuclear guanylyltransferase, were also synthetic lethal with \textit{xrn1}, whereas mutations in PRT1, encoding a subunit of eIF3, were not. Mutations in \textit{cdc33}, \textit{ceg1}, PRT1, \textit{pab1}, and TIF4631, encoding elf4G1, have been shown to lead to destabilization of mRNAs. Although such destabilization in \textit{cdc33}, \textit{ceg1}, and \textit{pab1} mutants can be partially suppressed by an \textit{xrn1} mutation, we observed synthetic lethality between \textit{xrn1} and either \textit{cdc33} or \textit{ceg1} and no suppression of the inviability of a \textit{pab1} null mutation by \textit{xrn1}. 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 \textit{mG} caps and for proper formation of the elf4E/ elf4G 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 Peitl 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 ('mG) cap. The synergistic stimulation of translation by 'mG cap and poly(A) is mediated by the interaction of elf4F 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 (elf4F) consists of elf4E, the cap-binding protein that is required for recruitment of the translation initiation complex to the 5'-end of the mRNA (Altman and Trachsel 1989; Lang et al. 1994; Vasiljevic et al. 1996; Tarun and Sachs 1997), and elf4G, which binds multiple factors including elf4E 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). elf4E, encoded by CDC33, Pab1p, encoded by PAB1, and either of the two different forms of elf4G, 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 elf4F suggested the model that reduced Pab1p binding to deadenylated mRNAs diminished elf4E 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 elf4G 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
in CDC33, TIF4631, PRT1, encoding a subunit of eIF3, and the eIF4A gene TIF1 have been shown to moderately destabilize certain mRNAs (Lin 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 xrn1Δ, mutations in SKL2 and SKI3 were identified (Johnson and Kolodner 1995). skl8 mutations are also synthetic lethal with xrn1 (Jacobs Anderson and Parker 1998; J. T. Brown and A. W. Johnson, unpublished results). Since SKL2, SKI3, and SKI8 are all required for normal 3'-5' exonucleolytic mRNA degradation (Jacobs Anderson and Parker 1998), synthetic lethality with xrn1Δ 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 ade3Δ72E7G:** To identify mutations synthetic lethal with xrn1Δ, UV mutagenesis was performed on yeast strain

### TABLE 1

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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 HindIII fragment containing CDC33 as the only intact open reading frame subcloned into YEp352 (yielding pAJ173) complemented the synthetic lethality of AJY816. The CDC33-containing HindIII fragment was moved from pAJ166 to HindIII-digested pRS406, a URA3-containing plasmid that lacks yeast replication sequences (Sikorski and Hieter 1989). The resultant plasmid (pAJ185) was linearized with Avrl 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.

Gap rescue and integration of cdc33E72G: pAJ173 was digested with SpeI and ligated to create a collapsed plasmid (pAJ178) lacking the CDC33 open reading frame. This plasmid was then linearized with SpeI and transformed into AJY816 to gap rescue the cdc33E72G allele onto the plasmid (yielding pAJ182). A cdc33E72G-containing HindIII fragment was moved from pAJ182 to HindIII-digested pRS406, a URA3-containing plasmid that lacks yeast replication sequences (Sikorski and Hieter 1989). The resultant plasmid (pAJ185) was linearized with Avrl 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 BamHI 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 xrn1Δ and wild-type yeast strains used in this report were constructed as follows. The mating type of yeast strain CH1305 was switched to Matα by the method of Her skowitz 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 xrn1Δ 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. Tetrad dissection of RKY1999 mated to AJY234 demonstrated synthetic lethality between xrn1::URA3 and cdc33E72G in an A. 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 xrn1Δ 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 xrn1Δ 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 xrn1Δ allele was scored by PCR. Tetrad dissection of RKY1976 mated with TP11B-2-2 and C3-2122-3 demonstrated the lack of synthetic lethality between xrn1::URA3 and both prl1-1 and prtl-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°C. 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 xrn1Δ pab1::HIS3 cross, AJY559 (xrn1Δ) was mated with

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<td>pRS306-ceg1-34</td>
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YAS1668 (pabl::HIS3 carrying PAB1 on a URA3 CEN vector). The resulting diploid was sporulated and tetrads were dissected. The pabl::HIS3 allele was scored by histidine prototrophy and the xrn1A allele was scored by PCR. All pabl::HIS3 and xrn1A pabl::HIS3 isolates were sensitive to 5-FOA.

**CAF20 disruption:** The CAF20 locus was amplified by PCR and ligated as a Sp6-EcoRI fragment into pRS416 (yielding pA167). A LEU2-containing BglII fragment of YEp13 was ligated into BglII-linearized pA167, deleting the CAF20 open reading frame from nucleotide 7 to 168 (yielding pA176). The c20::LEU2-containing XbaI-HindIII fragment of pA176 was transformed into AJY816. A Leu+ transformant (AJY225) was confirmed as a disruption integrant by PCR.

**Biochemical techniques:** 7-methyl-GTP (7mGTP) column chromatography was performed essentially as previously described (Johnson and Sachs 1996). Briefly, 2 liters of RKY2062 and AJY816 were grown to a density of 1.5 x 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 x g for 5 min each. The extracts (~10 ml) were then loaded onto separate 0.5-ml 7mGTP 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, 0.03% 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 µm GDP. Proteins were eluted with buffer B + 0.1 mm 7mGTP.

Extractions for analysis of eIF4G degradation were prepared as follows. Actively growing cultures of AJY201 and AJY202 at 26°C were split equally and plated at either 26°C or 37°C 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 pGAL-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 induction 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 thiolulin 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 xrn1A and cdc33:** A screen for mutations synthetic lethal with xrn1A (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 1998) 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).

**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
Genetic Interactions with \( xrn1 \Delta 

35Genetic Interactions with \( xrn1 \Delta 

D \) applied to \( 7mGTP \) Sepharose 4B columns (Pharmacia) and after extensive washing the bound proteins were eluted with free \( 7mGTP \). 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 \( 7mG \) column (Figure 2C). Since wild-type and mutant extracts contained similar levels of eIF4G (Figure 2D), the 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 \( 7mG \) 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 \( 4^\circ \) (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 appeared unchanged when performed with cells that were grown at these temperatures for an additional 5 hr (data not shown).

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 (\( xrn1 \Delta \) dc33E72G/\( pXRN1 \)) or parent strain RKY2062 (\( xrn1 \Delta \) CDC33/\( pXRN1 \)) were analyzed by colorimetric Western blot using antibody raised against eIF4E. Equal amounts of protein eluted off of \( 7mGTP \) 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 (dc33E72G) 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 dc33E72G 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 \( 7mGTP \) Sepharose 4B columns (Pharmacia) and after extensive washing the bound proteins were eluted with free \( 7mGTP \). 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 \( 7mG \) column (Figure 2C). Since wild-type and mutant extracts contained similar levels of eIF4G (Figure 2D), the 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 \( 7mG \) 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 \( 4^\circ \) (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.

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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-AD3 plasmid. Transformants were scored for the ability to lose the XRN1-AD3 plasmid, indicated by sectoring on low Ade plates. Indeed, high-copy mutant eIF4G1 prevented the loss of the XRN1-AD3 plasmid, indicated by the solid red colonies in the mutant eIF4G1 transformant (Figure 3).

**CAF20 does not mediate synthetic lethality between xrn1Δ and cdc33E72G.** eIF4E-binding proteins (eIF4Es) in higher eukaryotes and Caf20p in yeast are negative regulators of eIF4E function that bind eIF4E competitively with eIF4G (Haghhighat et al. 1995; Altman et al. 1997; Putskhina 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; Ber 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 (xrn1Δ cdc33E72G/pXRN1-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 'mG in vitro, disruption of its interaction with eIF4G may lead to reduced cap binding or translation initiation in vivo (Haghhighat and Sonenberg 1997; Putskhina 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 cdc3 have recently been shown to have varying modest effects on mRNA stability (Linz et al. 1997; Schwartz and Parker 1999). The stability of MATα1 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, preCYH2, 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 (t1/2 = 32 min in wild type and 18 min in the mutant) and for preCYH2 mRNA (t1/2 = 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 mRNAs.
Genetic Interactions with \textit{xrn1}\textsubscript{Δ}

**Figure 4.**—Stability of \textit{MATα1} and CYH2 transcripts in an \textit{eIF4E} mutant. (A) Cultures of \textit{AJY202 (CDC33)} and \textit{AJY201 (cdc33E72G)} carrying pGAL-\textit{MATα1} were transiently induced with galactose to produce \textit{MATα1} transcript. Temperature shift was for 2 hr. Northern blots were probed with a radiolabeled probe derived from random priming an EcoRV to HindIII digest of \textit{MATα1}. "R" indicates cells growing in rafinose, 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 \textit{AJY848 (CDC33)} and \textit{AJY846 (cdc33E72G)} at 30°C. Northern blots were probed with a radiolabeled probe derived from random priming a BamHI to EcoRI 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 \textit{ceg1} generate unstable transcripts that can be stabilized by deleting \textit{XRN1} (Schwer et al. 1998). In experiments to test the suppression of temperature-sensitive \textit{ceg1} mutations by \textit{xrn1Δ}, we found that \textit{ceg1} mutations were synthetic lethal with \textit{xrn1Δ} at 30°C, a temperature at which the \textit{ceg1} single mutants grew well (Figure 5). Thus even though deletion of \textit{XRN1} can suppress the transcript instability of \textit{ceg1} mutants, stabilization of the uncapped mRNAs resulting from the \textit{ceg1} mutation is lethal. In separate experiments to test the suppression of temperature-sensitive \textit{ceg1} mutations by temperature-sensitive mutations in \textit{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).

**Synthetic lethality with \textit{xrn1Δ} is specific to cap-related processes:** Because of the importance of 7mG cap in translation initiation, mutations in the cap-binding complex (\textit{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 \textit{xrn1Δ} mutants. We tested this idea by asking if temperature-sensitive alleles of \textit{PRT1} are synthetic lethal with \textit{xrn1Δ}. \textit{PRT1} encodes an essential subunit of the \textit{eIF3} complex that is involved in bringing the \textit{eIF2-GTP-Met-tRNA\textsubscript{f}} ternary complex to the 40S subunit of the eIF3 complex that is involved in bringing the \textit{eIF2-GTP-Met-tRNA\textsubscript{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 \textit{prt1-63} mutant shows destabilization of mRNAs (Schwartz and Parker 1999). Double mutants of \textit{xrn1::URA3} and either \textit{prt1-1} or \textit{prt1-63} were constructed by mating and dissecting the appropriate strains. The presence of temperature-sensitive Ura\textsuperscript{+} spore clones that were viable at all temperatures at which the single \textit{prt1} mutants were viable indicated no enhanced temperature sensitivity (Figure 6). Since the \textit{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 \textit{xrn1Δ} and \textit{cdc33}. Hence the genetic interaction is restricted to a subset of temperature initiation factors. Additionally, even though lesions in \textit{cdc33}, \textit{ceg1}, and \textit{prt1} accelerate the decay of some mRNAs, the genetic interaction with \textit{xrn1} is only...
observed if distinctly cap-specific processes are perturbed.

Disruption of PAB1 is not suppressed by xrn1Δ: Mutations in CDC33, CEG1, PRT1, and PAB1 destabilize mRNAs (Caponigro and Parker 1995; Fresco and Bratowsky 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, eIF4E/eIF4G interaction and renders cells temperature sensitive. This is consistent with the prior observation we expected mutations in these genes to show similar genetic interactions with xrn1Δ.

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 guanyltransferase responsible for adding the '7mG 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 '7mG cap. The cdc33Δ ceg1Δ mutation that we found from a synthetic lethal screen with xrn1Δ 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 cdc33Δ 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 xrn1Δ 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 elf4E binding was reduced. Supporting the idea that deletion of XRN1 enhances the requirement for elf4E/ elf4G interaction, high-copy expression of a tif4631 mutation that inhibits elf4E 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 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 elf4E/ elf4G cap-binding complex and not a result of reduced overall translational capacity since prt1 mutations showed no synthetic interaction with xrn1Δ. PRT1 encodes an essential subunit of the translation initiation factor 3 complex (Hanic-Joyce et al. 1987b; Naranda et al. 1994). elf3 is required for recruitment of 40S ribosomes and formation of the preinitiation complex (Chaudhuri et al. 1997), a step distinct from cap recognition by elf4F. We have found that several additional mutations that lead to substantially reduced 60S levels, including deletion of SPB2 or RA11, are also not synthetic lethal with xrn1Δ (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 elf4F complex on the 5’-cap are lethal in combination with an xrn1 mutation.

Suppression of RNA instability but not lethality by xrn1Δ. 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 following 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 xrn1Δ 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 elf4F/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 elf2F complex required for recycling elf2F, 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 Hinnenbush 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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