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Synthetic Lethality of *sep1* (*xrn1*) *ski2* and *sep1* (*xrn1*) *ski3* Mutants of *Saccharomyces cerevisiae* Is Independent of Killer Virus and Suggests a General Role for These Genes in Translation Control

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Strand exchange protein 1 (Sep1) (also referred to as exoribonuclease I [Xrn1]) from *Saccharomyces cerevisiae* has been implicated in DNA recombination, RNA turnover, karyogamy, and G4 DNA pairing among other disparate cellular processes. Using a genetic approach to study the role of *SEP1*/*XRN1* in mitotic yeast cells, we identified mutations in the genes superkiller 2 (*SKI2*) and superkiller 3 (*SKI3*) as synthetically lethal with an *sep1* null mutation. The *SKI* genes are thought to comprise an intracellular antiviral system controlling the expression of killer toxin from double-stranded RNA virus found in many yeast strains. However, the lethality of *sep1 ski2* and *sep1 ski3* mutants was independent of the L-A and M viruses, suggesting that the *SKI* genes act in a general cellular process in addition to virus control. We propose that Sep1/Xrn1 and Ski2 both act to block translation on transcripts targeted for degradation. Using a temperature-sensitive allele of *SEP1*/*XRN1*, we show that double mutants display a synthetic cell cycle arrest in late G_1 at Start.

Strand exchange protein 1 (Sep1) from the *Saccharomyces cerevisiae* has been implicated in various seemingly unrelated processes, including DNA recombination and RNA turnover. The *SEP1* gene is also referred to as *XRN1*, *DST2*, *KEM1*, and *RAR5* (reviewed in reference 27). We will identify this gene as *SEP1*/*XRN1* or as *SEP1* for clarity in this report. With respect to DNA recombination, Sep1/Xrn1 catalyzes the pairing of single-stranded circular and homologous linear duplex DNA (15, 31) a reaction that has been used extensively for the characterization of bacterial and phage recombination proteins (32). It also promotes the renaturation of single-stranded DNA (21), and its intrinsic 5′-to-3′ exonuclease is active on DNA as well as RNA (26, 52). More recently Sep1/Xrn1 has been shown to promote the formation of paranemic joints between single-stranded and duplex DNAs (6), a reaction that is thought to be specific for recombination proteins (32). Sep1/Xrn1 has also been described as a nuclease specific for G4 DNA, referred to as Kem1, suggesting a role in telomere function and/or G4 DNA-dependent meiotic chromosome pairing (35). Although the presence of strand exchange and 5′-to-3′ exonuclease activities of Sep1/Xrn1 are consistent with the protein having a direct role in recombination, *sep1*/*xrn1* mutants do not display significant defects in mitotic recombination (16, 30, 55), suggesting that the protein is important for a process other than DNA recombination in mitotic cells. On the other hand, *sep1*/*xrn1* mutants show significant recombination defects in meiosis which may arise from a direct role in meiotic DNA recombination or indirectly as the consequence of a defect in a different process (3, 16, 55, 56).

SEP1/*XRN1* has also been described as a 5′-to-3′ exoribonuclease important for RNA turnover (34, 52). The degradation of normal transcripts in yeast cells appears to be initiated by deadenylation followed by decapping, exposing the transcript to degradation by 5′-to-3′ exoribonucleases such as Sep1/Xrn1 (10). Consistent with this proposed role in RNA turnover, *sep1*/*xrn1* mutants accumulate unadenylated, uncapped transcripts (24) and display a decreased rate of 5′-to-3′ exonuclease degradation of the *MFA2* transcript following deadenylation and decapping (39). Furthermore, the rapid degradation observed for transcripts following premature translation termination is slowed in *sep1*/*xrn1* mutants (40). The Sep1/Xrn1 protein appears to act on a broad range of RNA substrates, since in addition to effects on mRNA, *sep1*/*xrn1* mutants accumulate internal transcribed spacer 1, a product of rRNA processing (20, 53).

Although Sep1/Xrn1 appears to be important for RNA turnover, it is not essential for viability. A second and essential 5′-to-3′ exoribonuclease in yeast cells, exonuclease II, encoded by the *HKE1* gene (28), shows considerable homology to Sep1/Xrn1. *HKE1* was also identified as *RAT1* in a screen for genes involved in the export of poly(A) RNA from the nucleus (2) and as *TAP1* in a screen for mutants that showed increased expression of a promoter-defective suppressor tRNA (12). The *rat1-1* allele accumulates an extended form of 5.8S RNA; however, this defect itself does not appear to cause lethality, and the role of *HKE1* in RNA turnover has not been described.

It is difficult to account for the various phenotypes and biochemical functions of Sep1/Xrn1 in a simple model. Indirect immunofluorescence studies have shown that the protein is localized predominantly to the cytoplasm (22, 29). The protein is abundant and appears to be expressed constitutively throughout the cell cycle. These results are consistent with the view that Sep1/Xrn1 is active in cytoplasmic RNA degradation, but they do not preclude additional activities of the protein.

To examine in the most general terms the in vivo role of Sep1/Xrn1 in mitotic cells, we carried out a synthetic lethal screen to identify mutants that in combination with an *sep1*/*xrn1* null mutant would be inviable. We hoped that such a screen would identify genes functionally redundant with *SEP1*/*XRN1* and lend insight to its cellular role(s). This report describes the identification of mutations in *SKI2* (superkiller 2) and *SKI3* (superkiller 3) as synthetically lethal with an *sep1*/*xrn1* null mutation. The *SKI* genes were originally identified as

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TABLE 1. Strains used in this work

Strain designation	Genotype	Plasmid	Source or reference
CH1305	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52*		C. Holm
CH1462	*MAT*α *ade2 ade3 leu2 his3 ura3-52*		C. Holm
MERX38	*MAT***a**/α *ADE5/ade5 met13-d/met13-c CYH2S/cyh2*^{r} *TRP5/trp5 leu1/LEU1 ADE6/ade6 CLY8/cly8 lys2-1/lys2-2 tyr1-1/tyr1-1 his7-2/his7-1 ade2-1/ade2-1 ura3-1/ura3-1 can1*^{r}*/CAN1*^{s} *SEP1/SEP1*		M. Esposito
RKY1308	*MAT***a**/α *ura3/ura3 lys2/lys2 ho*::*LYS2/ho*::*LYS2 leu2*::*hisG/leu2*::*hisG his4X/his4B*		
RKY1951	*MAT***a**/α *ADE5/ade5 met13-d/met13-c CYH2S/cyh2*^{r} *TRP5/trp5 leu1/LEU1 ADE6/ade6 CLY8/cly8 lys2-1/lys2-2 tyr1-1/tyr1-1 his7-2/his7-1 ade2-1/ade2-1 ura3-1/ura3-1 can-1*^{r}*/CAN-1*^{s} *sep1*Δ::*URA3/sep1*Δ::*URA3*		56
RKY1973	*MAT*α *ski2*Δ::*LEU2 LYS2*		
RKY1976	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ::*URA3*		
RKY1977	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ		
RKY1978	*MAT*α *ade2 ade3 leu2 his3 ura3-52 Gal2*$^+$ *sep1*Δ::*URA3*		
RKY1979	*MAT*α *ade2 ade3 leu2 his3 ura3-52 Gal2*$^+$ *sep1*Δ		
RKY1981	*MAT***a** *his5 ura3* L-A-o M-o L-BC *sep1*Δ::*URA3*		
RKY1982	*MAT*α *trp1 leu2*::*his3 pho3 pho5* L-A-o M-o *ski2*Δ::*LEU2*		
RKY1986	*MAT***a** *ade2 ade3leu2 lys2-801 ura3-52 ski2*Δ::*LEU2*		
RKY1997	*MAT***a** *leu2 ura3 trp1* L-A-o		
RKY1999	*MAT*α *leu2 ura3 sep1*Δ::*URA3* L-A-o		
RKY2032	*MAT*α *URA3 LEU2 trp1 ski2*Δ::*LEU2* L-A-o		
RKY2033	*MAT***a** *leu2 ura3 trp1 ski3*Δ::*TRP1* L-A-o		
RKY2034	*MAT*α *ski3*Δ::*TRP1 ura3 LEU2 trp1* L-A-o		
RKY2053	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ *ski2-14*		
RKY2055	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ *ski2-18*		
RKY2060	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ *ski3*		
RKY2062	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ	pRDK297	
RKY2063	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ *ski2*	pRDK252 (*SEP1*)	
RKY2064	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ *ski2*	pRDK252 (*sep1-10*^{ts})	
RKY2067	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ *ski3*	pRDK252 (*SEP1*)	
RKY2068	*MAT***a** *ade2 ade3 leu2 lys2-801 ura3-52 sep1*Δ *ski3*	pRDK252 (*sep1-10*^{ts})	
RW1074	*MAT***a** *leu1 kar1-1* L-A-HN M_1		R. Wickner
RW2889	*MAT***a** *his5 ura3* L-A-o M-o L-BC		R. Wickner
RW2966	*MAT*α *trp1 leu2*::*his3 pho3 pho5* L-A-o M-o		R. Wickner
RW5X47	*MAT***a**/*MAT*α K-R-		R. Wickner
YLL78	*MAT*α *ade2-101 his3*Δ*200 ura3-53 lys2-801 sep1-10*^{ts}		

regulators of killer toxin expression from cytoplasmic double-stranded RNA virus found in many laboratory yeast strains. Significantly, the lethality of *sep1 ski2* and *sep1 ski3* mutants is independent of L-A and killer double-stranded RNA viruses, suggesting a general role for *SKI2* and *SKI3*. We postulate that the Ski2 and Sep1/Xrn1 proteins work together to control translation of transcripts targeted for degradation.

MATERIALS AND METHODS

Strains. The yeast strains used are described in Table 1. Strains RKY1976, RKY1978, and RKY1981 contained *SEP1* disruptions (*sep1*Δ::*URA3*) and were constructed by homologous gene replacement by transforming strains CH1305, CH1462, and RW2889 with *Ase*I-digested pRDK227 (56) containing the *URA3* gene replacing the *SEP1* coding sequence between the *Eco*RI sites at nucleotides 108 and 3894. The correct disruptants were identified by PCR (46) using oligonucleotide primers 5′-AGTTTATTTTCTAAAGG, 5′-TTCTGCATCCATAGC GG, 5′-ATCGAAGTTTATCGCGG, and 5′-CTCCAGGAATAGGACCC, specific for *SEP1*, which give PCR products of 476 and 860 bp for wild-type *SEP1* and a single product of ~2,000 bp for the *URA3* disruption. RKY1977 (*sep1*Δ) and RKY1979 (*sep1*Δ), deleted of the *URA3* insertions in RKY1976 and RKY1978, respectively, were created by transformation with *Ase*I-digested pRDK285, containing the *SEP1* gene deleted for all coding sequence between the *Eco*RI sites at nucleotides 108 and 3894. Transformed cells were plated onto YPD plates, allowed to grow 24 h at 30°C, and then replica plated onto 5-fluoroorotic acid (5FOA) plates to select for the loss of the intervening *URA3* sequence. Correct disruptants were identified by the presence of a single 528-bp PCR product in reactions described above. RKY1982 (*ski2*Δ::*LEU2*) was created by transforming RW2966 with *Xba*I- and *Sst*I-digested pRDK310 and selecting for Leu$^+$ transformants. Correct disruptants were identified by an expected 3.5-kbp PCR product rather than the wild-type 764-bp and 1,065-bp products in PCRs using the oligonucleotide primers 5′-CTCAGAACGCCCATCGG, 5′-AT AAACCTTGACCATCC, 5′-TTTTAAGAATTGAGGCG, and 5′-GTGTGAC AATAGGAGGC. RKY2033 (*ski3*Δ::*TRP1*) was created by transforming RKY 1997 with *Bgl*II- and *Pst*I-digested pRDK321 and selecting for Trp$^+$ transformants. Correct disruptants were identified by a characteristic 1,246-bp product distinguished from the wild-type 2,059-bp PCR product in PCRs using the oligonucleotide primers 5′-CCACACGCGTGGTATGC and 5′-CGGAACCTGG AGAGAGC. All oligonucleotides were prepared by the Molecular Biology Core Facility, Dana-Farber Cancer Institute.

Plasmids. Plasmids novel to this study are listed in Table 2. All plasmid DNAs were propagated in *Escherichia coli* RDK1400 (54). pRDK297, a 2μm-based *SEP1 ADE3* vector used for the synthetic lethal screen, was constructed by

TABLE 2. Plasmids novel to this work

Name	Relevant information
pRDK285	Bluescript *sep1*Δ (deletion of *SEP1* from nucleotides 108–3894)
pRDK297	2μm *SEP1 URA3 ADE3*
pRDK307	pRS315 *SEP1*
pRDK308	pUC19 *SKI2*
pRDK310	pUC19 *ski2*Δ::*LEU2*
pRDK319	pUC19 *SKI3*
pRDK321	pUC19 *SKI3*Δ::*TRP1*
pRDK322	YCp50 *sep1-10*^{ts}
pRDK331	YEp351 *SKI2* subclone
pRDK329	YEp351 *SKI3* subclone

cloning the *ADE3 Bam*HI-to-*Sal*I fragment from pRDK255 (constructed by D. Koshland) into the same sites of pRDK242 (26). pRDK307 is an *SEP1 LEU2 ARSH4 CEN6* vector derived from pRS315 by replacing the *Xho*I-to-*Sst*I portion of the polylinker of pRS315 with oligonucleotides to give the sequence 5′-TCG ATTCTAGACCCGGGAAGCTTAAGTAGCTAGCT, thereby destroying the *Xho*I and *Sst*I sites. Subsequently an *SEP1 Xba*I-to-*Hin*dIII fragment from pRDK252 (55) was cloned into the *Xba*I and *Hin*dIII sites of the reconstructed polylinker. pRDK308 contains the *SKI2 Xba*I-to-*Sst*I fragment from the genomic clone p2-18/8-4 cloned into the *Xba*I-to-*Sst*I sites of pUC19. pRDK310, an *ski2*Δ::*LEU2* disrupter plasmid, was constructed from pRDK308 by replacing the *Eco*RV fragments from nucleotides −147 to 2596, containing the start codon and most of the coding sequence of *SKI2*, with the *LEU2 Hpa*I fragment from YEp13. pRDK319 contains the *SKI3* gene on a *Pst*I fragment from the subcloned genomic *SKI3* clone p3-21/1-2, inserted into the *Pst*I site of pUC18. pRDK321, an *ski3*Δ::*TRP1* disrupter plasmid, was made by replacing a portion of the *SKI3* coding sequence from nucleotides 208 to 2186 with a *TRP1 Bst*UI fragment from pRS424. pRDK322 contains a temperature-sensitive allele of *SEP1*, *sep1-10^{ts}*, recovered by gapped plasmid rescue and was made by transforming *Avr*II-digested pRDK252 (removing *SEP1* sequence from nucleotides −81 to 3112) into strain YLL78. DNA was prepared from the Ura$^+$ transformants, propagated in *E. coli*, and transformed back into RKY2053 to identify temperature-sensitive isolates. The 2μm *LEU2*-based yeast library used for cloning *SKI2* and *SKI3* was a yeast genomic *Hin*dIII partial digest cloned into the *Hin*dIII site of YEp213 and was obtained from B. Futcher (originally from S. Cameron and M. Wigler).

Media and genetic manipulations. Standard media and genetic techniques were as described previously (49). Low-pH methylene blue YPD plates for killer assays were prepared as described previously (60). Low-adenine Leu dropout plates contained, per liter, 6.7 g of yeast nitrogen base without Casamino Acids (Difco), 6 mg of adenine, 20 mg each of histidine, tryptophan, and uracil, 100 mg of lysine, 20 g of glucose, and 20 g of Bacto Agar. Yeast transformations were done as described elsewhere (18).

Isolation of mutants. Three independent UV mutagenesis experiments were carried out. In each case, RKY2062 was grown in Ura dropout medium to early stationary phase. The cells were washed once in H_2O, resuspended in 3 ml of H_2O at approximately 3×10^7 cells per ml, and sonicated briefly to disrupt cell aggregates. The cells were then placed in a plastic weigh boat and exposed for various times to UV radiation from a germicidal UV lamp with occasional swirling. A 4.5-min exposure resulted in 90% killing. Cells were diluted in H_2O and plated onto YPD plates to give an expected 300 colonies per plate. Colonies were scored for sectoring after 5 to 7 days at 30°C. In the first round, 15,000 colonies at 14% survival were screened and 22 potential nonsectoring colonies were picked. In the second round, 100,000 colonies at 19% survival yielded 32 candidate nonsectoring colonies. In the third round, 25,000 colonies at 10% survival yielded 13 potential nonsectoring colonies. All candidate mutants that were unable to grow on 5FOA (21 mutants) were mated with CH1462 to identify recessive mutants (19 mutants). The original haploid mutants were then transformed with pRDK307 to confirm that the nonsectoring phenotype was dependent on *SEP1* (15 mutants). To determine that the nonsectoring phenotype arose from a single mutant locus, candidate recessive mutants were mated with RKY1979 and sporulated, and the resulting tetrads were dissected onto YPD to score for sectoring. Strains that showed 2:2 segregation for white:solid red were picked for cloning of wild-type genes (8 mutants; 5 of the 15 were not carried through dissection because of poor growth or poor sporulation). A high percentage of random solid red spore clones compared with total viable clones (average 40%) was taken as a second indicator of segregation of a single allele.

Complementation of mutants was done by mating spore clones of the appropriate mating types on YPD and scoring for sectoring in the resulting diploids. The complementation analysis was confirmed by transformation with plasmid clones and scoring for sectoring.

Wild-type clones were identified from a 2μm *LEU2*-based yeast library containing large (15- to 20-kbp) inserts. Mutants transformed with library DNA were plated onto Leu dropout low-adenine plates and scored for sectoring. Sectored and white colonies were restruck to single colonies on the same medium and then scored for growth on YPG to eliminate petites and on 5FOA to confirm the loss of the *SEP1* plasmid. To eliminate *SEP1* clones, yeast plasmid DNA was prepared from candidate clones and was analyzed by PCR for wild-type *SEP1* sequences. All non-*SEP1* plasmids were transformed into *E. coli* and then transformed back into the original mutant strains to confirm that sectoring was restored by the plasmid. Two non-*SEP1* clones complementing RKY2055 and RKY2060, p2-18/8-4 and p3-21/1-2, respectively, were subcloned by digesting the plasmids with limiting amounts of *Sau*3A and size separating the partial digest products by electrophoresis on agarose gels. The gels were cut into portions containing 1 to 2, 2 to 4, 4 to 6, and 6 to 9 kbp of DNA. The DNA was purified by using Geneclean (Bio 101, Inc.), ligated into the *Bam*HI site of YEp351, and transformed into *E. coli*. All transformants from each size range were scraped from the plates, and plasmid DNA was prepared from them. The subcloned libraries were transformed back into the mutant strains, and complementing clones were identified as sectored colonies on Leu dropout low-adenine plates. Potential clones were scored for growth on 5FOA plates. Yeast plasmid DNA was prepared from the complementing clones obtained from the smallest-size-range pool, and the plasmids were recovered in *E. coli*. Plasmids were sequenced by the Dana-Farber Cancer Institute Molecular Biology Core Facility, using forward and reverse M13 sequencing primers.

Temperature shift experiments. Fresh overnight cultures grown in YPD at 22°C were diluted to an optical density at 600 nm of 0.2 in 25 ml of YPD. Cultures were grown at 22°C to an optical density at 600 nm of 0.5 to 0.7, at which time the cultures were shifted to 37°C and incubation at 37°C continued. At the indicated times, 3-ml samples were removed for photography, indirect immunofluorescence, and cell counting by a hemacytometer. The samples fixed with 3.7% formaldehyde for 30 min at 37°C, washed twice in 1.1 M sorbitol–0.1 M KPO_4 (pH 6.6), and stored overnight in the same buffer containing 0.02% NaN_3 at 4°C. For fluorescence-activated cell sorting (FACS) analysis, 2-ml samples were removed from the cultures, fixed with 2 ml of 95% ethanol, and stored at 4°C. Cells were prepared for FACS analysis as described previously (5), and the analysis was performed by the Dana-Farber Cancer Institute Cell Sorter Facility. 4′,6-Diamidino-2-phenylindole (DAPI) staining and indirect immunofluorescence for tubulin were done as described elsewhere (22a) except that monoclonal rat antitubulin (Serotec) diluted 1:200 and Texas red-conjugated goat anti-rat antibody (Jackson ImmunoResearch Laboratories, Inc.) diluted 1:1,000 were used as the primary and secondary antibodies, respectively. Antibodies were kindly provided by G. Schleinstedt and P. Silver.

Other methods. Double-stranded RNA was prepared as described previously (17). Small-scale yeast cell extracts were prepared as described previously (26). Extracts were electrophoresed through a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel, and the separated proteins were transferred to Immobilon-P (Millipore) (36). The membrane was blocked for 30 min in Tris-buffered saline (TBS; 10 mM Tris-HCl [pH 8], 150 mM NaCl) with 2% powdered milk (Bio-Rad). The membrane was incubated for 2 h with the mouse monoclonal anti L-A Gag antibody (1.6 μg/ml in TBS with 0.05% Tween 20 [TBST]) as the primary antibody. The blot was then processed as suggested in the ProtoBlot kit (Promega), using horseradish peroxidase-conjugated goat anti-mouse antibody (Promega) diluted 1:7,500 in TBST as the secondary antibody. Mouse monoclonal anti L-A Gag antibody (4) was a gift from A. Blanc and N. Sonenberg.

RESULTS

Screen for mutations synthetically lethal with an *sep1/xrn1* null mutation. A modification of a previously described synthetic lethal screen was used (33). Briefly, a haploid *ade2 ade3 sep1*Δ strain bearing a 2μm *ADE3 SEP1 URA3* plasmid was constructed. This strain yields solid red colonies when the plasmid is maintained and red and white sectored colonies without selection for the plasmid, since loss of the plasmid results in an *ade2 ade3* genotype. After mutagenesis, nonsectoring red colonies were picked, and those in which the plasmid-borne *SEP1/XRN1* had become essential due to a genomic mutation were identified. Because *CEN ARS* vectors bearing the *SEP1/XRN1* gene are stable in *sep1*Δ mutants, we constructed a 2μm *URA3 ADE3 SEP1* plasmid, pRDK297, which gave rise to sectored colonies in RKY1979. This strain was mutagenized with UV irradiation to 10 to 19% survival in three separate experiments. From 140,000 mutagenized colonies screened, eight recessive, *SEP1/XRN1*-dependent mutants were identified. Each segregated as a single locus as determined by tetrad analysis (see Materials and Methods). The mutants fell into three complementation groups with six mutants in one group.

Identification of the superkiller genes *SKI2* and *SKI3*. Complementing clones for two of the complementation groups were identified from a yeast genomic library and were partially sequenced. The DNA sequences obtained were compared against GenBank release 80 by BLAST (1). Matches were found with *SKI2* and *SKI3* (61) for clones from complementation groups 1 and 2, respectively. Figure 1 shows the regions of the *SKI2* and *SKI3* subclones that were sequenced and the locations of the gene replacements. Within the indicated regions, there was perfect identity in the DNA sequences of the subclones and published sequences. The *SKI2*-containing subclone contained the smallest genomic *Sau*3A fragment containing the entire *SKI2* gene, with 239 nucleotides upstream of the initiating ATG and 107 nucleotides downstream of the stop codon. *SKI2* was the only open reading frame encompassed by these sequences. The *SKI3* gene on chromosome XVI is bor-

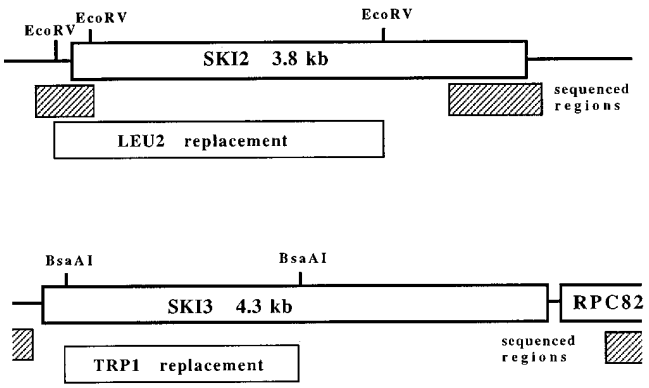

FIG. 1. *SKI2* and *SKI3*. Subclones pRDK331 and pRDK329 were partially sequenced as indicated. The locations of gene replacements used for disruption of the genomic *SKI2* and *SKI3* loci are shown.

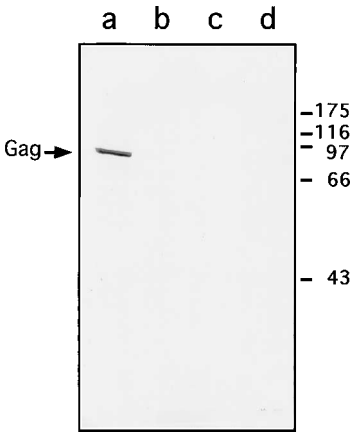

FIG. 2. Western blotting analysis of L-A gag protein. Ten micrograms of total soluble protein from each extract was separated by SDS-polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose and analyzed by Western blotting using a monoclonal antibody specific for the Gag protein encoded by L-A double-stranded RNA virus. Lanes: a, strain RKY1308 (killer$^+$); b, strain RKY1981 (*sep1*Δ::*URA3*); c, strain 1982 (*ski2*Δ::*LEU2*); d, strain RKY2033 (*ski3*Δ::*TRP1*). The strains used for tetrad disection shown in Fig. 3 were all derived from these strains. Molecular weight markers were Sep1 (175 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), and ovalbumin (43 kDa).

dered by the *RPC82* and *RPO26* genes (7) (GenBank release 80). Partial sequencing of the *SKI3* clone revealed nucleotide sequence from the region between *RPO26* and the 5′ end of *SKI3* and sequence from the 3′ end of *RPC82*. However, *SKI3* was the only complete open reading frame expected within these sequences. Complementing clones for the third complementation group have not been identified; however, it does not appear to be *HKE1*. Because a *ski2*-dependent superkiller phenotype could not be observed in this strain background after cytoduction of the M virus (data not shown), the third complementation group was not scored for the superkiller phenotype. The *SKI2* and *SKI3* genes had previously been identified in screens for mutants that increase the expression of killer toxin from the endogenous M double-stranded RNA virus present in many strains of *S. cerevisiae* (59).

Synthetic lethality does not depend on L-A or M double-stranded RNA viruses. To confirm the synthetic lethality between *sep1* and *ski2* and *sep1* and *ski3*, gene disruptions of *SEP1*, *SKI2*, and *SKI3* were made in haploid yeast strains. In addition, to test if synthetic lethality is independent of L-A and M viruses, the strains used were deficient for L-A and M. (See Discussion for a description of L-A and M). Whole-cell extracts of the haploid strains disrupted for *SEP1*, *SKI2*, or *SKI3* were analyzed by Western blotting (immunoblotting) for the L-A Gag protein, which forms the coat of the viral particle. As seen in Fig. 2, there was no detectable Gag protein in extracts made from the haploid parents used for all subsequent matings for tetrad dissection. As expected, a band of the appropriate size for the 86-kDa Gag protein was observed in an extract of the killer$^+$ strain RKY1308. In addition, preparations of double-stranded RNA from these putative L-A-deficient strains did not show the characteristic L-A genomic band on agarose gels, and these strains did not show killing in killer assays (data not shown).

The appropriate strains were mated, the resulting heterozygous diploids were sporulated, and tetrads were dissected onto YPD plates. Figure 3A shows tetrad analysis of the *ski2*Δ/*SKI2 SEP1*/*sep1*Δ heterozygous diploid in the virus-deficient background. The small colonies were all *sep1*Δ::*URA3*, demonstrating the slow growth of *sep1* mutants. All markers segregated 2:2. Leu$^+$ Ura$^+$ colonies were not recovered in 40 tetrads dissected, and spore inviability was approximately 25%, indicating that cosegregation of the *sep1* and *ski2* mutations was lethal. In all cases in which there was a dead clone, the spore had germinated and stopped growing as three or four large unbudded cells. Synthetic lethality between *sep1*Δ and *ski2*Δ was also observed in the background in which the synthetic lethal screen was done (CH1305 and CH1462, L-A$^+$ killer$^-$) and in the unrelated SK-1 (killer$^+$) background (data not shown). Figure 3B shows the results of dissecting a *ski3*Δ/*SKI3*

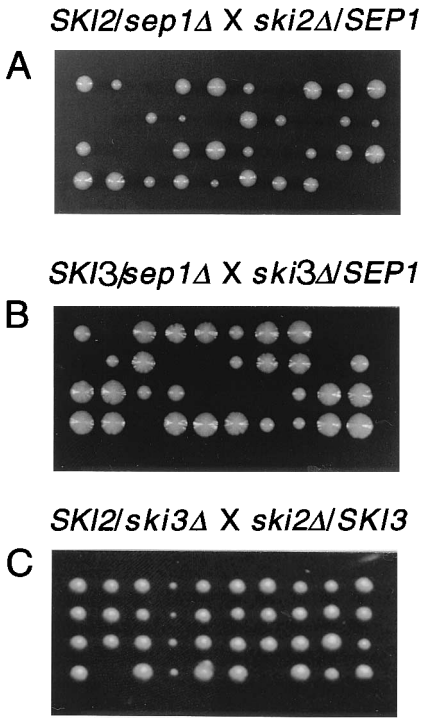

FIG. 3. Tetrad analysis of synthetic lethality. All strains were derived from L-A virus-deficient strains. (A) RKY1981 (*sep1*Δ::*URA3*) and RKY1982 (*ski2*Δ::*LEU2*) were mated and sporulated, and the resulting tetrads were dissected onto a YPD plate. The genotypes of the spore clones were determined by PCR and by replica plating. (B) As in panel A but with strains RKY1999 (*sep1*Δ::*URA3*) and RKY2033 (*ski3*Δ::*TRP1*). (C) As in panel A but with strains RKY2032 (*ski2*Δ::*LEU2*) and RKY2033 (*ski3*Δ::*TRP1*).

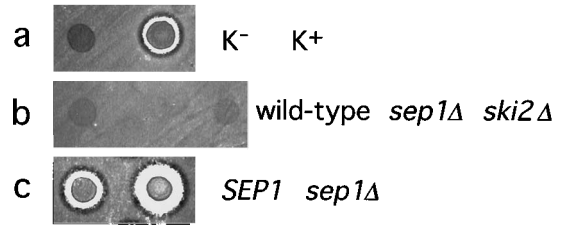

FIG. 4. Assay of various strains for killer function. A light lawn of the killer-sensitive diploid strain RW5X47 was spread onto a methylene blue, low-pH YPD plate. Strains to be tested for killer function were spotted onto the lawn from liquid cultures, and the plates were incubated at 22°C for 3 days. The dark rings surrounding the clear zones were dead cells stained with methylene blue. (a) Strains RW5X47 (killer$^-$) and RW1074 (killer$^+$); (b) strains CH1305, RKY1977 (*sep1*Δ), and RKY1986 (*ski2*Δ); (c) strains MERX38 (wild type) and RKY1951 (*sep1*Δ/*sep1*Δ).

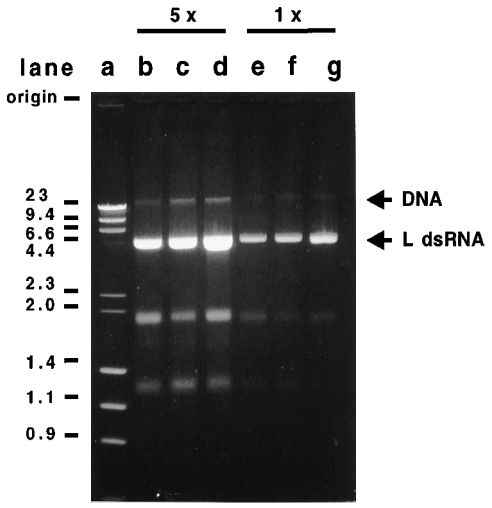

FIG. 5. Effects of *ski2* and *sep1* mutations on levels of double-stranded-RNA. Double-stranded RNA (dsRNA) was prepared as described in the text, analyzed by electrophoresis through 1% agarose gel in Tris-acetate-EDTA, and visualized by staining with ethidium bromide. Lanes: a, double-stranded DNA standards with sizes given in kilobase pairs; b through d, equal amounts of total RNA prepared from wild-type (CH1305), *sep1*Δ (RKY1977), and *ski2* mutant (RKY2053) strains, respectively; e through g, as for lanes b through g, respectively, but only one-fifth as much RNA was loaded. The position of the 4.3-kbp L-A genome is indicated. Minor RNA bands present are ribosomal 25S and 18S RNAs. tRNAs were not retained in the gel.

SEP1/*sep1*Δ diploid strain in the L-A- and M-deficient background. The *sep1*Δ::*URA3* disruption was identified by replica plating. To distinguish between *ski3*Δ::*TRP1* and the genomic *TRP1*, DNA was prepared from all Trp$^+$ spore clones and analyzed by PCR for disruption of the *SKI3* gene (data not shown). No *ski3*Δ::*TRP1 sep1*Δ::*URA3* clones were obtained, indicating that the *sep1 ski3* double mutant was lethal. As observed for the *sep1*Δ *ski2*Δ mutants, the *sep1*Δ *ski3*Δ spores germinated but stopped growing after two or three cell divisions. Interestingly, *ski2* and *ski3* together were not synthetically lethal, as *ski2 ski3* spore clones were readily identified from sporulation of a *ski2*Δ/*SKI2 SKI3*/*ski3*Δ strain and these double mutants did not display any obvious growth defects (Fig. 3c).

To confirm that the mutations initially identified from the synthetic lethal screen were in fact *ski2* and *ski3* mutations, strains RKY2053 (*sep1*Δ and putatively *ski2*, containing pRDK297) and RKY2060 (*sep1*Δ and putatively *ski3*, containing pRDK297) were mated with the deletion mutants RKY1973 (*SEP1 ski2*Δ::*LEU2*) and RKY2034 (*SEP1 ski3*Δ::*TRP1*), respectively. The resulting diploids were sporulated, and the tetrads were dissected. In these crosses, if the gene knockouts and original synthetic lethal mutations segregated independently, only half of the four-viable-spored tetrads (in which the *URA3 SEP1* plasmid pRDK297 had been maintained) would show 2:2 segregation of the *URA3* marker; the remainder would show 1:3 segregation (Ura$^+$:Ura$^-$). In fact, all four-spored tetrads displayed 2:2 segregation, indicating that the mutations originally identified were in the *SKI2* and *SKI3* genes (data not shown).

Further confirmation of the independence of synthetic lethality from killer virus came from a plate assay for killer function. Cells expressing killer toxin can kill neighboring toxin-sensitive cells. This phenomenon can be observed on plates, since killer strains give rise to colonies surrounded by a halo in which a sensitive strain is unable to grow and dead cells are stained with methylene blue. As seen in Fig. 4b, the strain in which the synthetic lethal screen was carried out was deficient for killer expression. As a control, a killer$^+$ strain, RW1074, showed the characteristic pattern of killing (Fig. 4a).

In addition to L-A and M, yeast cells harbor another minor double-stranded RNA virus, L-BC, which is the same size as L-A, and a single-stranded RNA replicon, 20S RNA. We believe that decontrol of these additional RNA species is not the cause of synthetic lethality for several reasons. (i) A *ski2* mutant containing a galactose-regulated *SEP1* gene on a single-copy *CEN ARS* plasmid showed no obvious overexpression of any RNA species when *SEP1* expression was reduced by shifting cells from galactose- to glucose-containing medium (data not shown). In addition, galactose-induced overexpression of *SEP1* from a high-copy-number plasmid did not lead to significant reduction in any RNA species (data not shown). (ii) Yeast cells appear to be able to tolerate high levels of RNA viruses: growth on acetate medium yields up to 10,000-fold amplification of 20S RNA without deleterious effect (37), and the major coat protein of L-A can account for up to 10 to 20% of the total cellular protein without negatively affecting cell growth (11).

An *sep1*/*xrn1* mutation confers a superkiller phenotype. The synthetic lethality between *sep1* and *ski2* mutations suggests that the two genes have similar functions in the cell. A prediction then is that *sep1* mutants would display a superkiller phenotype. When a wild-type killer strain and a *sep1*Δ mutant in the same genetic background were plated onto a lawn of a killer toxin-sensitive diploid, a larger zone of growth inhibition was observed for the *sep1* mutant (Fig. 4c). This phenotype was also observed in haploid yeast cells at 22 and 30°C (data not shown). Thus, by this criterion, *SEP1*/*XRN1* is a superkiller gene. The superkiller effects of *sep1* and *ski2* mutations were not observed in the unrelated background RKY1308, probably because wild-type SK-1 strains by themselves show a high level of killing obscuring the superkiller effect (data not shown). Of the known superkiller genes, the *ski1-1* mutation maps to the left arm of chromosome VII at a position indistinguishable from that of *SEP1*/*XRN1*, as determined from current map data (38).

The *ski1-1* mutant is the only superkiller that does not confer increased levels of L-A double-stranded RNA. Figure 5 shows agarose gel analysis of double-stranded RNA prepared from the wild type and *sep1* and *ski2* mutants. Similar amounts of total RNA were added in each set of lanes. Whereas the *ski2* mutant showed a modest increase in L-A levels as reported previously (61), the *sep1* mutant did not show increased L-A levels compared with the wild type. In addition, the *ski1-1*

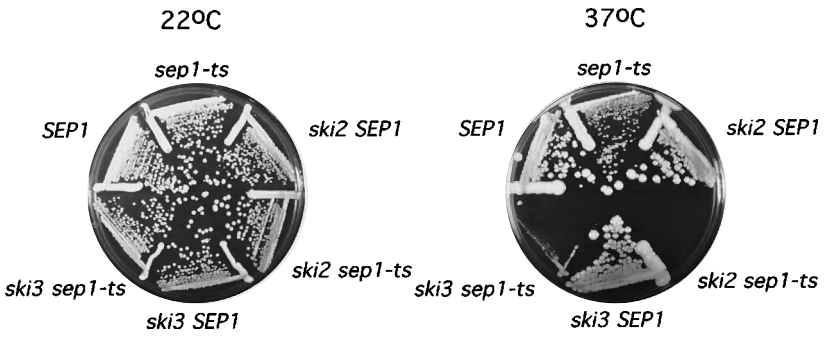

FIG. 6. A temperature-sensitive allele of *SEP1* is inviable at nonpermissive temperature in *ski2* and *ski3* mutants. The indicated strains were streaked for single colonies on YPD plates and incubated at room temperature and at 37°C (nonpermissive temperature) for 4 and 3 days, respectively.

mutation confers a slow-growth phenotype (57) which was partially complemented by an *SEP1*-bearing plasmid (data not shown). However, we were unable to demonstrate complementation of the superkiller phenotype by an *SEP1*-bearing plasmid because in our hands the superkiller phenotype of *ski1-1* could not be reliably scored. In addition, *ski1-1 sep1*Δ heterozygous diploids did not show the sporulation defect of homozygous *sep1* null mutants. The similar map positions and common phenotypes suggest that *ski1-1* is an allele of *SEP1/XRN1*; however, convincing genetic data are lacking. The *ski1-1* allele may be a partial function allele, since *ski1-1* strains express full-length Sep1/Xrn1 (data not shown).

***sep1 ski2* and *sep1 ski3* double mutants show a synthetic cell cycle arrest in late G_1.** The terminal phenotypes of the *sep1 ski2* and *sep1 ski3* double mutants were analyzed through the use of a temperature-sensitive allele of *SEP1/XRN1*. A strain temperature sensitive for the *SEP1/XRN1* gene had been identified (34a) by virtue of the accumulation of internal transcribed spacer 1 in *sep1* mutants (53). The *sep1-10^{ts}* allele was recovered from this strain by using gapped plasmid rescue. A *CEN ARS* vector containing the *sep1-10^{ts}* allele (pRDK322) or wild-type *SEP1/XRN1* (pRDK252) was then introduced into RKY1977 (*sep1*Δ), RKY2053 (*ski2 sep1*Δ), and RKY2060 (*ski3 sep1*Δ). At the permissive temperature (22°C), strains bearing the temperature-sensitive allele grew as well as wild-type strains (Fig. 6). At the restrictive temperature, RKY1977/pRDK322 (*sep1-10^{ts}*) grew more slowly than the same strain with a wild-type copy of *SEP1/XRN1*, characteristic of *sep1* mutants. The *sep1-10^{ts}* allele did not support growth at the restrictive temperature in the *ski2 sep1*Δ mutant, and minimal growth was observed in the *ski3 sep1*Δ mutant.

The terminal phenotypes of *sep1 ski2* and *sep1 ski3* mutants were analyzed in liquid medium by cell counting, optical density, and FACS. When an asynchronous culture of strain RKY2064 (*ski2 sep1-10^{ts}*) was shifted to the nonpermissive temperature, the cells arrested within one generation as large unbudded cells (Fig. 7A). There was a modest 50% increase in cell number after 20 h of incubation at the nonpermissive temperature, although the cells continued to grow (Fig. 7 and data not shown). The increase in cell number probably reflected the growth of small buds that were not counted as individual cells at the time of shift to the restrictive temperature. FACS analysis indicated that within 2 h, the arrested cells contained a 1n DNA content (Fig. 7C). DAPI staining and indirect immunofluorescence staining for tubulin indicated that the cells contained a single nucleus and a single focus of tubulin staining (data not shown). The same strain bearing a wild-type *SEP1/XRN1* gene continued to grow and divide, did not arrest as abruptly in G_1 following the temperature shift, and reached a high cell density after 20 h. These results indicate that the double mutant arrested in G_1. The *ski3 sep1* double mutant behaved similarly, but the arrest was not as complete; the arrested cells did not increase in size as rapidly as the arrested *ski2 sep1-10^{ts}* mutant cells, there was a greater increase in cell number, and the cells showed slight growth on plates at the nonpermissive temperature.

A G_1 arrest in which cells continue to grow is reminiscent of arrest at Start such as observed in *cdc28* mutants. Because a characteristic of cells arrested at Start is their ability to conjugate (45), the *sep1-10^{ts} ski2* and *sep1-10^{ts} ski3* mutants were tested for conjugation after the temperature shift. Cells were grown on YPD plates at the permissive temperature, shifted to 37°C for 2 h, and then mixed on plates with wild-type cells of the opposite mating type. After 3 h at 37°C, the cell mixtures were analyzed microscopically for the presence of shmoos and zygotes. In the mating of the *sep1-10^{ts} ski2* strain, the arrested cells were easily distinguished from the mating tester strain by their large size. Approximately 30% of the large arrested cells were zygotes and 22% were shmoos after 3 h. No shmoos or zygotes were seen in the absence of cells of the opposite mating type (data not shown). Similar results were obtained with *sep1-10^{ts} ski3* strain, although since the arrested cells were not uniformly large as in the case of *sep1-10^{ts} ski2*, determining the actual fraction of shmoos and zygotes relative to the total number of arrested cells was not possible. The ability of the arrested cells to mate places the arrest point at Start.

DISCUSSION

The many biochemical properties of purified Sep1/Xrn1 protein and the pleiotropy of *sep1/xrn1* mutants have led to multiple proposals for the function of Sep1/Xrn1 (reviewed in reference 27). We sought to address this dilemma from an approach that would minimize the presumptions made about the in vivo role of the protein. We carried out a screen for mutants that would be inviable in combination with an *sep1* null mutation. Such a screen is expected to identify genes that act in pathways parallel to that of Sep1/Xrn1 (19) and relies on the assumption that the function carried out by Sep1/Xrn1 is critical for a yeast cell but that there are other gene products that have functions in common with Sep1/Xrn1. For example, Sep1/Xrn1 is a 5′-to-3′ exonuclease that is argued to be important for RNA turnover (24, 34, 39, 40, 52). *sep1/xrn1* mutants display slower turnover of rapidly degraded messages (24, 39). However, these messages continue to be degraded at a slow rate, suggesting that there are other unidentified gene products important for transcript degradation (40). Such a redundancy for essential nucleases involved in RNA turnover

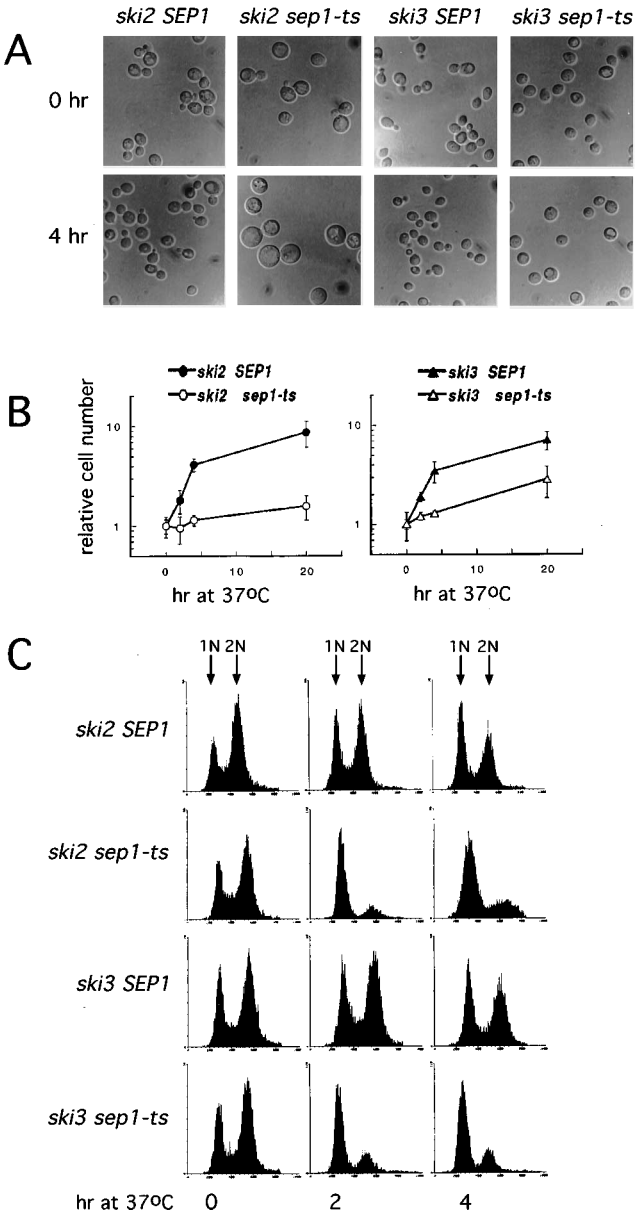

FIG. 7. Terminal phenotype of *ski2 sep1-10*^{ts} and *ski3 sep1-10*^{ts} mutants at the nonpermissive temperature. Log-phase cultures of strains RKY2063 (*ski2 SEP1*), RKY2064 (*ski2 sep1-10*^{ts}), RKY2067 (*ski3 SEP1*), and RKY2068 (*ski3 sep1-10*^{ts}) in liquid YPD medium were shifted to the nonpermissive temperature (37°C) at time zero. At the indicated times, samples were removed and photographed (A), cell density was determined microscopically by using a hemacytometer (B), and cells were analyzed FACS (C).

has been described for polynucleotide phosphorylase and RNase II in *E. coli* (13).

In the analysis described here, we identified mutations in the nuclear antiviral genes *SKI2* and *SKI3* that are synthetically lethal with an *sep1/xrn1* null mutation. Although it has been argued that the *SKI* genes comprise an intracellular antiviral defense system (see below), the synthetic lethality with *sep1/xrn1* mutants was independent of L-A and M double-stranded RNA viruses. We believe that these results are the first demonstration that the *SKI* genes play a general role in *S. cerevisiae* and are not specific for the killer virus.

It is somewhat surprising that this screen did not identify genes of other known RNases such as *HKE1* (28), considering the apparent role of *SEP1/XRN1* in RNA degradation. There are several interpretations of this result. First, it is possible that *SKI2* and *SKI3* encode such enzymatic activities. *SKI2* is a putative RNA helicase (61), but it has not been characterized in vitro for biochemical function. Because *SKI3* appears to be localized to the nucleus (47), it is less likely to act directly in cytoplasmic RNA turnover. Second, it is possible that the critical role of Sep1/Xrn1 is the regulation of gene expression by blocking translation on transcripts targeted for degradation rather than the degradation of RNA per se (see below). In this respect, it has been argued that the lethality due to mutations in polynucleotide phosphorylase and RNase II in *E. coli* results from the accumulation of RNA (13), but it is possible that lethality results from expression of mutant toxic proteins expressed from the partially degraded transcripts that accumulate in such mutants. For example, the disruption of translation in bacteria by streptomycin leads to the synthesis of toxic proteins (8). Lastly, it is likely that the screen described here has not been saturated, since only single representatives of two of the complementation groups were identified.

Yeast double-stranded RNA viruses and the *SKI* genes. Nearly all yeast strains contain various non-Mendelian genetic elements, including double-stranded RNA viruses such as L-A and M (59). These viruses apparently cannot transfect other yeast cells; they are transmitted horizontally by mating of haploid yeast cells. Nevertheless, these viruses display viral life cycles entirely within the cell (reviewed in reference 59). The L-A viral genome encodes two proteins, a Gag protein which provides the capsid protein of the viral particle and an RNA-directed RNA polymerase. The M virus, or killer virus, depends upon L-A for replication and packaging, since it lacks capsid and polymerase functions. The M virus encodes a killer toxin, the precursor form of which provides the cell with immunity to the mature secreted protein. Because killer strains of *S. cerevisiae*, those bearing the M virus, can kill yeast cells lacking the M virus, there is an environmental advantage to maintain the M virus.

Many nuclear yeast genes have been identified as controlling viral expression. These are the *MAK* (maintenance of killer) genes and the *SKI* (superkiller) genes (reviewed in reference 59). *SKI1*, *SKI2*, *SKI3*, *SKI4*, *SKI6*, *SKI7*, and *SKI8* were identified from genetic screens for mutants that lead to increased expression of killer toxin (48, 57). With the exception of *ski1* mutants, mutations in the *SKI* genes confer no phenotypes in the absence of killer virus but are essential for survival at low temperature in the presence of killer virus. Because of this apparent specificity to killer virus, the *SKI* genes are thought to comprise a cellular antiviral system (48, 59).

An *sep1/xrn1* null mutation also confers a superkiller phenotype in some strain backgrounds. Since the *ski1-1* mutation and the *SEP1/XRN1* gene map to the same position on chromosome VII and *ski1-1* and *sep1* mutants display common phenotypes, it is likely that *ski1-1* is an allele of *SEP1/XRN1*. However, the genetic confirmation of this has been ambiguous due to the weak phenotype of the *ski1-1* allele.

Sep1/Xrn1 and Ski2 in translation control. An important but poorly understood aspect of translation control is transcript degradation. Recently, a pathway for degradation of wild-type gene transcripts has been described in *S. cerevisiae* (9). Transcript turnover appears to be initiated by trimming the poly(A) tail down to a short oligo(A) tail. Subsequently, the 5′-m^{7}G cap is removed by a decapping enzyme, exposing the transcript to degradation by 5′-to-3′ exoribonucleases.

Sep1/Xrn1 was originally identified as a 5′-to-3′ exoribonuclease from a yeast ribosomal fraction in a search for RNA

degradative factors (52). Consistent with the view that Sep1/Xrn1 is important for RNA turnover, *sep1/xrn1* mutants show reduced rates of mRNA degradation (9, 39, 40) and an accumulation of uncapped unadenylated transcripts ranging from a twofold effect for the stable *PGK1* transcript to a six- to eightfold effect for the unstable *MFα1* transcript (24). In vitro, the exoribonuclease activity of Sep1/Xrn1 is blocked by the presence of a 5′-m^7G cap (51). Thus, Sep1/Xrn1 acts at a late step in transcript turnover, after the transcript has been targeted for degradation by deadenylation and decapping. Importantly, the uncapped, unadenylated RNAs that accumulate in *sep1/xrn1* mutants appear to persist in polysomes, suggesting that they are translated (24). In vivo translation of poly(A)-deficient mRNA (44) and in vitro cap-independent translation (25) have recently been found in *S. cerevisiae*. Such continued translation of transcripts targeted for degradation observed in *sep1/xrn1* mutants suggests that Sep1/Xrn1 plays an important role in translation control by removing these transcripts from the translation apparatus. The pleiotropy of *sep1* mutants may arise from the continued translation at inappropriate times in the cell cycle of mRNA targeted for degradation by decapping and deadenylation. Aberrant protein levels have been reported in *sep1/xrn1* mutants (34).

Ski2 also appears to be involved in translation control. Mutations in *SKI2* result in increased expression of killer toxin without increasing the levels of M double-stranded RNA encoding the toxin, suggesting that the wild-type gene product serves an antiviral role by blocking translation of the viral transcript (61). Interestingly, such control by *SKI2* was not observed when the M genome was transcribed by RNA polymerase II from a DNA plasmid clone (61). The virus is believed to be transcribed in the cytoplasm and apparently lacks a 5′ cap structure and a 3′ poly(A) tail (43), whereas the RNA polymerase II-transcribed RNA was expected to be polyadenylated and capped. Thus, the observed differential effects of a *ski2* mutation on the translation of viral transcripts versus plasmid transcripts suggests that Ski2 blocks translation on RNAs lacking 3′ poly(A) tracts or 5′ caps (61).

The substrate that Ski2 acts on, the uncapped unadenylated viral transcript, is reminiscent of the substrate of Sep1/Xrn1, an mRNA that has entered the degradation pathway and has been deadenylated and decapped. Since the lethality of *sep1 ski2* double mutants is independent of L-A and killer virus, Ski2 must have a more general role in the cell. We propose that both Ski2 and Sep1/Xrn1 act to block translation of transcripts that are targeted for degradation by deadenylation and decapping. Sep1/Xrn1 may block translation on such transcripts simply by degrading them. However, the uncapped unadenylated transcripts that accumulate in *sep1* mutants appear to remain in polysomes, suggesting that they are translated. Thus, Sep1/Xrn1 may compete with translation initiation factors for the 5′ ends of uncapped transcripts.

It is not yet clear how Ski2 functions to block translation. Perhaps Ski2 is a translation initiation factor that confers specificity for the 5′ cap. In this example, a lack of Ski2 function would relax the specificity of initiating on capped mRNAs. Alternatively, Ski2 may be important for the efficient translation of poly(A) mRNA (41), specifying translation on poly(A)-containing RNAs. Note that if Ski2 blocks translation of decapped and deadenylated transcripts, then it should act on all RNA polymerase II transcripts. However, the Ski2 effect was not seen when the M genome was transcribed from a plasmid cDNA clone (61). It is possible that Ski2 plays a minor role relative to Sep1/Xrn1 which is observed only in the absence of Sep1/Xrn1 or in the presence of killer virus. Lastly, Ski2 may affect translation indirectly by modifying ribosomal structure (61). Such an effect has been described for other putative RNA helicases (50). Considerably less is known about Ski3 and how it affects translation. Its apparent nuclear localization (47), however, would suggest an indirect role such as modifying ribosomal assembly in the nucleolus.

Synthetic cell cycle arrest in *sep1 ski2* and *sep1 ski3* mutants. Using a temperature-sensitive allele of *SEP1/XRN1*, we showed that *ski2 sep1-10^{ts}* and *ski3 sep1-10^{ts}* mutants arrested at Start similar to the arrest observed for the cell cycle mutants *cdc28* and *cdc37* (42) and other conditional mutants including RNA polymerase II (14) and *MAK16* (58). Because *sep1/xrn1* mutants have been shown to have modest effects on 5.8S RNA processing (20), it was conceivable that the arrest of *ski2 sep1* mutants arose from further disrupting rRNA processing. However, two findings suggest that this is not the case. First, yeast mutants defective for ribosome function arrest in early G_1 (23), unlike the late G_1 arrest observed for *ski2 sep1* mutants. Secondly, preliminary results indicate that the double mutant did not show any significant differences in rRNA species compared with the wild type (unpublished results).

Considering the probable role of Sep1/Xrn1 in RNA turnover (see above), there appear to be two general models that could explain this arrest. First, the arrest could be at a regulatory checkpoint, in response to a defect in some cellular process. Alternatively, there may be one or a small number of specific targets of Sep1/Xrn1 function that are critical for cell cycle progression past Start. For example, the double mutant may display stabilization of an mRNA species encoding a positive inhibitor of Start at the nonpermissive temperature. We are currently isolating suppressors of the *sep1 ski2* double mutant to better understand the mechanism of the arrest.

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