

Expanded View Figures

aa	Codon	Frequency/ 1000 (Number)	aa	Codon	Frequency/ 1000 (Number)	aa	Codon	Frequency/ 1000 (Number)	aa	Codon	Frequency/ 1000 (Number)
F	UUU	61.7 (458)	S	UCU	17.7 (131)	Y	UAU	104.8 (778)	C	UGU	10.9 (81)
	UUC	0.7 (5)		UCC	0.7 (5)		UAC	1.1 (8)		UGC	0.1 (1)
L	UUA	119.6 (888)		UCA	13.1 (97)	UAA		3.1 (23)	UGA		0.9 (7)
	UUG	1.6 (19)		UCG	0.4 (3)	UAG		-	W	UGG	3.9 (29)
L	CUU	0.5 (4)	P	CCU	10.2 (76)	H	CAU	9.4 (70)	R	CGU	1.9 (14)
	CUC	-		CCC	0.1 (1)		CAC	-		CGC	-
	CUA	1.4 (10)		CCA	3.6 (27)	Q	CAA	15.8 (117)		CGA	-
	CUG	-		CCG	0.1 (1)		CAG	1.2 (9)		CGG	-
I	AUU	70.1 (520)	T	ACU	14.3 (106)	N	AAU	150.5 (1117)	S	AGU	14.6 (108)
	AUC	1.1 (8)		ACC	0.1 (1)		AAC	0.9 (7)		AGC	-
	AUA	102.3 (759)		ACA	13.6 (101)	K	AAA	123.7 (918)	R	AGA	11.3 (84)
M	AUG	11.6 (86)		ACG	0.3 (2)		AAG	7.1 (53)		AGG	0.4 (3)
V	GUU	6.6 (49)	A	GCU	5.5 (41)	D	GAU	17.3 (128)	G	GGU	16.0 (119)
	GUC	-		GCC	0.1 (1)		GAC	0.3 (2)		GGC	-
	GUA	10.6 (79)		GCA	2.2 (16)	E	GAA	19.5 (145)		GGA	11.5 (85)
	GUG	0.1 (1)		GCG	-		GAG	1.1 (8)		GGG	1.8 (13)

Figure EV1. Codon usage by 30 proteins encoded in the *P. falciparum* apicoplast genome.

The table presents codon usage frequencies for all proteins encoded by the *P. falciparum* apicoplast genome. Frequencies are expressed as the number of occurrences of each codon per thousand codons, with the total number of occurrences provided in parentheses. Stop codons are shaded in gray. AUA (highlighted in yellow) is the most frequently used codon for isoleucine (I) in apicoplast-genome-encoded proteins. Single-letter amino acid (aa) codes are used. A dash (-) indicates codons that are not used in apicoplast-genome-encoded proteins.



**Figure EV2. The phylogenetic relationship among methionine- and isoleucine-decoding tRNAs.**

Apicoplast encoded tRNA_{CAU} are shown in blue font with PF3D7_API00600 (*pfal*_trnM-CAU2) sharing common ancestry with experimentally validated TiIS substrate tRNA^{Ile}_{CAU} from other species (in purple font). Bootstrap analyses (1000 replicates) were employed to assess the robustness of the branching patterns. The phylogenetic tree is drawn to scale, with branch lengths representing the estimated number of substitutions per site. Refer to Appendix Table S3 for tRNA sequences.

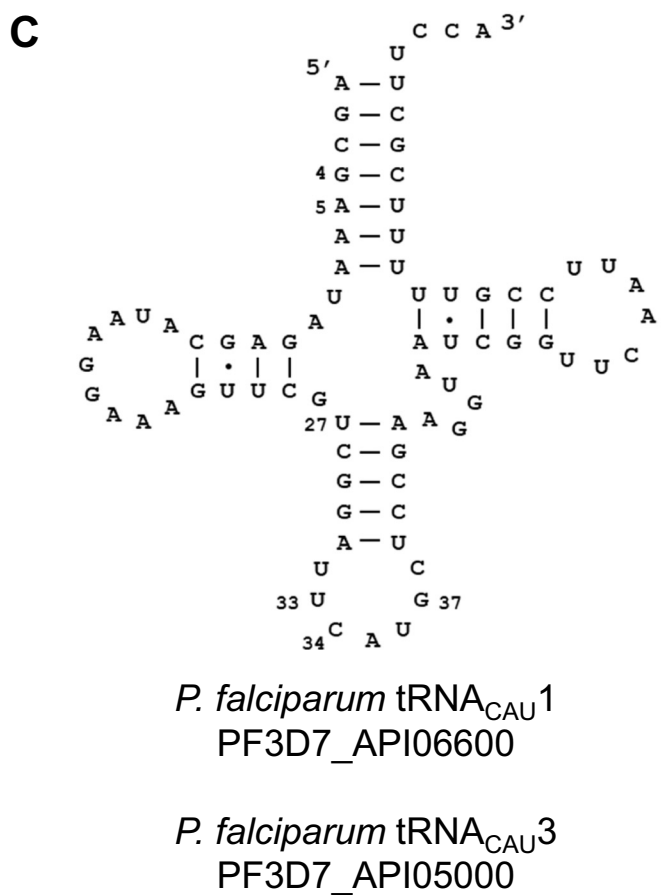
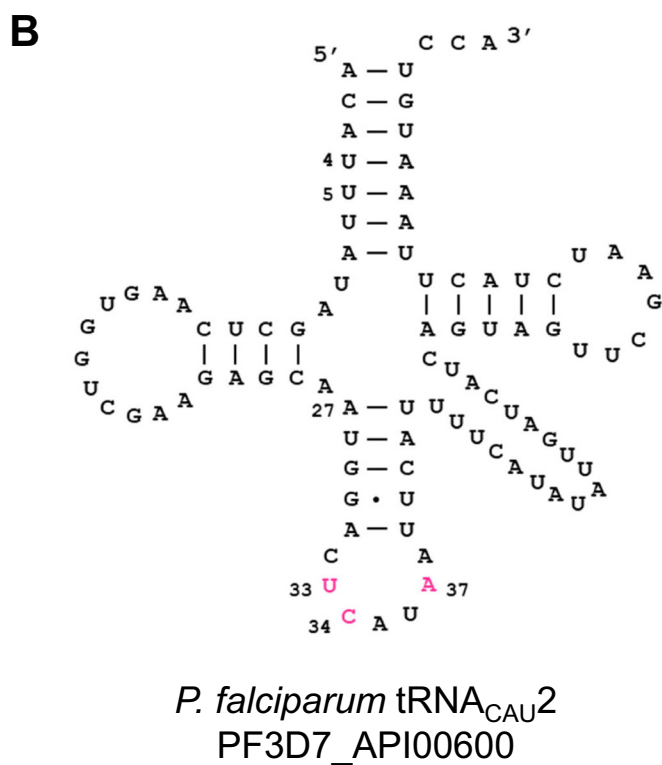
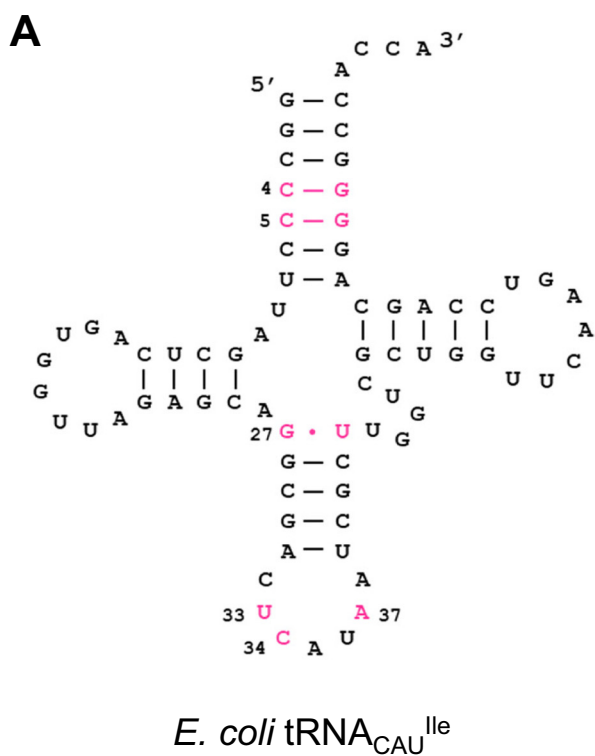




Figure EV3. Comparison of secondary structures between *Escherichia coli* tRNA^{Ile}_{CAU} and *Plasmodium falciparum* tRNAs from the apicoplast genome which are currently annotated as tRNA^{Met}_{CAU}.

(A) *E. coli* tRNA^{Ile}_{CAU}. (B) PF3D7_API00600. (C) PF3D7_API06600 and PF3D7_API05000 (identical sequences due to gene duplication). In (A) and (B), the positive determinants for Tis binding are shown in magenta. In all panels, the dash (-) indicates canonical base pairing and the dot (.) indicates GU or UU base pairing.