The Ski7 Antiviral Protein Is an EF1-α Homolog That Blocks Expression of Non-Poly(A) mRNA in *Saccharomyces cerevisiae*

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We mapped and cloned *SKI7*, a gene that negatively controls the copy number of L-A and M double-stranded RNA viruses in *Saccharomyces cerevisiae*. We found that it encodes a nonessential 747-residue protein with similarities to two translation factors, Hbs1p and EF1-α. The *ski7* mutant was hypersensitive to hygromycin B, a result also suggesting a role in translation. The *SKI7* product repressed the expression of nonpolyadenylated [non-poly(A)] mRNAs, whether capped or uncapped, thus explaining why Ski7p inhibits the propagation of the yeast viruses, whose mRNAs lack poly(A). The dependence of the Ski7p effect on 3′ RNA structures motivated a study of the expression of capped non-poly(A) luciferase mRNAs containing 3′ untranslated regions (3′UTRs) differing in length. In a wild-type strain, increasing the length of the 3′UTR increased luciferase expression due to both increased rates and duration of translation. Overexpression of Ski7p efficiently cured the satellite virus M₂ due to a twofold-increased repression of non-poly(A) mRNA expression. Our experiments showed that Ski7p is part of the Ski2p-Ski3p-Ski8p antiviral system because a single *ski7* mutation derepresses the expression of non-poly(A) mRNA as much as a quadruple *ski2 ski3 ski7 ski8* mutation, and the effect of the overexpression of Ski7p is not obtained unless other *SKI* genes are functional. *ski1/xrn1Δ ski2Δ* and *ski1/xrn1Δ ski7Δ* mutants were viable but temperature sensitive for growth.

The 5′ cap (m7G5′ppp5′Xp) and the 3′ poly(A) structures of eukaryotic mRNAs are essential for efficient translation and mRNA stability. The poly(A) tail acts synergistically with the 5′ cap structure in translation (11, 13; reviewed in reference 37). The first step in mRNA degradation is the shortening of this poly(A) tail, followed by a decapping reaction, producing an uncapped nonpolyadenylated [non-poly(A)] mRNA which is then subject to rapid 5′ → 3′ degradation (9, 29; reviewed in reference 16).

This intermediate of mRNA degradation resembles the mRNAs of the L-A and M double-stranded RNA (dsRNA) viruses of the yeast *Saccharomyces cerevisiae*, which naturally lack both the 5′ cap and the 3′ poly(A) tail (6, 46). The L-A and M mRNAs are nonetheless expressed, but their expression is sensitive to chromosomal mutations affecting pathways involving these 5′ or 3′ structures. The *ski* mutants were first isolated on the basis of their superkiller phenotype (35, 47). The *SKI2*, *SKI3*, *SKI4*, *SKI6*, *SKI7*, and *SKI8* products repress the copy number of L-A and M viruses, and mutations in these genes result in a higher level of expression of the secreted killer toxin encoded by M dsRNA (3, 35, 47, 53; reviewed in reference 50). The *SKI1* gene (47), later proved to be *XRN1*, encodes the 5′ → 3′ exoribonuclease specific for uncapped cellular mRNAs and is responsible for a major pathway of mRNA

decay (14, 18, 29, 43, 44). In a *ski1* mutant, uncapped viral mRNAs are more stable, causing, for example, increased toxin production (24). The *SKI2*, *SKI3*, and *SKI8* genes (26, 34, 53) encode a system that specifically blocks the expression of 3′ non-poly(A) mRNAs (24). The nuclear location of Ski3p (34) and the similarity of Ski2p to a nucleolar human homolog (21, 53) suggested that they carry out a function taking place in the nucleus and inducing specific repression of non-poly(A) mRNA translation (24).

Ski6p is homologous to bacterial RNase PH (4), an enzyme responsible for trimming a few nucleotides from the 3′ end of tRNA precursors and 5S rRNA during their maturation (22). Indeed, *ski6* mutants produce defective 60S subunits (4), and Ski6p (Rrp41p) was localized in a complex of 3′ → 5′ exoribonucleases involved in 5.8S rRNA processing (28). These abnormalities lead to derepressed expression of non-poly(A) mRNA in *ski6* mutants (4), with effects on both the initial rate and the duration of translation from electroporated non-poly(A) mRNAs. Mutations in the *SKI2*, *SKI3*, *SKI6*, or *SKI8* gene can also slow the 3′ → 5′ degradation of special mRNA decay intermediates whose degradation by the predominant 5′ → 3′ degradation system is blocked (1). Because the control of translation and mRNA turnover are multiply intertwined, distinguishing the primary effects of the *SKI* genes is difficult, but the overall effect on viral replication appears to be enhanced expression of the viral mRNA which lacks 3′ poly(A).

Since *ski* mutations (including, as we show here, *ski7*) primarily affect the expression of non-poly(A) mRNAs, we examined the role of the 3′ untranslated region (3′UTR) in non-poly(A) mRNA expression. The 3′UTR is known to have specific functions in many systems (49). Furthermore, all cellular mRNAs are believed to undergo deadenylation, and non-poly(A) mRNAs can be present in the polysomal fraction under special circumstances (14, 33). Thus, when the mRNA

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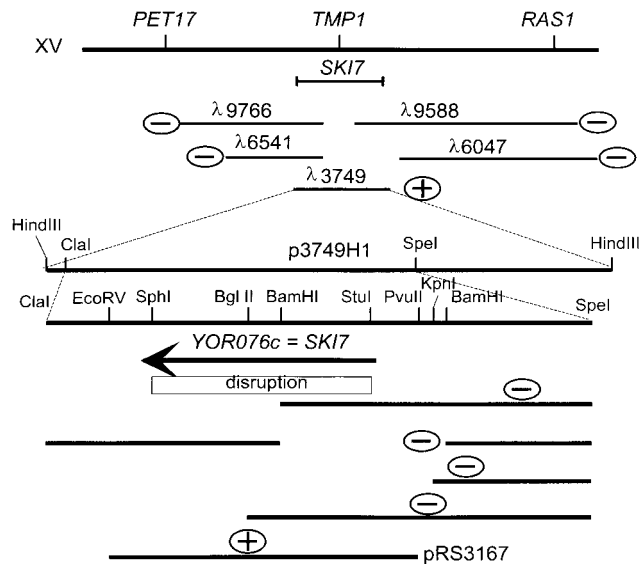
FIG. 1. Cloning of *SKI7*. The *ski7-1* mutation was localized by meiotic mapping to a region near *TMP1/CDC21*. Various clones from the Olson bank (36) were tested for complementation of *ski7-1*. Subcloning proved that *SKI7* was *YOR076c*; this finding was confirmed by the phenotype of the disruption and complementation of *ski7-1* by pRS3167 (see Materials and Methods).

TABLE 1. Strains of *Saccharomyces cerevisiae*

Strain	Description
RV603	*MAT**a** ura3 leu2 ski7-1 mkt1*; L-A-HN M$_2$
B117	*MAT**a** ura3 his3 trp1 ski7::HIS3*; M-o L-A-HN; from 2959
2959	*MAT**a** ura3 his3 trp1 SKI*; M$_2$ L-A-HN
B959	2959 K$^-$
3973	*MAT**a** ura3 his3 leu2 ski2::HIS3*
3974	*MAT**a** ura3 his3 leu2 ski3::URA3*
3975	*MATα ura3 his3 leu2 ski7::HIS3*
4602-2B	*MATα ura3 his3 leu2 trp1*
3963	*MATα ura3 his3 leu2 trp1 ski3::URA3 ski2::HIS3 ski8::URA3 ski7::HIS3*
RV493	*MAT**a** ura3 ade3 his leu2 trp1 ski6-2 mkt1*; L-A-HN M$_2$

remains capped and is non-poly(A), its 3′UTR may itself serve as an element influencing mRNA expression (reviewed in references 15 and 49). In CHO (Chinese hamster ovary) cells, increased 3′UTR length substantially improved the efficiency of translation initiation, suggesting a model in which the 3′UTR acts through more efficient ribosome recycling (45). A modest stabilization of mRNAs with longer 3′UTRs was also observed but was interpreted as a secondary phenomenon. There is also evidence for prokaryotes that events occurring during termination can affect the efficiency of ribosome recycling, the fourth step of translation (17, 32).

We show here that the *ski7* mutation increases both the initial rate and the duration of the expression of non-poly(A) mRNAs and that this effect is independent of the length of the 3′UTR. The effect of the *ski7* mutation on the expression of non-poly(A) mRNAs explains the superkiller phenotype of the mutant because the viral mRNAs lack 5′ poly(A). Overexpression of Ski7p cured the M$_2$ satellite RNA due to increased repression of the expression of non-poly(A) mRNAs. The increased block of the translation of non-poly(A) mRNAs and the resulting elimination of M$_2$ depended on the function of other *SKI* genes. This and other genetic experiments indicated that *SKI7* is part of the *SKI2-SKI3-SKI8* antiviral system.

MATERIALS AND METHODS

Genetic mapping of *SKI7*. M$_2$ dsRNA is a killer toxin-encoding satellite of the L-A virus (5, 27, 52). Above 32°C, M$_2$ propagation depends on two genes, *MKT1* and *MKT2* (maintenance of K$_2$), which are not needed by M$_1$ dsRNA (51, 52). *ski* mutations suppress this requirement of M$_2$ for *MKT* genes (35). Many laboratory strains carry *mkt1* mutations. We crossed *mkt1 ski7* M$_2$ strains with a set of multiply marked strains designed for genetic mapping (12), which also proved to be *mkt1*. We found that *ski7* was tightly linked to *pet17* (parental ditype [PD], 34; nonparental ditype [NPD], 0; tetratype [T], 8; 9.5 cM) and linked to *ade2* (PD, 24; NPD, 0; T, 22; 24 cM) on the right arm of chromosome XV. Analysis of individual tetrads showed that the order was *CEN15-PET17-SKI7*, placing *SKI7* very close to *TMP1/CDC21* (Fig. 1).

Cloning of *SKI7*. Strain RV603 (*MAT**a** ura3 leu2 ski7-1 mkt1*; L-A-HN M$_2$) is stably K$_2^+$ at either 25 or 32°C, but if it becomes *SKI$^+$*, then it will be K$_2^-$ after growth at 32°C (Table 1). Several λ clones of yeast DNA (36) located near *tmp1* were tested by cotransformation of λ clone DNA and pBM2240, the latter cut

with *Eco*RI and *Xho*I (10). Transformants were isolated at 25°C, grown at 25 or 32°C, and then tested for killer activity. Only λ3749 (ATCC 70213) gave recombinants which complemented *ski7-1* (Fig. 1). The pBM2240 derivative carrying the insert of λ3749 was isolated, and subclones were made by cleavage with *Hin*dIII and ligation to pRS316 cut with the same enzyme. One of these, p3749H1, containing an 8.9-kb insert, complemented *ski7-1*. Subclones of p3749H1 were made by cleavage with *Cla*I and *Spe*I and insertion into pRS316 cut with the same enzymes. One of these, p3749L, contained a 5-kb insert and complemented *ski7-1*. Deletions of p3749L were made by cutting with *Cla*I-*Bam*HI, *Bam*HI-*Not*I, *Bgl*II-*Cla*I, *Kpn*I, or *Bam*HI followed by ligation (Fig. 1). None of these deletion plasmids complemented *ski7-1*. The ends of several of these clones were sequenced and found to match a region of chromosome XV near *TMP1/CDC21* (48), as expected. The results indicated that the open reading frame *YOR076c* was responsible for the complementation of *ski7-1*. This finding was confirmed by cloning the 3,155-bp *Eco*RV-*Pvu*II fragment containing *YOR076c* into pRS316 cut with *Sma*I and showing that the resulting plasmid, pRS3167, complemented *ski7-1* (Fig. 1).

Plasmid constructions. The insert from pRS3167 (see above) was excised as a *Spe*I-*Hin*dIII fragment and inserted into pBluescript SK(+), creating pSKSKI7. To prepare a genomic disruption of *SKI7*, the *Stu*I-*Sph*I fragment including nearly all of the *SKI7* open reading frame was replaced with the *HIS3* gene on a *Sma*I-*Sph*I fragment from pJJ215 (19), creating pSKI7::HIS3. Multicopy YEpSKI7 was created by the insertion of the same *Spe*I-*Hin*dIII fragment containing *SKI7* into YEp351 cut with *Spe*I and *Hin*dIII.

Disruption of *SKI7*. A diploid strain, 2959 (*MAT**a** ura3 his3 trp1*; M$_2$ L-A-HN) × 2966 (*MATα leu2 his3 trp1 pho3 pho5*; L-A-o M-o), was transformed with linearized pSKI7::HIS3. His$^+$ diploids were selected, and the genomic replacement of *SKI7* was confirmed by Southern blotting. Positive diploids were sporulated, and the segregation observed was 2 His$^+$ Ski$^-$:2 His$^-$ Ski$^+$ (four tetrads). Tetrad analyses were confirmed by Southern blotting. As the *SKI7* disruptant was viable, strain B117 was constructed from strain 2959 by the same procedure.

Strain 5X47 was the toxin-sensitive strain used as an indicator in the killer assay (35).

Expression of luciferase mRNAs. The luciferase mRNA expression plasmids pT7-*luc* [no poly(A); untranslated region, 22 bases] and pT7-*luc*50A [50-mer poly(A) tail] (13) were linearized with *Sma*I and *Dra*I, respectively. Plasmids allowing the transcription of luciferase mRNAs with repeated copies of a 20-base 3′UTR (5′TCTACAGCATATCTGGATCTGGATCC3′) followed (or not) by a 50-mer poly(A) tail (5′CCA$_{25}$GTTATA$_{25}$TTTAAA3′) were kindly provided by Daniel Gallie (45). For RNA synthesis, pT7-*luc* with several copies of the above 3′UTR sequence was linearized with *Bam*HI, and pT7-*luc*(3′UTR)$_n$+50(A) was linearized with *Dra*I. Transcripts were synthesized with an Ambion MEGAscript transcription kit in the presence or absence of the cap analog 5′m7GpppG in accordance with the manufacturer's instructions. After DNase I treatment and precipitation with LiCl, RNAs were passed over G-50 columns (5 prime-3 prime SELECT-B) and quantitated by both measurement of the optical density (OD) at 260 nm and comparison with known concentration standards on agarose gels.

RNA electroporation was done as described previously (11) with minor modifications. Two micrograms of RNA was used for electroporation, and cells were assayed for luciferase activity after different times of growth at 30°C. Luciferase activity was assayed as previously described (24).

Drug sensitivity test. Isogenic wild-type and *ski7* cells grown in YPAD to the log phase were diluted to an OD at 600 nm of 0.15; 0.015-, 0.0015-, and 5-μl aliquots were spotted on YPAD plates with or without drug (the viabilities of both strains appeared to be identical and proportional to the measured OD on YPAD). The concentration of hygromycin B was 100 μg/ml, and that of cycloheximide was 100 ng/ml.

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EF-1                      *      * **
alpha:   7  HINVVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAELGKGSFKYAW---VLDKLK
Ski7p: 270  NLTCLFLGDTNAGKSTLLGHLLYDLNEISMSSMRELQKKSSNLDPSSSNSFKV--ILDNTK
Hbs1p: 170  HLSFVVLGHVDAGKSTLMGRLLYDLNIVNQSQLRKLQRESETMGKSSFK-FAW--IMDQTN

EF-1 :  65  AERERGITIDIALWKFET----PKYQVTVIDAPGH-RDFIKNMITG--TSQADCAILIIAG
Ski7p: 331  TERENGFSMFKKVIQVENDLLPPSSTLTLIDTPGSIKYFNKETLNSILTFDPEVYYLVIDC
Hbs1p: 231  EERERGVT----VSICTSHFSTHRANFTIVDAPGH-RDFVPNAIMGISQAD--MAILCVDC

EF-1 : 119  GVGEFEAGIS-KDGQTREHALLAFTLGVRQ-----LIVAVNKMDSVKWDESRFQEIVKETS
Ski7p: 390  NYDSWEKSLDGPNNQIYEILKVISYLNKNSACKKHLITLLNKADLISWDKHRL-EMIQSEL
Hbs1p: 280  STNAFESGFDL-DGQTKEHMLLASSLGIHN-----LIIAMNKMDNVDWSQQRF-EEIKSKL
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FIG. 2. Ski7p is similar to Hbs1p (31) and yeast EF-1α (Tef1p or Tef2p) (40). Black boxes show residues identical to those in Ski7p; dashes show gaps.

RESULTS

The *SKI7* product is similar to translation factors. Like other *ski* (superkiller) mutations, *ski7* confers cold sensitivity for growth on cells carrying M dsRNA, indicating the importance in nature of the *SKI* genes. We genetically mapped *ski7* close to *CDC21/TMP1* on chromosome XV by using the suppression by *ski7* of a defect in propagation of the M₂ virus. We obtained λ clones including this small area and cloned the gene from one of them (see Materials and Methods) (Fig. 1). Since the clone complementing the *ski7* mutation was obtained from DNA that maps close to *CDC21/TMP1*, we have cloned *SKI7* and not a suppressor. Deletion analysis (Fig. 1) proved that *SKI7* is identical to *YOR076c*, previously identified in the yeast genome project (48). A deletion-substitution *ski7* mutation produced the superkiller phenotype but did not produce a growth defect, showing that *SKI7* is not essential (see Materials and Methods).

The 747-residue protein Ski7p is similar to yeast Hbs1p (24.4% identity and 58% similarity in 582 amino acids), a factor whose overexpression suppresses the growth defect of an *ssb1 ssb2* double mutant. *SSB1* and *SSB2* products are known to be associated with the ribosome during peptide synthesis (31). However, overexpression of Hbs1p does not suppress *ski7-1* (data not shown). Ski7p also resembles the translation elongation factors EF-Tu and EF-1α (45 identities in a 154-residue region of similarity) (Fig. 2) and the yeast translation termination factor Sup35p (data not shown), including a GTPase consensus sequence. These similarities suggest that Ski7p is involved in translation.

Ski7p specifically blocks the expression of non-poly(A) mRNAs. Because the *ski7* mutation, like *ski1*, *ski2*, *ski3*, *ski6*, and *ski8*, increases the expression of viral uncapped non-poly(A) mRNAs, we used electroporation of luciferase mRNAs to study the effect of a *SKI7* disruption (Table 2). In a wild-type strain, capped (C⁺) polyadenylated (A⁺) mRNA was expressed 38 to 42 times better than C⁺ non-poly(A) (A⁻) mRNA (Table 2). In the isogenic *SKI7* disruptant, C⁺ A⁺ mRNA was expressed only 2 to 5.5 times better than C⁺ A⁻ mRNA. Thus, the *SKI7* disruption increases the expression of C⁺ A⁻ mRNA 7- to 20-fold. A similar effect on uncapped (C⁻) A⁻ mRNA expression was also seen, with a seven- to ninefold increase in expression in the *ski7::HIS3* disruptant (Table 2). In contrast, no increased expression of C⁻ A⁺ mRNA in the mutant was seen; a decrease of two- to threefold was observed (Table 2). This modest effect of the absence of a cap in the mutant might also explain why C⁻ A⁻ mRNA expression is not enhanced in the *ski7* mutant as strongly as is that of C⁺ A⁻ mRNA.

In a *ski7* mutant, the lack of a 3′ poly(A) structure appears less critical. The poly(A) structure is well known to be important in both the initial rate and the duration of translation of mRNAs. Since 3′UTRs can also play roles in translation (45) and in mRNA stability (30), it is possible that the *ski7* mutation derepresses 3′UTR function itself, compensating for the loss of the poly(A) tail or its natural absence on viral mRNAs. We thus examined the effect of the length of the 3′UTR on mRNA expression in a wild-type strain or an isogenic *ski7* mutant.

3′UTR length influences non-poly(A) mRNA expression, and the *ski7* mutation has an additive effect. We used constructs allowing the production in vitro of capped luciferase mRNAs with a 3′UTR sequence composed of repeats of a 20-nucleotide sequence (45) (see Materials and Methods). Expression from these mRNAs was measured 2 h after their electroporation into spheroplasts prepared from wild-type and

TABLE 2. Ski7p blocks the expression of non-poly(A) mRNAs[a]

Expt	Strain	Expression of the following luciferase mRNA:			
		C⁺ A⁺	C⁻ A⁺	C⁺ A⁻	C⁻ A⁻
1	Wild type	33.4 (100)	2.9 (8.8)	0.88 (2.6)	0.19 (0.6)
	ski7::HIS3	26.3 (100)	1.26 (4.8)	13.5 (51)	1.76 (6.7)
	Ratio of *ski7* to wild type	0.79 (1.0)	0.43 (0.54)	15.3 (20)	9.3 (11)
2	Wild type	26.9 (100)	2.25 (8.4)	0.64 (2.4)	0.12 (0.4)
	ski7::HIS3	30 (100)	0.80 (2.7)	5.4 (18)	0.81 (2.7)
	Ratio of *ski7* to wild type	1.1 (1.0)	0.35 (0.32)	8.4 (7.5)	6.8 (6.7)

[a] Wild-type (B959) and isogenic *ski7::HIS3* (B117) strains were electroporated with 2 μg of the indicated mRNA made from pT7-*luc* [for non-poly(A) mRNAs] or pT7-*luc50*A (for polyadenylated mRNAs). C⁺ and C⁻ indicate mRNAs with and without the 5′ cap, respectively; A⁺ and A⁻ indicate mRNAs with and without the 3′ poly(A), respectively. Luciferase was measured 2 h after electroporation as described in Materials and Methods and is reported in light units per microgram of protein. Numbers in parentheses for strain data are percentages of the C⁺ A⁺ value. Numbers in parentheses are ratios assuming a ratio of 1.0 for C⁺ A⁺ mRNA.
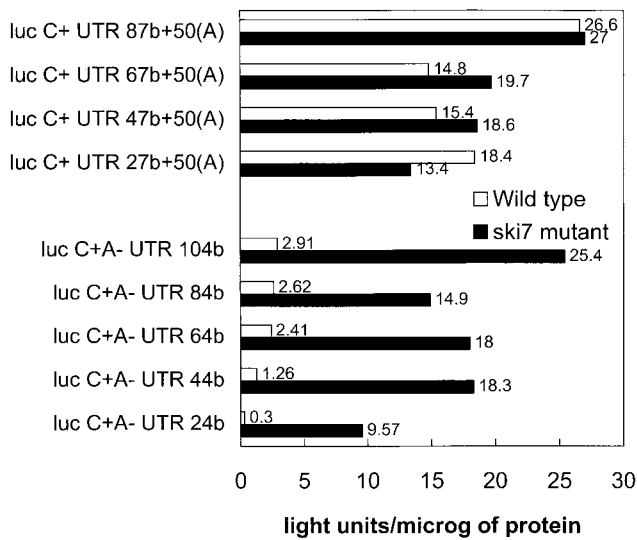
FIG. 3. Luciferase expression in isogenic wild-type (B959) and *ski7*Δ mutant (B117) strains 2 h after electroporation with 2 μg of the indicated mRNAs. All mRNAs had 5′ cap structures. The lengths of the 3′UTRs are indicated (one to five identical 20-bp units were inserted 3′ of the *luc* coding region) (45) (see Materials and Methods). microg, microgram.

isogenic *ski7* strains (Fig. 3). Increasing the length of the 3′UTR from 24 bases to 104 bases resulted in a 9.7-fold increase in expression in the wild-type strain (Fig. 3). With a poly(A) tail, the difference between these mRNA constructs was at most twofold (Fig. 3). We confirmed with yeast cells the observation made with CHO cells (45) that increasing the length of the 3′UTR improves expression when mRNAs are non-poly(A).

The *ski7* mutation increased expression from all of these non-poly(A) mRNAs, regardless of the length of the 3′UTR (Fig. 3 and Table 3). As in the wild-type strain, the expression of polyadenylated mRNAs in a *ski7* strain was not strongly dependent on the length of the 3′UTR (Fig. 3).

From the time course of luciferase accumulation, the luc-64b-3′UTR or luc-104b-3′UTR mRNAs were found to induce a 3- to 4-fold-higher initial rate and a 2.5-fold-increased dura-

TABLE 3. 3′UTR length and *SKI7* effects on mRNA translation and stability[a]

Strain	$C^+ A^-$ RNA[b]	Initial rate	$Ft_{1/2}$	Maximum activity
Wild type	UTR 4b	0.9 ± 0.1	3.7 ± 0.1	0.15 ± 0.01
	UTR 24b	2.9 ± 0.1	3.2 ± 0.2	0.2 ± 0.01
	UTR 64b	10.3 ± 2.4	7.1 ± 0.1	1.9 ± 0.6
	UTR 104b	10.8 ± 0.6	8.4 ± 0.4	1.7 ± 0.3
ski7::HIS3	UTR 4b	3.9 ± 0.5	13.7 ± 0.3	1.4 ± 0.1
	UTR 24b	16.3 ± 3.1	17.2 ± 2.5	4.4 ± 0.1
	UTR 64b	18.0 ± 3.3	31.6 ± 0.3	8.6 ± 0.2
	UTR 104b	21.2 ± 4.8	34.4 ± 3.5	13.7 ± 0.5

[a] Wild-type (B959) and isogenic mutant (B117) strains were electroporated with the indicated $C^+ A^-$ luciferase mRNAs. From the time course of luciferase synthesis, we determined the initial rate of luciferase accumulation (initial rate) (in light units [×100] per minute per microgram of protein) and functional stability ($Ft_{1/2}$) (in minutes required to reach one-half maximum luciferase activity). Maximum activity is reported in light units per microgram of protein. The control had 0.005 light unit/μg of protein. Errors indicate the variation of results within one experiment with identical preparations of RNA and cells.

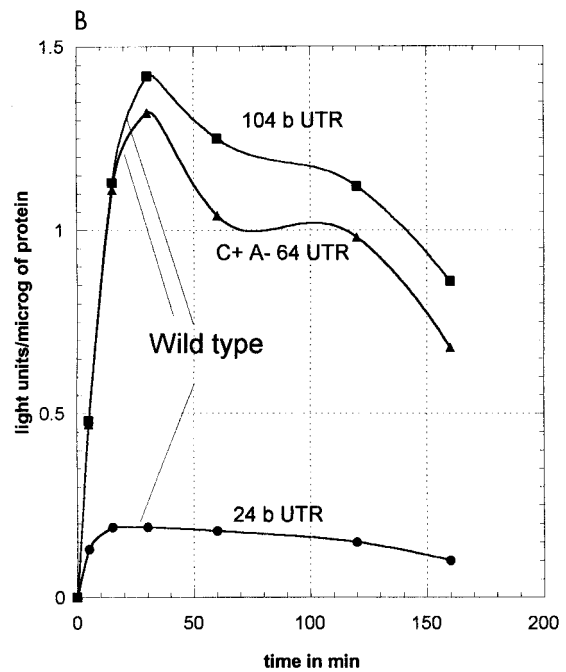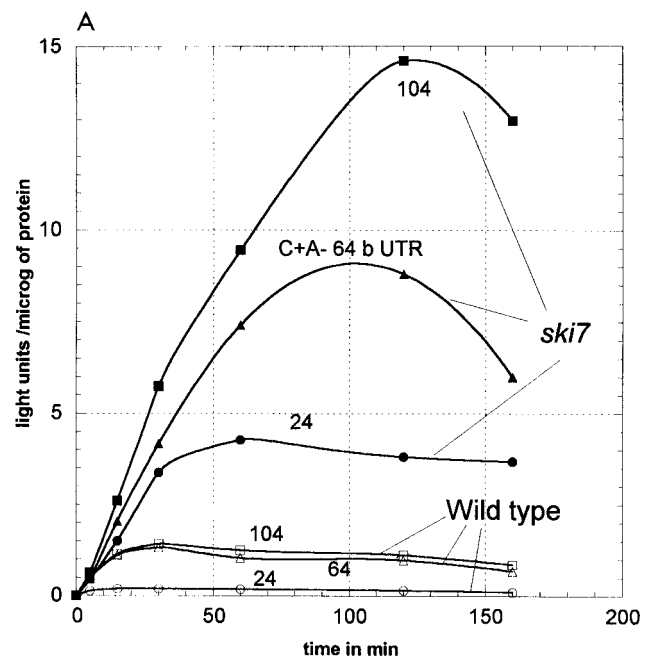[b] 4b, 24b, etc., indicate the number of bases in the 3′UTR.

FIG. 4. Time course of luciferase accumulation in cells electroporated with 2 μg of $C^+ A^-$ mRNAs with various lengths of 3′UTRs. Isogenic wild-type (B959) and *ski7*Δ mutant (B117) strains were used. (B) Wild-type results on an expanded scale. microg, microgram.

tion of expression compared to the luc-4b-3′UTR mRNA (Table 3), leading to a maximum luciferase activity that was 10-fold higher (Table 3).

The initial rate of expression for the luc-24b-3′UTR mRNA was 5.5-fold higher in the mutant than in the wild type (Fig. 4B and Table 3). The functional half-life was increased five times, and overall expression was 24-fold higher. Similarly, the initial rate for the luc-4b-3′UTR mRNA was increased fourfold in the *ski7* mutant. Thus, the *ski7* mutation clearly increases both
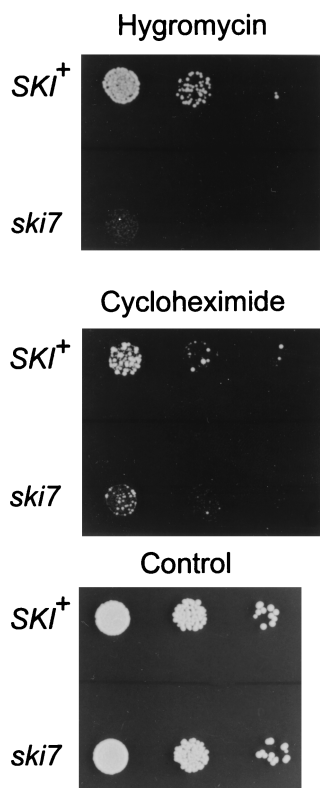
FIG. 5. Hypersensitivity of the $ski7\Delta$ mutant to hygromycin B and cycloheximide. Dilutions of suspended wild-type (B959) and $ski7\Delta$ mutant (B117) strains were spotted on rich medium containing hygromycin B, cycloheximide, or neither drug (control).

TABLE 4. $SKI7$ present in a high copy number cures M_2 dsRNA[a]

Pladmid used with $ski7$-1 mutant RV603	Killer toxin secretion at the following temp (°C):	
	25	32
pRS316 (single-copy vector)	++++	+++
pRS3167 (+ $SKI7$)	+	−
YEp351 (high-copy vector)	++++	+++
YEpSKI7 (+ $SKI7$)	−	−

[a] The $sci7$-1 strain RV603 was transformed at 25°C with the single-copy plasmids pRS316 and pRS3167 and with the high-copy-number plasmids YEp351 and YEpSKI7. Transformants were streaked for single colonies at the indicated temperature, and the colonies were then tested for toxin secretion at 20°C.

the initial rate of expression and the duration of expression of these RNAs.

One test of whether the $ski7$ mutation produces its effects by derepressing the activity of the 3′UTR is to examine an mRNA that has almost no 3′UTR. We used a C⁺ A⁻ mRNA with a 3′UTR only 4 nucleotides long. We still saw a 4-fold increase in the initial expression rate in the $ski7$ strain and a 3.7-fold increase in the functional half-life (Table 3). These results show that Ski7p does not act by repressing the activity of the 3′UTR. Conversely, a comparison of luc-4b-3′UTR and luc-64b-3′UTR mRNAs in the $ski7$ strain showed increases in the initial expression rate and in the functional half-life comparable to those seen in the wild-type host (Table 3). These results indicate that the 3′UTR effect is not dependent on Ski7p.

We also noted that while functional half-lives and initial expression rates are undoubtedly connected, there is no one-to-one relationship. For example, the initial expression rates for luc-24b-3′UTR in a wild-type strain and luc-4b-3′UTR in a $ski7$ mutant were similar, but their duration of expression differed by fourfold. Likewise, the functional half-lives for luc-24b-3′UTR and luc-4b-3′UTR in the $ski7$ mutant were similar, but their initial expression rates were fourfold different.

Antibiotic sensitivity of the $ski7$ mutant. Although the $ski7$ mutant had an apparently normal polysome profile (data not shown), many mutations affecting components of the translation apparatus are found to be hypersensitive to hygromycin B (7, 38) and other drugs that increase translational errors (23). Figure 5 shows that the $ski7$ mutant strain was hypersensitive to hygromycin B and slightly hypersensitive to cycloheximide (but

not to paromomycin; data not shown), supporting the notion that $SKI7$ affects ribosome function.

$SKI7$ is a regulator of non-poly(A) mRNA expression. We examined the effect of the overproduction of Ski7p on viral propagation and non-poly(A) mRNA expression. $SKI7$ was subcloned on a multicopy plasmid and used to transform RV603, a $ski7$-1 mutant. ski mutants are able to propagate M_2 dsRNA at 25 and 32°C and have a superkiller phenotype (Table 4) (see Materials and Methods). When the $ski7$ mutation was complemented by a single copy of $SKI7$ (plasmid pRS3167), the strain remained a killer at 25 but lost the virus at 32°C. This result is expected from a wild-type strain containing LA-HN and M_2 viruses (35). Interestingly, $SKI7$ present on a multicopy plasmid (YEpSKI7) induced the loss of killer toxin production and M_2 dsRNA at every temperature (Table 4 and data not shown).

We further analyzed the expression of non-poly(A) mRNAs in a $ski7$ mutant or wild-type strain in the presence or absence of $SKI7$ on a multicopy plasmid. Figure 3 shows that a $ski7$ mutant strain expressed luc-24b-3′UTR, luc-64b-3′UTR, and luc-104b-3′UTR 31.9-, 7.5-, and 8.7-fold better than a wild-type strain, respectively. Ski7p overproduction in a wild-type strain (or a $ski7$ mutant strain) reduced the expression of C⁺ A⁻ mRNAs, particularly those with luc-64b-3′UTR and luc-104b-3′UTR, to levels twofold below those seen in a wild-type strain with the vector alone (Table 5). We speculate that this stronger repression of expression of non-poly(A) mRNAs was responsible for the observed loss of the virus after several cell divisions.

Thus, inactivation of Ski7p produces derepression and its overproduction produces repression of C⁺ A⁻ mRNA expression. We conclude that the level of expression of Ski7p is critical for regulating non-poly(A) mRNA expression and M_2 virus propagation.

Ski7p activity in different ski backgrounds. Single deletions of $SKI2$, $SKI3$, $SKI7$, or $SKI8$ do not affect cell viability (34, 42, 53; this work). Within this group, multiple ski deletion strains were easily constructed. For example, the cross 4592-1C (MATa $leu2$ $ura3$ $his3$ $ski8::URA3$ $ski7::HIS3$) × 4593-6B (MATα $trp1$ $ura3$ $his3$ $ski3::URA3$ $ski2::HIS3$) gave 90% spore germination at 30°C. A quadruple disruptant (strain 3963) was confirmed by a subsequent cross. Overexpression of $SKI7$ in strain 3963 did not modify the expression of luciferase mRNAs (Table 5). Nor did overproduction of Ski7p alter expression in single $ski2$, $ski3$, or $ski6$ strains. Further, adding $ski2$, $ski3$, and $ski8$ mutations to a $ski7$ mutant did not alter either the initial rates or the duration of translation of different luciferase mRNAs (Table 6). These results suggest that the different Ski proteins are related in function.

$ski1/xrn1$ $ski7$ and $ski1/xrn1$ $ski2$ double mutants are viable but temperature sensitive for growth. The cold-sensitive and

TABLE 5. Overproduction of Ski7p blocks the expression of non-poly(A) mRNAs[a]

Strain	C[+] A[−] RNA	Luciferase expression with:		Ratio (YEp351/YEpSKI7)
		YEp351	YEpSKI7	
ski7::HIS3 3975	UTR 24b	12.7	0.40	31.9
	UTR 64b	14.9	1.33	11.2
	UTR 104b	20.9	1.33	15.7
Wild type 4602-2B	UTR 24b	0.16	0.11	1.5
	UTR 64b	2.9	1.2	2.4
	UTR 104b	2.2	1.0	2.2
ski6-2 RV493	UTR 24b	10.0	7.9	1.3
	UTR 64b	10.5	11.5	0.9
	UTR 104b	11.2	9.2	1.2
ski2::HIS3 3973	UTR 24b	19.0	17.3	1.1
	UTR 64b	30.2	31.2	1.0
	UTR 104b	29.9	26.6	1.1
ski3::URA3 3974	UTR 24b	12.2	20.2	0.6
	UTR 64b	25.9	25.4	1.0
	UTR 104b	24.6	33.5	0.7
Quadruple mutant 3963 (*ski2 ski3 ski7 ski8*)	UTR 24b	15.7	18.4	0.8
	UTR 64b	17.1	16.3	1.1
	UTR 104b	16.6	16.6	1.0

[a] The indicated C[+] A[−] luciferase RNAs were electroporated into strains with different *SKI* genes and in the presence of *SKI7* on a multicopy plasmid or the vector alone. Luciferase expression is reported in light units per microgram of protein. The control had less than 0.005 light unit/μg of protein. Values are averages of measurements from two independent experiments. Each measurement was made in duplicate.

temperature-sensitive growth of *ski2*, *ski3*, *ski4*, *ski6*, *ski7*, or *ski8* mutants depends on M dsRNA (35) and is believed to be due to its high copy number. However, a *ski2* mutation is known to also result in increased copy numbers of L-A, L-BC, and 20S RNAs (3, 25). It has been reported that *ski1/xrn1 ski2* and *ski1/xrn1 ski3* double mutants are lethal even if they lack L-A and M dsRNAs (18). Since *ski1/xrn1* mutants were first isolated based on their increased expression of viral mRNAs (47) and should derepress the expression of L-A, L-BC, 20S, and 23S RNAs, all of which apparently lack 5′ caps and are found in nearly all laboratory strains, it is possible that the reported lethality of *ski1 ski2* or *ski1 ski3* is due to viral effects.

We crossed deletion mutants of *ski1/xrn1* marked with *URA3* with *ski7::HIS3* and *ski2::HIS3* strains. The doubly heterozygous diploids were subcloned several times at 39°C in an attempt to cure viruses before sporulation (41), but this procedure cured only L-A and M dsRNAs. Germination at 30°C produced no viable double mutants, as was previously observed for *ski1/xrn1 ski2* and *ski1/xrn1 ski3*, but germination at 20°C produced almost the expected proportions of viable *ski1/xrn1*

ski7 and *ski1/xrn1 ski2* segregants (Table 7). The double-mutant segregants were unable to grow at 30°C, as expected, and showed prominent L-BC dsRNA bands (data not shown). Because there is no known method for curing 20S RNA or 23S RNA and heat curing of L-BC dsRNA is variable and ineffective in these strains, it remains unclear whether the growth defect in these double mutants was due to effects of these RNA replicons or to translation and mRNA stability effects on the host.

DISCUSSION

Viral mRNAs, 3′UTRs, and *SKI7*. Mutation of *SKI7* produces an increased copy number of L-A and M viruses and an associated superkiller phenotype. We demonstrate that this mutation specifically induces the expression of non-poly(A) luciferase mRNAs, thus explaining its effect on viruses, since L-A and M viral mRNAs are non-poly(A). Thus, the *SKI7* product is a repressor of non-poly(A) mRNA expression. This effect on luciferase or viral mRNAs having only a 3′UTR [but

TABLE 6. A quadruple *ski* mutant is similar to an *ski7* mutant[a]

Strain	C[+] A[−] RNA	Initial rate	Ft$_{1/2}$	Maximum activity
Quadruple mutant 3963 (*ski2::HIS3 ski3::URA3 ski7::HIS3 ski8::HIS3*)	UTR 24b	18.6	18	5.0
	UTR 64b	25.7	41	9.7
	UTR 104b	26.2	32.5	11.0
Single mutant 3975 (*ski7::HIS3*)	UTR 24b	17.9	18.5	4.3
	UTR 64b	24.5	32	8.8
	UTR 104b	30.2	32.5	13.2

[a] Strain 3963 or 3975 was electroporated with the indicated C[+] A[−] mRNAs, and the kinetics of luciferase synthesis were measured as described in Materials and Methods. Initial rate, initial rate of luciferase expression in light units (×100) per minute per microgram of protein. Ft$_{1/2}$, functional stability in minutes. Maximum activity is reported in light units per microgram of protein. The control had 0.005 light unit/μg of protein.

TABLE 7. Viability of *xrn1/ski1 ski2* and *xrn1/ski1 ski7* mutants[a]

Cross	Parents	Temp (°C) of germination	No. of *xrn1 ski* spores/ total no. of spores
4605	*xrn1 × ski2*	20	7/48 (12/48 expected)
4596	*xrn1 × ski2*	30	0/70
4602	*xrn1 × ski7*	20	5/24 (6/24 expected)
4597	*xrn1 × ski7*	30	0/76

[a] In each cross, *ski1*, *ski2*, and *ski7* were detected in the meiotic segregants by auxotrophy. Cross 4596 was D223-2A (*MAT**a** ura3 his3 trp1 xrn1::URA3*) × 4588-6C (*MATα ura3 his3 trp1 ski2::HIS3*); crosses 4597 and 4602 were D223-2A × 4587-1A (*MATα ura3 his3 trp1 leu2 ski7::HIS3*); and cross 4605 was 4602-3C (*MATα trp1 his3 ura3 xrn1::HIS3*) × 4591-2B (*MAT**a** leu2 his3 ura3 ski2::HIS3*).

no poly(A)] led us to question its possible involvement in this regulation by *SKI* genes. As has been shown in other systems (e.g., 45, 49), we find that 3′UTRs are not passive elements in mRNA expression but elevate both the initial rates and the duration of translation of electroporated mRNAs. 3′UTRs are also known to be the locations of certain specific signals affecting the processes of translation and mRNA turnover. We confirm that general 3′UTR actions can be detected only when the mRNA has been deadenylated, because the addition of a poly(A) tail largely overwhelms 3′UTR effects on translation.

The poly(A) structure and the poly(A) binding protein, Pab1p, act synergistically with the factors associated with the 5′ cap in initiation. The poly(A) structure may also play roles in translation termination, in reinitiation, and in protecting mRNA from degradation from both 3′ and 5′ ends. The 3′UTRs could affect any of these functions.

Knowing the 3′UTRs can promote mRNA expression in the absence of poly(A), we considered the possibility that Ski7p is normally a repressor of such a 3′UTR function. *ski7* mutants are enhanced for the functions to which the 3′UTR contributes, with increased initial rates and duration of translation. However, mRNAs which have essentially no 3′UTR also show a similar effect of *ski7* mutations. This fact indicates that Ski7p and the 3′UTR both affect related events but that Ski7p does not act by repressing 3′UTR function.

Translation efficiency and mRNA stability. Translation and mRNA turnover are intertwined in complex and poorly understood ways. Translation may either protect an mRNA or lead to its decay. For example, in nonsense-mediated decay, failure of translation of an mRNA leads to mRNA degradation (16). In contrast, there are several cases in which mRNA degradation requires translation of the mRNA to be degraded (2, 39). The 5′ cap and 3′ poly(A) structures each promote translation and protect the mRNA from degradation.

If one assumes that initial rates of translation of electroporated mRNA reflect translation efficiency and that functional half-life reflects mRNA stability, the experiments reported here show effects of *ski7* mutations on both. Which is the primary effect? The relationship of translation kinetics to initiation rates and mRNA degradation depends on several assumptions, not directly tested here. Arguing for an effect on translation efficiency are the Ski7p effect on initial rates of translation, the similarity of Ski7p to the known translation components HBS1p and EF1-α, and the fact that Ski7p inactivation produces hypersensitivity to drugs known to affect ribosomal function. Even with longer 3′UTRs, which would be expected to protect an mRNA from 3′ degradation, a substantial effect of a *ski7* mutation is seen. However, *ski2*, *ski3*, *ski7*, and *ski8* mutations also affect the functional half-life of mRNA (24; this work), effects which could be primary, as suggested by

others (1), or secondary to translation effects. It is also possible that *ski7* affects both translation efficiency and mRNA turnover. For example, the *UPF* genes have recently been found to function both in translation elongation (e.g., in maintenance of the reading frame) and in nonsense-mediated decay (8, 16). Further work is necessary to distinguish these possibilities.

Relationships of *SKI* genes. Although *ski1Δ* leads to slowed growth (20) and *ski6Δ* is lethal (4), *ski7Δ* does not affect cell growth if M dsRNA is absent. Even a quadruple *ski2 ski3 ski7 ski8* mutant is healthy and has kinetics of translation of C⁺ A⁻ luciferase mRNA similar to those of any of the single mutants. This finding indicates that these four Ski proteins are functionally related, perhaps part of a common complex or part of a common pathway. Ski7p may be the limiting component, since its overexpression can eliminate M₂ from a normal strain, but its overexpression does not suppress other *ski* mutations.

Although *xrn1/ski1Δ ski2Δ*, and *xrn1/ski1Δ ski3Δ* double mutants have been reported to be lethal (18), we find that at least *xrn1/ski1Δ ski2Δ* and *xrn1/ski1Δ ski7Δ* are viable at 20°C, although they do fail to grow at 30°C, the temperature at which the earlier experiments were performed. Several yeast RNA replicons, because they lack both the 5′ cap and 3′ poly(A), are normally subject to repression by the *SKI* genes. It is thus possible that this conditional lethal phenotype is due to these other replicons. There is no known method of curing 20S RNA or 23S RNA, and the ability of L-BC to be cured is variable and difficult at best, making testing of this question impractical at present.

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