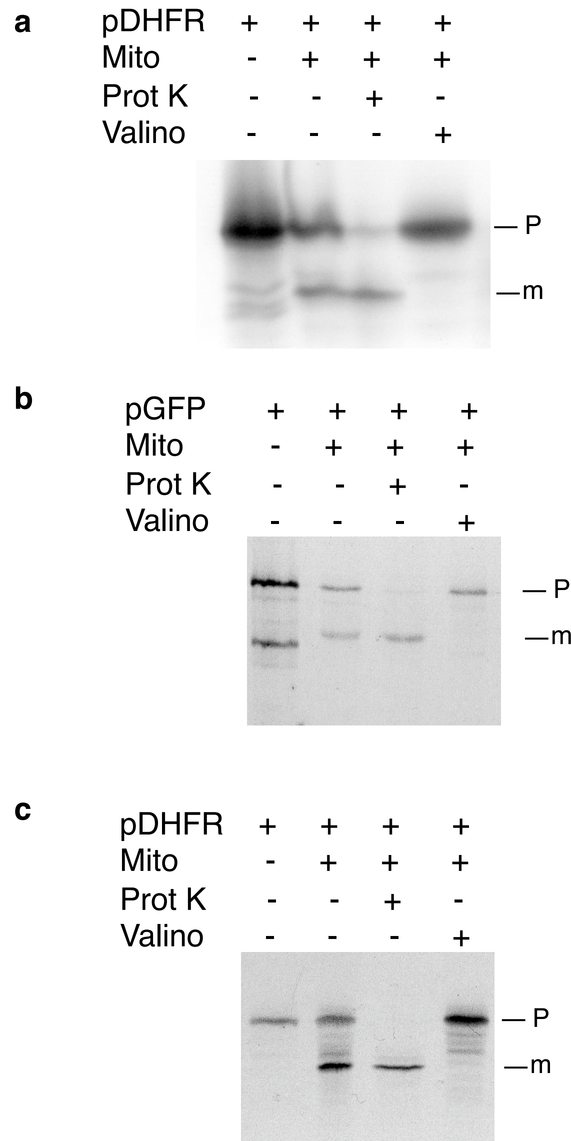
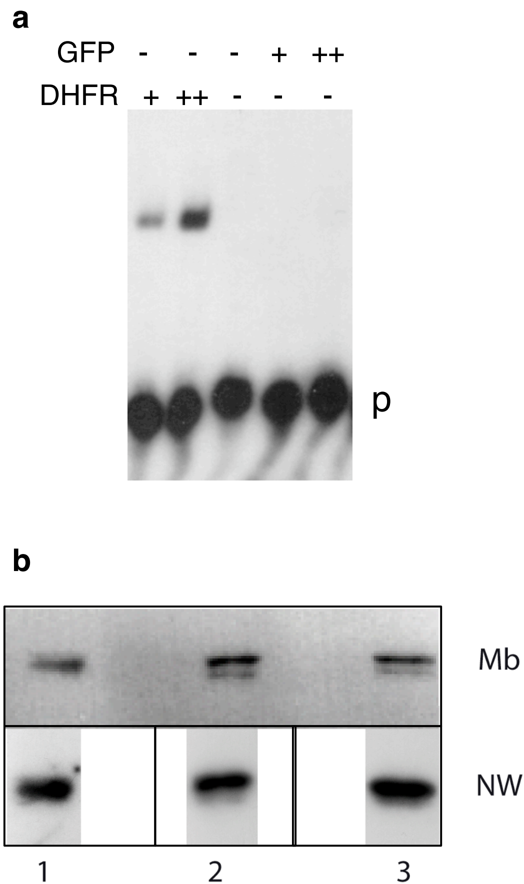


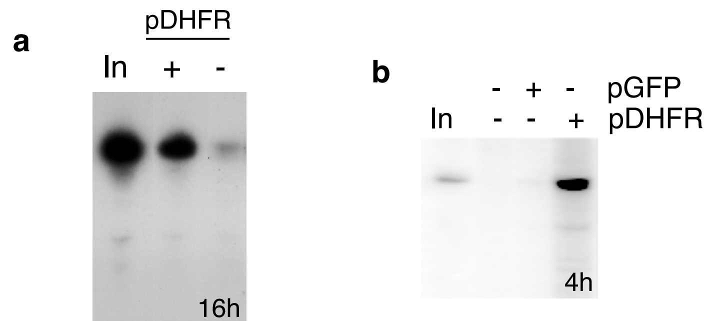
Supplementary Figure S1

pDHFR and pGFP are *in vitro* imported into isolated mitochondria. Radioactive pre-proteins were obtained by *in vitro* transcription/translation of the DNA constructs carried out with a TNT Coupled Reticulocyte Lysate System (Promega) in the presence of [^{35}S]methionine. Import of the proteins into purified potato or human mitochondria was performed according to (6). (**a**) and (**b**) pDHFR and pGFP import into isolated potato mitochondria respectively. (**c**) pDHFR import into isolated human mitochondria. When the pre-proteins are incubated with isolated mitochondria, smaller proteinase K-resistant peptides appear, showing that both pDHFR and pGFP are imported into mitochondria and processed into the mature form (m). When mitochondria are preincubated with valinomycine (Valino), protein import is inhibited and the formation of mature protein is prevented.



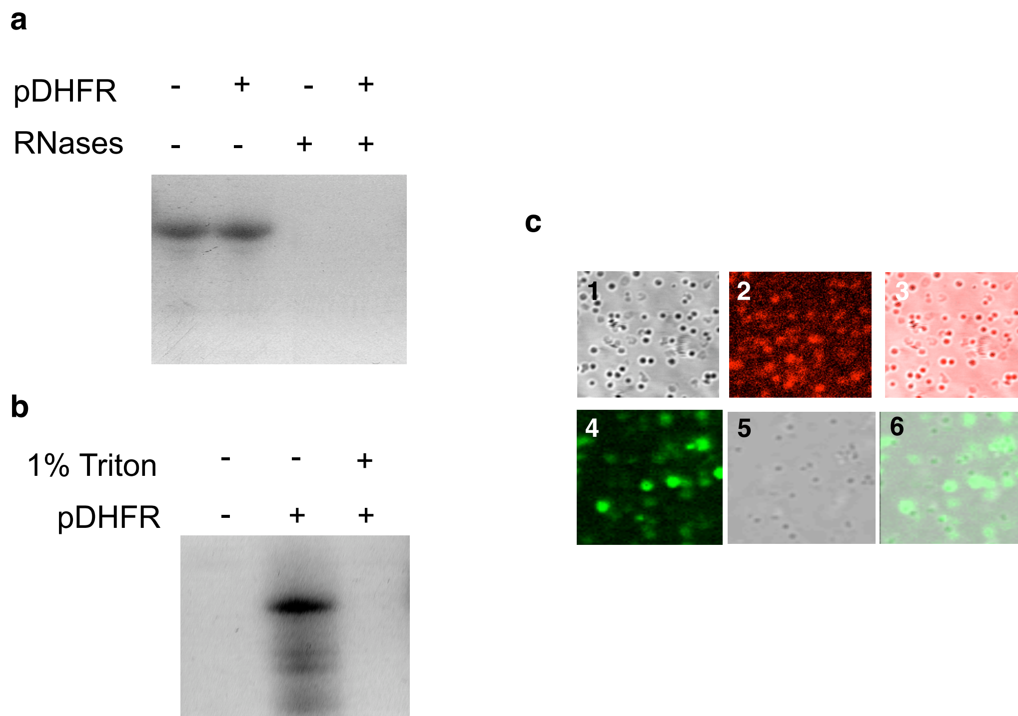
Supplementary Figure S2

Mouse DHFR interacts with RNA *in vitro*. (**a**) Gel-shift assay performed as described in (2) with labeled plant cytosolic $tRNA^{Ala}$ as a probe (p) in the absence (-) or in the presence (+ and ++) of increasing amounts of His-tagged purified DHFR protein. His-tagged purified GFP (2) was used as a negative control. + (8 pmol) and ++ (12 pmol) of purified DHFR or GFP. (**b**) Northwestern blot analysis of the interaction between DHFR and labeled $tRNA^{Ala}$, $tRNA^{His}$ precursor and atp9 mRNA. To allow the comparison, similar amount (1 μg) of DHFR was fractionated in triplicate on a 12% polyacrylamide gel. After transfer, the stained membrane (Mb) was cut into three pieces. For Northwestern blot analysis, the 3 membranes, after DHFR renaturation, were incubated with labeled *in vitro* transcribed $tRNA^{Ala}$ (1), precursor of $tRNA^{His}$ (2) and atp9 mRNA (3) according to the protocol described in (Salinas *et al*., Proc Natl Acad Sci 103, 18362-18367). The specific activity of the three labeled RNA was the same. Time of exposure of the autoradiography is 30 min.



Supplementary Figure S3

pDHFR, but not pGFP, efficiently enhances $tRNA^{Ala}$ import into isolated potato mitochondria. (**a**) Same experiments as in Fig. 1a, but with a 16 hours exposure (16h) of the corresponding autoradiography. The weak signal observed in the absence of pDHFR corresponds to the intensity of the signal previously obtained in {Delage, 2003 #2350}. (**b**) ^{32}P-labeled *in vitro*-transcribed $tRNA^{Ala}$ was incubated with isolated potato mitochondria in the absence (-) or presence (+) of 35 pmol of pDHFR or pGFP. In, input RNA (2 fmol). RNAs were fractionated on a denaturing 15% (w/v) polyacrylamide gel and visualized by autoradiography (4h, 4 hours exposure).



Supplementary Figure S4

The pDHFR recombinant protein facilitates the targeting of RNA to mitochondria. (**a**) Thirty minutes incubation of labeled tRN^{Ala} transcript in the import medium (without mitochondria) in the presence (+) or not (-) of 35pmol of pDHFR. After incubation the mix was treated with RNases (+) or not (-) as for a classical import assay. RNAs were phenol extracted before polyacrylamide gel analysis. (**b**) Labeled tRNA^{Ala} transcript was incubated with potato mitochondria in the absence (-) or presence (+) of 35 pmol of pDHFR. Following standard import conditions, Triton X-100 (1%) was added (+) or not (-) prior to the RNAse treatment. After extraction, RNAs were fractionated on a denaturing polyacrylamide gel. (**c**) Visualization under confocal microscope. Isolated mitochondria used for the *in vitro* import experiments observed under visible light (1) and stained with mitotracker (2), (3) merged image of 1 and 2, (4) visualization of Alexa Fluor-labeled tRNA^{Ala} transcript incubated (20 minutes) with potato mitochondria in the presence of 35 pmol of pSu9-DHFR, (5) image of the mitochondria analyzed in 4 under visible light and (6) merged picture of the images shown in 4 and 5.

GCGAGTATAGACGTGTCTAAG**GGCGAGCATAACCCAATAGGTCAGAGTGTCAGATTG**
TGAATTCGAAAACACGGGTTCGAATCCCGTTATTCGCCGCACCCCATTGTCGACCAG
GCCCTCTCCCCTAGCCTATGCTTTGCATGAACATCTCAATGTCCAAGATAAAAAGAA
CGAGGGGAAGAATCGACGAGGCGAGTGTTCTCGAAGAGAAAATCGTGATGGAAAAAG
CGTGAGGAGAATTCGAAACTCGAGATGTTAGAAGGTGCAAAATCAATGGGTGCAGGA
GCTGCTACAATTGCTTCAGCGGGAGCTGCTATCGGTATTGGAAACGTCCTTAGTTCC
TCGATTCATTCCGTGGCGCGAAATCCATCATTGGCAAAACAATTATTTGGTTATGCC
ATTTTGGGCTTTGCTCTAACCGAAGCTATTGCATCGTTTGCCCCAATGATGGCCTTT
TTGATCTCATTCGTATTCCAAGTTCGTTAGTAATCGTTTACGGTGGGTGGATAAGCA
GGAAGGGATCCCTGTGGTTAGACTAACTGGCCGAGAAGGCTAGTGAGGTTCCTGCTA
TGGTGAAGTGAAAGATCTTTCACTATAGTGGGAAGAAGACAGGTGGGAGCGAGCCGA
GCGAGAGCAAAGCAAGTTTCAGTGGTGGGCTGTCTTCGCGGTCCCATT

Supplementary Figure S5

Nucleotide sequences of the cDNA construct coding for the edited form of the larch mitochondrial tRNA^{His} precursor fused to the unedited form of potato mitochondrial *atp9*. The tRNA^{His} coding sequence is in bold and underlined, its flanking sequences in black. The atp9 open reading frame is in blue, the 5'- and 3'- UTR are written in gray. The C residues converted into U residues by post-transcriptional editing (5) are in red. The 4 editing sites found edited upon uptake of the unedited *atp9* mRNA into isolated potato mitochondria (see **Fig. 3d**) are underlined.