Supplementary Material for

Reconstitution of the Human tRNA Splicing Endonuclease Complex: insight into the regulation of pre-tRNA cleavage.

Cassandra K. Hayne[1], Casey A. Schmidt[2], Maira I. Haque[1,3], A. Gregory Matera[2,4,5], and Robin E. Stanley[1*]

[1]Signal Transduction Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, 111 T. W. Alexander Drive, Research Triangle Park, NC 27709, USA

[2]Curriculum in Genetics & Molecular Biology and Integrative Program for Biological and Genome Sciences, University of North Carolina, Chapel Hill, NC 27599, USA.

[3]Department of Biology, North Carolina State University, Raleigh, NC 27695, USA.

[4]Departments of Biology and Genetics, University of North Carolina, Chapel Hill, NC 27599, USA.

[5]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599, USA.

This PDF file includes:

Figure S1 to S6

Tables S1 to S5

Supplemental References

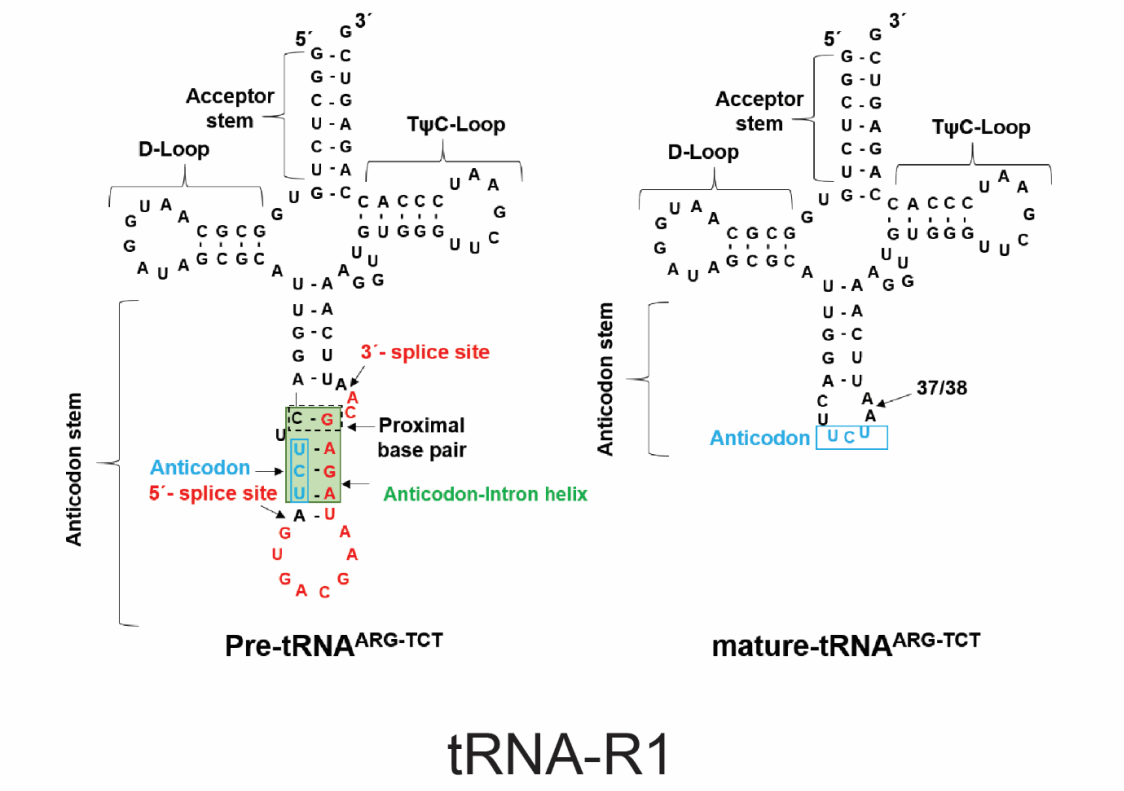

Figure S1. Human tRNA-R1 substrate used in this study. Transfer RNA tRNA-R1 is shown here as pre-tRNA (left) and mature tRNA (right). Key regions of the tRNA are labelled.

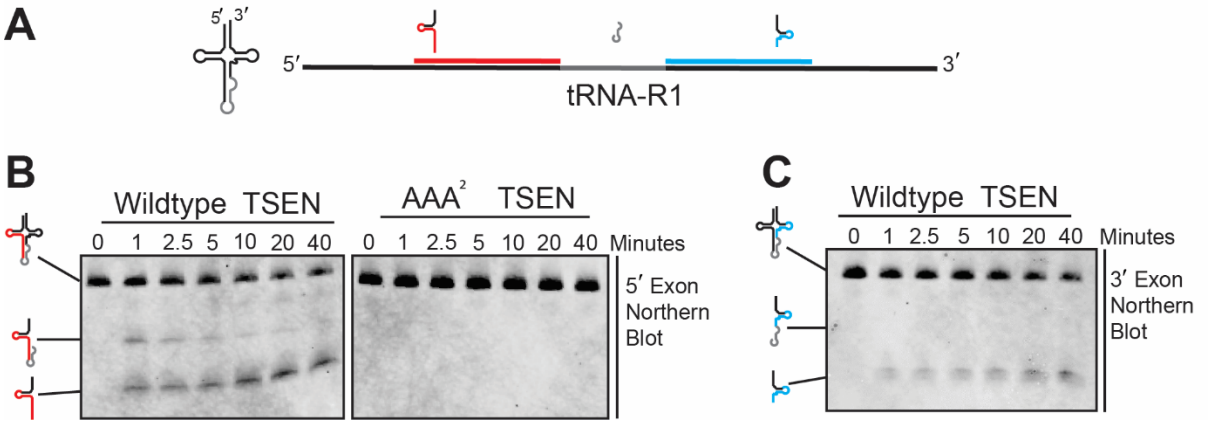

Figure S2. Northern blot validation of time course experiments. (A) Fluorescently labelled DNA oligos specific to tRNA-R1 were designed to bind to the 5′ (red) or 3′ (blue) exons in the regions flanking the intron. A time course of the human TSEN complex (1.5 μM) cleaving tRNA-R1 (1.7 μM) was performed, and samples were analyzed by Northern blotting. Northern blots to the 5′ **(B)** and 3′ **(C)** exons were performed.

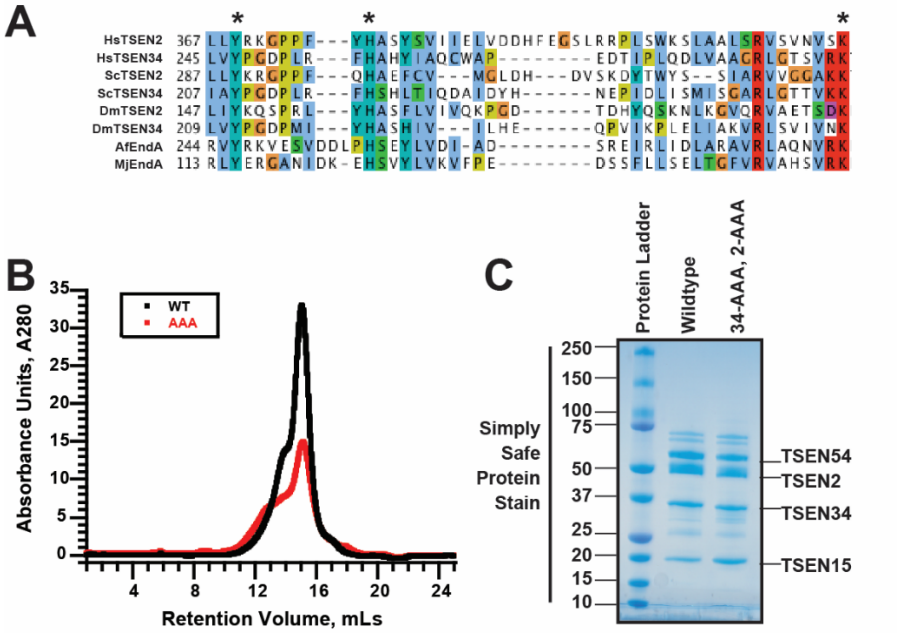

Figure S3. Active site conservation of the TSEN endonucleases. (A) A multiple sequence alignment of the active sites for the TSEN2 and TSEN34 endonuclease subunits from *Homo Sapiens (Hs)*, *Saccharomyces Cerevisiae (Sc),* and *Drosophila Melanogaster (Dm)* shows the conservation of the active site with two archaea (*A.fulgidus (Af) and Methanococcus Jannaschii (Mj))* endonucleases. **(B)** Superdex 200 10/300 Increase (GE Healthcare) gel filtration profiles of wildtype (black) and catalytic dead TSEN (red). **(C)** SDS-PAGE of 1.875 μg of total protein of Wildtype and catalytic dead TSEN complexes concentrated from the gel filtration peak shown in B.

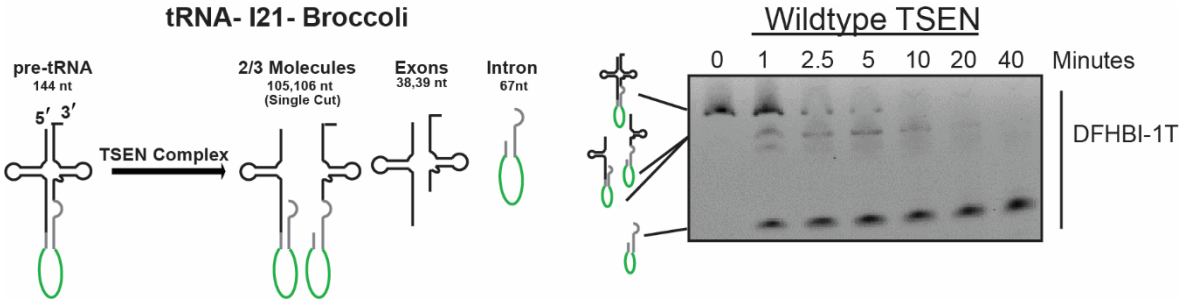

Figure S4. RNA cleavage time course of tRNA substrate tRNA-I21-Broccoli by the human TSEN complex. The *E. coli* recombinant TSEN complex efficiently cleaves the tRNA-I21 with a Broccoli RNA aptamer engineered into its native intron. A time course (0, 1, 2.5, 5, 10, 20, 40 minutes) is shown for the cleavage of the tRNA substrate by 1.5 μM TSEN complex. RNA cleavage products were separated on a TBE-Urea gel, refolded in the gel, stained with 10 μM DFHBI-1T, and Broccoli-containing RNA was visualized using a typhoon imager set to 488 nm.

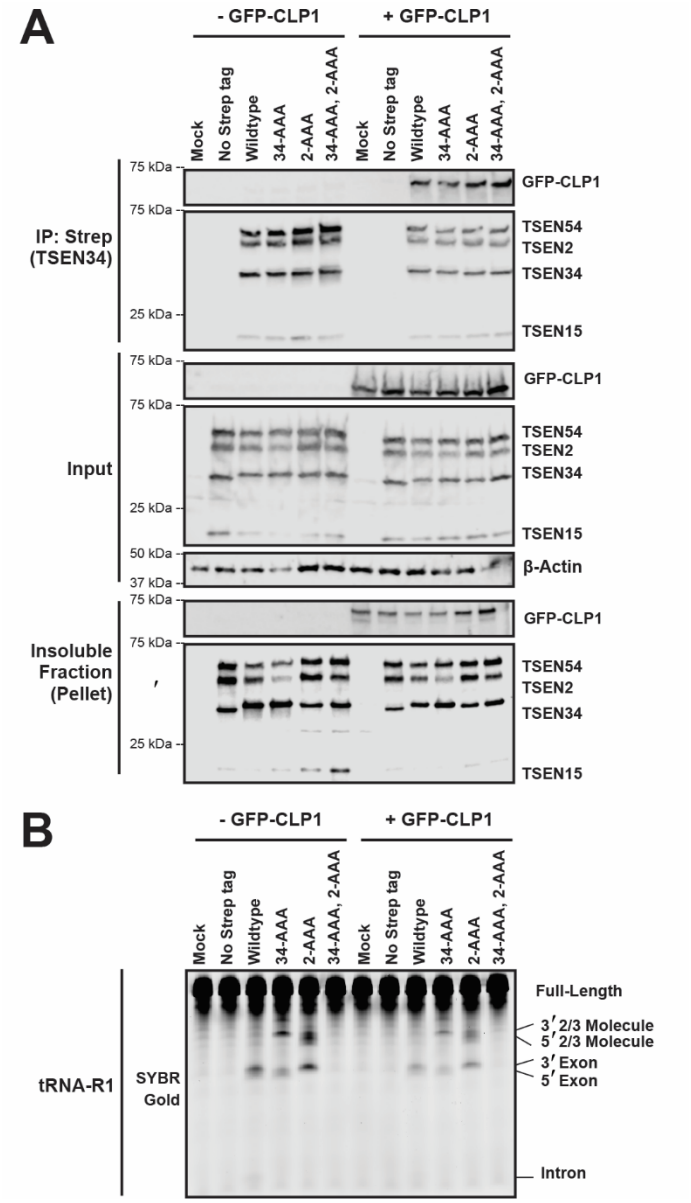

Figure S5. Characterization of tRNA cleavage with TSEN34 and TSEN2 catalytic mutants
(A) HEK293 cells were transiently co-transfected with plasmids for FLAG-tagged TSEN subunit variants, including wild-type and endonuclease dead variants of TSEN2 and TSEN34. Samples were co-transfected with or without GFP-CLP1. Samples were immunoprecipitated using Anti-Strep resin to target Strep-FLAG-TSEN34. **(B)** Immunoprecipitated proteins were assayed for cleavage of tRNA-R1. Samples were analyzed by denaturing TBE-Urea gels stained with SYBR Gold to visualize all tRNA products.

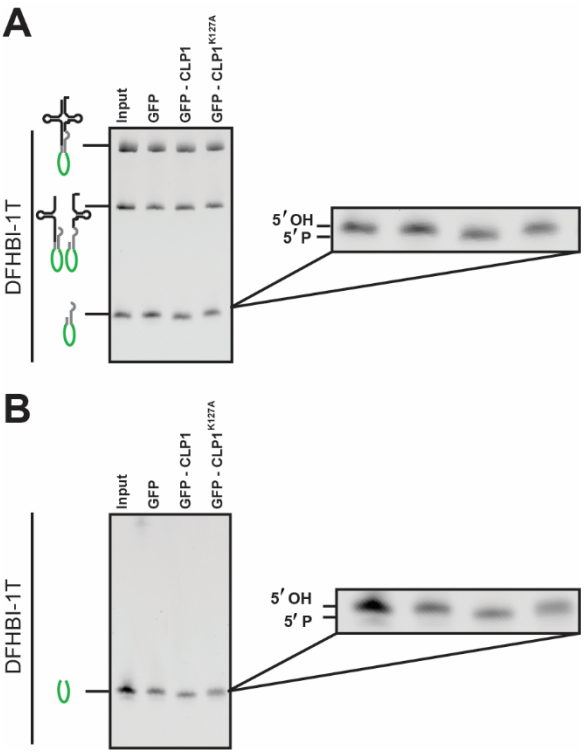

Figure S6. Wildtype GFP-CLP1 isolated from HEK cells is active on RNA substrates. HEK cells were transfected with GFP, GFP-CLP1, or GFP-CLP1 K127A. GFP immunoprecipitated complexes were assayed for kinase activity on both **(A)** tRNA-R1 containing broccoli in the intron was pre-cleaved by the *E. coli* TSEN complex and **(B)** purified broccoli RNA (synthesized by IDT). Samples were separated on a TBE-Urea gel and stained by DFHBI-1T to visualize broccoli-containing complexes.

Table S1. Plasmids used in this study.

Expression Plasmids used in this study
pCDNA3.1-GFP-CLP1
pCDNA3.1-GFP-CLP1 (K127A)
pLexM--TSEN54
pCDNA3.1-TSEN2-FLAG
pLexM-TSEN-FLAG
pLexM-TSEN2 (Y369A, H377A, K416A)-FLAG
pCAG-OSF(OneStrepFLAG)-TSEN34
pCAG-OSF(OneStrepFLAG)-TSEN34 (Y247A, H255A, K286A)
pLexM-FLAG-TSEN34
pCDNA3.1-TSEN15-FLAG
pTric-U6-HsTyr-Broccoli (1)
pST39-TSEN34-TSEN54-TSEN2-TSEN15-HIS
pST39-TSEN34(Y247A, H255A, K286A)-TSEN54-TSEN2(Y369A, H377A, K416A)-TSEN15-HIS

Table S2. tRNA DNA templates for *in vitro* transcription. Constructs were cloned into pUC19 vectors using BamHI or EcoR1/EcoRV. Vectors are engineered as follows: *BamHI/EcoR1 site* -T7 promoter – **5′ exon** – intron (Broccoli) – **3′ exon** – *EcoRV site (*blunt cut site).* The anticodon is shown in blue.

tRNA-R1	*GGATCC*TAATACGACTCACTATAGGG**GGCTCTGTGGCGCAATGGAT AGCGCATTGGACTTCTA**GTGACGAATAGAGCA**ATTCAAAGGTTGTG GGTTCGAATCCCACCAGAGTCG***GAT*ATC*
tRNA-I21-Broccoli	*GAATTC*GAGCTCGGTACCTAATACGACTCACTATAGGG**GCTCCAGT GGCGCAATCGGTTAGCGCGCGGTACTTATA**AGACAGTGCAAGACG GTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTGGGCTCCTG TGAGCA**ATGCCGAGGTTGTGAGTTCAAGCCTCACCTGGAGCA***GAT* ATC*

Table S3. Northern blot probes

Dm - Dual reporter:	TCCGAGCCGGAATCGAACCGG
Dm - tric31905	AAGCCTCGGAAACTTTCAACATATCGC
Dm - U1:	GAATAATCGCAGAGGTCAACTCAGCCGAGGT
5′- Exon (Hs-tRNA-R1)	TAGAAGTCCAATGCGCTATCC/3Cy3Sp/
3′- Exon (Hs-tRNA-R1)	TCGAACCCACAACCTTTGAAT/3Cy3Sp/

Table S4. Primers for dsRNA knockdowns.

cbc RNAi F:	TAATACGACTCACTATAGGGTGAAGAAAAAGCAGTACGAGTTCGG
cbc RNAi R:	TAATACGACTCACTATAGGGGCTTGTTGCTATTCAGAGATTGCAGT
Gaussia Luciferase (negative control) Neg ctrl RNAi F:	TAATACGACTCACTATAGGGTTCAACATCGTGGCCGTGGC
Gaussia Luciferase (negative control) Neg ctrl RNAi R:	TAATACGACTCACTATAGGGGTTGGCAAGCCCTTTGAGGCA

Table S5. RT-PCR Primers.

cbc RT F:	CCAAAGTGCTGGTGTCCCTGG
cbc RT R:	GAGTACACAGCCCTGGTACGTG
5S rRNA F:	AACAACACGCGGTGTTCCCAAGC
5S rRNA R:	GCCAACGACCATACCACGCTGAA

Supplemental References

1. Schmidt, C.A., Noto, J.J., Filonov, G.S. and Matera, A.G. (2016) A Method for Expressing and Imaging Abundant, Stable, Circular RNAs In Vivo Using tRNA Splicing. *Methods in enzymology*, **572**, 215-236.