

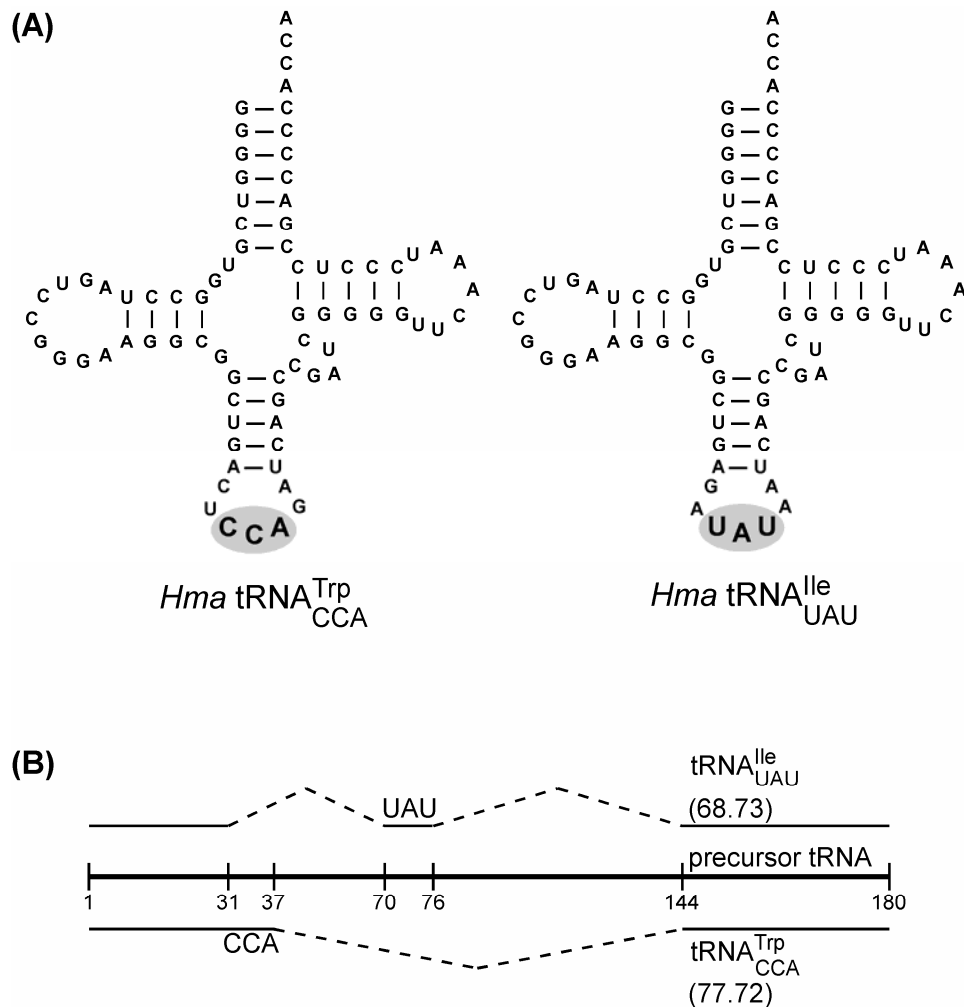
**Identification and characterization of a tRNA decoding the rare AUA codon in *Haloarcula marismortui***

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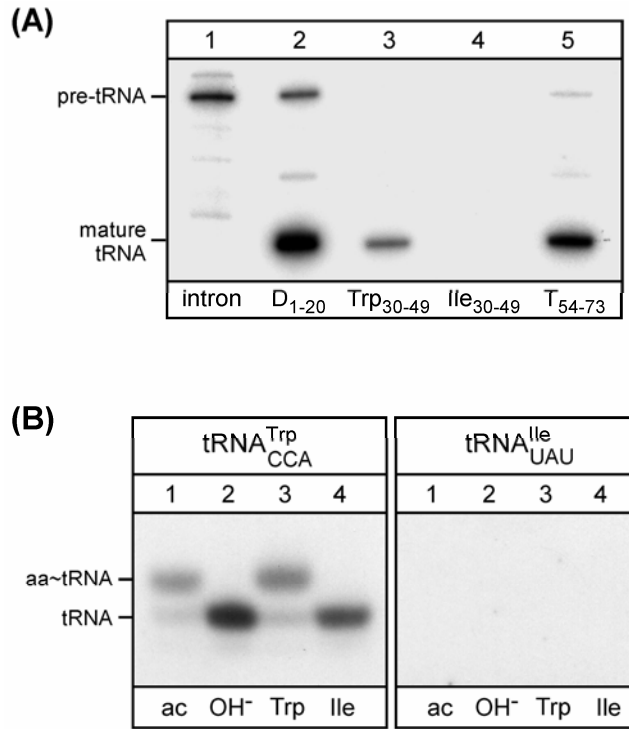
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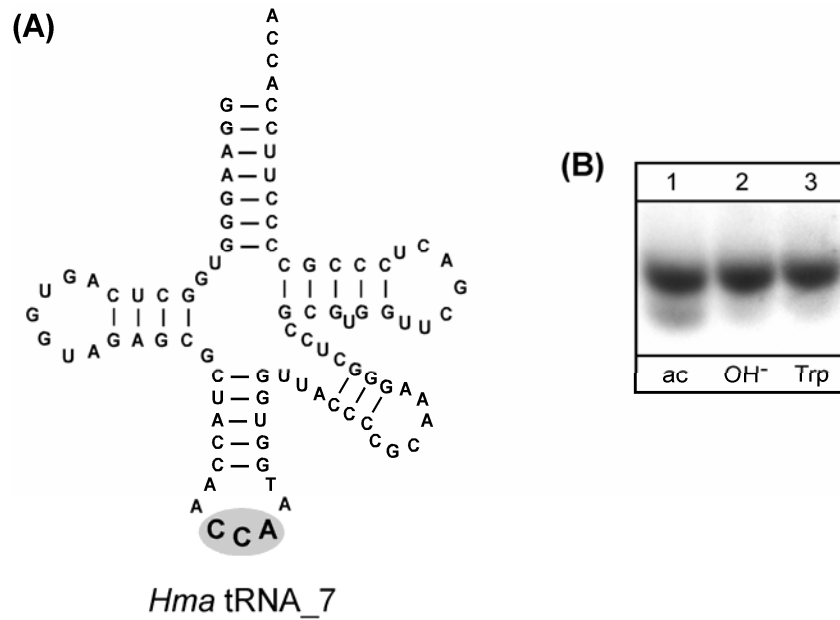
**SUPPLEMENTARY FIGURES**



**SUPPLEMENTARY FIGURE 1.** *In silico* prediction of alternative splicing of tRNA genes containing non-canonical introns in archaea. **(A)** Cloverleaf structures of tRNA<sup>Trp</sup><sub>CCA</sub> and tRNA<sup>Ile</sup><sub>UAU</sub> derived from the putative composite isoleucine-tryptophan gene in *H. marismortui* as proposed by Ghosh et al. (2006). **(B)** Schematic representation of proposed canonical and non-canonical splicing, accompanied by COVE scores of mature tRNAs, based on a standard covariance model calculated by tRNAscan-SE (Lowe and Eddy 1997). It was proposed that the single intron-containing gene produces two different tRNAs by means of alternative splicing, tRNA<sup>Trp</sup><sub>CCA</sub> through canonical splicing at position 37/38, and tRNA<sup>Ile</sup><sub>UAU</sub> through non-canonical splicing with splice sites at 31/32 and canonical splicing with splice sites at 37/38. Introns are indicated by dotted lines; exons, full lines.



**SUPPLEMENTARY FIGURE 2.** Northern hybridization of tRNAs encoded by *H. marismortui* chromosome 1 loci 646,395 – 646,575, which contains the putative composite isoleucine-tryptophan tRNA gene encoding tRNA<sup>Trp</sup><sub>CCA</sub>, annotated as tRNA<sub>5</sub>, and the newly predicted tRNA<sup>Ile</sup><sub>UAU</sub>. **(A)** Analysis of total RNA on a 10% polyacrylamide gel under denaturing conditions followed by Northern hybridization using probes targeting precursor (pre-tRNA) and mature tRNAs. Lane 1, intron-specific probe; lane 2, probe directed against the 5'-terminal region of tRNA<sup>Trp</sup><sub>CCA</sub>/tRNA<sup>Ile</sup><sub>UAU</sub> (D<sub>1-20</sub>); lanes 3 and 4, anticodon stem-loop-specific probes for tRNA<sup>Trp</sup><sub>CCA</sub> (Trp<sub>30-49</sub>) and tRNA<sup>Ile</sup><sub>UAU</sub> (Ile<sub>30-49</sub>), respectively; lane 5, probe directed against the 3'-terminal region of tRNA<sup>Trp</sup><sub>CCA</sub>/tRNA<sup>Ile</sup><sub>UAU</sub> (T<sub>54-73</sub>). **(B)** Analysis of total RNA by acid urea PAGE/Northern hybridization using probes directed against the anticodon stem-loop of tRNA<sup>Trp</sup><sub>CCA</sub> (left panel; Trp<sub>30-49</sub>) and tRNA<sup>Ile</sup><sub>UAU</sub> (right panel; Ile<sub>30-49</sub>). Lanes 1 and 2, tRNA isolated under acidic conditions before (ac) and after deacylation by base-treatment (OH<sup>-</sup>); lanes 3 and 4, *in vitro* aminoacylation of deacylated tRNA with tryptophan using human TrpRS (Trp) or isoleucine using *E. coli* IleRS (Ile), respectively. aa-tRNA, aminoacyl-tRNA.



**SUPPLEMENTARY FIGURE 3.** Analysis of tRNA<sub>7</sub>, an alternative tRNA<sub>CCCA</sub><sup>Trp</sup> encoded by *H. marismortui* chromosome I region 984,958 – 985,059. (A) Cloverleaf structure of tRNA<sub>7</sub> as predicted by tRNAscan-SE. (B) Acid urea PAGE analysis of total RNA isolated from *H. marismortui* under acidic conditions followed by Northern hybridization using a probe specific for tRNA<sub>7</sub> (positions 54 – 73); lanes 1 and 2, tRNA before (ac) and after deacylation by base-treatment (OH<sup>-</sup>); lane 3, *in vitro* aminoacylation of deacylated tRNA with tryptophan using human TrpRS (Trp).