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The tRNA Splicing Endonuclease Complex Cleaves the Mitochondria-localized *CBP1* mRNA*

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Background: The yeast tRNA splicing endonuclease (Sen) complex is located on the mitochondrial outer membrane and splices precursor tRNAs.
Results: The Sen complex cleaves the mitochondria-localized *CBP1* mRNA *in vivo* and *in vitro*.
Conclusion: Mitochondrial localization of the Sen complex is required for the cleavage of the *CBP1* mRNA.
Significance: This study shows a novel role of the tRNA splicing endonuclease complex in the cleavage of mitochondria-localized mRNA.

The tRNA splicing endonuclease (Sen) complex is located on the mitochondrial outer membrane and splices precursor tRNAs in *Saccharomyces cerevisiae*. Here, we demonstrate that the Sen complex cleaves the mitochondria-localized mRNA encoding Cbp1 (cytochrome *b* mRNA processing 1). Endonucleolytic cleavage of this mRNA required two *cis*-elements: the mitochondrial targeting signal and the stem-loop 652–726-nt region. Mitochondrial localization of the Sen complex was required for cleavage of the *CBP1* mRNA, and the Sen complex cleaved this mRNA directly *in vitro*. We propose that the Sen complex cleaves the *CBP1* mRNA, which is co-translationally localized to mitochondria via its mitochondrial targeting signal.

Cells have translation-dependent surveillance systems that eliminate aberrant mRNAs to prevent the production of potentially harmful protein products. The nonstop decay quality control system rapidly degrades aberrant mRNAs lacking a termination codon (nonstop mRNAs), which are produced mainly by premature polyadenylation within an ORF (1, 2). Perturbation of translation elongation by stable RNA secondary structures, rare codons, or positively charged nascent peptides induces both no-go decay (NGD),[4] in which mRNAs in the vicinity of stalled ribosomes undergo endonucleolytic cleavage (3, 4), and ribosome quality control, in which the arrested protein product is co-translationally degraded by the proteasome (5–12).

In *Saccharomyces cerevisiae*, Cbp1 (cytochrome *b* mRNA processing 1) is a nuclear-encoded protein that is imported into the mitochondria. It was proposed that Cbp1 plays a role in post-transcriptional regulation and translation of the mRNA encoding cytochrome *b* by interacting with its 5′-UTR (13, 14). The *CBP1* nonstop mRNA, which is produced by polyadenylation in the ORF, is rapidly degraded by nonstop decay (2). The ratio of nonstop to normal *CBP1* mRNA is regulated by the carbon source and is increased during the switch to respiratory growth (15), suggesting that the production of nonstop mRNA plays a role in down-regulation of the *CBP1* mRNA and protein, as well as the mRNA encoding cytochrome *b*, during the induction of respiration (14).

The tRNA splicing endonuclease (Sen) complex is localized to the nucleus in vertebrates (16) and the mitochondria in *S. cerevisiae* (17, 18). The yeast Sen complex splices tRNAs on the mitochondrial surface; although nuclear-localized Sen complex mutants are capable of tRNA splicing in yeast (18, 19), they cannot complement the lethality caused by depletion of the native Sen complex (19). Hence, the biological significance of mitochondrial localization of this complex is still largely unknown. Here, we report that the Sen complex cleaves the *CBP1* mRNA on the mitochondrial outer membrane. To our knowledge, this study is the first description of the potential correlation between mitochondrial localization of mRNAs and their endonucleolytic cleavage.

Experimental Procedures

Strains and Other Methods—The yeast gene disruption mutants were constructed using *kanMX4* (20) or *natMX4* (21), as described previously (22). The strains and plasmids used in this study are listed in Table 1, and the oligonucleotides used for plasmid construction are listed in Table 2. Northern and Western blot analyses were performed as described previously (22). The DIG-labeled probes used in this study are listed in Table 3.

Primer Extension Experiments—Primer extension experiments were performed using a 5′-fluorescently labeled primer

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[4] The abbreviations used are: NGD, no-go decay; ECIS, endonucleolytic cleavage-inducible sequence; MTS, mitochondrial targeting signal; Sen, tRNA splicing endonuclease; DIG, digoxigenin.

TABLE 1
Yeast strains and plasmids used in this study

Strain/plasmids	Genotype/plasmid	Source
Strains		
W303-1a	*MAT*a *ade2 his3 leu2 trp1 ura3 can1*	Lab stock
YIT2013	W303-1a *ski2*Δ::*kanMX*	Ref. 6
YIT2019	W303-1a *xrn1*Δ::*kanMX*	Ref. 29
YIT2020	W303-1a *xrn1*Δ::*kanMX dom34*Δ::*hygtMX*	Ref. 4
TYSC360	W303-1a *sen54*Δ::*HIS3*, p314–54 *CEN6-ARSH4 TRP1 SEN54*	Ref. 17
TYSC361	W303-1a *sen54*Δ::*HIS3*, p314–200 *CEN6-ARSH4 TRP1 sen54-*Δ*200–232*	Ref. 17
COSC05	W303-1a *sen2*Δ::*LEU2*, pCOSC05 *CEN6-ARSH4 TRP1 SEN2*	Ref. 17
COSC06	W303-1a *sen2*Δ::*LEU2*, pCOSC05 *CEN6-ARSH4 TRP1 sen2–41*	Ref. 17
YTS1612	W303-1a *sen54*Δ::*HIS3*, p314–54 *CEN6-ARSH4 TRP1 SEN54*, *ski2*Δ::*kanMX*	This study
YTS1613	W303-1a *sen54*Δ::*HIS3*, p314–200 *CEN6-ARSH4 TRP1 sen54-*Δ*200–232*, *ski2*Δ::*kanMX*	This study
YTS1614	W303-1a *sen54*Δ::*HIS3*, p314–54 *CEN6-ARSH4 TRP1 SEN54*, *xrn1*Δ::*kanMX*	This study
YTS1615	W303-1a *sen54*Δ::*HIS3*, p314–200 *CEN6-ARSH4 TRP1 sen54-*Δ*200–232*, *xrn1*Δ::*kanMX*	This study
YTS1616	W303-1a *sen2*Δ::*LEU2*, pCOSC05 *CEN6-ARSH4 TRP1 SEN2*, *ski2*Δ::*kanMX*	This study
YTS1617	W303-1a *sen2*Δ::*LEU2*, pCOSC05 *CEN6-ARSH4 TRP1 sen2–41*, *ski2*Δ::*kanMX*	This study
YTS1638	W303-1a *sen2*Δ::*LEU2*, pCOSC05 *CEN6-ARSH4 TRP1 SEN2*, *xrn1*Δ::*kanMX*	This study
YTS1639	W303-1a *sen2*Δ::*LEU2*, pCOSC05 *CEN6-ARSH4 TRP1 sen2–41*, *xrn1*Δ::*kanMX*	This study
YTS1798	W303-1a *tom20*Δ::*natMX*	This study
YTS1801	W303-1a *tom20*Δ::*natMX ski2*Δ::*kanMX*	This study
YTS1803	W303-1a *puf3*Δ::*hygMX ski2*Δ::*kanMX*	This study
YTS1806	W303-1a *tom20*Δ::*hygMX xrn1*Δ::*kanMX*	This study
Plasmids		
p416*GPDp*	URA3 CEN	Ref. 30
p416*GAL1p*	URA3 CEN	Ref. 30
p415*GAL1p*	LEU2 CEN	Ref. 30
p414*GAL1p*	TRP1 CEN	Ref. 30
p413*GAL1p*	HIS3 CEN	Ref. 30
yCplac33	URA3 CEN	Ref. 31
pSA144	p416*GPDp-GFP-FLAG-HIS3*	Ref. 32
pTYSC155	CEN6-ARSH4 URA3 *SEN54*	Ref. 17
pTS152	p416*GAL1p-CBP1*	This study
pTS220	p416*GAL1p-CBP1-*Δ*652–678*	This study
pTS221	p416*GAL1p-CBP1-*Δ*679–702*	This study
pTS223	p416*GAL1p-CBP1-*Δ*703–726*	This study
pTS256	p416*GAL1p-CBP1-*Δ*727–738*	This study
pTS257	p416*GAL1p-CBP1-*Δ*739–762*	This study
pTS258	p416*GAL1p-CBP1-*Δ*763–789*	This study
pTS343	p416*GAL1p-CBP1-FS (+652–726)*	This study
pTS344	p416*GAL1p-CBP1-FS (+727–789)*	This study
pTS244	p416*GAL1p-CBP1-M1*	This study
pTS247	p416*GAL1p-CBP1-M2*	This study
pTS184	p416*GAL1p-stemloop-CBP1*	This study
pTS260	p416*GAL1p-stemloop-CBP1-*Δ*MTS*	This study
pTS179	p416*GAL1p-CBP1-*Δ*MTS*	This study
pTS225	p416*GAL1p-CBP1-*Δ*uORF*	This study
pTS173	p416*GAL1p-CBP1-MMH2*	This study
pTS229	p416*GPDp-GFP-CBP1 (+643–738)-FLAG-HIS3*	This study
pTS231	p416*GPDp-CBP1 (+1–99)-GFP-FLAG-HIS3*	This study
pTS236	p416*GPDp-CBP1 (+1–99)-GFP-CBP1 (+643–738)-FLAG-HIS3*	This study
pTS324	p416*GPDp-CBP1 (+1–99)-GFP-CBP1 (FS 643–738)- FLAG-HIS3*	This study
pTS332	p416*GPDp-CBP1 (+1–99)-GFP-CBP1 (M1 643–738)- FLAG-HIS3*	This study
pTS318	p415*GAL1p-SEN2*	This study
pTS299	p415*GAL1p-His6-flag-SEN2*	This study
pTS300	p415*GAL1p-His6-flag-sen2 H297A*	This study
pTS305	p413*GAL1p-SEN15*	This study
pTS314	p414*GAL1p-SEN54*	This study
pTS320	p416*GAL1p-SEN34*	This study
pTS351	yCplac33*TOM20p-TOM20*	This study

(LI-COR Biosciences) complementary to the +786–815-nt region of the *CBP1* mRNA. PrimeScript reverse transcriptase (TaKaRa) was used to synthesize a single-stranded DNA toward the 5′ end of the RNA. The reaction contained 5 μg of RNA and 0.1 pmol of 5′-fluorescently labeled primer. The size of the labeled single-stranded DNA was determined relative to a sequencing ladder on a sequencing gel.

Western Blotting—Yeast cells were grown in minimal medium containing 2% glucose or galactose. The cells were harvested when the culture reached an A_{600} of 0.6. The protein products of various *GFP-HIS3* reporter genes were detected by Western blotting using an anti-GFP antibody (sc-9996; Santa Cruz Biotechnology) and an anti-mouse IgG, horseradish peroxidase-conjugated secondary antibody (GE Healthcare). The intensities of the bands on the blots were quantified using the LAS4000 imaging system and Multi-Gauge version 3.0 software (Fujifilm). The relative levels of the protein products were determined by comparison with a standard curve prepared using a series of dilutions.

In Vitro tRNA Splicing Assay—FLAG-tagged wild-type Sen2 and FLAG-tagged Sen2-H297A were purified from cell extracts and examined for endonucleolytic activity. Nontagged wild-type Sen2 was used as a negative control. The RNA products were separated on a polyacrylamide gel containing 7 M urea, dried, and then exposed to an imaging plate (Fujifilm). Identification of all RNA species was confirmed by UTP labeling of

TABLE 2
List of oligonucleotides used for plasmid construction

Description	Sequence
CBP1	5′-GCACTAGTATTTTGCACGTTTCCCTTTCCATGCAATGT-3′ 5′-GCGTCGACATATCGTAAATGTGCGTTTGGCCGTTCATC-3′
CBP1-FS(+652–726)	5′-ATTAGAAAGGAGTTTCTAATTGAAACCTCGTTAACTTTATTGACCGCCTCCAAGGTTATACTTTCGAACAGAGAGTTCAG-3′ 5′-CGAGGTTTCAATTAGAAACTCCTTTCTAATAAGGCCCTCGGCTTACGTAATGAAGCCACCGCAATTATGCAAGGCTATAC-3′
CBP1-FS(+727–789)	5′-AAAGAATAGTTTATATTCATCAGTTTGTTCAGTATAGCCTTGCATAATTGCGGTGGCTTCATTACTTAAGCCGAGGGCCT-3′ 5′-GGCTATACTGAACAAACTGATGAATATAAACTATTCTTTGACTATACGATATTGAAGCTTCGGGATGATCAAGTGCTGCT-3′
CBP1-M1	5′-CAACTGAACTCTCTGTTCGAAAGTATAACCGGAGGCGGAATAAAGTTAACCAGATTTCAATTAGAAACTCTTAGTAATAAAGC CTTAGGCTTAAGTAATGAAGCCCCGCAATTATGC-3′ 5′-GGTTATACTTTCGAACAGAGAGTTCAGTTG-3′
CBP1-M2	5′-CAACTGAACTCTCTGTTCGAAAGTATAACCGGAGGTGGAATAAAGTTAACTAGATTCCAGCTAGAGACATTGAGTAACAAAGC CTTAGGATTAAGTAATGAAGCCCCGCAATTATGCAAG-3′ 5′-GGTTATACTTTCGAACAGAGAGTTCAGTTG-3′
CBP1-ΔMTS	5′-GACTAGTATGGATCCAATTCAAAAACAGGTCTTGGC-3′ 5′-GCGTCGACATATCGTAAATGTGCGTTTGGCCGTTCATC-3′
CBP1-Δupstream ORF	5′-GACTAGTATGTTTTTACCTCGTCTCGTTCGG-3′ 5′-GCGTCGACATATCGTAAATGTGCGTTTGGCCGTTCATC-3′
CBP1-MMH2	5′-GATCTGCAGCGAGATGTTCTGGCAGCTGACCCCCGAGTACTACTGCAACAACCCACTGATCCTGCCGGCCATCATCGACTTC ATCACCAAGCAGGACAGCCTCACCATGGCCAAGGAACTCATGCAGAACATTAACAGATAC-3′ 5′-GTCAGCTGCCAGAACATCTCGCTGCAGATCGATCGGTAGGCCCGGTTGTGGGTGCAGATCTTGAAGACCTTGTAGATGATCC TGCTGTCGTGCGCGCTAATGGAAACATAGTGCTCTTTGATTTTGTTCC-3′
CBP1(+643–738)	5′-CTAGAAGTATAACCGGAGGCGGAATAAAGTTAACGAGGTTTCAATTAGAAACTCTTTCTAATAAGGCCCTCGGC TTAAGTAATGAAGCCCCGCAATTATGCA-3′ 5′-CTAGTGCATAATTGCGGGGCTTCATTACTTAAGCCGAGGGCCTTATTAGAAAGAGTTTCTAATTGAAACCTCGTTAACTTTA TTCCGCCTCCGGTTATACTT-3′
CBP1(+1–99)	5′-CTAGAATGTTTTTACCTCGTCTCGTTCGGTACAGGACCGAGAGGTTTATAAAAATGGTACCTACCAGGACCTTGCGA CGAATCAACCACAGCAGCAGGGATCCAA-3′ 5′-CTAGTTGGATCCCTGCTGCTGTGGTTGATTCGTCGCAAGGTCCTGGTAGGTACCATTTTTATAAACCTCTCGGTCCTGTACC GAACGAGACGAGGTAAAAACATT-3′
FS 643–738	5′-CTAGTAAGTATAACTCGGAGGCGGTCAATAAAGTTAACGAGGTTTCAATTAGAAACTCCTTTCTAATAAGGCCCTC GGCTTACGTAATGAAGCCCCGCAATTATGCAT-3′ 5′-CTAGATGCATAATTGCGGGGCTTCATTACGTAAGCCGAGGGCCTTATTAGAAAGGAGTTTCTAATTGAAACCTCGTTAACTT TATTGACCGCCTCCGAGTTATACTTA-3′
M1 643–738	5′-CTAGAAGTATAACCGGAGGCGGAATAAAGTTAACCAGATTTCAATTAGAAACTCTTAGTAATAAAGCCTTAGGCTTAAGTA ATGAAGCCCCGCAATTATGCA-3′ 5′-CTAGTGCATAATTGCGGGGCTTCATTACTTAAGCCTAAGGCTTTATTACTAAGAGTTTCTAATTGAAATCTGGTTAACTTTA TTCCGCCTCCGGTTATACTT-3′
His6-flag-SEN2	5′-GACTAGTATGCATCACCATCACCATCACCATCACGACTACAAGGACGACGATGACAAGATGTCTAAAGGGAGGvGTCAATCA GAAGCGTTAC-3′ 5′-CCCAAGCTTCTAGTCTCTATTTCTTCCGGGAACCCATCTCTTATAC-3′
His6-flag-sen2 H297A	5′-GATTATTTATTATATAAGAGAGGGCCACCATTTCAAGCCGCTGAATTTTGTGTTATGGGTCTTGAC-3′ 5′-GTCAAGACCCATAACACAAAATTCAGCGGCTTGAAATGGTGGCCCTCTCTTATATAATAAATAATC-3′
Tom20 −500F and +500R	5′-CGAGCTCGGCAGCTATGCCATCGTTAAGTGTGGATTTCCC-3′ 5′-CCGCTCGAGATAAAATTGGCGGTAAGAAAGGATCTGAAGG-3′

TABLE 3
List of oligonucleotides used for DIG-labeled probes

Probe name	DIG labeling method	Oligonucleotide sequences
GFP	Internal label (PCR)	5′-GCTCTAGAATGAGTAAAGGAGAAGAACTTTTCAC-3′ 5′-GGACTAGTTTTGTATAGTTCATCCATGCCA-3′
HIS3	Internal label (PCR)	5′-GCTCTAGATGACAGAGCAGAAAGCCCTAG-3′ 5′-CGGGATCCCATAAGAACACCTTTGGTGG-3′
CBP1 168–210	5′ end label	5′-CCGAAGACTTTTGTAGTCCGCCATGTCAGACCAATATTTCCG-3′
CBP1 457–504	5′ end label	5′-CAGCAGGAAAAGGTCGGCAGCCATAACTATGTCTCCTGT GCCGATGGC-3′
CBP1 786–835	5′ end label	5′-CAATGGACTTGTACGCAAGCAGCACTTGATCATCCCGAA GCTTCAAATCG-3′
CBP1 1189–1258	5′ end label	5′-GTTAAGCCAGACAATATGATGGTTTTCGGGTAAGTGTAT CTGTTAATGTTCTGCATGAGTTCCTTGGCC-3′
CBP1 −26–889	Internal label (PCR)	5′-GCACTAGTATTTTGCACGTTTCCCTTTCCATGCAATGT-3′ 5′-TGCTCTTTGATTTTGTTCCAA-3′
CBP1 1101–1965	Internal label (PCR)	5′-CATGCAGAACATTAACAGATACAC-3′ 5′-GCGTCGACATATCGTAAATGTGCGTTTGGCCGTTCATC-3′
CBP1 1–300	Internal label (PCR)	5′-GACTAGTATGTTTTTACCTCGTCTCGTTCGG-3′ 5′-TGAGGTTTTTGTGCGATTGATGAAACTG-3′

precursor RNA substrates. All procedures were performed as described previously (23, 24).

Plasmid Construction—Recombinant DNA procedures were performed as described previously (25). Plasmids expressing various *CBP1* mRNAs were constructed as follows: To construct pTS152 (p416*GAL1p-CBP1*), a SpeI-SalI fragment was amplified by PCR using the two *CBP1* primers listed in Table 2 and then inserted into the SpeI-SalI sites of p416*GAL1*p. To construct the *CBP1* deletion constructs pIT220, 221, 223, and 256–258, deletions were introduced by PCR-based site-directed mutagenesis. An oligonucleotide (5′-GATCCGATATCCCGTGGAGGGGCGCGTGGTGG-CGGCTGCAGCCGCCACCACGCGCCCCTCCACGGGA-TATCG-3′) was self-annealed and inserted into the SpeI site of pTS152 or pTS179 to create the pTS184 or pTS260 reporter, respectively.

The plasmids expressing various *GFP-HIS3* reporters were constructed as follows: The two *CBP1(+643–738)* oligonucleotides listed in Table 2 were annealed and inserted into the SpeI site of pSA144 (p*GPDp-GFP-FLAG-HIS3-CYC1ter*) to create the pTS229 (p416*GPDp-GFP-CBP1(+643–738)-FLAG-HIS3*) reporter. The two *CBP1(+1–99)* oligonucleotides were annealed and inserted into the XbaI site of pSA144 and pTS229 to create pTS231 (p416*GPDp-CBP1(+1–99)-GFP-FLAG-HIS3*) and pTS236, respectively. Two *FS 643–738* or *M1 643–738* oligonucleotides were annealed and inserted into the SpeI site of pTS231 to create pTS324 and pTS332, respectively.

The plasmids expressing various Sen complex proteins were constructed as follows: To construct pTS318, 305, 314, and 320, the ORF fragments of each protein were amplified by PCR and inserted into p415*GAL1p*, p413*GAL1p*, p414*GAL1p*, or p416*GAL1p*, respectively. To construct pTS299, the SpeI-HindIII fragment of *SEN2* was amplified by PCR using the two *His6-flag-SEN2* primers listed in Table 2 and then inserted into the SpeI-HindIII sites of p415*GAL1p*. To construct pTS300, a point mutation was introduced by PCR-based site-directed mutagenesis using the *His6-flag-sen2 H297A* primers.

The plasmids expressing the Tom20 protein were constructed as follows. To construct pTS351, a SacI-XhoI ORF fragment including *TOM20* was amplified by PCR using the *Tom20 −500F and +500R* primers listed in Table 2 and then inserted into the SacI-XhoI sites of yCplac33.

Results

The CBP1 mRNA Undergoes Endonucleolytic Cleavage—The *CBP1* nonstop mRNA is an endogenous target of nonstop decay that is rapidly degraded by the exosome, a 3′ to 5′ exoribonuclease complex (1, 2). The Dom34-Hbs1 complex stimulates the degradation of nonstop mRNAs by dissociating ribosomes that are stalled at the 3′ ends of mRNAs (Fig. 1*A*) (4). The *CBP1* nonstop mRNA was more stable in *xrn1*Δ*dom34*Δ ($t_{½}$ = 19.2 min) cells than *xrn1*Δ cells ($t_{½}$ = 12.2 min) (Fig. 1*B*), indicating that the Dom34-Hbs1 complex facilitates the degradation of the *CBP1* nonstop mRNA. Unexpected transcripts were detected in *ski2*Δ or *xrn1*Δ mutant cells, but not wild-type cells, indicating that endonucleolytic cleavage may occur within the ORF of the *CBP1* nonstop mRNA (Fig. 1*B*). Northern blotting with probes corresponding to various regions of the *CBP1* mRNA revealed that the 700-nt 5′ fragment (5′ cleavage product) was detected even in *ski2*Δ mutant cells that were deficient in 3′ to 5′ degradation pathways (Fig. 1, *A–C*). Two 3′ fragments of 1100 nt (3′ cleavage product) and 300 nt (3′ cleavage product of nonstop) were detected only in *xrn1*Δ mutant cells that were deficient in 5′ to 3′ degradation pathways (Fig. 1, *A–C*). These results indicate that endonucleolytic cleavage occurs in the vicinity of the 600–780-nt region of the *CBP1* ORF. The 5′ cleavage product lacked a termination codon, and the Dom34-Hbs1 complex facilitated its degradation as expected ($t_{½}$ > 32.0 min and $t_{½}$ = 22.6 min in *xrn1*Δ*dom34*Δ and *xrn1*Δ cells, respectively; Fig. 1*B*).

To determine whether translation is required for endonucleolytic cleavage of the *CBP1* mRNA, translation was inhibited by adding cycloheximide to the cells or inserting a stem-loop structure into the 5′-UTR of the mRNA. After the induction of *CBP1* transcription from the *GAL1* promoter in the presence of cycloheximide, the 3′ and 5′ cleavage products were only barely observed in *xrn1*Δ cells (Fig. 1*D*) and *ski2*Δ cells (data not shown), respectively. Endonucleolytic cleavage of the *CBP1* mRNA was also abrogated by the insertion of a stem-loop structure into the 5′-UTR (Fig. 1*E*, *lanes 3* and *4*). To identify the region of the *CBP1* mRNA that must be translated to allow cleavage, *CBP1* mutants that lacked the mitochondrial targeting signal (MTS) or upstream ORF were generated. The 3′ cleavage product was detected in mutants that lacked an upstream ORF, but not in those that lacked the MTS (Fig. 1*F*, *lanes 1–6*). These results indicate that translation of the region of the *CBP1* mRNA encoding the MTS is required for its endonucleolytic cleavage. The 3′ cleavage product was also detected in an MMH2 mutant that was defective in premature polyadenylation in the ORF (Fig. 1*F*, *lanes 7* and *8*) (14), indicating that polyadenylation within the *CBP1* ORF to produce nonstop mRNA is dispensable for its endonucleolytic cleavage.

The Secondary Structure of the cis-Element in the CBP1 mRNA Is Crucial for Its Cleavage—A deletion analysis revealed that the +652–726 nt region of the *CBP1* ORF is indispensable for cleavage of the mRNA (Fig. 2*A*). Hence, this region was named the endonucleolytic cleavage-inducible sequence (ECIS). Next, a primer extension analysis was performed to analyze the 5′ ends of the 3′ cleavage products. The major cleavage sites, +720 and +721 nt, were located close to the 3′ end of the ECIS (*C2* and *C1* in Fig. 2*B*), and additional minor cleavage sites were located upstream of the major sites (*C3*, *C4*, and *C5* in Fig. 2*B*). To address the significance of nascent peptides to the cleavage of *CBP1*, a frameshift mutation was introduced at the ECIS or the +727–789-nt region. The 3′ and 5′ endonucleolytic cleavage products derived from the frameshift *CBP1* mutants were detected (Fig. 2*C* and data not shown), indicating that the translational product of the ECIS is not essential for the mRNA cleavage. Analysis of the secondary structure of the ECIS using the MFOLD program (26) identified a stem-loop structure (Fig. 2*D*); therefore, the *CBP1* mRNA sequence was mutated (M1 and M2) to disrupt the secondary structure but retain the amino acid sequence of the ECIS (Fig. 2*D*). The 3′ endonucleolytic cleavage products derived from the M1 and M2 mutants were not detected in *xrn1*Δ cells (Fig. 2*E*), indicating that the secondary structure of the *CBP1* mRNA is indispensable for its cleavage.

To determine whether they are able to induce endonucleolytic cleavage of the *CBP1* mRNA, reporter genes containing the MTS and/or the ECIS were generated. The +643–738-nt sequence containing the ECIS (+652–726 nt) was inserted between the *GFP* and *HIS3* genes, and the MTS was inserted at the 5′ end of *GFP* to construct the *MTS-GFP-ECIS-HIS3* fusion (Fig. 2*F*). Northern blotting with a DIG-labeled *GFP* probe to detect the 5′ cleavage product (data not shown) or a DIG-labeled *HIS3* probe to detect the 3′ cleavage product (Fig. 2*G*) revealed that insertion of both the ECIS and the MTS of *CBP1,* but not either alone, induced the endonucleolytic cleavage of the *GFP-HIS3* reporter gene.

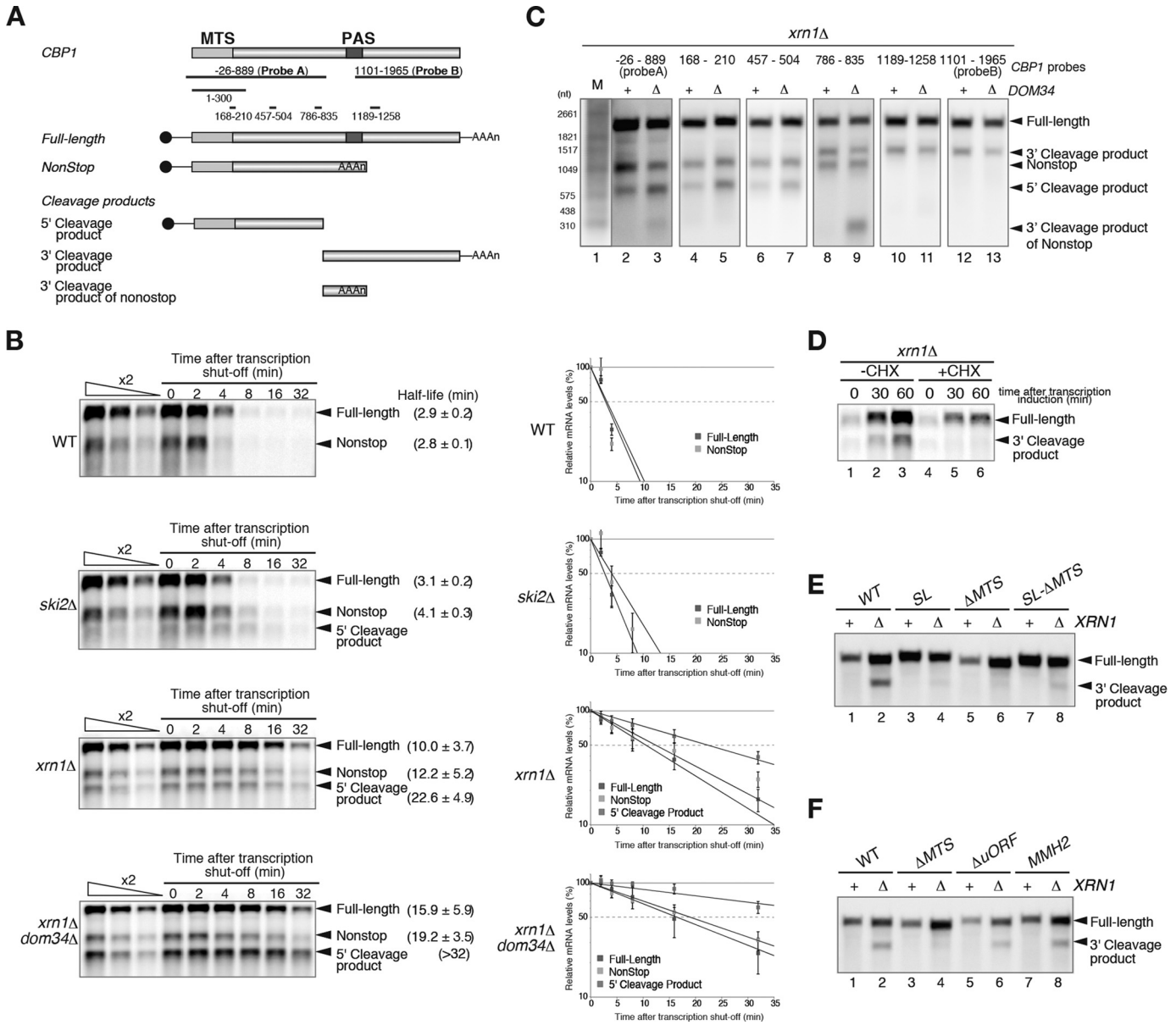
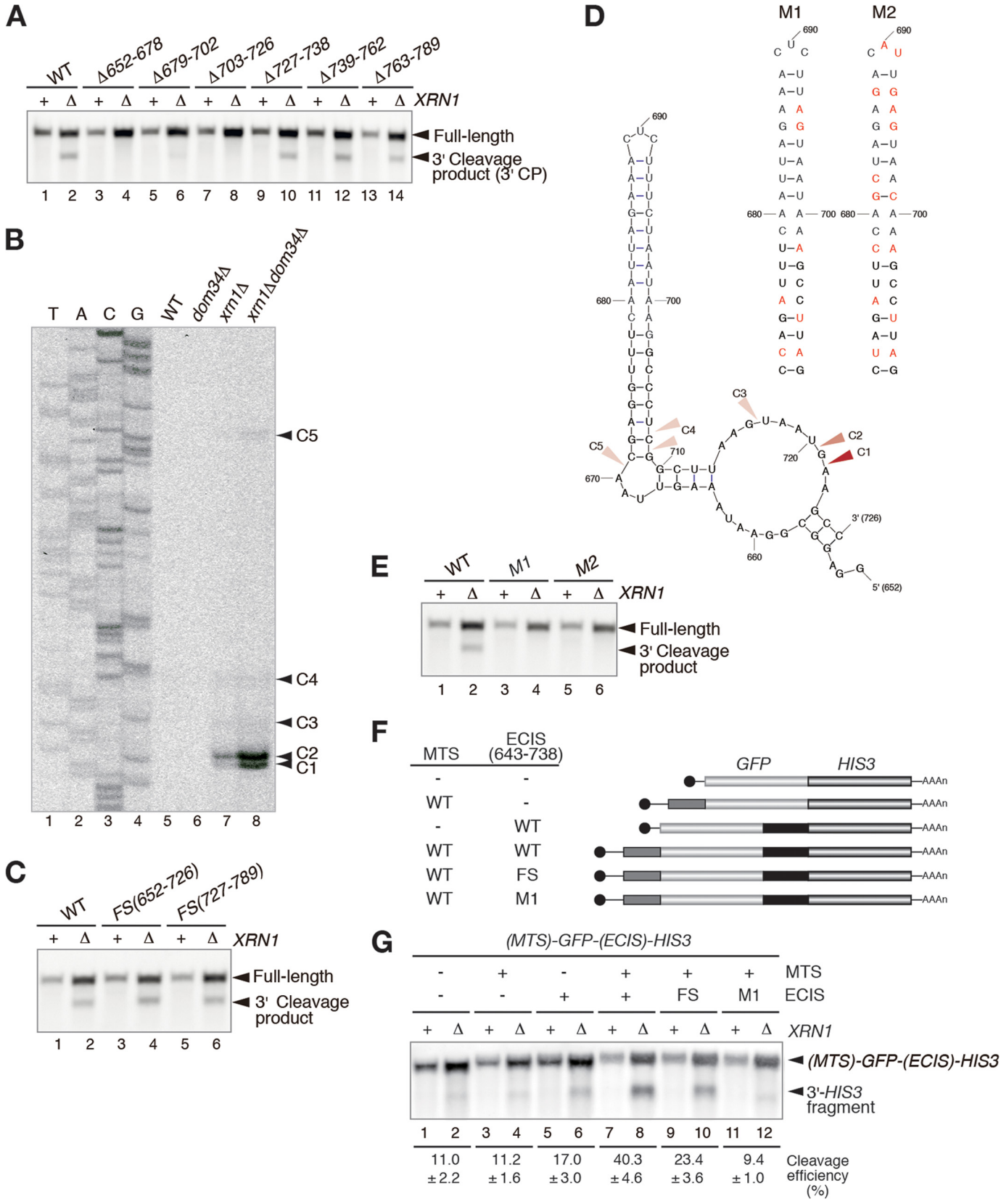


FIGURE 1. **Translation of the MTS is required for endonucleolytic cleavage of the *CBP1* mRNA.** *A*, a schematic illustration of the *CBP1* mRNA. The *filled box* indicates the ORF, the *line* represents nontranslated regions, and the tract of *A*s denotes the poly(A) tail. The MTS region encodes the mitochondrial targeting signal. *PAS* indicates the polyadenylation site. *B*, the *CBP1* mRNA undergoes endonucleolytic cleavage. *Left panels*, the half-lives of the mRNAs are shown as the means ± standard deviation of three independent experiments. The detection of the 5′ cleavage product by Northern blotting with a DIG-labeled *CBP1* 1–300 probe. *Right panels*, graphs of the half-life analysis. The relative amounts of the mRNAs in the indicated cells are shown as the means ± standard deviation of three independent experiments. *C*, Northern blotting with probes corresponding to various regions of the *CBP1* mRNA identified a number of cleavage products. W303*xrn1*Δ and W303*xrn1*Δ*dom34*Δ mutants were grown in minimal medium containing 2% galactose. RNA samples were analyzed by Northern blotting with the DIG-labeled *CBP1* probes depicted in *A*. The results shown are representative of three independent experiments. *D*, translation is required for endonucleolytic cleavage of the *CBP1* mRNA. After translation inhibition by the addition of cycloheximide for 10 min, transcription from the *GAL1p-CBP1* plasmids was induced by the addition of galactose. The cells were harvested at the indicated time points after induction. The 3′ cleavage product was detected by Northern blotting with a DIG-labeled *CBP1* 1101–1965 probe, which is depicted as probe B in *A*. The results shown are representative of three independent experiments. *E*, the mitochondrial targeting signal of the *CBP1* mRNA is required for its cleavage. W303 and W303*xrn1*Δ mutant cells were transformed with the indicated *CBP1* plasmids. *SL*, the stem-loop structure was inserted into the 5′-UTR. Δ*MTS*, deletion of the mitochondrial targeting signal. The 3′ cleavage product was detected as described for *D*. The results shown are representative of three independent experiments. *F*, neither the upstream ORF nor polyadenylation within the ORF are required for cleavage of the *CBP1* mRNA. *MMH2*, *CBP1* mutant defective in polyadenylation within the ORF. Δ*uORF*, deletion of the upstream ORF. The 3′ cleavage product was detected as described for *D*. The results shown are representative of three independent experiments.

The Sen Complex Is Responsible for Cleavage of the CBP1 mRNA in Vivo and in Vitro—The MTS of the *CBP1* mRNA was required for its endonucleolytic cleavage, suggesting that this mRNA is cleaved on the mitochondrial surface. Because the Sen complex localizes to the mitochondria in *S. cerevisiae*, we examined its role in cleavage of the *CBP1* mRNA. Endonuclease activity is impaired severely in *sen2–41* temperature-sensitive mutant cells at the restrictive temperature (18); therefore, *sen2*Δ*ski2*Δ and *sen2*Δ*xrn1*Δ mutants harboring either wild-type *SEN2* or *sen2–41* mutant plasmids were generated. Total RNAs were prepared from cells grown at the permissive (30 °C) or restrictive (37 °C) temperature and then analyzed by North-


A
WT
Δ652-678
Δ679-702
Δ703-726
Δ727-738
Δ739-762
Δ763-789
+ Δ + Δ + Δ + Δ + Δ + Δ + Δ
XRN1
Full-length
3' Cleavage product (3' CP)
1 2 3 4 5 6 7 8 9 10 11 12 13 14
B
T A C G
WT
dom34Δ
xrn1Δ
xrn1Δdom34Δ
C5
C4
C3
C2
C1
1 2 3 4 5 6 7 8
C
WT
FS(652-726)
FS(727-789)
+ Δ + Δ + Δ
XRN1
Full-length
3' Cleavage product
1 2 3 4 5 6
D
M1
M2
690
680
700
710
720
670
660
C1
C2
C3
C4
C5
3' (726)
5' (652)
E
WT
M1
M2
+ Δ + Δ + Δ
XRN1
Full-length
3' Cleavage product
1 2 3 4 5 6
F
MTS
ECIS (643-738)
- -
WT -
- WT
WT WT
WT FS
WT M1
GFP
HIS3
AAAn
G
(MTS)-GFP-(ECIS)-HIS3
- + - + + + MTS
- - + + FS M1 ECIS
+ Δ + Δ + Δ + Δ + Δ + Δ + Δ
XRN1
(MTS)-GFP-(ECIS)-HIS3
3'-HIS3 fragment
1 2 3 4 5 6 7 8 9 10 11 12
11.0 ±2.2
11.2 ±1.6
17.0 ±3.0
40.3 ±4.6
23.4 ±3.6
9.4 ±1.0
Cleavage efficiency (%)

ern blotting with *CBP1* probes. Endonucleolytic cleavage was abrogated in *sen2–41* cells under both conditions, indicating that the Sen complex cleaves the *CBP1* mRNA *in vivo* (Fig. 3*A*, *lanes 2* and *6*).

The *sen54*-Δ(200–232) mutant is defective in mitochondrial localization of the Sen complex (16). Neither the 3′ cleavage products (Fig. 3*B*, *lane 4*) nor the 5′ cleavage products (Fig. 3*B*, *lane 10*) of the *CBP1* mRNA were detected in *sen54*-Δ(200–232) mutant cells. These defects were complemented by transforming the cells with a plasmid harboring wild-type *SEN54* (Fig. 3*B*, *lanes 6* and *12*). These results indicate that endonucleolytic cleavage of the *CBP1* mRNA requires mitochondrial localization of the Sen complex. Complementary results were also obtained using the *MTS-GFP-ECIS-HIS3* reporter gene (Fig. 3*C*), indicating that the reporter mRNA localized to mitochondria by the *CBP1* MTS underwent cleavage at the ECIS by the Sen complex.

Next, we examined whether the Sen complex cleaves NGD-targeted mRNAs. NGD caused by translation arrest-inducing sequences was intact in *sen2–41* and *sen54*-Δ(200–232) mutant cells (data not shown). Moreover, cleavage of NGD target mRNAs was not stimulated by the presence of a MTS at the amino terminus (data not shown), suggesting that the *CBP1* mRNA and NGD-targeted mRNAs might be cleaved by different mechanisms. To determine whether it cleaves the *CBP1* mRNA directly *in vitro*, the Sen complex was affinity-purified from cells expressing FLAG-tagged wild-type Sen2 or a FLAG-tagged Sen2-H297A mutant, which is deficient in 5′ endonuclease activity toward tRNA (23, 24) (Fig. 3*D*). The partially purified Sen complexes were tested in endonucleolytic cleavage assays using [^{32}P]UTP-labeled RNAs. In the assays using the wild-type Sen complex, fragments of 41 nt were cleaved from every RNA species containing the ECIS sequence (Fig. 3*E*, *lanes 7* and *11*). We speculated that these fragments might correspond to the stem-loop region of the ECIS and that the cleavage site may correspond to the C4 site shown in Fig. 1*D*. Cleavage of these fragments did not occur in assays using the Sen2-H297A complex (Fig. 3*E*, *lanes 8* and *12*), indicating that this mutant is deficient in endonucleolytic cleavage of the 3′ region of the ECIS (Fig. 3*F*). Overall, these results are consistent with the *in vivo* data and indicate that the Sen complex cleaves the *CBP1* mRNA directly *in vitro*. We noticed that the *in vitro* experiment using the mutant Sen complex produced a longer cleavage product, which may have resulted from the catalytic activity of Sen34, the Sen subunit harboring the other endonucleolytic center. In addition, the 3′ cleavage site *in vitro* is different from the major site *in vivo* (Figs. 2 and 3). These results suggest that processing of the ECIS *in vivo* requires the collaboration of several endonucleolytic activities, including the Sen activities.

Recognition of the Cbp1-translating Ribosome-mRNA Complex by Components of the Mitochondrial Translocation Machinery Is Crucial for Cleavage of the CBP1 mRNA—The MTS of a nascent peptide emerging from the ribosome is recognized by the mitochondrial translocator component Tom20, which is a MTS receptor that targets the translating mRNA to the mitochondrial outer membrane (7, 27). In addition, the Pumilio family protein Puf3 binds to the ORF and the 3′-UTR of specific mRNAs to localize them to the mitochondrial outer membrane (7). To determine whether Tom20 or Puf3 affects endonucleolytic cleavage of the *CBP1* mRNA, *tom20*Δ or *puf3*Δ mutant cells were constructed in a *ski2*Δ or *xrn1*Δ mutant background. Cleavage of the *CBP1* mRNA was reduced in both the *tom20*Δ*xrn1*Δ and *tom20*Δ*ski2*Δ mutant cells, and this defect was complemented by transformation of the cells with a plasmid expressing Tom20 (Fig. 4*A* and data not shown). In addition, cleavage of the *MTS-GFP-ECIS-HIS3* reporter mRNA was reduced in the *ski2*Δ*tom20*Δ mutant cells, but not in the *ski2*Δ*puf3*Δ mutant cells (Fig. 4*B*, *lane 4*). These results suggest that Tom20 is required for mitochondrial localization of the *CBP1* mRNA, thereby facilitating its endonucleolytic cleavage by the Sen complex.

Discussion

The Cytoplasmic CBP1 mRNA Undergoes Endonucleolytic Cleavage by the tRNA Splicing Complex on the Outer Membrane of the Mitochondria—The results presented here demonstrate that the tRNA splicing endonuclease Sen complex cleaves the *CBP1* mRNA on the mitochondrial outer membrane. The *CBP1* mRNA was endonucleolytically cleaved *in vivo* (Fig. 1). Deletion analyses revealed that a MTS and an ECIS, the latter of which is located at the +652–726-nt region and forms a secondary structure, were indispensable for the cleavage (Figs. 1 and 2). In support of this finding, translation inhibition of the MTS and ECIS inhibited the endonucleolytic cleavage of the *CBP1* mRNA (Fig. 1). Furthermore, insertion of the +643–738-nt region of the *CBP1* mRNA, which included the ECIS, into a reporter mRNA induced endonucleolytic cleavage when a MTS was also present at the amino terminus (Fig. 2). Cleavage of the *CBP1* mRNA was eliminated in a temperature-sensitive *sen2* mutant that lacked endonuclease activity, and in a *sen54* mutant displaying a defect in localization of Sen54 to the mitochondria (Fig. 3). *In vitro* tRNA splicing assays

FIGURE 2. **Endonucleolytic cleavage of the *CBP1* mRNA requires a stem-loop structure in the ORF.** *A*, the +652–726-nt sequence of the *CBP1* mRNA is indispensable for its cleavage. RNA samples extracted from W303 and W303*xrn1*Δ cells harboring the indicated deletion plasmids were analyzed by Northern blotting with DIG-labeled probe B, as described for Fig. 1*D*. *B*, analysis of the 5′ ends of the *CBP1* mRNA 3′ cleavage products. The *arrowheads* indicate the 5′ ends of the 3′ endonucleolytic cleavage products derived from the reporter genes. *C*, the amino acid sequence encoded by the ECIS is not essential for cleavage of the *CBP1* mRNA. The 3′ cleavage product was detected by Northern blotting with DIG-labeled probe B, as described for Fig. 1*D*. *D*, the predicted secondary structure of the *CBP1* ECIS (*left*). The *arrowheads* indicate the positions of the 5′ ends of the 3′ endonucleolytic cleavage products determined in the primer extension experiments. The secondary structure of the RNA was predicted using MFOLD (26). The *CBP1* nucleotides that were mutated in the M1 (*middle*) and M2 (*right*) plasmids are shown in *red*. *E*, analysis of the 3′ endonucleolytic cleavage products derived from the M1 and M2 mutants. RNA samples extracted from W303 and W303*xrn1*Δ cells harboring the mutant plasmids were analyzed by Northern blotting with DIG-labeled probe B, as described for Fig. 1*D*. *F*, schematic illustration of the *GFP-HIS3* reporter mRNA. The *boxes* indicate the open-reading frames, the *lines* represent nontranslated regions, and the tract of *A*s denotes the poly(A) tail. The *gray* and *black boxes* indicate the MTS region encoding the mitochondrial targeting signal and the ECIS region that is essential for the endonucleolytic cleavage, respectively. *G*, the ECIS of *CBP1* is sufficient for its endonucleolytic cleavage. Northern blotting with a DIG-labeled *HIS3* probe to detect the 3′ cleavage product. The levels of full-length mRNAs and 3′ cleavage products are shown as the mean values of three independent experiments. All results shown in the figure are representative of three independent experiments.

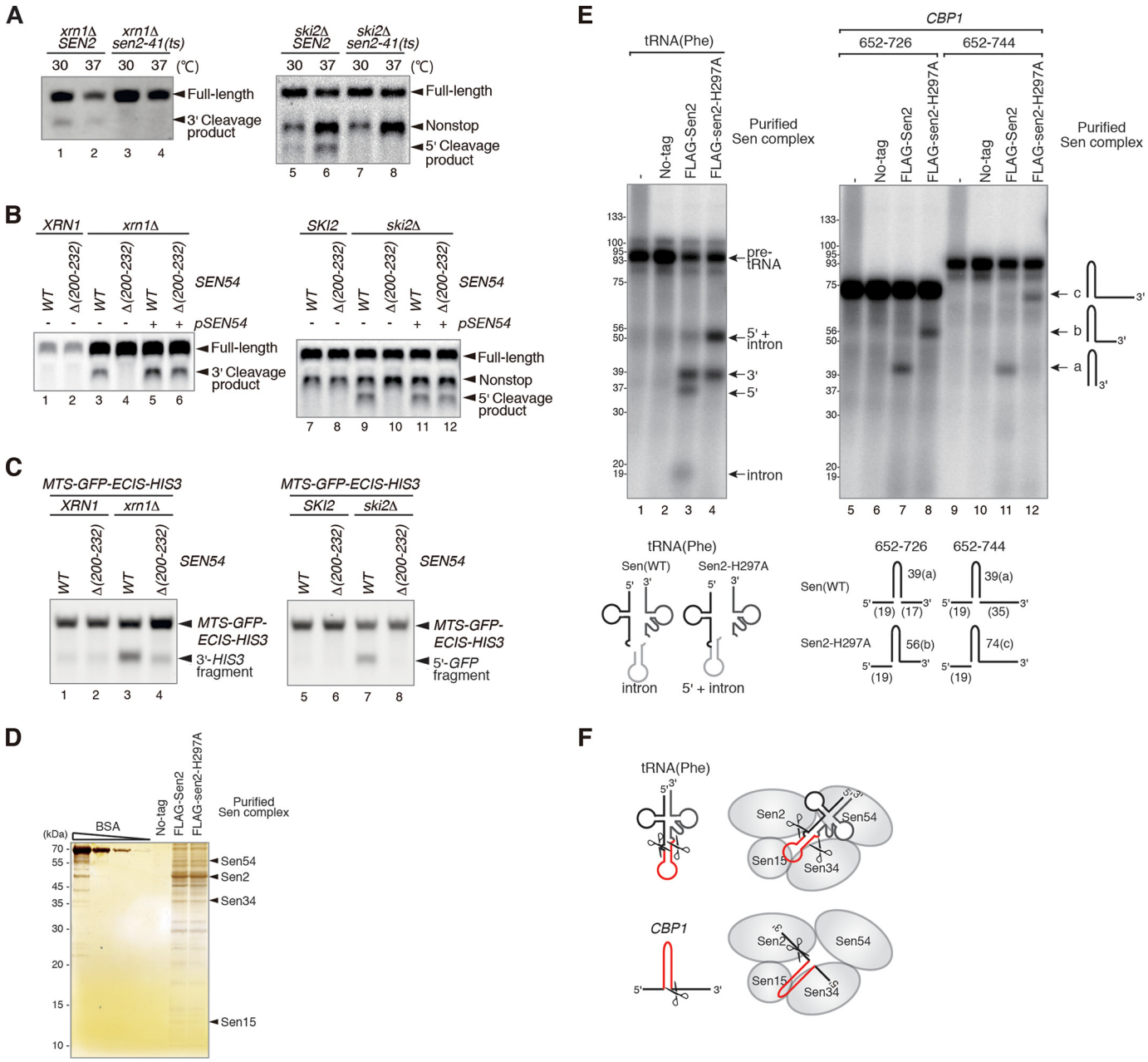


FIGURE 3. **The Sen complex cleaves the *CBP1* mRNA.** *A*, the Sen complex cleaves the *CBP1* mRNA *in vivo*. *Left panel*, W303*sen2*Δ*xrn1*Δ cells harboring wild-type *SEN2* or the temperature-sensitive *sen2–41* mutant were grown under normal (30 °C) or restrictive (37 °C) conditions. The 3′ cleavage product was detected by Northern blotting with DIG-labeled probe B. *Right panel*, Northern blot analyses of RNA samples from W303*sen2*Δ*ski2*Δ mutants harboring the wild-type *SEN2* or *sen2–41* plasmids. The cells were grown under normal conditions (30 °C) or were grown under normal conditions and then shifted to the restrictive temperature (37 °C) for 2 h prior to analysis. Northern blotting was performed using probe A (depicted in Fig. 1*A*) to detect the 5′ cleavage product. *B*, the mitochondrial location of Sen54 is required for cleavage of the *CBP1* mRNA. Northern blot analyses of the indicated mutant cells were performed as described for *A*. A complementation assay was also performed. *C*, cleavage of the ECIS-containing reporter mRNA requires mitochondrial localization of the Sen complex. *Left panel*, Northern blotting using a *HIS3* probe to detect the 3′ cleavage product. *Right panel*, Northern blotting using a *GFP* probe to detect the 5′ cleavage product. *D*, purification of the Sen complex. Samples were affinity-purified from cell extracts expressing Flag-Sen2-WT or Flag-Sen2-H297A and then subjected to 15% SDS-PAGE followed by silver staining. *E*, the Sen complex cleaves the ECIS *in vitro*. Analyses of the tRNA splicing activities of FLAG-tagged wild-type Sen2 and a FLAG-tagged Sen2-H297A mutant. *F*, a model for cleavage of the precursor tRNA or ECIS of the *CBP1* mRNA by the Sen complex. All results shown in the figure are representative of three independent experiments.

revealed that the Sen complex cleaves the *CBP1* mRNA directly (Fig. 3). In addition, the endonucleolytic cleavage of this mRNA was regulated by Tom20 (Fig. 4). These results suggest that the *CBP1* mRNA is localized to the mitochondria to enable cleavage by the tRNA splicing endonuclease Sen complex and that the mechanism of localization to the mitochondria is mediated by the MTS nascent peptide and Tom20 (Fig. 4*C*).

A Model for Endonucleolytic Cleavage of the CBP1 mRNA—Based on the results presented here, we propose a model of the mechanism of endonucleolytic cleavage of the *CBP1* mRNA (Fig. 4*C*). In this model, Tom20 recognizes the MTS, an amino-terminal region of the nascent peptide derived from the *CBP1* mRNA, and localizes the translation elongation complex composed of the ribosome, mRNA, and nascent polypeptide to the

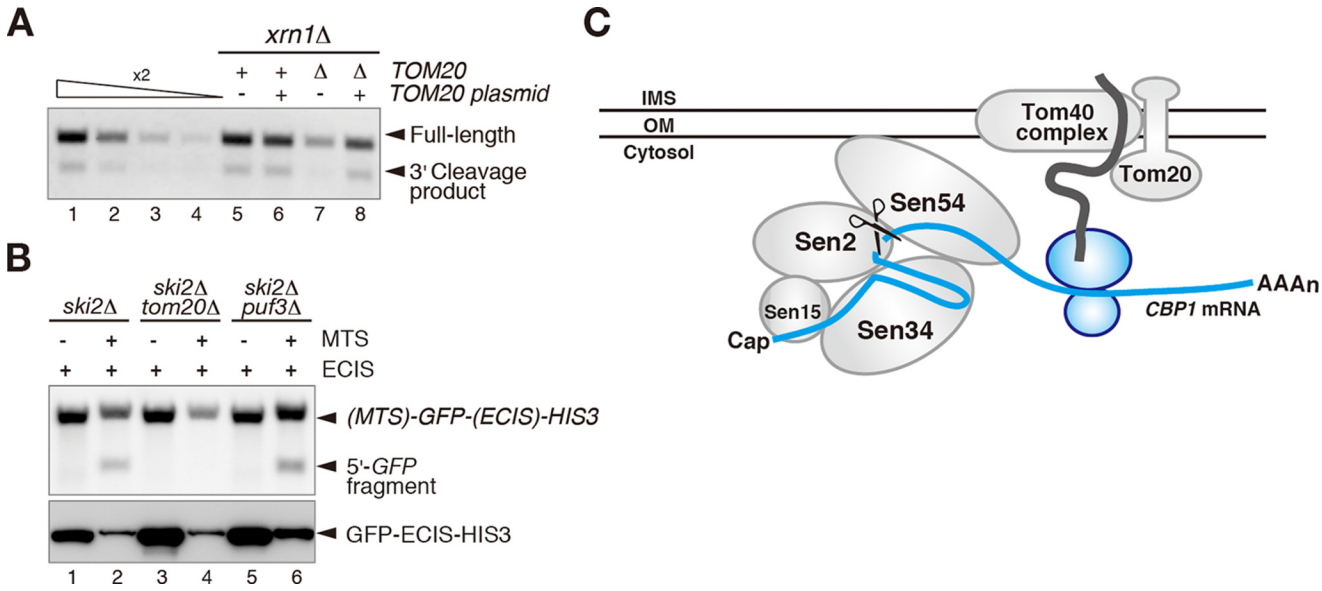


FIGURE 4. **Tom20 is involved in endonucleolytic cleavage of the *CBP1* mRNA.** *A*, cleavage of the *CBP1* mRNA is reduced in *tom20*Δ cells. W303*xrn1*Δ and W303*tom20*Δ*xrn1*Δ cells harboring a control or wild-type *TOM20* plasmid were analyzed by Northern blotting with probe B to detect the 3′ cleavage product, as described for Fig. 1*D*. *B*, cleavage of the *MTS-GFP-ECIS-HIS3* reporter mRNA requires Tom20. RNA samples were analyzed by Northern blotting with a *GFP* probe (*upper panel*), and protein samples were analyzed by Western blotting with an anti-GFP antibody (*lower panel*). *C*, proposed model showing the mechanism by which the Sen complex controls the endonucleolytic cleavage of the *CBP1* mRNA on the mitochondrial outer membrane. All of the results shown in the figure are representative of three independent experiments.

mitochondrial outer membrane. The Sen complex then recognizes and cleaves the secondary structure of the ECIS in the *CBP1* mRNA. Cleavage by the Sen complex requires formation of the stem-loop structure and translation of the ECIS sequence (Fig. 2). Alternatively, translation of the ECIS may be required to dissociate a putative factor that binds to this region and protect it against cleavage by the Sen complex. The Sen complex splices tRNA on the mitochondrial surface in *S. cerevisiae*, and nuclear-localized Sen complex mutants are also able to splice tRNA (19). However, depletion of endogenous mitochondrial Sen proteins cannot be complemented by nuclear-localized Sen protein mutants (19). Therefore, the biological significance of localization of the Sen complex to the mitochondria is still unknown.

Overall, the results presented here demonstrate that the Sen complex localized on mitochondria cleaves the *CBP1* mRNA. To our knowledge, this study is the first description of a correlation between mitochondrial localization and endonucleolytic cleavage of mRNAs. A previous study showed that the *CBP1* mRNA has a mitochondria localization ratio of ~20% (7). Because the efficiency of endonucleolytic cleavage of *CBP1* mRNA is less than 20% (Figs. 1–4), we suspect that only a proportion of the mitochondria-targeted *CBP1* mRNA undergoes endonucleolytic cleavage by the Sen complex localized on mitochondria. Genome-wide analyses identified a number of mitochondria-targeted mRNAs (7, 28), and the identification of mitochondria-localized mRNAs cleaved by the Sen complex will help to reveal the biological roles of this complex.

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References

1. van Hoof, A., Frischmeyer, P. A., Dietz, H. C., and Parker, R. (2002) Exosome-mediated recognition and degradation of mRNAs lacking a termination codon. *Science* **295,** 2262–2264
2. Frischmeyer, P. A., van Hoof, A., O'Donnell, K., Guerrerio, A. L., Parker, R., and Dietz, H. C. (2002) An mRNA surveillance mechanism that eliminates transcripts lacking termination codons. *Science* **295,** 2258–2261
3. Doma, M. K., and Parker, R. (2006) Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation. *Nature* **440,** 561–564
4. Tsuboi, T., Kuroha, K., Kudo, K., Makino, S., Inoue, E., Kashima, I., and Inada, T. (2012) Dom34:hbs1 plays a general role in quality-control systems by dissociation of a stalled ribosome at the 3′ end of aberrant mRNA. *Mol. Cell* **46,** 518–529
5. van den Elzen, A. M., Henri, J., Lazar, N., Gas, M. E., Durand, D., Lacroute, F., Nicaise, M., van Tilbeurgh, H., Séraphin, B., and Graille, M. (2010) Dissection of Dom34-Hbs1 reveals independent functions in two RNA quality control pathways. *Nat. Struct. Mol. Biol.* **17,** 1446–1452
6. Kuroha, K., Akamatsu, M., Dimitrova, L., Ito, T., Kato, Y., Shirahige, K., and Inada, T. (2010) Receptor for activated C kinase 1 stimulates nascent polypeptide-dependent translation arrest. *EMBO Rep.* **11,** 956–961
7. Saint-Georges, Y., Garcia, M., Delaveau, T., Jourdren, L., Le Crom, S., Lemoine, S., Tanty, V., Devaux, F., and Jacq, C. (2008) Yeast mitochondrial biogenesis: a role for the PUF RNA-binding protein Puf3p in mRNA localization. *PLoS One* **3,** e2293
8. Shoemaker, C. J., and Green, R. (2011) Kinetic analysis reveals the ordered coupling of translation termination and ribosome recycling in yeast. *Proc. Natl. Acad. Sci. U.S.A.* **108,** E1392–E1398
9. Shao, S., von der Malsburg, K., and Hegde, R. S. (2013) Listerin-dependent nascent protein ubiquitination relies on ribosome subunit dissociation. *Mol. Cell* **50,** 637–648
10. Lyumkis, D., Doamekpor, S. K., Bengtson, M. H., Lee, J. W., Toro, T. B., Petroski, M. D., Lima, C. D., Potter, C. S., Carragher, B., and Joazeiro, C. A. (2013) Single-particle EM reveals extensive conformational variability of the Ltn1 E3 ligase. *Proc. Natl. Acad. Sci. U.S.A.* **110,** 1702–1707
11. Matsuda, R., Ikeuchi, K., Nomura, S., and Inada, T. (2014) Protein quality control systems associated with no-go and nonstop mRNA surveillance in yeast. *Genes Cells* **19,** 1–12
12. Brandman, O., Stewart-Ornstein, J., Wong, D., Larson, A., Williams, C. C.,

Li, G. W., Zhou, S., King, D., Shen, P. S., Weibezahn, J., Dunn, J. G., Rouskin, S., Inada, T., Frost, A., and Weissman, J. S. (2012) A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell* **151,** 1042–1054

13. Islas-Osuna, M. A., Ellis, T. P., Marnell, L. L., Mittelmeier, T. M., and Dieckmann, C. L. (2002) Cbp1 is required for translation of the mitochondrial cytochrome *b* mRNA of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **277,** 37987–37990
14. Sparks, K. A., Mayer, S. A., and Dieckmann, C. L. (1997) Premature 3′-end formation of CBP1 mRNA results in the downregulation of cytochrome *b* mRNA during the induction of respiration in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **17,** 4199–4207
15. Sparks, K. A., and Dieckmann, C. L. (1998) Regulation of poly(A) site choice of several yeast mRNAs. *Nucleic Acids Res.* **26,** 4676–4687
16. Paushkin, S. V., Patel, M., Furia, B. S., Peltz, S. W., and Trotta, C. R. (2004) Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3′ end formation. *Cell* **117,** 311–321
17. Yoshihisa, T., Yunoki-Esaki, K., Ohshima, C., Tanaka, N., and Endo, T. (2003) Possibility of cytoplasmic pre-tRNA splicing: the yeast tRNA splicing endonuclease mainly localizes on the mitochondria. *Mol. Biol. Cell* **14,** 3266–3279
18. Yoshihisa, T., Ohshima, C., Yunoki-Esaki, K., and Endo, T. (2007) Cytoplasmic splicing of tRNA in *Saccharomyces cerevisiae*. *Genes Cells* **12,** 285–297
19. Dhungel, N., and Hopper, A. K. (2012) Beyond tRNA cleavage: novel essential function for yeast tRNA splicing endonuclease unrelated to tRNA processing. *Genes Dev.* **26,** 503–514
20. Wach, A., Brachat, A., Pöhlmann, R., and Philippsen, P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* **10,** 1793–1808
21. Goldstein, A. L., and McCusker, J. H. (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* **15,** 1541–1553
22. Inada, T., and Aiba, H. (2005) Translation of aberrant mRNAs lacking a termination codon or with a shortened 3′-UTR is repressed after initiation in yeast. *EMBO J.* **24,** 1584–1595
23. Trotta, C. R., Miao, F., Arn, E. A., Stevens, S. W., Ho, C. K., Rauhut, R., and Abelson, J. N. (1997) The yeast tRNA splicing endonuclease: a tetrameric enzyme with two active site subunits homologous to the archaeal tRNA endonucleases. *Cell* **89,** 849–858
24. Trotta, C. R., Paushkin, S. V., Patel, M., Li, H., and Peltz, S. W. (2006) Cleavage of pre-tRNAs by the splicing endonuclease requires a composite active site. *Nature* **441,** 375–377
25. Sambrook, J., and Russell, D. W. (2006) The Condensed protocols. From *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31,** 3406–3415
27. Abe, Y., Shodai, T., Muto, T., Mihara, K., Torii, H., Nishikawa, S., Endo, T., and Kohda, D. (2000) Structural basis of presequence recognition by the mitochondrial protein import receptor Tom20. *Cell* **100,** 551–560
28. Marc, P., Margeot, A., Devaux, F., Blugeon, C., Corral-Debrinski, M., and Jacq, C. (2002) Genome-wide analysis of mRNAs targeted to yeast mitochondria. *EMBO Rep.* **3,** 159–164
29. Tsuboi, T., and Inada, T. (2010) Tethering of poly(A)-binding protein interferes with non-translated mRNA decay from the 5′ end in yeast. *J. Biol. Chem.* **285,** 33589–33601
30. Mumberg, D., Müller, R., and Funk, M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* **156,** 119–122
31. Gietz, R. D., and Sugino, A. (1988) New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. *Gene* **74,** 527–534
32. Dimitrova, L. N., Kuroha, K., Tatematsu, T., and Inada, T. (2009) Nascent peptide-dependent translation arrest leads to Not4p-mediated protein degradation by the proteasome. *J. Biol. Chem.* **284,** 10343–10352