

Dual targeting of the tRNA nucleotidyltransferase in plants – not just the signal.
Serena Schmidt von Braun, Antonino Sabetti, Pamela J. Hanic-Joyce, Jun Gu, Enrico Schleiff and Paul B.M. Joyce

Supplementary material

Supplementary Figure S1. Complementation of a temperature-sensitive defect in the *S. cerevisiae* *CCA1* gene coding for tRNA nucleotidyltransferase by its *Arabidopsis* homologue. Cells carrying plasmids of interest were transformed into the temperature-sensitive yeast strain (E189F) and grown at 22°C. These cells were patched on SC-His plates and replica plated to 22°C and 37°C. The yeast expression vector alone (G131-2) does not complement the defect, but the plasmid carrying the *Arabidopsis* cDNA coding for tRNA nucleotidyltransferase (*attRNA*) does.

Supplementary Figure S2. Comparison of the proteins encoded by splice variants of At1g22660

The cDNAs for the two splice variants deposited to the database were translated *in silico* and aligned using Tcoffee (C.Notredame, D. Higgins, J. Heringa, T-Coffee: A novel method for multiple sequence alignments; Journal of Molecular Biology, 2000, 302, 205-217) at standard setup. The mismatch is highlighted in bold. The additional amino acids encoded by the 5' extension identified but not present in the database are underlined. The sequence with similarity to a bipartite nuclear localization signal is given in bold and italic. Amino acids changed are indicated above the sequence and the name of the variant protein is given.

Supplementary Figure S3. Homology Modeling

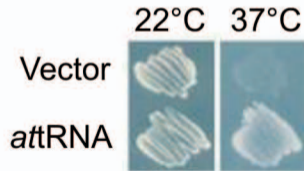
The homology model of AT1G22660.1 based on the structure of a tRNA nucleotidyltransferase from *Aquifex aeolicus* (PDB:1VFG) was produced as described

in materials and methods. The model shown starts (N) at amino acid 105 and therefore does not contain the N-terminal targeting signal. The C-terminal ~150 amino acids could not be modeled safely by homology, because of a very low sequence identity to the template within this region (<<10%). The Glu 399 (first amino acid of the altered motif) is indicated by N' and the altered region is shown in yellow. The tRNA and ATP analogue were added to the template to better illustrate the RNA-binding site. The homology model confirms that the altered region is exposed to the surface.

Supplementary Figure S4. The distribution of GFP tagged tRNA-NT, TP-tRNA-NT and TP-tRNA-NT-2.

The constructs described in Figure 2 fused to GFP were expressed in onion cells and GFP fluorescence analyzed by confocal fluorescence microscopy. The left panel shows the representative distribution of GFP fluorescence. In the middle the mitotracker staining and on the right the overlay image is shown. Please note, for tRNA-NT a cell was chosen with localization of the protein in both, mitochondria and plastids.

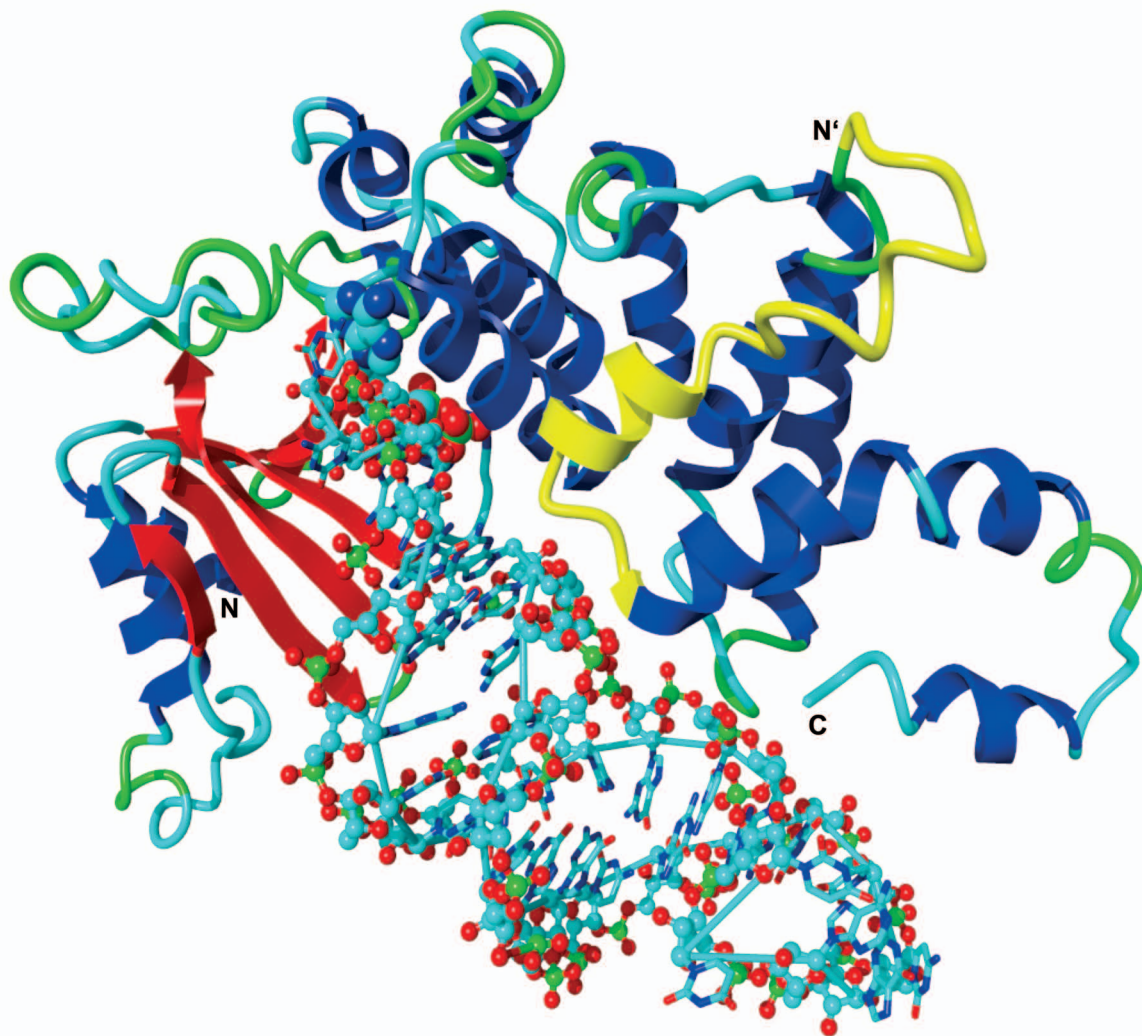
"Dual targeting of the tRNA nucleotidyltransferase in plants not just the signal"; Serena Schmidt von Braun, Antonino Sabetti, Pamela J. Hanic-Joyce, Jun Gu, Enrico Schleiff and Paul Joyce; Supplementary Figure S1



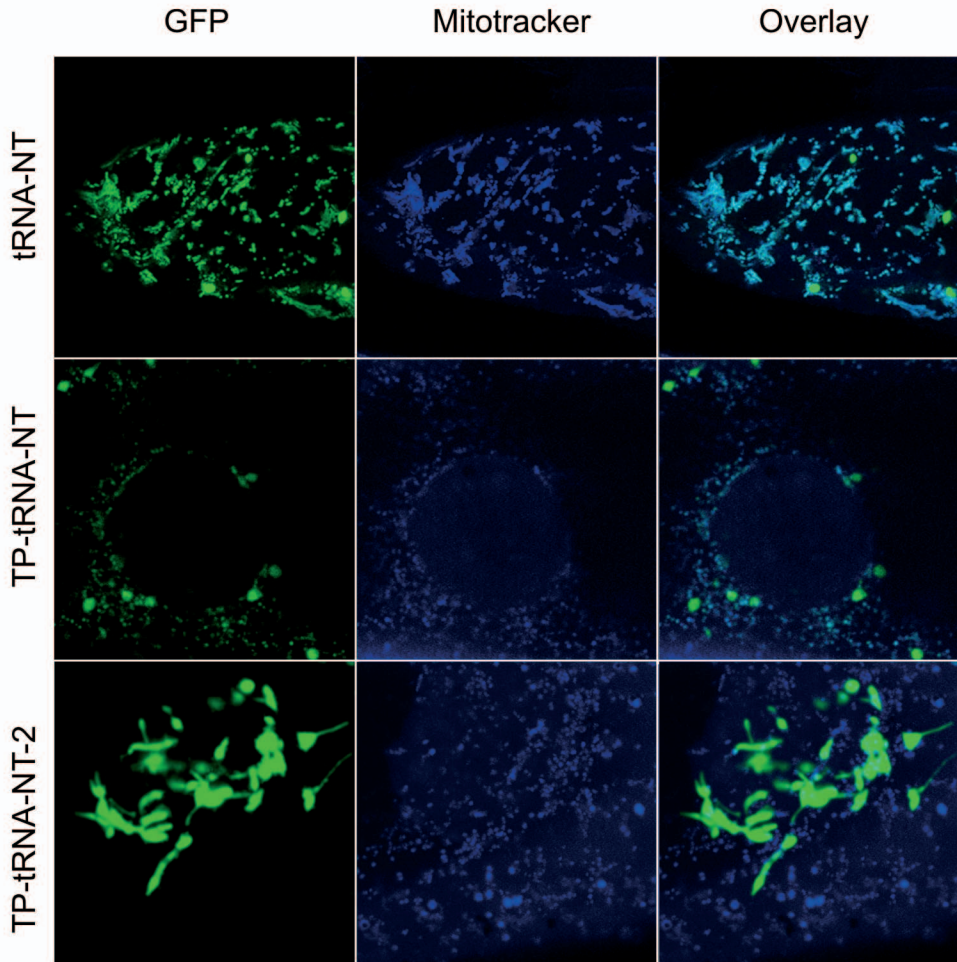
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AT1G22660.1	MILKT	
AT1G22660.2	MILKT	
	L	ATG2
AT1G22660.1	MRLSSLPINTLINLPKSLFLISPFRRNLNRS�TVASRISSTLLRVSG	
AT1G22660.2	MRLSSLPINTLINLPKSLFLISPFRRNLNRS�TVASRISSTLLRVSG	
AT1G22660.1	VSSRPCGYWFSTNAAMTNVGEEDKQSIPSIELKENIELTDKERKIFDR	
AT1G22660.2	VSSRPCGYWFSTNAAMTNVGEEDKQSIPSIELKENIELTDKERKIFDR	
AT1G22660.1	LLSTLRYCNLDTQLRVAGGWVRDKLLGKESDDIDIAIDNMSGSEFLDK	
AT1G22660.2	LLSTLRYCNLDTQLRVAGGWVRDKLLGKESDDIDIAIDNMSGSEFLDK	
AT1G22660.1	FKEYLSSRDEEVQGDTVIERNPQSKHLETAKLRIYDQWIDFVNLRS	
AT1G22660.2	FKEYLSSRDEEVQGDTVIERNPQSKHLETAKLRIYDQWIDFVNLRS	
AT1G22660.1	EYTENSRIPTMKFGTAKDDAFRRDLTINSLFYNINS GAVEDLTERGID	
AT1G22660.2	EYTENSRIPTMKFGTAKDDAFRRDLTINSLFYNINS GAVEDLTERGID	
AT1G22660.1	DLKSGKIVTPLPAKATFLDDPLRVLRVRFGARFGFTLDEELKEAASS	
AT1G22660.2	DLKSGKIVTPLPAKATFLDDPLRVLRVRFGARFGFTLDEELKEAASS	
AT1G22660.1	EEVRVALGEKISRERIGNEIDLMSGNGPVSAVTYLSDLKLFVVFAL	
AT1G22660.2	EEVRVALGEKISRERIGNEIDLMSGNGPVSAVTYLSDLKLFVVFAL	
AT1G22660.1	PSSAEPSPENCGLSLSQSYLEAMWSLLKTPRPGKFSGEQRRALALYAAM	
AT1G22660.2	PSSAEPSPENCGLSLSQSYLEAMWSLLKTPRPGKFSGEQRRALALYAAM	
	E E E E EME	MatMu
AT1G22660.1	FLPFRKTVY KDTRGKS IPVNVHIF KFSMKRK TSDAETVMNIHQTTTERF	
AT1G22660.2	FLPFRKTVY KDTRGKS IPVNVHIF KFSMKRK TSDAETVMNIHQTTTERF	
AT1G22660.1	RSLIPSLEVKKDVELDEL'TWAADILEHWKSITLNDPVI PATSKIRVLT	
AT1G22660.2	RSLIPSLEVKKDVELDEL'TWAADILEHWKSITLNDPVI PATSKIRVLT	
AT1G22660.1	GFLLRDIKDFWRVSLTSLLLSATVDGSNDHQDIGQLDFQLERMRETY	
AT1G22660.2	GFLLRDIKDFWRVSLTSLLLSATVDGSNDHQDIGQLDFQLERMRETY	
AT1G22660.1	LTVEATIHGLDKIWDKPLVNGREIMQIAELKGGSRILIREW QQ KLL	
AT1G22660.2	LTVEATIHGLDKIWDKPLVNGREIMQIAELKGGSRILIREW -- KLL	
AT1G22660.1	TWQLAYPNGTAECKEWMRDIKAKRQRIE	
AT1G22660.2	TWQLAYPNGTAECKEWMRDIKAKRQRIE	

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Supplementary Tables

Supplementary Table S1. Listed are the species names, name codes and sequence accession numbers for the sequences used in phylogenetic analysis

	Species	Name	Accession number
Plant	<i>Arabidopsis thaliana</i>	Atha	At1g22660
	<i>Lupinus albus</i>	Lalb	gi 1139585
	<i>Populus trichocarpa</i>	Ptri	eugene3.00410098
	<i>Physcomitrella patens</i>	Ppat	jgi Phypa1_1 21288 gw1.13.65.1
	<i>Oryza sativa</i>	Osat	gnl tigr cds.tigr_LOC_Os12g07350.1:1-600
	<i>Ostreococcus lucimarinus</i>	Oluc	jgi Ost9901_3 34467 e_gwEuk.3.120.1_
Fungi	<i>Schizosaccharomyces pombe</i>	Spom	gi 4539282 emb CAB39906.1 SPCC645.10
	<i>Saccharomyces cerevisiae</i>	Scer	gi 6321016 ref NP_011095.1
	<i>Neurospora crassa</i>	Ncra	gi 85108987 ref XP_962692.1
	<i>Pichia guilliermondii</i>	Pgui	gi 146416643 ref XP_001484291.1
Metazoa	<i>Danio rerio</i>	Drer	gi 50344974:1-405 tRNA
	<i>Equus caballus</i>	Ecab	gi 149728339 ref XP_001496927.1
	<i>Xenopus laevis</i>	Xlae	gi 147900819 ref NP_001089848.1
	<i>Apis mellifera</i>	Amel	gi 110764826 ref XP_397254.3
	<i>Plasmodium falciparum</i>	Pfal	gi 23508403 ref NP_701072.1
Cyanobacteria	<i>Nostoc sp. PCC 7120</i>	Nsp	ref NP_488029.1 :1-904
	<i>Anabaena variabilis ATCC 29413</i>	Avar	gi 75907929 ref YP_322225.1
	<i>Nodularia spumigena</i>	Nspu	gi 119512844 ref ZP_01631911.1
Proteobacteria	<i>Erythrobacter sp. NAPI</i>	Esp	gi 85707865 ref ZP_01038931.1
	<i>Geobacter sulfurreducens PCA</i>	Gsul	gi 39996681 ref NP_952632.1
	<i>Pelobacter carbinolicus DSM 2380</i>	Pcar	gi 77918901 ref YP_356716.1

Supplementary Table S2. Listed is the smallest sum probability after WU-BLAST (<http://www.arabidopsis.org/cgi-bin/wublast>; e.g. Gish, Warren, and David J. States (1993). Identification of protein coding regions by database similarity search (*Nature Genetics* **3**:266-72.) using the sequences of the identified homologues (supp. Table 1) as bait. The results for two polynucleotide adenylyltransferase family proteins and the investigated sequence At1g22660 are given.

	At1g22660	At1g28090	At2g17580
Hsap	4.1e-11	1.6e-11	3.0e-14
Drer	2.3e-11	5.4e-15	1.4e-14
Ecab	6.6e-12	4.3e-11	4.3e-14
Xlav	9.9e-10	1.1e-11	5.8e-13
Amel	1.1e-09	2.7e-11	3.2e-09
Atha	0.0	3.7e-08	4.3e-09