

Supplementary Data

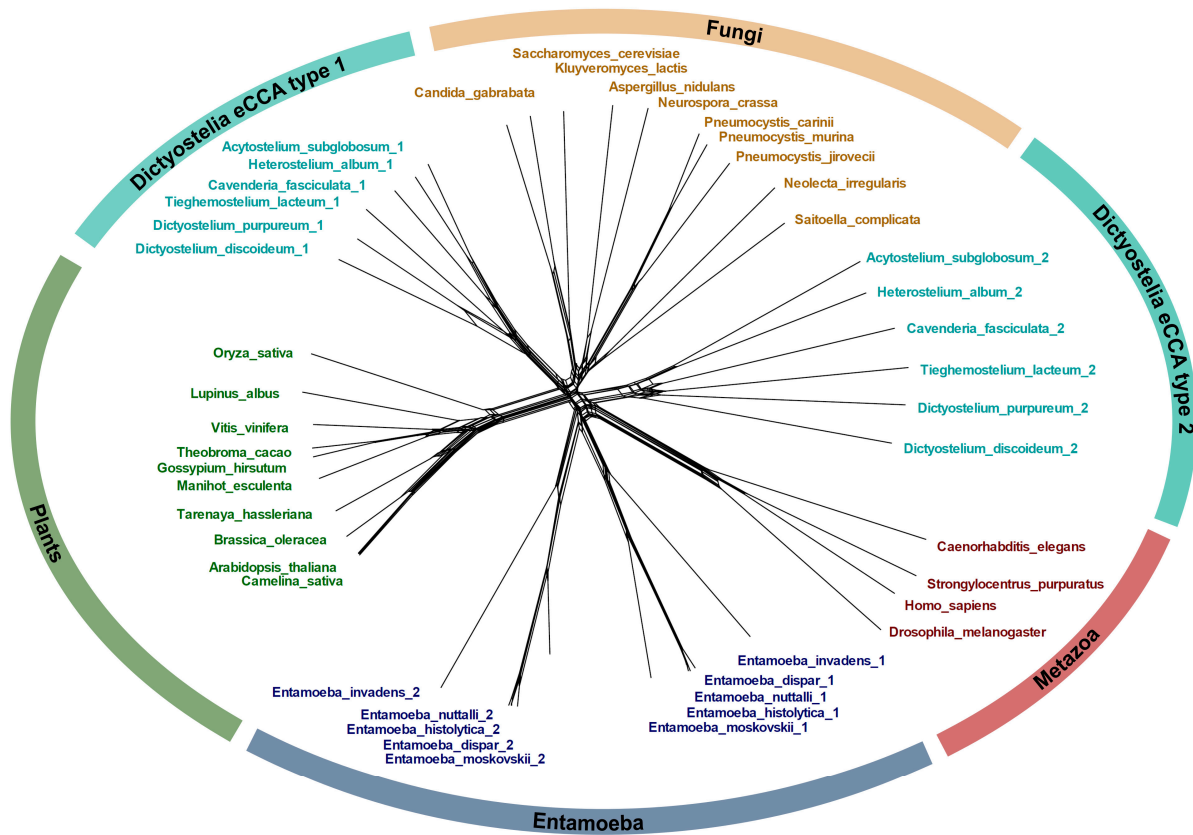


Figure S1. Phylogenetic analysis of the two Dictyostelia CCA-adding enzyme types. Protein sequences of Dictyostelia, Fungi, Metazoa, Plants and Entamoeba CCA-adding enzymes were aligned using Clustal Omega [1] with standard settings. The alignment was used for phylogenetic tree generation using the program SplitsTree 4 [2]. The phylogenetic tree indicates that both types of Dictyostelia CCA-adding enzymes are only distantly related because they do not share a common branch, but have separate splits. The Dictyostelia eCCA1 (ancestral eukaryotic CCA-adding enzyme type 1) enzymes branch more closely with Plants and Fungi enzymes, whereas enzymes of type 2 (eCCA2) branch more closely to the metazoan enzymes.

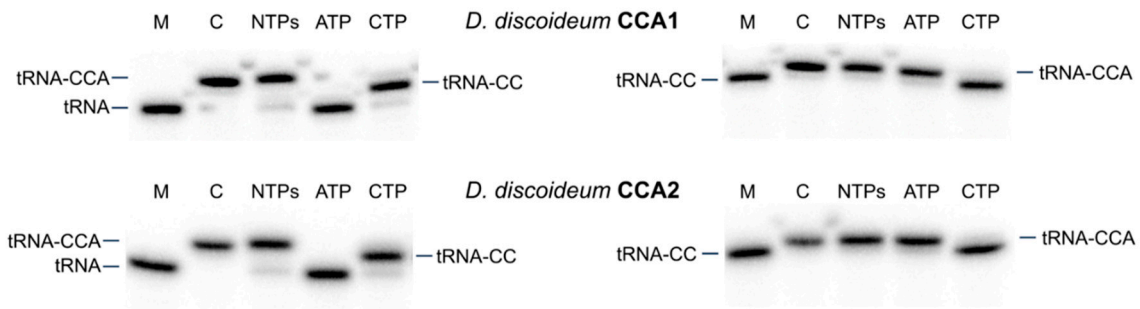


Figure S2. Both CCA1 and CCA2 synthesize full CCA-ends. Recombinantly expressed CCA-adding enzymes of *D. discoideum* were incubated with a α -³²P-labeled tRNA substrate that lacks the CCA-terminus (tRNA) and a tRNA substrate that carries a CC-end, where only the terminal A residue is missing (tRNA-CC). Left panel: In the presence of only CTP, both enzymes add two residues to the tRNA lacking the CCA-end, resulting in tRNA-CC. In contrast, ATP is not incorporated at all. The same result is observed in the presence of all four nucleotides (NTPs). Right panel: On tRNA-CC, both enzymes add a terminal A residue when ATP (or NTPs) is offered. These results prove that CCA1 as well as CCA2 are bona fide CCA-adding enzymes and do not represent tRNA nucleotidyltransferases with partial activities as found in some other organisms [3–7].

In these assays, yeast tRNA^{Phe} was used as substrate. As the structure of this *in vitro* transcript is highly similar to that of the native and fully modified tRNA [8], it is frequently used as a standard substrate for the investigation of *in vitro* tRNA processing events [9–13]. M – mock incubation of labeled tRNA transcripts without enzyme. C, migration control consisting of labeled tRNA carrying a CCA-end.

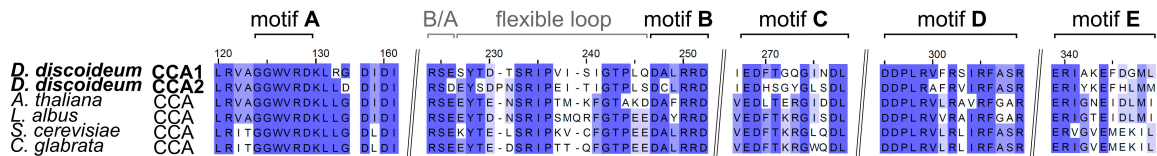


Figure S3. Sequence alignment of the two *D. discoideum* CCA-adding enzymes with eukaryotic CCA-adding enzymes with known CCA-adding activity. The two protein sequences of *D. discoideum* CCA-adding enzymes were aligned with protein sequences of the eCCA enzymes of *A. thaliana* (AC: Q94K06), *L. albus* (AC: Q42867), *S. cerevisiae* (AC: P21269) and *C. glabrata* (AC: Q9P4S5) using Clustal Omega [1] with standard parameters. All aligned protein sequences share the same conservation of the core motifs A – E, the basic/acidic motif and the flexible loop element. These motifs characterize the *D. discoideum* CCA-adding enzymes as ancestral eukaryotic type enzyme (eCCA).

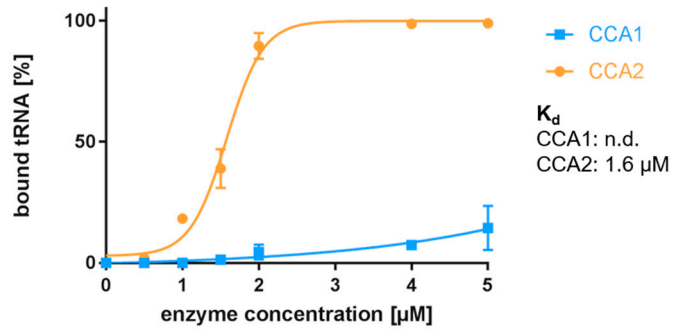


Figure S4. Interaction of CCA1 and CCA2 with tRNA-CC is not affected by ATP. Gel shifts were performed after preincubation of enzymes with 1mM ATP γ S. While tRNA binding of CCA2 shows no significant difference to the interaction without ATP γ S (Fig. 5 in the main text), CCA1 shows almost no interaction, as seen in the absence of ATP γ S.

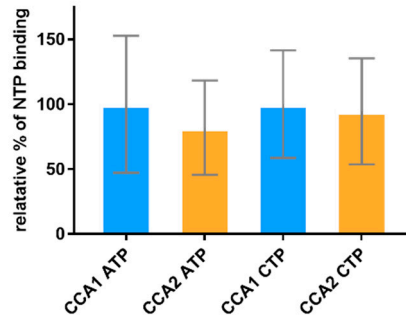


Figure S5. CTP and ATP binding behavior of CCA1 and CCA2. Nucleotide binding of CCA1 was set to 100%. The binding behavior of CCA2 is shown relative to CCA2, indicating only marginal deviations. NTP interaction was quantified in a comparative nucleotide binding assay according to Lizano *et al.* [14].

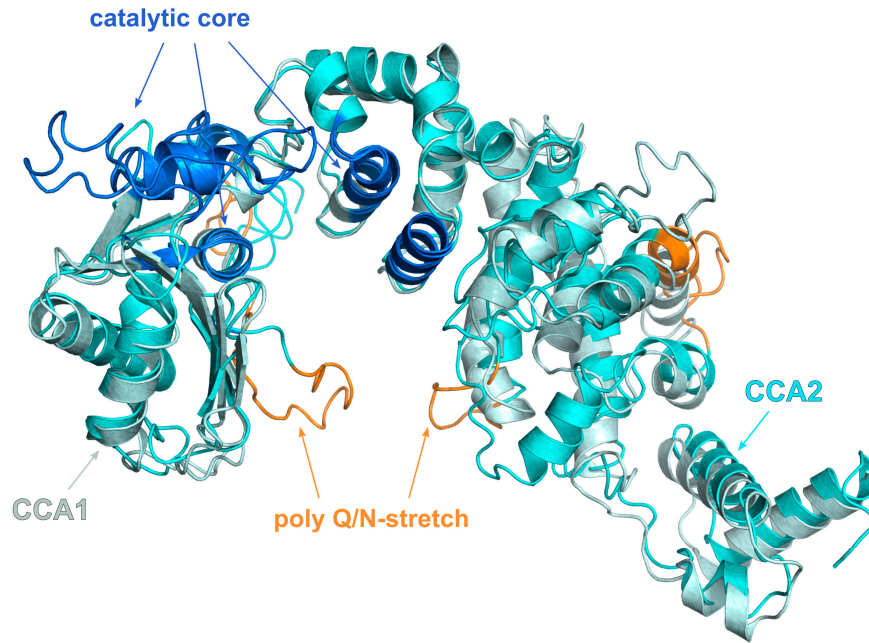


Figure S6. Structure prediction of the two *D. discoideum* CCA-adding enzymes. The structures of the CCA-adding enzymes were calculated using iTasser [15]. The core motifs of the two enzymes are colored in blue. The CCA-adding enzyme CCA2 (cyan) harbors several Q/N stretches (orange) that are not found in CCA1 (grey).

Accession numbers of the protein sequences used in Figure S1:

	Species	Accession number
Metazoa	<i>Caenorhabditis elegans</i>	Q93795
	<i>Drosophila melanogaster</i>	AAF51964
	<i>Homo sapiens</i>	BAB70662
	<i>Strongylocentrotus purpuratus</i>	XP_784774.4
Fungi	<i>Aspergillus nidulans</i>	KZN89459
	<i>Candida glabrata</i>	Q9P4S5
	<i>Kluyveromyces lactis</i>	Q6CSU9
	<i>Neoelecta irregularis</i>	(prosplign)
	<i>Neurospora crassa</i>	Q7SAL9
	<i>Pneumocystis jirovecii</i>	XP_018230996.1
	<i>Pneumocystis carinii</i>	XP_018225359.1
	<i>Pneumocystis murina</i>	XP_007874977.1
	<i>Saccharomyces</i>	P21269
	<i>Saitoella complicata</i>	XP_019025108.1
Plants	<i>Arabidopsis thaliana</i>	Q94K06
	<i>Brassica oleracea</i>	XP_013637841.1
	<i>Camelina sativa</i>	XP_010477526.1
	<i>Gossypium hirsutum</i>	XP_016698247.1
	<i>Lupinus albus</i>	Q42867
	<i>Manihot esculenta</i>	XP_021601417.1
	<i>Oryza sativa subsp japonica</i>	Q2QX33
	<i>Tarenaya hassleriana</i>	XP_010534110.1
	<i>Theobroma cacao</i>	XP_007020008.2
	<i>Vitis vinifera</i>	A7QGJ5
Entamoeba	<i>Entamoeba dispar</i> 1	XP_001739538.1
	<i>Entamoeba dispar</i> 2	XP_001740657.1
	<i>Entamoeba histolytica</i> 1	XP_652076.2
	<i>Entamoeba histolytica</i> 2	XP_654783.1
	<i>Entamoeba invadens</i> 1	XP_004183347.1
	<i>Entamoeba invadens</i> 2	XP_004257247.1
	<i>Entamoeba moskovskii</i> 1	EMO_031410-1
	<i>Entamoeba moskovskii</i> 2	EMO_113580-1
	<i>Entamoeba nuttalli</i> 1	XP_008860114.1
	<i>Entamoeba nuttalli</i> 2	XP_008854811.1
Dictyostelia	<i>Acytostelium subglobosum</i> 1	XP_012758000.1
	<i>Acytostelium subglobosum</i> 2	XP_012749843.1
	<i>Cavenderia fasciculata</i> 1	XP_004361592.1
	<i>Cavenderia fasciculata</i> 2	XP_004359300.1
	<i>Dictyostelium discoideum</i> 1	DDB_G0293504
	<i>Dictyostelium discoideum</i> 2	DDB_G0271378
	<i>Dictyostelium purpureum</i> 1	XP_003291342.1
	<i>Dictyostelium purpureum</i> 2	XP_003283778.1
	<i>Heterostelium album</i> 1	XP_020429327.1
	<i>Heterostelium album</i> 2	XP_020431701.1
	<i>Tieghemostelium lacteum</i> 1	KYQ91040.1
	<i>Tieghemostelium lacteum</i> 2	KYQ90023.1

Sequences of the Dictyostelia tRNA nucleotidyltransferases used in our analysis

>Acytostelium subglobosum 1

MEHSDKKIKTKHIPITLISNDYKHDNKFHKSCTKTQAQATSTPTLTQQVNTPTPTTPTPDTKCNDEVNMMNNN
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IALDNLMGFAFASLVNQYLQDHNQETHRIGVIQSNPSQSKHLETATVRI FDMWIDFVNLRSEYTESRIPTIQI
GTPQEDAFRRDLTINSLFYNNINENKIEDFTGNGINDLMCGIVRTPPLPQTTFLLDDPLRVLR SIRFATRLLYYAIDH
KLVEAASSPKVKEALKSKVSHERVGIELEGMLSGPRPD LAIHLIHDGLGDFDHI FSLPEGVTI KEKNYRFIATENI
LNMMRFINWGVANEEVPTKRLRLVSSLLQVFKDYNNTTKKNRQLPLIQHLMVEFLKFSNKDYDDVLNVLECAEKF
KQHVANLDIKGTF SRKDVGLIMYKAGPLWRTALANSLISELPKYNRNLSYPLHKPELIDGVPSPSSPMHDFHLHS
HNPHAHHPPLCEASKAIVAKYDNFVASVASHDLIGVWNIKKHLDGKMMEMMLNMKPGKWLAVLQEI FEWQLDNP
KLDQEHCKQWVLNKFSSLPKQ

>Acytostelium subglobosum 2

MFVLRPGHWHQLQQQHNSIRSI INSSINSI INHRQYTTTTTTTTTRKTKTKTAQTEPRLLVPOQQELDASSAIV
VSRLSNREKQFFDMFKSVASHYGGNIHLRVSGGWVRDKLLTTTSANPKVDDIDIAIDGITGREFVDLIERYRVE
KGLSQQKYLIRSNPEKSKHLETA AIKIDGCTFDFTGLRAEDYDQHSRI PKI SAGTLESDAHRRDITINALYFNLN
TYMVEDYTGKGLDDLRKGI VRTPLEPMITLLEDPLRALRVIRFATRFNFQIHPALYKSLTSSDVRKSLELKVSK
RIYVEFTSMMSSFKSGAIQYYRYLVGTGLVDAVFPFDSMVKQPVEMIPRHLEKEI ILSLLLLPLYIKYGKSDNIN
MEQLLMDFKLPKKEIRAVLSIIQSQEI IAKSVSSMLMISPDTLYHPDQFETSLRPLLEWLRHNQYWQAGLWLVSSV
HFKLKHFSPDFNKDQFWIEFEKLIQNSLEKMANQQYCLDGHEIKDALSIPQEESKPI TNIDE LLIWQWKYNICH
DKITKDDAKHFVRGLWAQQQLPHQ

>Cavenderia fasciculata 1

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GEAFAALVNKYLEDDHHQETHRIGVIQSNPDQSKHLETATVKI FDSWVDLVNLRSEYSSNSRIPEIKIGTPEQDA
FRDLTINSLFYNNENRIEDFTGNGIVDLKCGIVRTPPLPQTTFLLDDPLRVLR SIRFATRLLYYAIDHKLVEAAS
NPTVKEALKSKVSHERIGIELDGMLNGPRPD LAIQLIYEFGLFDHVINIPDSIEIQDKEYKYKSI LNVLNMMRFI
NWGTQAEAEASTRKRLLASLCANIYGYTFKNPLKKNKDTPI LQHVLDVYLKVSNEKCEDEITNVLECAKLFRLVN
QFYTNHVF SRKSVGLVIYKAGPFWKSSLAIALISELPEFQHNLVYPLHKEEINNSPSHDFYLHLSHPNHHHPPLC
DESKDIEIKYDQFCATVANHDLVGVWNIKKLLNGKQVMDMLQRKPGEWLTPLLQDVFEWQLDNPQLGEKECKEYV
LSKFSPK

>Cavenderia fasciculata 2

MYLNRIGSFCINSSKRLNFSTGTITSSSTTSATTSSGSPFFIIPPSSSSTSSSTSSPSEFRKITNPLKSR LN
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IQGGTDIDIAIDNLTGHEFVQMIQKYYSINNQILFRNYI IKSNEKSKHLETASLVMKDGLTFDFTLGRSEYDE
FSRI PNIVKGT LISDSHRDITINSLYNNLMTTRVEDYTLMGLPDLENKI VRTPLSPTLTLCEDPLRALRVLRFS
NRFSFSIESQLYESLCSDRVRD LGLKVSRRVHTEFTSMMSYKHAIPYFKTLVDTGLIHSIFPTQGLITTTQDW
YQSLHYLEQSQHVSNI VPDQSIQEFILSSI FLPLKLYQNHLDVFKILLDLKYSKKI CFNVSSTLLSYEILKDP I
QLITSRPLEHLIDDNQLFEQSVLPLLKWLRHNQLWKNLWTLARLYFYVNHHPVLSRDDQI WIDAQQI IEKEIMPI
SNTKFCFEARAVMDALGIVNSCDIRPAIDQIMVYQWKNKSTSFSLDHI VKNQFFKIT

>Dictyostelium discoideum 1

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EPYLSIELINKFGLFDCLFSLPTVGLDETYIEQLKNSNKDSEKYCEIADSWIGMGKQYEEIDTKRQLI LSALMIP
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WIVVIIISFIKIYQRPNKLQTLFSRYDKPENEKYTI FHKFVGEIKEHDLIGIWNIKRLLNGKQVQD L LNRKPGTW
LAP
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>Dictyostelium discoideum 2

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DYLDITGLINSIIDFDKIINDHEI IKELFKKSLKYLKILINQNNNNNDNLFNIDFYKCCIFLPPFIDAHLGFKDN
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HELKNCKAGGLQWNFPNLSISIVYLLEKYPDRENEILDVLNQLTKLFSDDNFTFEKVSLLVDGRKIKNLFPDQSID
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>Dictyostelium purpureum 1

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ITYLNANKKNKETSIVIHMIIEYLKLSNKDNDIEISLVLDTAYQIRPHVLKYKEEGPSSFSRKNVGLI IHKCGVLW
RAALAIALILDLPKYNFNHFNFPPTLCDESNI IINHYNLFCDEI KEQDLIGIWKIKRMLNGKQVQDLLNRKPGEWL
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>Dictyostelium purpureum 2

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>Heterostelium album 1

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ERVGIELDGMLSGPRPDLAIHLINHFGLFDHIFSLPEGVTI KEKNYQYIAAENILNMMRFISWGSTTEELPTRKI
RLLSLLQVFHNYTFTTKNRTIPAIQYTI VEFLKFSNKDYDDVLNVLECADRFKPEVQKLNNDNGSFSRDKVGLI
VYKAGPLWRVALANALISELPKYNRSIVYFPQRLENPDGSPVPRSPMHD FHLHSHNPHVHHPPLCEDAKKIVSKY
DNFCASVASHNLVGIWNTKLLDGKQVMELLLKKKPGAWLAPVLQEVFEWQLENPNNSNEEQCKQWVLNKF S

>Heterostelium album 2

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DLIEHYRIEHGLSQKKYLVRNPNPKSKHLETASIVIDGYTFDFTGLRAEQYDTHTRIPQIVDGTPTTDAFRRDIT
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EMQEDFILGVLMLPLKLYHDALNLEYLLEHFKSSKKQVRTVLSIISPEILINQVIKTLLLPSMTITKESLTQP
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>Tieghemostelium lacteum 1

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TVKVYDMWIDFVNLRSETYTEGSRIPITIDIGTPLEDALRRDLTINSLFFNINENLIQDYIGTAIKDLQLGIIRTP
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QLGLYQYLFALPPGYNELSKINYIDASCNYINQMMLAHWGENSEELSTKRVRLLSALMIPFHLLTYKNPQNKNR
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>Tieghemostelium lacteum 2

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DITINTLFYNIHTQEIEDHTQQGLKDLSECLIRTPPLSPFETLLEDPLRAFRVLR FATKFHFAIEPQLMGV IKSVD
FRKTVLKKVSRERINTEFNKLMTKPAYALQYFKYLVETGLVEIVYHHTPNPSLPFQNSLPYLELGNSQSVNTKVL
DTLEFRHSLIFYPMVLDNLLSLTDITKILRDFKSPNKEISQTL SILRLSQVLSTIKSHYTQDTQHTNTSIESYLI

DSPTNFKTHLFPLLLELRKSHQFLLSIHLCRIHSQINDNNNNNNSEQFWDVEALISIKCYPLSKIYSLTQEDV
FENVEIVKSGKCSGTGFTSLMNELIYHQFSKHNFKSNHIQPIISKQEAIQFLNSLPNK

References

1. Sievers, F.; Wilm, A.; Dineen, D.; Gibson, T.J.; Karplus, K.; Li, W.; Lopez, R.; McWilliam, H.; Remmert, M.; Söding, J.; et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **2011**, *7*, 539, doi:10.1038/msb.2011.75.
2. Huson, D.H.; Bryant, D. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **2006**, *23*, 254–267, doi:10.1093/molbev/msj030.
3. Tomita, K.; Weiner, A.M. Collaboration between CC- and A-adding enzymes to build and repair the 3'-terminal CCA of tRNA in *Aquifex aeolicus*. *Science* **2001**, *294*, 1334–1336, doi:10.1126/science.1063816.
4. Bralley, P.; Chang, S.A.; Jones, G.H. A phylogeny of bacterial RNA nucleotidyltransferases: *Bacillus halodurans* contains two tRNA nucleotidyltransferases. *J. Bacteriol.* **2005**, *187*, 5927–5936, doi:10.1128/JB.187.17.5927-5936.2005.
5. Reid, N.E.; Ngou, J.S.; Joyce, P.B.M. *Schizosaccharomyces pombe* contains separate CC- and A-adding tRNA nucleotidyltransferases. *Biochem. Biophys. Res. Commun.* **2019**, *508*, 785–790, doi:10.1016/j.bbrc.2018.11.131.
6. Preston, M.A.; Porter, D.F.; Chen, F.; Buter, N.; Lapointe, C.P.; Keles, S.; Kimble, J.; Wickens, M. Unbiased screen of RNA tailing activities reveals a poly(UG) polymerase. *Nat. Methods* **2019**, *16*, 437–445, doi:10.1038/s41592-019-0370-6.
7. Erber, L.; Franz, P.; Betat, H.; Prohaska, S.J.; Mörl, M. Divergent Evolution of Eukaryotic CC- and A-Adding Enzymes. *IJMS* **2020**, *21*, 462, doi:10.3390/ijms21020462.
8. Shi, H.; Moore, P.B. The crystal structure of yeast phenylalanine tRNA at 1.93 Å resolution: a classic structure revisited. *RNA* **2000**, *6*, 1091–1105, doi:10.1017/s1355838200000364.
9. Oommen, A.; Li, X.Q.; Gegenheimer, P. Cleavage specificity of chloroplast and nuclear tRNA 3'-processing nucleases. *Mol. Cell. Biol.* **1992**, *12*, 865–875, doi:10.1128/mcb.12.2.865.
10. Loria, A.; Pan, T. The 3' substrate determinants for the catalytic efficiency of the *Bacillus subtilis* RNase P holoenzyme suggest autolytic processing of the RNase P RNA in vivo. *RNA* **2000**, *6*, 1413–1422, doi:10.1017/s1355838200000959.
11. Hoffmeier, A.; Betat, H.; Bluschke, A.; Günther, R.; Junghanns, S.; Hofmann, H.-J.; Mörl, M. Unusual evolution of a catalytic core element in CCA-adding enzymes. *Nucleic Acids Res.* **2010**, *38*, 4436–4447, doi:10.1093/nar/gkq176.
12. Tretbar, S.; Neuenfeldt, A.; Betat, H.; Mörl, M. An inhibitory C-terminal region dictates the specificity of A-adding enzymes. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 21040–21045, doi:10.1073/pnas.1116117108.
13. Ernst, F.G.M.; Erber, L.; Sammler, J.; Jühling, F.; Betat, H.; Mörl, M. Cold adaptation of tRNA nucleotidyltransferases: A tradeoff in activity, stability and fidelity. *RNA Biol.* **2018**, *15*, 144–155, doi:10.1080/15476286.2017.1391445.
14. Lizano, E.; Schuster, J.; Müller, M.; Kelso, J.; Mörl, M. A splice variant of the human CCA-adding enzyme with modified activity. *Journal of Molecular Biology* **2007**, *366*, 1258–1265, doi:10.1016/j.jmb.2006.12.016.
15. Yang, J.; Zhang, Y. I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Res.* **2015**, *43*, W174–81, doi:10.1093/nar/gkv342.