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Function of the Ski4p (Csl4p) and Ski7p Proteins in 3′-to-5′ Degradation of mRNA

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One of two general pathways of mRNA decay in the yeast *Saccharomyces cerevisiae* occurs by deadenylation followed by 3′-to-5′ degradation of the mRNA body. Previous results have shown that this degradation requires components of the exosome and the Ski2p, Ski3p, and Ski8p proteins, which were originally identified due to their superkiller phenotype. In this work, we demonstrate that deletion of the *SKI7* gene, which encodes a putative GTPase, also causes a defect in 3′-to-5′ degradation of mRNA. Deletion of *SKI7*, like deletion of *SKI2*, *SKI3*, or *SKI8*, does not affect various RNA-processing reactions of the exosome. In addition, we show that a mutation in the *SKI4* gene also causes a defect in 3′-to-5′ mRNA degradation. We show that the *SKI4* gene is identical to the *CSL4* gene, which encodes a core component of the exosome. Interestingly, the *ski4-1* allele contains a point mutation resulting in a mutation in the putative RNA binding domain of the Csl4p protein. This point mutation strongly affects mRNA degradation without affecting exosome function in rRNA or snRNA processing, 5′ externally transcribed spacer (ETS) degradation, or viability. In contrast, the *csl4-1* allele of the same gene affects rRNA processing but not 3′-to-5′ mRNA degradation. We identify *csl4-1* as resulting from a partial-loss-of-function mutation in the promoter of the *CSL4* gene. These data indicate that the distinct functions of the exosome can be separated genetically and suggest that the RNA binding domain of Csl4p may have a specific function in mRNA degradation.

Gene expression is a process that can be regulated at multiple steps. One important control point is the regulation of the decay rate of mRNA. In recent years two general pathways of mRNA decay in the yeast *Saccharomyces cerevisiae* have been characterized and some of the proteins required for these pathways have been identified (reviewed in reference 32). Both general pathways of mRNA degradation in yeast involve the shortening of the poly(A) tail as the initial step. Subsequently, the mRNA can be degraded in either the 5′-to-3′ direction or the 3′-to-5′ direction. Degradation in the 5′-to-3′ direction involves removal of ^{7m}GDP from the ^{7m}GpppN cap structure by the Dcp1p decapping enzyme. Following decapping the remaining mRNA is rapidly digested by the 5′-to-3′ exonuclease Xrn1p. In the 3′-to-5′ pathway the mRNA is degraded by a complex of 3′-to-5′ exonucleases named the exosome (17).

Although the pathways of mRNA decay in other eukaryotes have not been worked out in detail, it appears likely that both 5′-to-3′ and 3′-to-5′ degradation of mRNA operates in other eukaryotes (reviewed in reference 32). Although both general pathways of mRNA decay are probably conserved in other eukaryotes, it appears likely that some organisms, cell types, or mRNAs may preferentially use one or the other pathway. Indeed, yeast cells appear to degrade the majority of their mRNA through the 5′-to-3′ pathway. It has been estimated that in wild-type cells the 5′-to-3′ degradation of the PGK1 mRNA is approximately twofold faster than 3′-to-5′ degradation (25).

The 3′-to-5′ mRNA degradation pathway requires the exosome (17). The exosome is a protein complex that contains multiple 3′-to-5′ exoribonucleases and RNA binding proteins (1, 23). The exosome is also required for a number of nuclear RNA 3′ processing reactions (2, 7, 22, 34). The exosome is located both in the nucleus and in the cytoplasm (1, 19, 23, 37). Therefore, it appears likely that the exosome contains the nuclease(s) that carries out both the mRNA degradation and the RNA processing reactions for which it is required. This raises several questions such as how the exosome recognizes the wide variety of its substrates and why it processes some RNAs to shorter forms while it completely degrades others. One possible explanation is that the exosome requires specific additional proteins to act on the various substrates. Candidates for these proteins include the products of the *MTR4* and *SKI2* genes. The *MTR4* gene encodes a nuclear RNA helicase that is required for the processing of a variety of RNA species by the exosome (2, 13, 34), and *SKI2* encodes a homologous cytoplasmic RNA helicase that is required for 3′-to-5′ mRNA degradation (10, 17).

The *SKI* genes were first identified because mutations in them cause a "superkiller" phenotype (31). Ski2p was later found to be in a cytoplasmic complex that also contains Ski3p and Ski8p (10), and all three proteins are required for 3′-to-5′ mRNA decay by the exosome (17). Similarly, Ski6p was found to be one of the 3′-to-5′ exonucleases in the exosome (23), and mutations in *SKI6* inhibit 3′-to-5′ degradation of mRNA (17). Therefore mutations in at least four *SKI* genes affect 3′-to-5′ degradation of mRNA. In contrast, *SKI1* encodes the Xrn1p enzyme involved in 5′-to-3′ mRNA decay (18).

Superkiller strains are yeast strains that are especially effective in killing other yeast strains. Many yeast strains contain killer viruses that encode a secreted toxin and kill strains not infected by the virus. The killer virus replicates as an RNA molecule that lacks both a cap and a 3′ poly(A) tail (reviewed in references 35 and 36). In these respects this RNA is similar to cellular mRNA that has been deadenylated and decapped and is about to be completely degraded. The superkiller phe-

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notype is obtained when either pathway of mRNA degradation is blocked. One simple explanation of these observations is that in the absence of either degradation pathway the uncapped unadenylated killer toxin mRNA is more stable and therefore more killer toxin protein is produced per killer toxin mRNA. Mutations in the *SKI2*, *SKI3*, *SKI6*, *SKI7*, and *SKI8* genes also result in increased production of luciferase activity from unadenylated luciferase RNA introduced into yeast by electroporation (7, 8, 21). It was proposed that this increased luciferase production is caused by a reduced ability of the translation machinery of *ski* mutant cells to distinguish between polyadenylated and unadenylated mRNA. In this case, the defect in 3′-to-5′ mRNA degradation might be a secondary effect of the defect in translation. Alternatively, the difference in luciferase production in the electroporation experiments might be a result of the decreased degradation of mRNA in *ski* mutants. The latter possibility is supported by the observation that the increased luciferase production is at least partially due to an increased functional half-life of the luciferase mRNA (7, 8, 21).

To further characterize the role of the *SKI* genes, we have analyzed the effects of the *ski4* and *ski7* mutations on 3′-to-5′ mRNA degradation. The possible roles of Ski4p and Ski7p in mRNA decay are largely unexplored. The *SKI4* gene remains unidentified, and beyond the superkiller phenotypes they produce little is known about the effect of *ski4* mutations. *SKI7* has recently been cloned and was found to encode a putative GTPase (8). Mutations in the *SKI7* gene resemble *ski2*, *ski3* and *ski8* mutations in two aspects. First, deletion of any of these four genes does not have a detectable effect on growth (except at very low temperatures, e.g., 8°C). Second, all four deletions affect the yield of luciferase activity when capped but unadenylated luciferase mRNA is introduced into yeast cells by electroporation (8, 21). In contrast to the common phenotypes of *ski2*, *ski3*, *ski7*, and *ski8* mutants is the observation that the Ski2p, Ski3p, and Ski8p proteins form a complex. This complex does not appear to include Ski7p, nor is Ski7p required for complex formation (10). The latter observation may suggest that the roles of Ski7p and the Ski2p-Ski3p-Ski8p complex might be different.

In the experiments described here we address the roles of both Ski4p and Ski7p in cellular mRNA degradation. Both proteins are required for 3′-to-5′ degradation of yeast mRNA. We have also identified the *SKI4* gene and found that it is allelic to *CSL4*, which encodes one of the subunits of the exosome. The *ski4-1* mutation appears to block the degradation of mRNA by the exosome but has little or no effect on other exosome reactions, suggesting possible roles for Csl4p/Ski4p in 3′-to-5′ degradation of mRNA.

MATERIALS AND METHODS

Yeast strains. The genotypes of the strains used are in Table 1. The *ski4-1* strain 2373 was generously supplied by R. Wickner. The *CSL4* gene from this strain was amplified by PCR using primers oRP943 (ATGAGCTTATGGTAC GGCATG) and oRP944 (GTTTAATCACGTTCCCGCTTC). The products from two independent PCRs were gel purified and sequenced directly using the same oligonucleotides. Sequence analysis from −80 of the start codon to +100 of the stop codon revealed eight nucleotide differences compared to the sequence deposited in the yeast data base (SGD; http://genome-www.stanford.edu /Saccharomyces/; G-3-5 to C, T-5 to C, G194 to A, T199 to C, G258 to A, C363 to T, T540 to C, and G758 to A, numbered from the AUG start codon). These changes result in two changes in the predicted amino acid sequence as detailed in Results. The mutation that alters glycine 253 also results in the destruction of a recognition site for restriction endonuclease *Sty*I.

The *ski4-1* mutation was introduced into the yRP840 genetic background by repeated backcrossing. In these backcrosses the segregation of the *ski4-1* allele was determined either by Northern blotting or by PCR amplification of the *CSL4* gene followed by either sequencing or *Sty*I digestion.

The *ski7*Δ mutation was introduced into the yRP840 genetic background by PCR amplification and homologous recombination. The *ski7*Δ::*NEO* cassette from a *ski7*Δ strain obtained from Research Genetics (record no. 1852) was amplified using oligonucleotides oRP922 (TAGCGTCCTCAGCTGTCAC) and oRP923 (GTGTCACAATCTGCTCCCG). The PCR product was gel purified and used to transform (R. Agatep, R. D. Kirkpatrick, D. L. Parchaliuk, R. A. Woods, and R. D. Gietz, Technical tips online [http://tto.trends.com], 1998) a yRP840/yRP841 diploid. Transformants were selected on yeast extract-peptone-dextrose plates containing 150 mg of Geneticin/liter. The resulting diploid transformants were sporulated, and *ski7*Δ::*NEO* haploids were selected. Successful gene disruption was confirmed by PCR analyses of the resulting haploids.

The mutation in *csl4-1* was identified by PCR amplification of *csl4-1* DNA using oligonucleotides 40-519 (CGACACTTATGGAGAATTCG) and 40-1053b (TTCCGTACCGTACTGTGGGC) followed by direct DNA sequencing of the purified PCR product. To confirm that this substitution caused the *csl4-1* phenotype, the C-172T mutation was introduced into a wild-type *CSL4*/*SKI4* strain by "pop-in/pop-out" gene replacement (28). A *CSL4*/*SKI4* integration plasmid analogous to pRB289 except containing the C-172T substitution was constructed as described previously (5). The plasmid was then cleaved with *Nru*I and introduced into a wild-type *CSL4*/*SKI4* strain (pop-in); URA3$^+$ transformants were selected, and correct integration at the *CSL4*/*SKI4* locus was verified by PCR of genomic DNA. The C-172T mutation was detected by the presence of a linked *Bgl*II restriction fragment length polymorphism (RFLP) located at position −115. One of the C-172T integrants was grown nonselectively and plated on 5-fluoroorotic acid to select plasmid pop-outs, which were then screened for retention of the mutation by *Bgl*II analysis of PCR-amplified genomic DNA. (The *Bgl*II RFLP by itself has no detectable phenotype.)

The *CSL4*::*lacZ* reporter strain V15E4 was obtained from M. Snyder (27), and the *URA3* marker was changed to *LEU2* (5). The C-172T mutation (*csl4-1*) was introduced by pop-in–pop-out recombination as described above. A *cep1*::*URA3* gene disruption in the V15E4 genetic background was obtained by one-step gene replacement as described previously (4). Haploid segregants of *csl4-1*::*LacZ* and *cep1*::*URA3* were obtained by sporulating the parental diploids. A *CSL4*/*SKI4* plasmid (pRB306) (5) was introduced into the diploids before sporulation to provide *CSL4*/*SKI4* function to *cls4-1*::*LacZ* segregants.

Double-mutant strains were made by crossing the respective single-mutant strains, sporulating the resulting diploids, and dissecting tetrads.

Plasmids. pRP1000 (i.e., pCSL4) was constructed by digestion of pRB289 with *Xba*I and *Xho*I (5) and isolation of the 1.5-kb fragment. This fragment was ligated into CEN URA3 plasmid pRS416 (29), also digested with *Xba*I and *Xho*I. The resulting plasmid was sequenced to confirm its identity. The sequence proved identical to the one deposited in SGD. Two independent constructions of this plasmid were tested and gave identical results.

pRP1001 (i.e., pcsl4-G253E) was constructed from pRP1000 by site-directed mutagenesis as described by Kunkel et al. (20) using the antisense oligonucleotide oRP977 (GCTCTGGCGAACACGACC**T**CAAGGTCATTCC [mutation in boldface]). This mutation is identical to the one in *ski4-1* that changes G253 to E and destroys a *Sty*I site. Resulting plasmids were screened using digestion with *Sty*I. Sequencing was used to confirm that the plasmid contained the desired mutation and no other mutations. Two independent constructions of this plasmid were tested and gave identical results.

The plasmids encoding MFA2pG and PGK1pG have been described previously (13, 24). They were introduced for the experiment shown in Fig. 1 into strain 2373 using standard transformation procedures (Agatep et al., http://tto.trends.com). For the experiment shown in Fig. 9 the MFA2pG plasmid was introduced into *csl4-1* strain R95-1-1, which already contained plasmid pAP2, and into isogenic *CSL4* strain BM3-40a (5).

RNA isolation and analyses. mRNA and fragment half-lives were determined in duplicate as described by Jacobs Anderson and Parker. (17). The half-lives given in Fig. 2 are for the experiment shown. A duplicate experiment gave identical half-lives. Similarly, the half-lives given in Fig. 4 are for the experiment shown, and a duplicate experiment yielded essentially the same results (wild type, 5 min; *ski7*Δ strain, 4 min; *ski4-1* strain, 3 min; *dcp1-2* strain, 7 min; *dcp1-2 ski7*Δ strain, 38 min; *dcp1-2 ski4-1* strain, >90 min). RNA isolation and Northern blotting were done as described previously (34). The oligonucleotides used as probes were as follows: MFA2pG, oRP140 (ATATTGATTAGATCAGGAAT TCC); PGK1pG, oRP141 (AATTGATCTATCGAGGAATTCC); signal recognition particle (SRP), oRP100 (GTCTAGCCGCGAGGAAGG); 7S pre-rRNA, oJA003 (TGAGAAGGAAATGACGCT); 5.8S rRNA, oRP924 (TTTCGCTGC GTTCTTCATC); pre-U4 snRNA, oRP768 (CAGTCCCTTTGAAAGAATGA AT); U4 snRNA, oRP756 (CGGACGAATCCTCACTGATA); 5′ externally transcribed spacer (ETS), oRP993 (CGAACGACAAGCCTACTCG).

Localization of Csl4p/Ski4p. The *GFP-CSL4* allele was constructed by inserting DNA encoding green fluorescent protein (GFP)-Bex1 (3) into the naturally occurring *Sph*I site at codon 2 of *CSL4*/*SKI4*. The GFP-Bex1 insert was generated by PCR using primers containing in-frame *Sph*I sites. The *GFP-CSL4* allele was introduced into yeast by pop-in/pop-out recombination as described above, replacing the endogenous gene. Localization of GFP fused to Ski4-1p was done using a *ski4-1* strain as the starting strain for the pop-in/pop-out recombination. The pop-out recombination yielded isogenic *SKI4*::*GFP* and *ski4-1*::*GFP*, depending on the site of resolution of the Holliday junction. *SKI4*::*GFP* and *ski4-1*::*GFP* isolates were identified by PCR and *Sty*I digestion as described

TABLE 1. Strains used in this study

Strain	Genotype	Source or reference
2373	*MAT***a** *ura3 ski4-1*	
BM3-40A	*MAT***a** *leu2 lys2 ura3 his4-580 ade3 cep1*Δ *cyh2 cry1 ade2-1 tyr1 SUP4-3 csl4-1* [*CEP1/LEU2/ADE3*]	5
R95-1-1	*MAT***a** *leu2 lys2 ura3 his4-580 ade3 cep1*Δ *cyh2 cry1 ade2-1 tyr1 SUP4-3* [*CEP1/LEU2/ADE3*]	5
p170	*MAT***a** *trp1 leu2*Δ*1 ura3 his3*Δ*200 gal2 gal108 csl4*::*HIS3*::*GAL*::*csl4*	1
6000	*MAT***a** *leu2*Δ*0 ura3*Δ*0 his3*Δ*1 met15*Δ*0 hbs1*Δ::*NEO*	Research Genetics
BY4742	*MAT*α *leu2*Δ*0 ura3*Δ *his3*Δ*1 lys2*Δ*0*	Research Genetics
V15E4	*MAT***a**/*MAT***a** *leu2-98/leu2-98 cry1*^{R}/*CRY1 ade2-101/ade2-101 HIS3/his3-200 ura3-52/ura3-52 lys2-801/lys2-801 can1*^{R}/*CAN1 trp1-1/TRP1 CYH2/cyh2*^{R} *CSL4/CSL4*::*mTn-3xHA-LacZ* [*Cir*^{0}]	27
yRP840	*MAT***a** *leu2-3,112 his4-539 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG*	16
yRP841	*MAT***a** *leu2-3,112 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG*	16
yRP1195	*MAT***a** *leu2-3,112 his4-539 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski2*Δ::*LEU2*	17
yRP1070	*MAT***a** *leu2-3,112 his4-539 trp1 ura3 cup1*::*LEU2/PGK1pG/MFA2pG dcp1*Δ::*URA3*	6
yRP1204	*MAT***a** *leu2-3,112 his4-539 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski6*Δ::*URA3* [*ski6-100/LYS2*]	17
yRP1340	*MAT***a** *leu2-3,112 his4-539 lys2-201 trp1 ura3 dcp1-2*::*TRP1*	14
yRP1346	*MAT***a** *leu2-3,112 his4-539 lys2-201 trp1 ura3 cup1*::*LEU2/PGK1pG/MFA2pG dcp2*Δ::*TRP1*	14
yRP1501	*MAT*α *leu2-3,112 lys2-201 trp1 ura3-52 dcp2-7*::*URA3*	14a
yRP1515	*MAT*α *leu2-3,112 his4-539 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG dcp1-2*::*TRP1*	Dunkley and Parker
yRP1516	*MAT***a** *leu2-3,112 his4-539 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG dcp2-7*::*URA3*	Dunkley and Parker
yRP1533	*MAT*α *trp1 leu2-3,112 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski7*Δ::*NEO*	This study
yRP1534	*MAT*α *leu2-3,112 his4-539 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski2*Δ::*LEU2 ski3*Δ::*TRP1 ski7*Δ::*NEO ski8*Δ::*URA3*	This study
yRP1535	*MAT***a** *leu2-3,112 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG dcp1-2*::*TRP1 ski4-1*	This study
yRP1536	*MAT*α *leu2-3,112 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG dcp1-2*::*TRP1 ski7*Δ::*NEO*	This study
yRP1537	*MAT***a** *leu2-3,112 his4-539 lys2-201 trp1 ura3-52 dcp2-7*::*URA3 ski4-1*	This study
yRP1538	*MAT*α *leu2-3,112 his4-539 lys-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG dcp2-7*::*URA3 ski7*Δ::*NEO*	This study
yRP1539	*MAT***a** *leu2-3,112 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski4-1*	This study
yRP1540	*MAT***a** *leu2-3,112 his4-539 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski4-1*	This study
yRP1541	*MAT***a** *leu2-3,112 his4-539 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski4-1* [*URA3*]	This study
yRP1542	*MAT***a** *leu2-3,112 his4-539 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski4-1* [*CSL4/URA3*]	This study
yRP1543	*MAT*α *leu2-3,112 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG* [*URA3*]	This study
yRP1544	*MAT*α *leu2-3,112 lys2-201 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG* [*CSL4/URA3*]	This study
yRP1545	*MAT***a** *leu2-3,112 his4-539 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski4-1* [*csl4-G253E/URA3*]	This study
yRP1570	*MAT*α *leu2-3,112 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG ski4-1*::*GFP*	This study
yRP1571	*MAT*α *leu2-3,112 trp1 ura3-52 cup1*::*LEU2/PGK1pG/MFA2pG SKI4*::*GFP*	This study
R314-3B	*MAT***a** *ade2-1 his4-3 lys2*^{0} *trp1*^{a} *SUP4-3*^{ts} *ura3 CSL4*::*GFP*	This study
R318	*MAT***a**/*MAT*α *leu2-98/leu2-98 cry1*^{R}/*CRY1 ade2-101/ade2-101 HIS3/his3-200 ura3-52/ura3-52 lys2-801/lys2-801 can1*^{R}/*CAN1 trp1-1/TRP1 CYH2/cyh2*^{R} *CSL4/csl4-1*::*mTn-3xHA-LacZ cep1*::*URA3/CEP1* [*Cir*^{0}] [*URA3/CSL4*]	This study

above. The steady-state MFA2pG mRNA phenotypes of *SKI4*::*GFP* and *ski4-1*::*GFP* were indistinguishable from those of *SKI4* and *ski4-1* strains, respectively.

For microscopy, *GFP-CSL4* cells were grown in yeast extract-peptone (YEP) medium containing 2% glucose to a cell density of 0.5×10^7 to 1×10^7 cells/ml and fixed by the addition of 1/10 volume of 37% formaldehyde. After continued incubation for 45 min under growth conditions, the fixed cells were washed once in standard phosphate-buffered saline (PBS), incubated on ice for 15 min in ethanol containing 1 μg of 4′,6′-diamidino-2-phenylindole (DAPI)/ml, washed twice with PBS, resuspended in PBS, and kept on ice. Microscopy was performed either with a Nikon microscope equipped with epifluorescence optics and charge-coupled device (CCD) camera (Santa Barbara Instrument Group) or with a Leica microscope and Hamamatsu CCD camera. Collected images were adjusted for contrast and brightness and colorized using Adobe Photoshop.

RESULTS

The *ski4-1* and *ski7*Δ lesions affect the metabolism of an mRNA fragment. In order to determine whether the *ski4* and *ski7* mutants affected 3′-to-5′ mRNA degradation, we first examined the effect of the *ski4-1* allele and a *ski7*Δ mutant on the degradation of an mRNA fragment. In these experiments we used two reporter mRNAs, PGK1pG and MFA2pG. Each of these mRNAs contains a poly(G) tract inserted into its 3′ untranslated region. This poly(G) tract forms a stable secondary structure that effectively blocks 5′-to-3′ degradation by Xrn1p (24, 26). Thus, 5′-to-3′ degradation of these mRNAs produces an intermediate that stretches from the 5′ end of the poly(G) tract to the 3′ end of the mRNA (Fig. 1A). This fragment is normally degraded by the 3′-to-5′ mRNA degradation pathway (17). Therefore, mutants that are defective in 3′-to-5′ decay accumulate higher levels of this fragment and show a slow digestion from the 3′ end, leading to the appearance of a ladder phenotype (17). As shown in Fig. 1B, both the *ski4-1* allele and the *ski7*Δ mutant cause alterations in the amount and structure of the MFA2pG fragment similar to those seen in the *ski2*Δ mutant known to affect 3′-to-5′ mRNA turnover (similar data for PGK1pG not shown). This observation strongly suggests that Ski4p and Ski7p are involved in 3′-to-5′ degradation of mRNA.

If Ski7p acts in the same pathway as Ski2p, Ski3p, and Ski8p, then the deletion of the *SKI7* gene and deletion of the *SKI2*, *SKI3*, or *SKI8* gene should not have additive effects. As shown in Fig. 1B the phenotype of the *ski2*Δ *ski3*Δ *ski7*Δ *ski8*Δ quadruple mutant is no more severe than the phenotype of the *ski2*Δ single-mutant strain (additional data not shown). This observation suggests that the Ski7p protein acts in the same

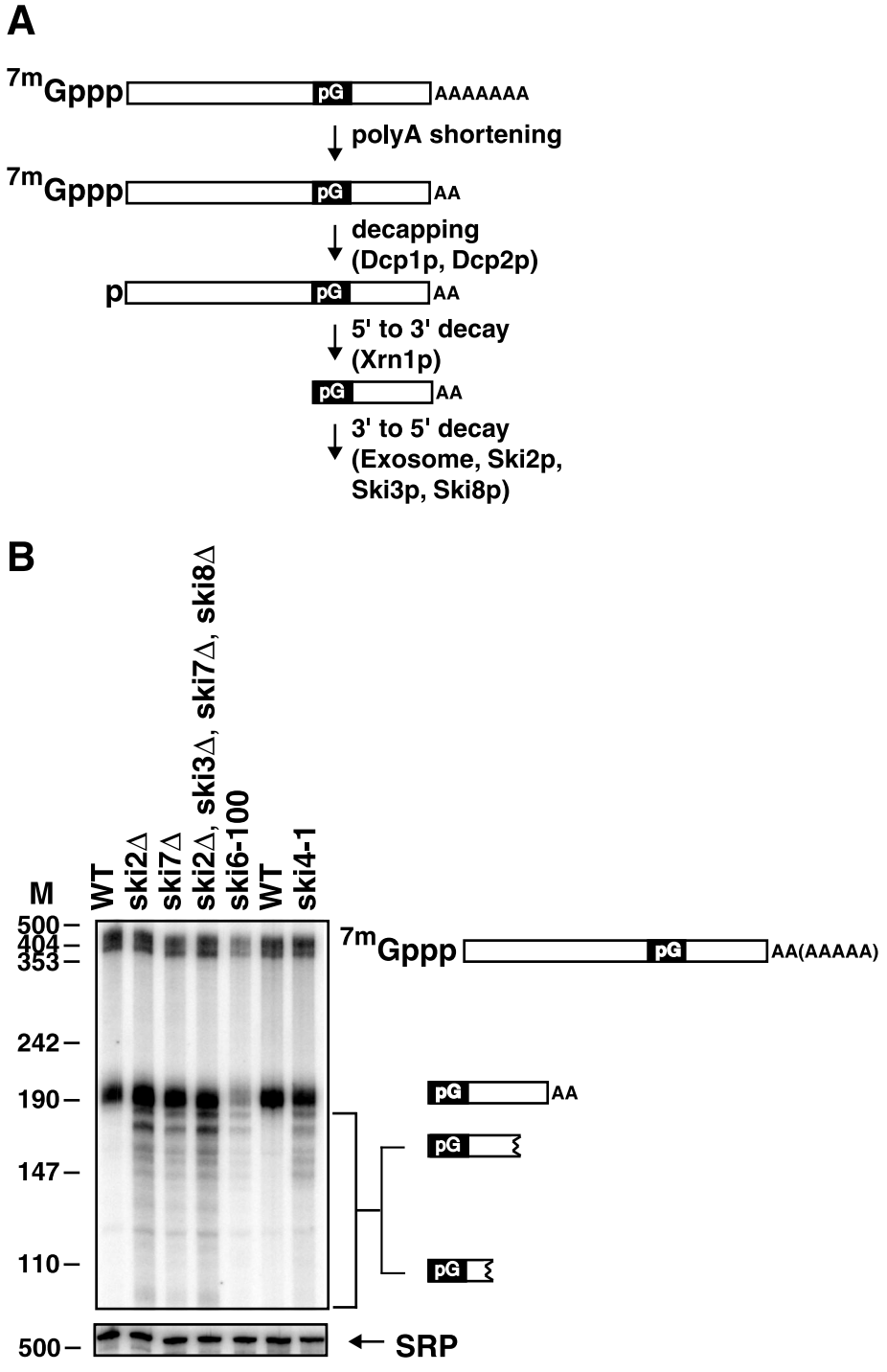

FIG. 1. The *ski4-1* and *ski7*Δ mutations affect the metabolism of a degradation intermediate of MFA2pG mRNA. (A) The degradation of MFA2pG mRNA through the 5′-to-3′ pathway is initiated by decapping, which requires Dcp1p and Dcp2p. Decapping is followed by digestion by the 5′-to-3′ exonuclease Xrn1p. This 5′-to-3′ exonuclease cannot proceed through a stable secondary structure formed by the poly(G) insert. The resulting poly(G)-to-3′-end fragment is therefore degraded by the exosome in a process that requires Ski2p, Ski3p, and Ski8p. (B) The metabolism of the poly(G)-to-3′-end fragment of MFA2pG is altered in *ski4-1* and *ski7*Δ strains. Shown is a polyacrylamide Northern blot of the indicated strains probed with an oligonucleotide that hybridizes just 3′ of the poly(G) insert of MFA2pG mRNA. The cartoons to the right of the Northern blot indicate the identities of the various species detected. The first five lanes (from the left) contain RNA from strains containing the MFA2pG gene integrated into the genome grown in YEP containing 2% Gal. The MFA2pG reporter was introduced on a plasmid into a *ski4-1* strain. To maintain this plasmid, the *ski4-1* strain used for the last lane was grown in medium lacking uracil but containing 2% Gal. The wild-type (WT) strain in the sixth lane was grown in the same medium supplemented with uracil. All strains were grown at 30°C with the exception of the *ski6-100* strain, which was grown at 24°C and shifted to 37°C for 2 h. The length of *Hpa*II fragments of pUC18 is given in nucleotides. M, nucleotides.

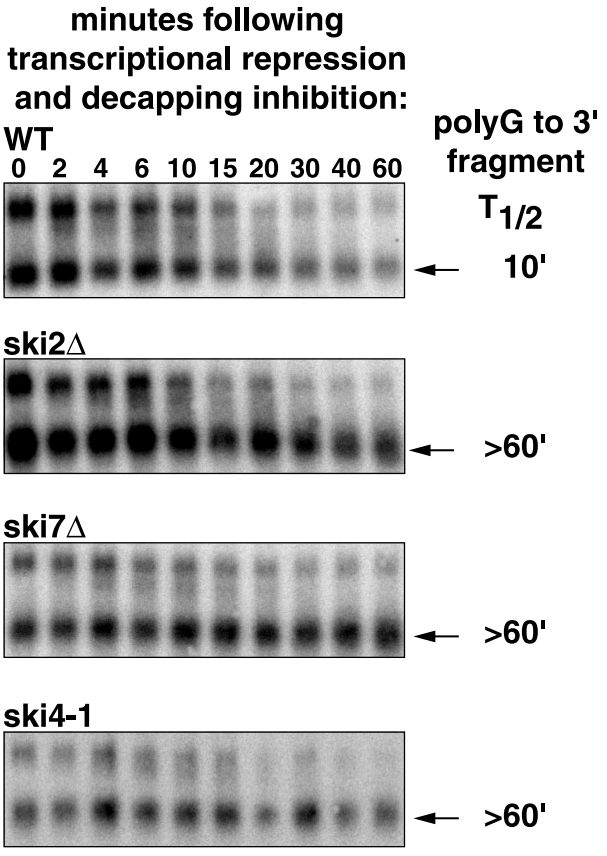

FIG. 2. The *ski4-1* and *ski7*Δ mutations stabilize a degradation intermediate of MFA2pG mRNA. Shown are agarose Northern blots of the indicated strains with the poly(G)-to-3′-end degradation intermediate of the MFA2pG mRNA (arrows). Each strain was grown in YEP containing 2% Gal. At the beginning of the experiment glucose and cycloheximide were added to inhibit transcription from the Gal promoter and the decapping enzyme, respectively. Samples were harvested at the indicated times after the beginning of this treatment and analyzed. The signals were quantitated using a phosphorimager and corrected for loading using the SRP RNA. Half-lives were calculated and are indicated on the right.

pathway of 3′-to-5′ degradation of mRNA as Ski2p, Ski3p, and Ski8p.

In order to confirm that the *ski4* and *ski7* lesions affected 3′-to-5′ degradation of the poly(G)-to-3′-end mRNA fragment, we directly measured its decay rate. In this experiment we monitored the loss of the decay fragment over time after blocking the production of new fragment. We utilized a combination of cycloheximide and glucose to inhibit production of fragment. Cycloheximide is known to inhibit decapping rapidly (6, 17), while glucose represses transcription from the Gal promoter. We used a *ski2*Δ strain as a control in this experiment, because *SKI2* was previously shown to be required for the normal 3′-to-5′ degradation of the poly(G)-to-3′-end fragment (17). As shown in Fig. 2, like the *ski2*Δ lesion, both the *ski4-1* and *ski7*Δ lesions caused a decrease in the rate at which the MFA2 fragment is degraded, leading to an increase in its half-life from about 10 min to more than 60 min. Similar results were obtained for the PGK1pG mRNA (data not shown), indicating that this effect is not specific for the MFA2 mRNA. Therefore, like Ski2p, Ski3p, and Ski8p, Ski4p and Ski7p are likely to affect the 3′-to-5′ degradation of a wide variety of mRNAs.

The *ski4-1* and *ski7*Δ lesions are synthetically lethal with mutations affecting decapping. The above results suggested that the *ski4-1* and *ski7*Δ lesions affected 3′-to-5′ mRNA degradation. One property of mutations affecting this process is that they are synthetically lethal with lesions that block the other major decay pathway of decapping and 5′-to-3′ degradation (17; T. Dunckley and R. Parker, personal communication). In order to test if *ski4-1* and *ski7*Δ mutations are synthetically lethal with mutations blocking 5′-to-3′ decay, we crossed the *ski4-1* and *ski7*Δ strains to *dcp1*Δ and *dcp2*Δ strains, which are defective in decapping. In all four crosses we observed a reduced viability of spores and failed to recover any double mutants. This suggests that the *ski4-1* and *ski7*Δ mutations are synthetically lethal with the *dcp1*Δ and *dcp2*Δ alleles. We confirmed this conclusion by crossing both the *ski4-1* and *ski7*Δ strains to strains containing temperature-sensitive alleles of either *DCP1* or *DCP2* (i.e., *dcp1-2* and *dcp2-7* [14a, 30]). Because the 5′-to-3′ decay pathway is not essential in our strain background, these temperature-sensitive alleles of *DCP1* and *DCP2* do not by themselves affect viability but are synthetically lethal at the restrictive temperature with mutations affecting 3′-to-5′ decay of mRNA (14a, 17). In crosses of *dcp1-2* or *dcp2-7* strains with *ski4-1* or *ski7*Δ strains we were able to recover double mutants by germinating the spores at low temperature (i.e., 23°C). In each case these double mutants were unable to grow at the temperature restrictive for the relevant *dcp* mutation (Fig. 3). This conditional lethality supports a role of the Ski4p and Ski7p proteins in 3′-to-5′ degradation of mRNA.

The *ski4-1* and *ski7*Δ lesions affect the rate of 3′-to-5′ mRNA degradation. The *dcp1-2 ski4-1* and *dcp1-2 ski7*Δ double-mutant strains allowed us to examine if the *ski4-1* and *ski7*Δ alleles affect the 3′-to-5′ decay of a full-length mRNA by comparing them to a *dcp1-2* strain. In a *dcp1-2* strain mRNA degradation at the restrictive temperature occurs (almost) exclusively through the 3′-to-5′ pathway (17). To measure mRNA decay rates in the double mutants, these strains were grown at the permissive temperature and shifted to a restrictive temperature for 1 h. Subsequently, transcription of the reporter MFA2pG mRNA was inhibited by addition of glucose, allowing monitoring of the loss of mRNA over time. Both the *ski4-1* and *ski7*Δ mutations cause a severe decrease in the rate of degradation of the MFA2pG mRNA under these conditions (Fig. 4). The half-life of the MFA2pG mRNA increased from 7 min in the *dcp1-2* strain to 36 and more than 90 min in the *dcp1-2 ski7*Δ and *dcp1-2 ski4-1* strains, respectively. This implies that the *SKI4* and *SKI7* gene products are required for the 3′-to-5′ degradation of mRNA.

Based on all of these results we conclude that the Ski4p and Ski7p proteins are required for 3′-to-5′ mRNA degradation as are the components of the exosome and the Ski2p, Ski3p, and Ski8p proteins.

The *SKI4* gene is identical to the *CSl4* gene. In order to understand the function of the Ski4p protein in 3′-to-5′ degradation, it was important to examine the properties of the encoded gene product. The *SKI4* gene has not been cloned. However, we hypothesized that the *SKI4* gene might be identical to the *CSL4* gene for two reasons. First, the mapping of the *ski4-1* allele placed it within a few map units of the *KEX2* gene (31), which is about 10 kb from the *CSL4* gene. Second, the Csl4p protein is a component of the exosome (1), and therefore defects in this protein might be expected to affect 3′-to-5′ mRNA degradation.

In order to test if the *CSL4* gene was identical to the *SKI4* gene, we first determined if a plasmid carrying the wild-type *CSL4* gene would complement the defect in mRNA decay in a *ski4-1* strain. We constructed a low-copy-number plasmid containing *CSL4* and introduced it into a *GAL*::*csl4* strain. The

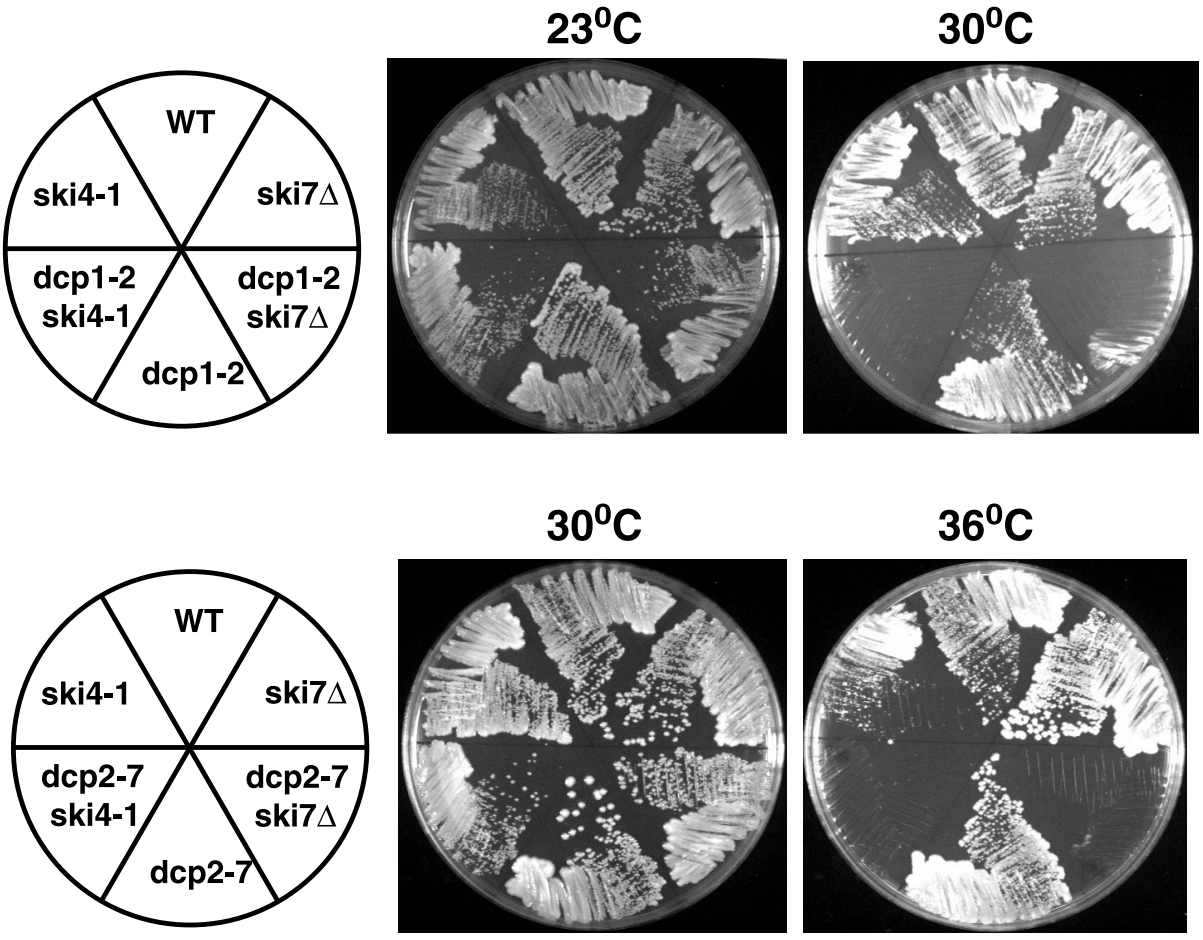

FIG. 3. Strains containing either *ski4-1* or *ski7*Δ in combination with *dcp1-2* or *dcp2-7* are not able to grow under conditions restrictive for the decapping defect. Shown are plates of YEP–2% glucose containing the indicated strains grown at the indicated temperatures.

GAL::*csl4* gene is not expressed in the presence of glucose. Since the *CSL4* gene is an essential gene, the *GAL*::*csl4* strain cannot grow on plates containing glucose (1). The low-copy-number *CSL4* plasmid complemented this conditional growth defect (Fig. 5A), indicating that the plasmid carried a functional *CSL4* gene. As shown in Fig. 5B, this plasmid also complements the mRNA decay defect of a *ski4-1* strain, indicating that *SKI4* and *CSL4* are the same gene. In addition the same plasmid complements the temperature-sensitive growth defect of a *dcp1-2 ski4-1* strain (Fig. 5C). These results, in combination with the prior linkage data strongly argue that the *ski4-1* allele represents a mutation in the *CSL4* gene.

To further test the hypothesis that *ski4-1* and *csl4* are allelic and to identify the specific sequence change in *ski4-1*, we amplified the *CSL4* gene from the *ski4-1* strain by PCR. Sequence analysis of two independent PCRs each revealed that there were two amino acid residues changed in Csl4p. One of these (R65K) is a conservative substitution in the N-terminal part of the Csl4p. This N-terminal domain is apparently not conserved in any of the Csl4p homologs. The other amino acid change (G253E) affects a glycine that is conserved in Csl4p homologs from mammals, *Schizosaccharomyces pombe*, and plants and is part of the S1 RNA binding domain (Fig. 6A). We suspected that the latter change is responsible for the phenotypes of *ski4-1*. We tested this hypothesis by introducing a coding sequence change producing the G253E change into a plasmid containing a wild-type *CSL4* gene. This pcsl4-G253E plasmid no longer was able to complement the RNA phenotype of a *ski4-1* strain (Fig. 6B). Therefore, we conclude that the G253E change is responsible for the mRNA degradation defect of *ski4-1*. Based on the mapping, complementation, and sequencing data we conclude that the *ski4-1* allele represents a mutation in the *CSL4* gene.

The *ski4-1* allele genetically separates the functions of the exosome in rRNA processing and mRNA decay. The Csl4p/Ski4p protein is a component of the exosome and is essential for viability (1, 5). Depletion of this protein from cells leads to a variety of defects including defects in 5.8S rRNA and U4 snRNA processing (1) (data not shown). Moreover, Csl4p/Ski4p copurifies with two different complexes that presumably correspond to the nuclear and cytoplasmic exosomes (1). That Csl4p/Ski4p is required for 5.8S rRNA processing reactions explains why *CSL4*/*SKI4* is an essential gene. All these data suggest that Csl4p/Ski4p functions in both the nucleus and cytoplasm; however, we sought to confirm this hypothesis by localizing the Csl4p/Ski4p protein. We generated an N-terminal fusion of GFP to Csl4p/Ski4p. The *GFP*::*CSL4* gene was integrated into the genome at the *CSL4* locus under the control of the *CSL4* promoter and 3′ untranslated region (see Materials and Methods). The resulting strains containing *GFP-CSL4* as the sole source of Csl4p were viable and showed no detectable mRNA phenotype, indicating that the fusion pro-

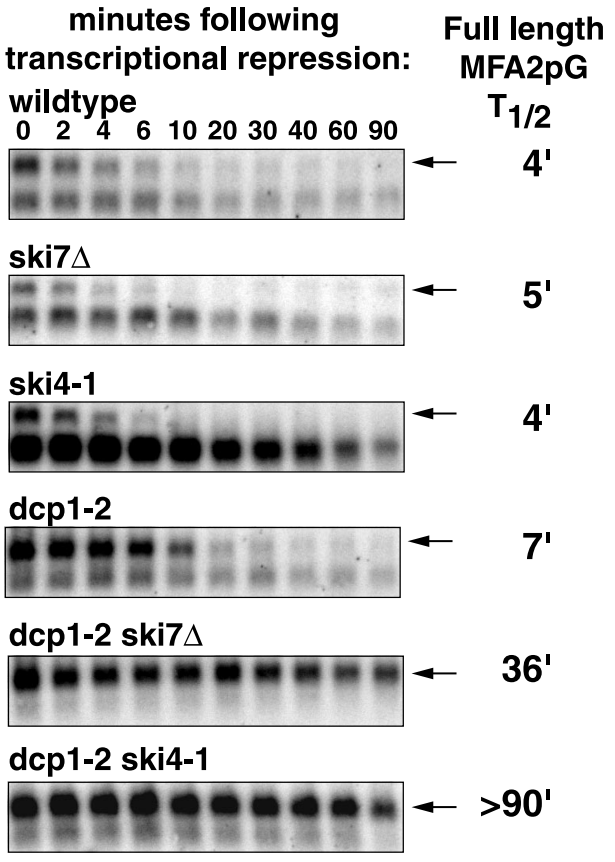

FIG. 4. The *ski4-1* and *ski7*Δ mutations inhibit 3′-to-5′ degradation of the MFA2pG mRNA. In a *dcp1-2* strain at the restrictive temperature mRNA degradation occurs (almost) exclusively 3′ to 5′. Shown are agarose Northern blots of the indicated strains with the MFA2pG mRNA (arrows). Each strain was grown in YEP containing 2% Gal at 24°C and incubated for 1 h at 37°C. After this incubation, glucose was added to inhibit transcription from the Gal promoter. Samples were harvested at the indicated times after the addition of glucose and analyzed. The signals were quantitated using a phosphorimager and corrected for loading using the SRP RNA. Half-lives were calculated and are indicated on the right.

tein was functional. The nucleus was highly fluorescent, and some fluorescence was also observed in the cytoplasm (Fig. 7). Within the nucleus, most of the fluorescence was in a region adjacent to the region stained with DAPI. This region of high fluorescence most likely corresponds to the nucleolus. We conclude that Csl4p/Ski4p is localized to the nucleus and to the cytoplasm, which is consistent with previous data on the function of the *CSL4* gene and purification of the exosome (1). Therefore the observation that the *ski4-1* mutation affects mRNA degradation by the exosome without affecting other reactions carried out by the exosome (see below) cannot be explained by Csl4p/Ski4p being present only in the cytoplasmic exosome. In addition, we localized GFP fused to the Ski4-1p. The localization of this mutant protein was indistinguishable from that of the wild-type GFP-Ski4p (Fig. 7). We conclude that the G253E mutation does not grossly alter the subcellular distribution of Csl4p/Ski4p.

The *ski4-1* allele of *CSL4*/*SKI4* is not lethal, and the cells grow normally under a variety of conditions, as do other mutants defective solely in 3′-to-5′ degradation of mRNA (e.g., the *ski2*Δ mutant). However, temperature-sensitive mutations in core exosome components, such as the *ski6-100* mutation, and depletion of core exosome components, such as Csl4p, are known to inhibit several essential RNA-processing events. These events include 3′ trimming of 5.8S rRNA and U4 snRNA precursors (1, 2, 22, 34) (data not shown). In addition, depletion of Csl4p and other exosome components leads to accumulation of the 5′ ETS of the rRNA primary transcript (2). Given this, we determined if the *ski4-1* allele of *CSL4* causes a defect in either 5.8S rRNA or U4 snRNA processing or in the degradation of the 5′ ETS. As shown in Fig. 8, we observe no accumulation of either the 5′ ETS or 3′-extended forms of 5.8S rRNA or U4 snRNA in the *ski4-1* strain. These data suggest that the *ski4-1* lesion might disrupt the function of the Csl4p/Ski4p protein in mRNA decay but not its function in RNA processing.

The defect of *ski4-1* in mRNA degradation but not in RNA processing can be explained in two ways. First, it is possible that the *ski4-1* mutation effectively lowers the exosome activity and that mRNA degradation requires more activity than RNA processing. This hypothesis seems unlikely, because partial-loss-of-function mutations in other exosome components such as those encoded by *mtr3-1*, *rrp4-1*, *ski6-2*, and *ski6-100* disrupt both mRNA degradation and RNA processing (1, 2, 17, 22, 34). Second, it is possible that the G253E substitution in the product of *ski4-1* specifically affects mRNA degradation. To try to distinguish between these two possibilities, we characterized the defect in a second allele of *CSL4*/*SKI4*, *csl4-1*. The *CSL4* gene was initially identified because the *csl4-1* mutation is synthetically lethal with a deletion of the *CEP1* gene, which encodes a transcription factor. The *csl4-1* locus was amplified by PCR and sequenced. The only nucleotide change observed was a C-to-T substitution at position −172 of the *CSL4* 5′-flanking DNA. We verified that this mutation gives rise to the *cep1*Δ synthetic lethality phenotype of *csl4-1* by introducing the same mutation into a wild-type *CSL4* strain and testing synthetic lethality with *cep1*Δ by tetrad analysis. No viable double mutants were recovered in this cross. The *csl4-1* mutation is a substitution in a putative Reb1p binding site in the promoter region of *CSL4*/*SKI4*. This substitution lowered the expression of a CSL4-LacZ fusion protein fivefold (data not shown; see Discussion). The identity of the specific sequence change, combined with the reduction in LacZ reporter activity, indicates that *csl4-1* introduces a partial loss of function mutation in the promoter that reduces the expression of Csl4p and presumably also reduces the number of functional exosome complexes.

The *csl4-1* mutation leads to a reproducible increase in the level of the 7S precursor to the 5.8S rRNA, indicating that the processing of 5.8S rRNA is affected by the *csl4-1* mutation (Fig. 9). We did not observe any intermediates between 7S pre-rRNA and 5.8S rRNA in the *csl4-1* strain like those seen in the *ski6-100* strain (Fig. 8) and cells depleted of Csl4p (1). The signals for the 7S pre-rRNA were quantitated and normalized for the amount of RNA loaded using the RNA subunit of the SRP. The SRP RNA is not known to be processed by the exosome, and previous analysis has shown that its levels are not affected by various exosome mutations (34). This experiment revealed that 7S pre-rRNA was about 2.5-fold more abundant in the *csl4-1* strain than in an isogenic wild-type strain. This result was confirmed by repeating the experiment with either the U6 snRNA or the RNA subunit of RNase MRP as the loading control. Like the SRP RNA subunit, these two RNAs have previously been shown not to be affected by exosome mutations (34). These results indicate that processing of 5.8S rRNA is affected by the *csl4-1* mutation but that this processing is still relatively efficient. In contrast to the defect seen for 5.8S rRNA processing, we did not observe a defect in the degradation of the MFA2pG fragment under the same conditions (Fig. 9). However, we cannot exclude the possibility that *csl4-1* has

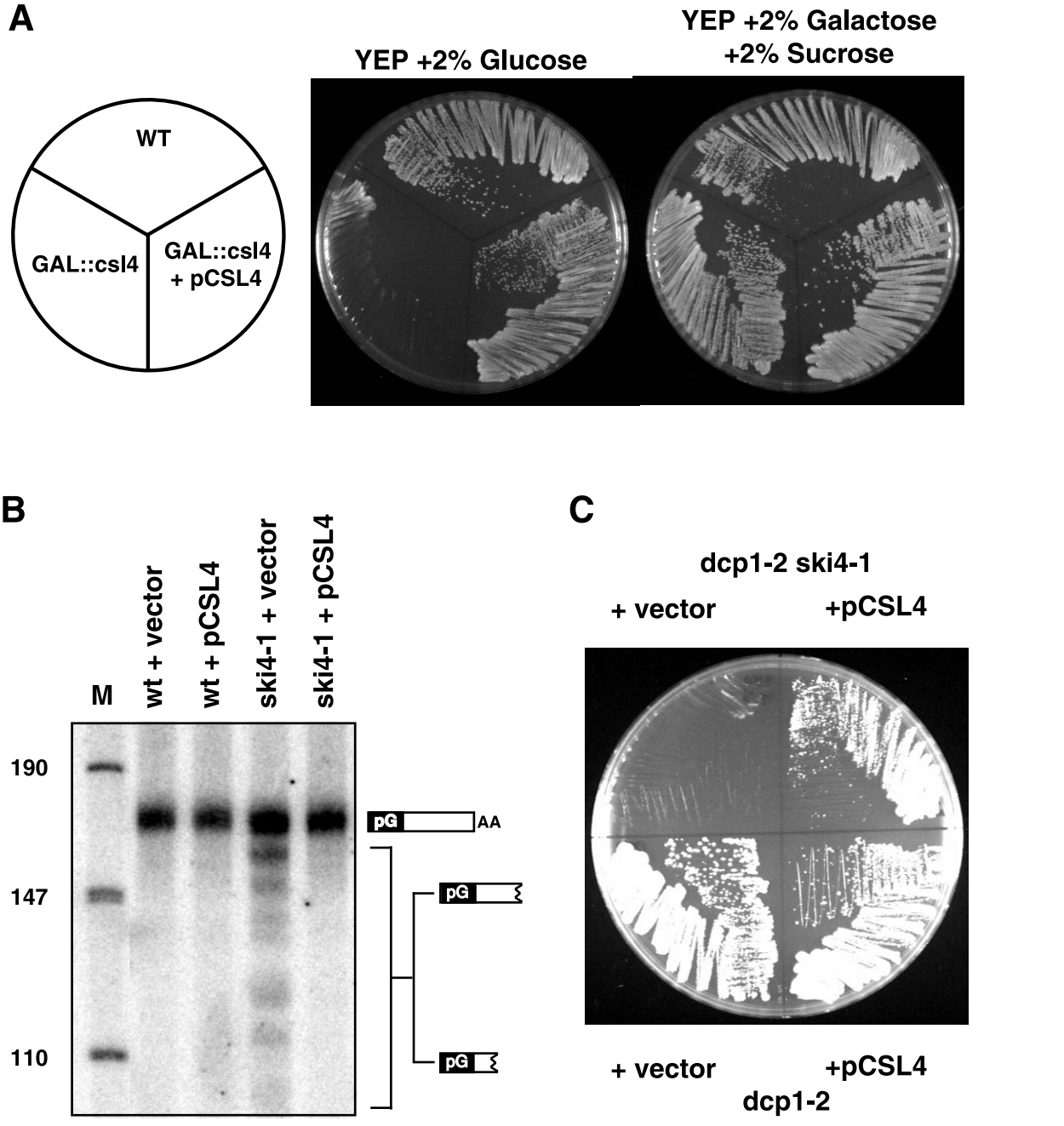

FIG. 5. The *ski4-1* mutation is complemented by a wild-type (WT) *CSL4* gene. (A) Plates of YEP containing either 2% glucose or 2% Gal and 2% sucrose. The *GAL*::*csl4* strain fails to grow on plates containing glucose but grows on plates containing Gal and sucrose, because the only copy of the essential *CSL4* gene has been placed under the control of the Gal promoter. This conditional growth defect is corrected by pCSL4. (B) Polyacrylamide Northern blot of RNA from wild-type and *ski4-1* strains containing either pCSL4 or the empty vector. The blot was probed with an oligonucleotide that detects the poly(G)-to-3′-end fragment of PGK1pG mRNA. The detected species are indicated with cartoons on the right. The length of *Hpa*II fragments of pUC18 is givne in nucleotides. M, nucleotide marker lane. (C) Plates containing YEP–2% glucose. The indicated strains were grown at 33°C.

a small effect on 3′-to-5′ mRNA decay. In either case, these data show that a simple partial-loss-of-function mutation in *CSL4*/*SKI4* affects rRNA processing more severely than mRNA degradation. The combination of the effects of *csl4-1* and *ski4-1* suggests that the *ski4-1* mutation separates the function of Csl4p/Ski4p in mRNA degradation from its other functions.

The SKI7 gene is specifically required for mRNA degradation by the exosome. The exosome is required both for 3′-to-5′ degradation of mRNA and for various RNA-processing reactions. In contrast, the Ski2p, Ski3p, and Ski8p proteins are specifically required for 3′-to-5′ degradation of mRNA. Given this we tested whether a *ski7*Δ strain showed defects in 5′-ETS degradation or processing of 5.8S rRNA or U4 snRNA. As shown in Fig. 10, these reactions are not affected by the *ski7*Δ mutation. We therefore conclude that Ski7p is specifically required for mRNA degradation by the exosome.

One possible reason why Ski7p is not required for RNA-processing reactions by the exosome is that another protein may replace Ski7p in this reaction. This would be similar to

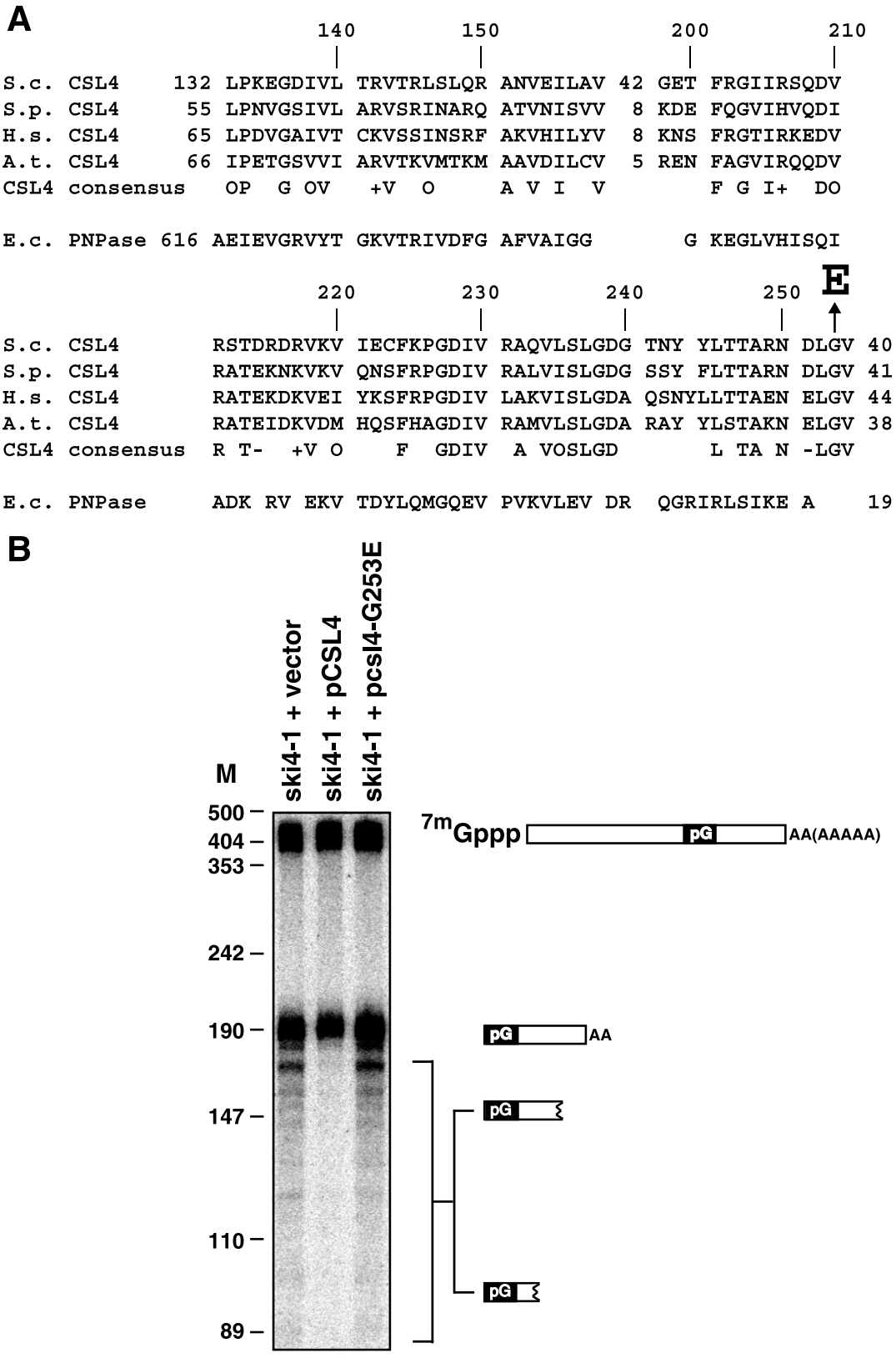

FIG. 6. A mutation in the conserved S1 RNA binding domain of Csl4p is responsible for the *ski4-1* phenotypes. (A) Alignment of the S1 RNA binding domain of Csl4p (S.c.) with putative homologs from *S. pombe* (S.p.) (accession no. T41654), *Homo sapiens* (H.s.) (5), and *Arabidopsis thaliana* (A.t.) (deduced from genomic sequence under accession no. AB009048). Included is a consensus of residues conserved in all four CSL4 sequences (O, hydrophobic residue, I, L, V, or M; +, positively charged residue R, K, or H; −, negatively charged residue D or E). Also included is the alignment of the S1 RNA binding domain from *Escherichia coli* (E.c.) PNPase, which is the only S1 RNA binding domain for which the structure has been determined (11). The alignment was generated using a hidden Markov model for the S1 RNA binding domain (I. S. Mian, personal communication). The *Arabidopsis* sequence was added manually based on BLAST results after removal of putative introns. Arrow, G-to-E change in the product of *ski4-1* and *csl4-G253E* (B) Polyacrylamide Northern blot probed with an oligonucleotide that detects the MFA2pG species indicated to the right. A *ski4-1* strain was transformed with the indicated plasmids. The resulting strains were grown at 30°C in medium lacking uracil and containing 2% Gal. The length of *Hpa*II fragments of pUC18 is given in nucleotides. M, nucleotides.

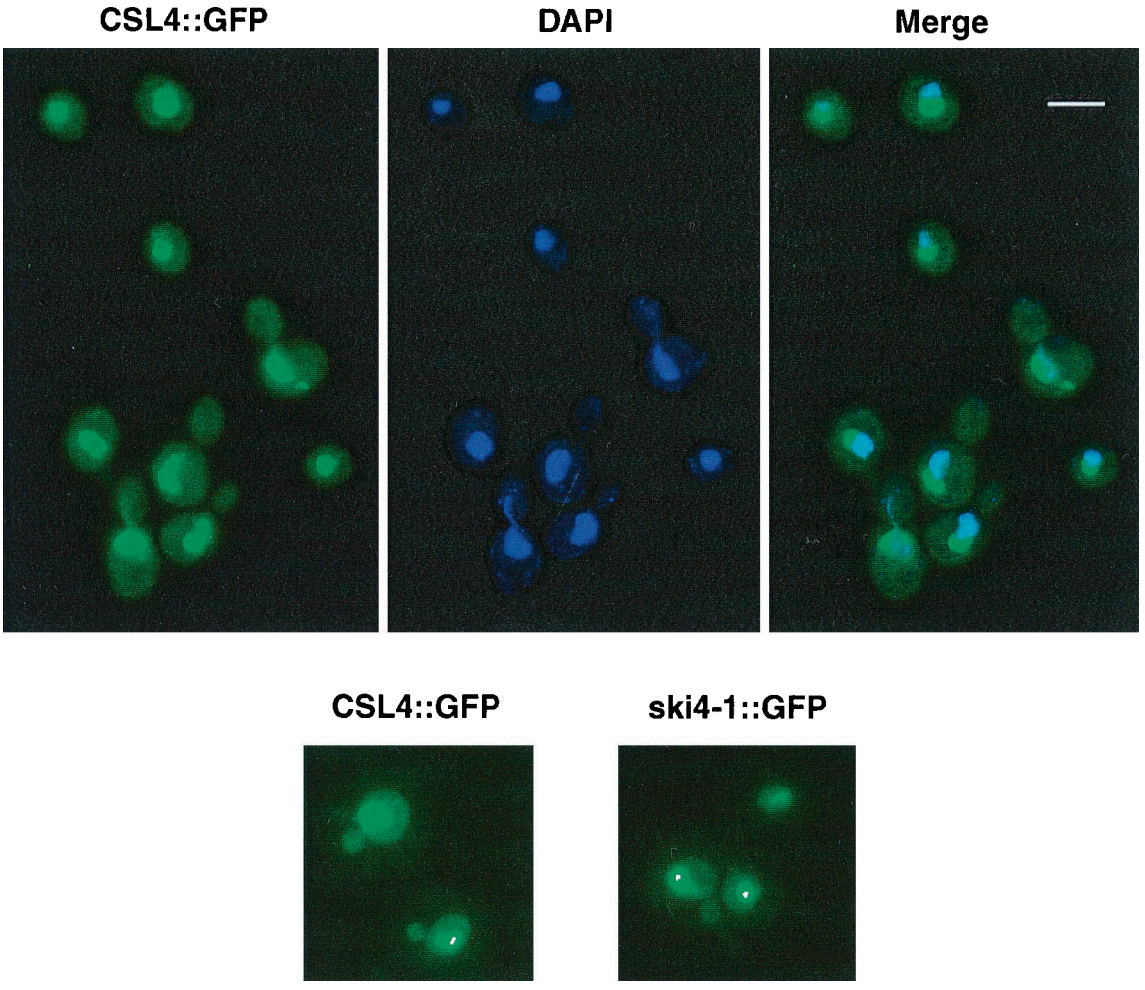

FIG. 7. Csl4p/Ski4p is localized in the nucleus and the cytoplasm. A gene encoding a GFP-CSL4 fusion protein was integrated into the genome at the *CSL4* locus. This yielded GFP fused to wild-type Csl4p/Ski4p or to the mutant Ski4-1p as indicated. Cells were fixed and stained for DNA using DAPI. Bar, 5 μm.

what has been found for Ski2p. Ski2p is only required for mRNA degradation, and Mtr4p, a close homolog of Ski2p, is required for all other exosome-mediated reactions. Interestingly, the *HBS1* gene codes for a protein that is homologous to Ski7p. We therefore tested whether an *hbs1*Δ mutation affected 5′-ETS degradation or processing of 5.8S rRNA or U4 snRNA. As shown in Fig. 10, none of these reactions are significantly affected by a deletion of the *HBS1* gene, suggesting that Hbs1p does not function in exosome-mediated RNA-processing reactions.

DISCUSSION

The Ski4p and Ski7p proteins are required for mRNA degradation. Multiple observations indicate that both Ski4p and Ski7p are required for the degradation of mRNA through the 3′-to-5′ pathway. First, both *ski4-1* and *ski7*Δ mutations result in a decrease in the 3′-to-5′ decay rate of mRNA. This is evident for full-length mRNA under conditions where the 5′-to-3′ decay pathway is blocked in *trans*, as well as for the poly(G)-to-3′-end fragment whose 5′-to-3′ degradation is blocked in *cis*. Second, both *ski4-1* and *ski7*Δ mutations are synthetically lethal with mutations that block decapping. Third, the *ski4-1* mutation is a mutation in the *CSL4*/*SKI4* gene and the *CSL4*/*SKI4* gene product is a component of the exosome (1) which is known to be required for 3′-to-5′ degradation of mRNA (17). Fourth, the *ski7*Δ mutation does not act synergistically with *ski2*Δ, *ski3*Δ, and *ski8*Δ mutations, indicating that all four mutations likely affect the same pathway.

Several functions for the Ski proteins have been proposed. We have previously suggested that the Ski proteins, with the exception of Ski1p, function directly in 3′-to-5′ mRNA degradation (17, 33, 34). An alternative explanation that has been proposed is that the Ski proteins function in ribosome biogenesis in the nucleolus, such that *ski* mutants contain altered ribosomes that have a reduced ability to discriminate between polyadenylated and nonadenylated mRNA molecules (7, 8, 21). According to this intriguing hypothesis, the altered translation of mRNAs would affect mRNA degradation as a secondary effect.

Several observations now combine to argue that the Ski2p, Ski3p, Ski7p, and Ski8p proteins and the exosome function directly in cytoplasmic mRNA degradation. First, the exosome has the required 3′-to-5′ exonucleolytic activity (22, 23). Second, the exosome and the Ski2p and Ski3p proteins are known to be localized to the cytoplasm (1, 10, 19, 23, 37). Third, the *ski2*Δ, *ski3*Δ, *ski7*Δ, *ski8*Δ, and exosome mutations all affect the degradation of a poly(G)-to-3′-end mRNA fragment, which is no longer translated at the time it is degraded (17). Fourth, the

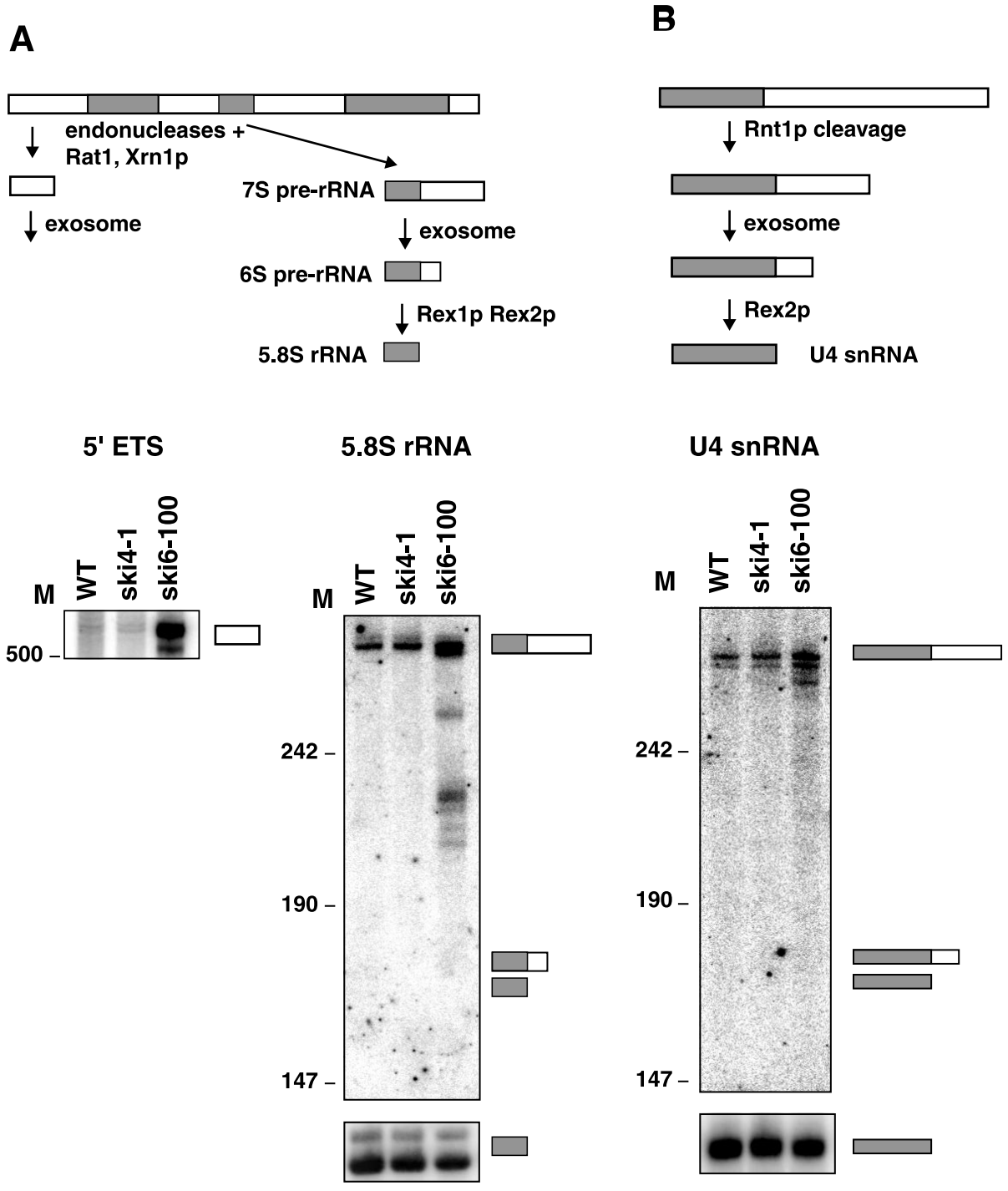

FIG. 8. The *ski4-1* mutation does not affect all Csl4p functions. (A) 5.8S rRNA processing and 5′ ETS degradation are not affected by *ski4-1*. Shown is a diagram of part of the processing pathway of the 35S pre-rRNA. This processing pathway yields the 5′ ETS (white box) and 5.8S rRNA (gray box), which are substrates for the exosome. The 35S pre-rRNA also yields 18S and 25S rRNA and several other spacer fragments, which are not shown. Also shown is a polyacrylamide Northern blot probed for 5′ ETS (left) and 3′ extended precursors of 5.8S rRNA (top right) and reprobed for the mature 5.8S rRNA (bottom right). The wild-type (WT) and *ski4-1* strains were grown at 30°C. The *ski6-100* strain was grown at 24°C and incubated for 1 h at 37°C. (B) U4 snRNA processing is not affected by *ski4-1*. Same as panel A except that U4 snRNA probes were used. The length of *Hpa*II fragments of pUC18 is given in nucleotides. M, nucleotides.

*ski2*Δ, *ski3*Δ, *ski7*Δ, *ski8*Δ, and *ski4-1* mutations affect mRNA degradation without affecting any other known function of the exosome (17, 36). The simplest explanation of all of these observations is that the exosome is the catalytic complex that degrades mRNA 3′ to 5′ in the cytoplasm and that the Ski2p, Ski3p, Ski7p and Ski8p proteins function to allow or promote this exosome function. In this view the increased luciferase expression from electroporated unadenylated mRNA in *ski* mutants may reflect a competition between exosome-mediated degradation and entry into the translatable pool.

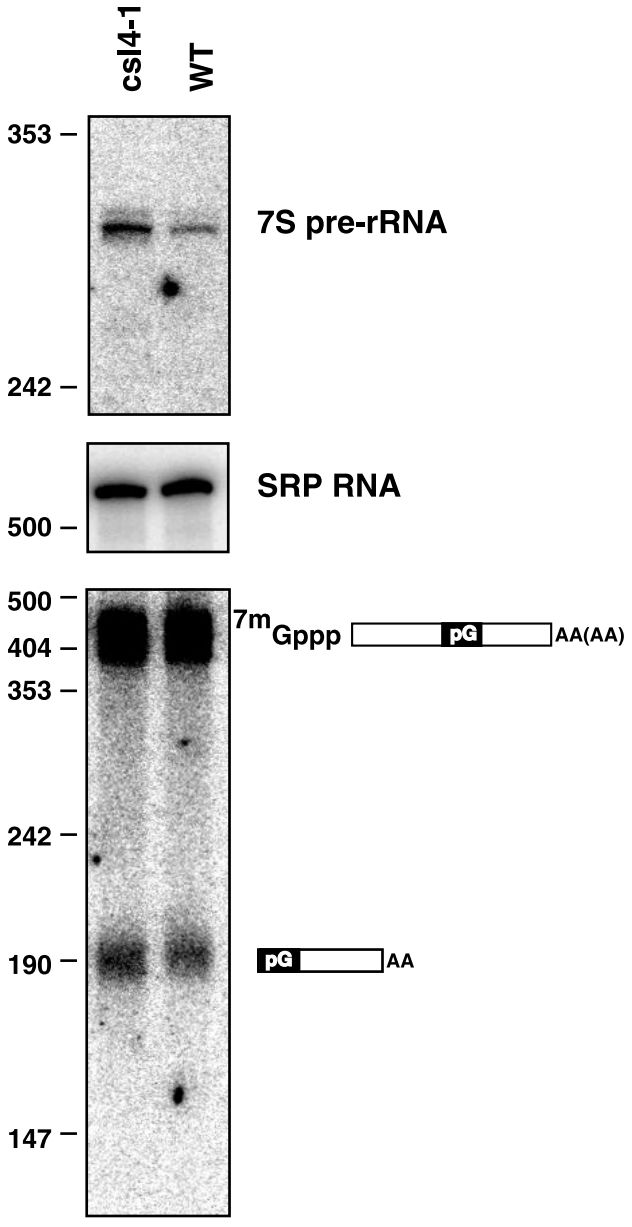

FIG. 9. The *csl4-1* mutation affects rRNA processing but not mRNA degradation. Shown are polyacrylamide Northern blots probed for 3′ extended precursors of 5.8S rRNA (top), the RNA subunit of the SRP (middle), or MFA2pG mRNA (bottom). The indicated strains were grown at 30°C in medium lacking leucine and uracil and containing 2% sucrose and 2% Gal. The length of *Hpa*II fragments of pUC18 is given in nucleotides. Numbers on the left indicate nucleotides. WT, wild type.

The role of Ski7p in mRNA degradation. Ski7p has significant sequence similarity to translation elongation factor EF1A (8). However, it is not clear whether Ski7p has a role in translation. Interestingly, the sequence similarity of Ski7p and EF1A appears to be limited to their GTPase domains. The similarity between Ski7p and EF1A is reminiscent of what has been observed for Snu114p and EF2. EF2 is another GTPase involved in translation elongation, and Snu114p is very similar to EF2. In this case the homology is not limited to the GTPase domain but covers all of EF2 (15). Despite this extensive homology, Snu114p is part of the U5 snRNP particle and functions in pre-mRNA splicing, with no apparent role in transla-

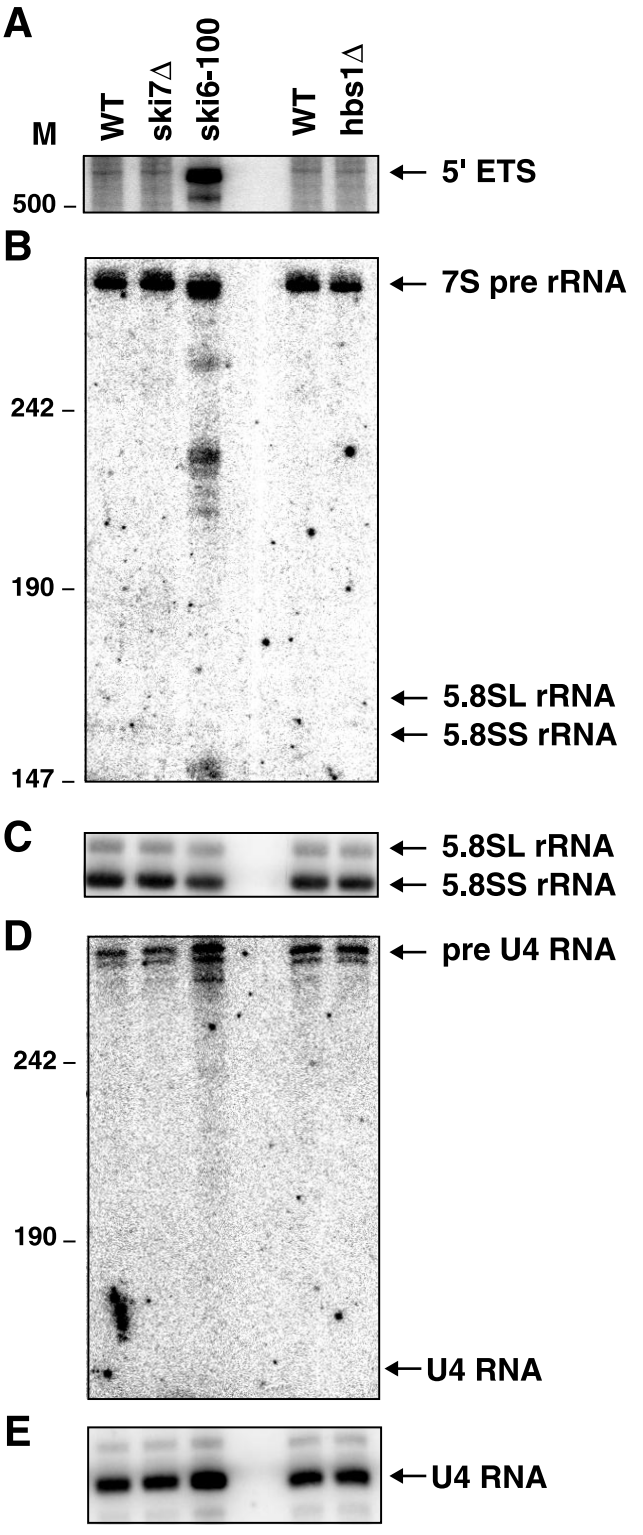

FIG. 10. The *ski7*Δ and *hbs1*Δ mutations do not affect 5.8S rRNA or U4 snRNA processing or 5′ ETS degradation. Shown are polyacrylamide Northern blots. The wild type (WT), *ski7*Δ, and *hbs1*Δ strains were grown at 30°C in YEP medium containing 2% Gal. The *ski6-100* strain was grown in the same medium at 24°C and incubated at 37°C for 1 h. (A) A Northern blot was probed for the 5′ ETS. (B) The Northern blot in panel A was stripped and reprobed for 3′ extended precursors of 5.8S rRNA. (C) The Northern blot in panel B was stripped and reprobed for 5.8S rRNA. (D) Northern blot probed for 3′ extended precursors of U4 snRNA. (E) The Northern blot in panel D was stripped and reprobed for U4 snRNA. M, nucleotides.

tion (15). This argues that despite similarity to EF1A, Ski7p may not function in translation.

Ski7p belongs to the family of GTPases whose members can bind GTP and hydrolyze it to GDP. This transition from the GTP-bound state to the GDP-bound state is often associated with large conformational changes in the protein, resulting in alterations of protein-interacting and/or RNA-interacting surfaces (11). Therefore, one hypothesis is that Ski7p functions to bring two or more macromolecules together and uses GTPase activity as a source of energy for a conformational change. Under this hypothesis possible interacting molecules would be the substrate mRNA, Ski2p, Ski3p, Ski8p, and the exosome. Previous studies have failed to detect an interaction between Ski7p and the Ski2p-Ski3p-Ski8p complex (10). However, it appears likely that the interactions of Ski7p are dependent on whether it is in a GTP-bound state or a GDP-bound state, and therefore an interaction may only be detectable in vitro in the presence of GDP, GTP, or a GTP analog.

Why is Ski7p required for mRNA degradation by the exosome but not for 5.8S rRNA processing by the exosome? One possibility is that a different protein substitutes for Ski7p in 5.8S rRNA processing. This would be similar to what has been found for Ski2p and Mtr4p (see reference 33 for a review on the possible roles of Ski2p and Mtr4p). The most likely candidate for a second GTPase required for exosome function appeared to be Hbs1p. Hbs1p and Ski7p are more similar to each other over a longer stretch of both proteins than either protein is to other GTPases (8; our unpublished observations). However, an *hbs1*Δ strain does not have an obvious growth defect, as might be expected for a strain defective in rRNA processing, nor does it have an obvious defect in the processing of 5.8S rRNA or U4 snRNA or the degradation of the 5′ ETS of the 35S pre-rRNA. Therefore, it appears unlikely that Hbs1p plays a role in RNA processing by the exosome.

Implications for the function of Csl4p/Ski4p in mRNA degradation. The observation that the *ski4-1* mutation affects mRNA degradation by the exosome without affecting other functions of the exosome is strong support for a direct role of the exosome in mRNA degradation. There are several explanations why the one amino acid change in Csl4p could affect mRNA degradation specifically. One appealing model is that Csl4p/Ski4p is an RNA binding protein that specifically binds mRNA molecules that are substrates for the exosome. The mutation in the RNA binding domain of *CSL4*/*SKI4* in the *ski4-1* allele would alter the specificity or affinity of Csl4p/Ski4p for mRNA under this hypothesis. Alternatively the *ski4-1* mutation might disrupt a protein interaction between Csl4p/Ski4p and some other protein. This protein-protein interaction would be required for mRNA degradation by the exosome. Interestingly, the *ski4-1* mutation does not affect the degradation of the 5′ ETS of the pre-rRNA. Therefore the mutation disrupts an mRNA-specific interaction and not an interaction involved in distinguishing degradation substrates of the exosome from processing substrates.

Synthetic lethality of *csl4-1* and *cep1*Δ mutations. The *csl4-1* mutation was originally identified in a screen for mutations that were synthetically lethal with the *cep1*Δ mutation. The *CEP1* gene encodes the kinetochore protein/transcription factor CP1/Cbf1p (5). The identification of the *csl4-1* mutation as a mutation in the *CSL4*/*SKI4* promoter sheds light on the synthetic lethality of this mutation with a *cep1*Δ mutation. The *csl4-1* mutation likely disrupts the binding of transcription factor Reb1p to the *CSL4* promoter. Disruption of this binding is insufficient to affect the viability of the yeast strains (5). The *CSL4*/*SKI4* promoter also contains a binding site for the general regulatory factor Cep1p. Disruption of this binding by deletion of the *CEP1* gene or the Cep1p binding site also is insufficient to affect viability (5). However, we hypothesize that disruption of both Reb1p binding and Cep1p binding reduces expression of the essential *CSL4*/*SKI4* gene so much that it no longer is sufficient to support viability. The importance of Reb1p and Cep1p binding was tested by analyzing the expression of a CSL4-LacZ fusion protein. The CSL4-LacZ reporter gene in this case contained either a wild-type *CSL4* promoter or a promoter containing the *csl4-1* mutation. This analysis showed that the *csl4-1* mutation lowers the expression of the CSL4-LacZ fusion by about fivefold. Moreover, the *cep1*Δ mutation further lowers the expression of the reporter gene driven by the *csl4-1* promoter to 29-fold below wild-type levels (R. E. Baker, unpublished results). Thus, the synthetic lethality of *csl4-1* and *cep1*Δ mutations is likely the result of a strongly reduced expression of the essential *CSL4*/*SKI4* gene.

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