SUPPLEMENTARY METHODS:

List of probes and primers

tRNA and rRNA northern probes:

ND0050 (tRNA^Glu^veG) 5'-CTCTCCATAGGGGCTCGAACACTTCCGTTATCCTTCTGAAGAAAATCGGCT-3'
ND0081 (tRNA^Arg^veG) 5'-GGGGCCAGCTTGGGAAATTGACCCGGGCTCCTCG CATGCTTTTGTTCCTTCTTTAAATCGG-3'
ND0062 (tRNA^Asp^veG) 5'-TGAGACCTAAGGGCTCAACCCTTGATCGGAGAATGTAAGGTTTTATAGTTAATCCAC-3'
ND0063 (tRNA^Sur^veG) 5'-CGTACAGAGACCAGATCGACGGTAAACCAATGCTAATTTGCTTGAGG-3'
ND0064 (tRNA^Leu^veG) 5'-GCGAATCTTGTTGGATCGAACACGACCTCAGGACAGTACGTTGGACAGAATTTTT-3'
WHIT32 (tRNA^Ser^veG) 5'-CAGCAAGAAGACCCCAAGAGATAGCAGACTTTTTTATTCCA-3'
WHIT50 (tRNA^Gly^veG) 5'-CAGGAATAGCAAACCTCGGCCCTGACCAAC-3'

Supplementary Figure Legends

Fig. S1: Localization of tRNA splicing endonuclease subunits. Yeast expressing plasmids encoding tRNA splicing endonuclease subunits Sen2-GFP, Sen15-GFP, Sen34-GFP, or Sen54-GFP were assessed. Tom20-mCherry signals are from mitochondrial outer membrane protein Tom20. Merge panels depict merged GFP and mCherry panels. Size bar = 4 μm.

Fig. S2: Ability of nuclear localized tRNA splicing machinery to complement of (A) los1Δ and (B) temperature sensitive rna1-1 splicing defect. Wild-type (wt), tRNA nuclear export mutant (los1Δ), or RAN GAP mutant (rna1-1) cells were transformed with either vectors (V) or a set of Gal-inducible plasmids encoding the nuclear SEN complex and nuc/cyt ligase (N). Transformed cells were assessed for nuclear intron removal in the presence (+) or absence (-) of galactose induction and in the case of rna1-1 cells, at the permissive (23°C) or non-permissive (37°C) temperatures. p; initial transcript pre-tRNA; i; end-matured intron-containing pre-tRNA; m; mature tRNA; SS; loading control.

Fig. S3: Northern analyses assessing ability of nuclear localized tRNA splicing machinery to catalyze tRNA splicing for all families of intron-containing tRNA genes (labeled below each panel on the figure). sen2-42 cells were transformed with either vectors (V) or plasmids encoding nuclear localized tRNA splicing machinery (N) at permissive (23°C) or np (37°C) temperatures and probes assessing nine families of tRNAs (labeled) encoded by intron-containing tRNA genes were utilized. Lane 1: sen2-42 transformed with empty vector in the absence of galactose at 23°C; 2: sen2-42 transformed with vectors in the absence of Gal after a shift to 37°C; 3: sen2-42 transformed with vectors in the presence of galactose at 23°C; 4: sen2-42 transformed with vectors in the presence of galactose after a shift to 37°C; 5: sen2-42 transformed with plasmids encoding the nuclear tRNA splicing machinery in the absence of Gal at 23°C; 6: sen2-42 transformed with plasmids encoding the nuclear tRNA splicing machinery in the absence of Gal after a shift to 37°C; 7: sen2-42 transformed with plasmids encoding the nuclear tRNA splicing machinery in the presence of Gal at 23°C; 8: sen2-42 transformed with plasmids encoding the nuclear tRNA splicing machinery in the presence of Gal after a shift to 37°C. p; initial transcript pre-tRNA; i; end-matured intron-containing pre-tRNA; m; mature tRNA.

Fig. S4: Nuclear NLS-Trl1-2GFP can rescue ts growth phenotype of rgl1-4. Temperature sensitive tRNA ligase mutant rgl1-4 expressing either NLS-Trl1-2GFP (Nuc) or vector control (V) was assessed for growth. The assay was accomplished by placing an equal number of serially diluted cells on selective media containing Gal and incubated at the permissive (23°C) or the np (37°C) temperature for 3 d.
Fig. S5: Northern analyses assessing pre-rRNA processing in growth-inhibited cdc6-1 mutants. RNAs were extracted at 23°C or after a 2 hr shift to np (37°C) temperature, resolved on a polyacrylamide gel, transferred to a membrane, and the membrane was probed for the 27SA2, 27S, and 20S pre-rRNAs and 25S and 18S rRNAs (Kressler et al. 1997). In addition, sen2-42 cells were also assessed for pre-rRNA processing as described in Fig. 8.

SUPPLEMENTARY BIBLIOGRAPHY