# Supporting Information

Rudinger-Thirion et al. 10.1073/pnas.1103698108

A

5'UTR
ORF
3'UTR
(167 or 633 nts)
(A)19
AAAAAAA
1790 or 2256 nts
249 or 715 bp
115 or 580 bp
(1) **RT**
(2) **PCR #1**
(3) **PCR #2**

B

Brain
Muscle
Breast
Second
poly A signal
580 bp
First
poly A signal
115 bp

C

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Alu-J-right-arm        -----------GCCACTGCACTCCAGCCTGGGCGACAGAGCGAGACCCTGTCTCAAAAAA
Homo-sapiens           -ATTCACACTTTGCCACTTAACTCCAGTGTGGATGACAGAGCGAGACCCTGCCTCAAAAAAAAAAAAAAAAAAA------GAAAGCCACACTTATTCTTT
Pan-troglodytes        -ATTCACACTTTGCCACTTAACTCCAGTGTGGATGACAGAGCGAGACCCTGCCTCAAAAAAAAAAAAAAAAAAAA--AAAGAAAGCCACACTTACTCTTT
Macaca-mulatta         -ATTCACATCTTGCCTCTGAACTCCAGTGTGGATGACAGAGCGAGACCCTGCCTCAAAAAAAACAAAAAAGAAA----------GCCACACTTACTCTTT
Callithrix-jacchus     -AGCCACACTTTGCCAGTGAACTCCAGTGTGGATGACAGAGCGCGGCCCTGCCTCAAAAGAAGAAAGAAAGAAA----------GCCACACTTACTCTTT
Pongo-abelii           -ATTCACACTTTGCCACTTAACTCCAGTGTGGATGACAGAGCAAGACCCTGCCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCCACACTTATTCTTT
Gorilla-gorilla        -ATTCACACTTTGCCACTTAACTCCAGTGTGGATGACAGAGCAAGACCCTGCCTCAAAAAAAAAAAAAAAAAAAAAG-AAAGCAAGCCACACTTACTCTTT
Ratus-norvegicus       -AGCCACACTTT--------------------------------------------------------------------------------TTATTCTCT
Mus-musculus           -AGCCACACTTT--------------------------------------------------------------------------------TTATTCTCT

Homo-sapiens           TCAGTAACCTGCTAGTGCACAGGCTGTACTTTAGGTACTTAAAATATGCACTAGAATAAATTTGCAAGGCCCTAAAATATCACTGTTATTTTTGGAGTAA
Pan-troglodytes        TCAGTAACCTGCTAGTGCACAGGCTGTATTTTAGGTACTTAAAATATGCACTAGAATAAATTTGCAAGGCCCTAAAATATCACTGTTATTTTTGGAGTAA
Macaca-mulatta         CCAGTAACCTGCTAGTGCACAGGCTGTACTTTAGGTACTTAAAATATGCACTAGAATAAATTTGCAAGGCCCTAAAGTATCACTGTTATTTTTGGAGTAA
Callithrix-jacchus     CCAGTAACCTGCTATTGCACAGGCTGTACTTTAGGTACTTAAAATATGCACTAGAATAAATTTGCAAGACCCTAAAGTATCACTGTTATTTTTGGAGTAA
Pongo-abelii           TCAGTAACCTGCTAGTGCATAGGCTGTACTTTAGGTACTTAAAATATGCACTAGAATAAATTTGCAAGGCCCTAAAGTATCACTGTTATTTTTGGAGTAA
Gorilla-gorilla        TCAGTAACCTGCTAGTGCACAGGCTGTACTTTAGGTACTTAAAATATGCACTAGAATAAATTTGCAAGGCCCTAAAATATCACTGTTATTTTTGGAGTAA
Ratus-norvegicus       CT------------TTGATTAGCTTATACCTGAGGTACTTAAGATATGCAGTAGAATTAATGCATTA----CTAAAGTGCCACAATTATTTTTTAAGTAG
Mus-musculus           CCAGGAACCTGCTATTGATTAGGTTATACCTGAGGTACTTAAAATATGCAGTAGAATTAATGTCTTA----CTAAAGTGCCACAATTATTTTTTAAGTAG

Homo-sapiens           TTCAGTATAGGTTCGTTTAAAAGAGA-----TTTTTATAACTTCAGACATGCATCAGTAGGAAATAACT-----------TGAGAAATTCATATGGTTAT
Pan-troglodytes        TTCAGTATAGGTTCGTTTAAAAGAGA-----TTTTTATAACTTCAGACATGCATCAGTAGGAAATA-CT-----------TGAGAAATTCATATGGTTAT
Macaca-mulatta         TTCGGTATAGATATGTTTAAAAGAGA-----TTTTTATAACTTCAGACGTGCGTCAGTAGGAAATA-CT-----------TGAGAAATTAATATGGTTAT
Callithrix-jacchus     TTCAGTATAGATTCGTTTAAAAGAGAGAAGATTTTTATAACTTCAGACATGCATCAGTAGGAAATA-CT-----------TGAGAAATTCATATGGTTAT
Pongo-abelii           TTCAGTGTAGGTTCATTTAAAAGAGA-----TTTTTATAACTTCAGATATGCATCAGTAGGAAATA-CT-----------TGAGAAATTCATATGGTTAT
Gorilla-gorilla        TTCAGTATAGGTTCGTTTAAAAGAGA-----TTTTTATAACTTCAGACATGCATCAGTAGGAAATA-CT-----------TGAGAAATTCATATGGTTAT
Ratus-norvegicus       CTCAATACGAATTCATTTAAGTCAAAA---CGTTTTAGAACTTCAGATATGCTTAAATAGGAAATACTTGAACATTTTAATGAGAAATTTCTATGGTTG-
Mus-musculus           CTCAGTACAAATTAATTTAAGTAAAAAA--CGTTTTAGAATTTCAAATATCCTTAAATAGGAAATACTTGAACGTTTTAATGAGAAATTTCTATGGTTG-

Homo-sapiens           GTTACAAATTCATATTCTGTTACTACAGTAAACGTTAA---G-AGTTTTAAACAGTT-AAGATTGTACAATTTTTCTT-CTTTTCTATATTACAAGGGCC
Pan-troglodytes        GTTACAAATTCATATTCTGTTACTACAGTAAACGTTAA---G-AGTTTTAAACAGTT-AAGATTGTACAATTTTTTTT-CTTTTCTATATTACAAGGGCC
Macaca-mulatta         GTGAAAAATTCATATTCTGTTACTACAGTAAATGTTAC---G-AGTTTTAAACAGTT-AAGATTGTACAACTTTTCTT-CTTTTCTATATTACAAGGGCC
Callithrix-jacchus     GTTAAAAATTCATATTCTGTTACTGCAGTAAATGTTAACAAG-AGTTTTAAACAGTT-AAGATTGTACAATTTTTTTC-CTTT----TATTACAAGGGCC
Pongo-abelii           GTTAAAAATTCATATTCTGTTACTACAGTAAACGTTAA---G-AGTTTTAAACAGTT-AAGATTGTACAATTTTTTTT-CTTTTCTATATTACAAGGGCC
Gorilla-gorilla        GTTACAAATTCATATTCTGTTACTACAGTAAACGTTAA---G-AGTTTTAAACAGTT-AAGATTGTACAATTTTTTTT-CTTTTCTATATTACAAGGGCC
Ratus-norvegicus       -----AAATTCATAGTCTATCACTATAGGAAATGTTAA-GGA-CAATTTAAACAGTTTAGGATTATATAATTTTTTTTTCTTTTCTATATTGTGGTGACC
Mus-musculus           -----AAATTCATAGTCTTTCACTATAGGAAATGTTAA-GAA-AAATTTAAACAGTTTAAGATTATACAATTTTTTTTTCTTTTCTATATTGAGGTGGCC

Homo-sapiens           CCAGTGTTAATGTCTTAGATTTTCAGTATTTGAACTTATTTTTTTAAATTCTGTCATTGAGATAAGAATAATTCAGGTAGCATCTGAAATTTTAATGAAT
Pan-troglodytes        CCAGTGTTAATGTCTTAGATTTTCAGTATTTGAACTTATTTTTTTAAATTCTGTCATTGAGATAAGAATAATTCAGGTAGCATCTGAAATTTTAATGAAT
Macaca-mulatta         CCAGTGTTAATGTCTTAGATTTTCAGTATTTGAACTTATTTTTTTAAATTCTGACATTGGAATAAGAATA-TTCAGGTAGCATCTGAAATTTTAATGATT
Callithrix-jacchus     CCGATGTTAATGTCTTAGATTTTCAGTATTTGAACTTATTTTT--AAATTATGACATTGAGATAAGAATAATTCAGGTAGTATCTGAAATTTTAATGAAT
Pongo-abelii           CCAGTGTTAATGTCTTAGATTTTCAGTATTTGAACTTATTTTTTTAAATTCTGACATTGGGATAAGAATAATTCAGGTAGCATCTGAAATTTTAATGAAT
Gorilla-gorilla        CCAGTGTTAATGTCTTAGATTTTCAGTATTTGAACTTATTTTTTTAAATTCTGTCATTGAGATAAGAATAATTCAGGTAGCATCTGAAATTTTAATGAAT
Ratus-norvegicus       ACCATACTAATATCCTAAATTTTCAATATTTGAACTT------TTAACTCTTGACATTGAGA-AAAAAAAATTGGAGAAATTACCAAGATTTTAACATAT
Mus-musculus           ACCATACTAATATTCTAAATTTTCAATATTTGAACTT------TTAACTCTTGACATTGAAA-AAAAAGGATTGGAGAAATTACCAAGATTTTAACATCT

Homo-sapiens           GTATAATTGGCATATCATGGAAAATTAACCAGAAAGTATCAGTTCTTAAAAGTTATGCCTAGAAATTATGTAAAGCT----AAACTACTGGTTAGAAAGT
Pan-troglodytes        GTATAATTGGCATATCATGGAAAATTAACCAGAAAGTATCAGTTCTTAAAAGTTATGCCTAGAAATTATGTAAAGCT----AAACTACTGGTTAGAAAGT
Macaca-mulatta         GTATAATTGGCATATCATGGAAGATTAACCAGAAAGTATCAGTTCTTAAAAGTTATGTCTAGAAATTATGTAAAGCAGGCTAAAATACTGGTTAGAAAGT
Callithrix-jacchus     GTGTAATTGGCATATCATGGAAAATTAGCCAGAAAGTAACAGTTCTTAAAAGTTATGCCTAGAAAT-ATGTAAAGCAGGCAAAAATGCTGGTTAGAAAGT
Pongo-abelii           GTATAATTGGCATATCATGGAAAATTAACCATAAAGTATCAGTTCTTAAAAGTTATGCCTAGAAATTATGTAAAGCT----AAACTACTGGTTAGAAAGT
Gorilla-gorilla        GTATAATTGGCATATCATGGAAAATTAACCAGAAAGTATCAGTTCTTGAAAGTTATGCCTAGAAATTATGTAAAGCT----AAACTACTGGTTAGAAAGT
Ratus-norvegicus       ATAAAACTGGTATGTC-----------------------------TTAAAAGTTACTTTCAGAAATCATGTAAAGTATCTGAAAACACTGGCTAGAA---
Mus-musculus           ATAAAATTGGTATGTC-----------------------------TTAAAAGTTACTTTCAAAAATCCTGTGAAGCATCTGAAA---CTGGCTAGAA---

Homo-sapiens           ATTCAGTGTAATATTGTATTAATTTGTTA--AATTCTAAACTTGAATTTCAATAAAATTTTAAAGCT---------------------------------
Pan-troglodytes        ATTCAGTGTAATATTGTATTAATTTGTTA--AATTCTAAACTTGAATTTCAATAAAATTTTAAAGCTAATTGTAGTTTTTGTGTAACTTTGTGGCCAACT
Macaca-mulatta         ATTCAGTGTAATCTTGTATTAAATTGTTA--AATTCTAAACTTGAATTTCAATAAAATTTTAAAGCTAATT-----------------------------
Callithrix-jacchus     ATTCAGTGTAATATTGTATTAAATTGTTA--AATTCTAAACTTGAATTTCAATAAAATTTTAAAGCT---------------------------------
Pongo-abelii           ATTCAGTGTAATATTGTATTAATTTGTTA--AATTCTAAACTTGAATTTCAATAAAATTTTAAAGCT---------------------------------
Gorilla-gorilla        ATTCAGTGTAATATTGTATTAATTTGTTA--AATTCTAAACTTGAATTTCAATAAAATTTTAAAGCTAATTGTAGTTTTT--------------------
Ratus-norvegicus       --GCAGT--AACACTGAA--ACCTTGTTATCAATTCTGAGGCTGAAATTCAATAAAAACTTAAAGCT---------------------------------
Mus-musculus           --CCAGT--AACACTGAG--ATGTTGTTACCAGTCCTGAGGCTGAGATTCAATAAAAACTTACAGCTGAAAAAAAAAAAAAAAAAAAAAAA---------
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**Fig. S1.** Identification and analysis of the different 3′ UTR characterizing the human aspartyl-tRNA synthetase (AspRS) mRNA. (*A*) Principle of RNA ligase mediated (RLM)-RACE experiment. The first strand cDNA was synthesized from total RNA using a "3′ RACE adapter" (gcgagcacagaattaatacgactcactatagg$(t)_{12}$). The cDNA is then used in two consecutive PCR reactions using a primer complementary to the 3′ RACE adapter and primers specific for the region of interest. At each step of the process, the expected sizes of the amplified products from the long or the short AspRS mRNAs are indicated. (*B*) Results of RLM-RACE experiments on AspRS mRNA 3′ UTRs: three different human cDNA samples (brain, muscle, and breast) have been tested by RLM-RACE to isolate the different populations of AspRS mRNA 3′ UTR. Two bands were detected; the shortest one (115 bp) corresponds to maturation of the transcript at position 167 in the 3′ UTR and the longest band (580 bp) to the full-length mRNA transcript polyadenylated at position 633 in the 3′ UTR. Maturation of the AspRS mRNA at the first polyadenylation signal produces a very instable transcript, which could be detected only by this specific technique. Therefore we can not measure either the polyadenylation efficiency or the stability of the shortest mRNA, and any comparison between the two transcripts is possible. (*C*) Sequence alignments of 3′ UTRs of AspRS mRNAs from primates, compared to other mammals: primate cDNA sequences corresponding to *Homo sapiens*, *Pan troglodytes*, *Macaca mulatta*, *Callithrix jacchus*, *Pongo abelii*, and *Gorilla gorilla* are compared to cDNA sequences from two other mammals, namely *Ratus norvegicus* and *Mus musculus* (http://www.ncbi.nlm.nih.gov/). Primate specific insertions are boxed and the similarities of the first insertion with the Alu-J right arm sequence is highlighted. Core elements of both the proximal and the distal polyadenylation signals are indicated in gray; they exhibit the standard hexanucleotide AAUAAA and U/GU-rich motif upstream and downstream the cleavage sites (arrows), respectively, and the additional motif UGUA, binding site for the pre-mRNA cleavage factor I (CFIm) (1).

1. Millevoi S, Vagner S (2010) Molecular mechanisms of eukaryotic pre-mRNA 3′ end processing regulation. *Nucleic Acids Res* 38:2757–2774.

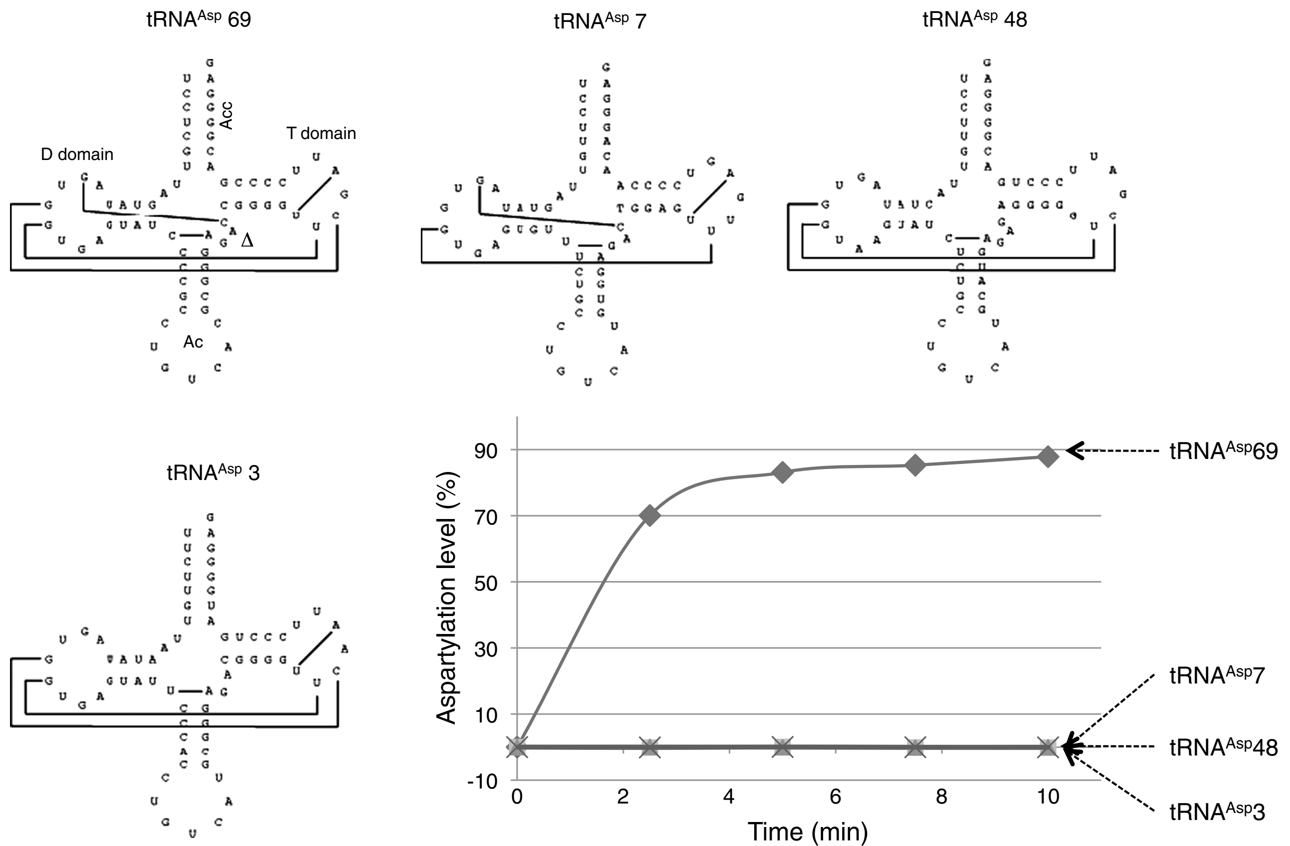


**Fig. S2.** Cloverleaf representations of the reference tRNA$^{Asp}$69 and of the three most deviating tRNA$^{Asp}$ isodecoders: tRNA$^{Asp}$7, 48, and 3 and their aminoacylation capacities. Acc, D, T, and Ac domains and Δ are indicated on the reference tRNA$^{Asp}$. Plain lines correspond to conserved long-range tertiary interactions (1). Aminoacylation activities of each tRNA isodecoders were determined in vitro, in the presence of 50 nM human AspRS at 37 °C.

1. Giegé R, Puglisi JD, Florentz C (1993) tRNA structure and aminoacylation efficiency. *Prog Nucleic Acid Res Mol Biol* 45:129–206.

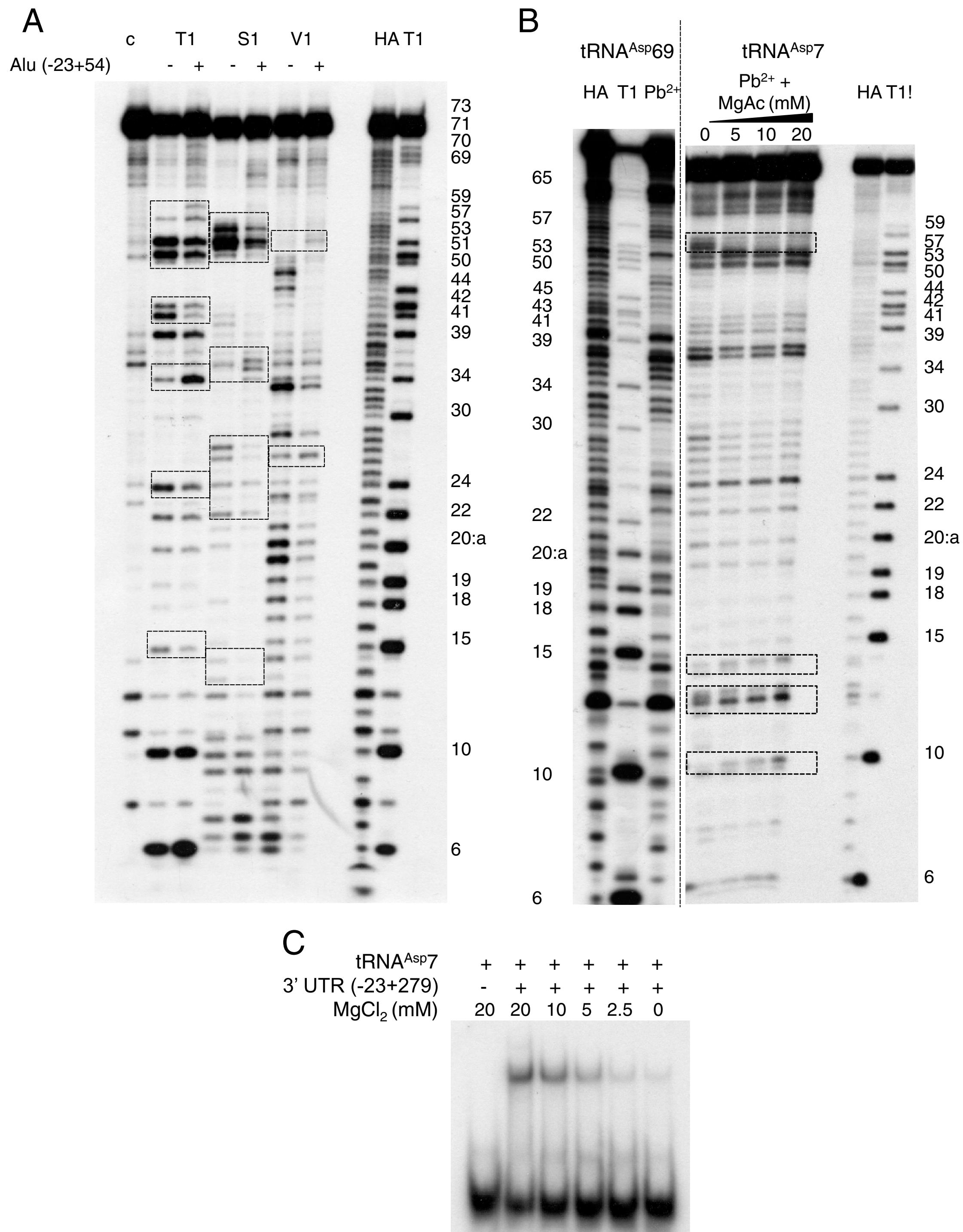


**Fig. S3.** tRNA$^{Asp}$7 structure and function depends on the concentration of $Mg^{2+}$ ions. (*A*) Footprinting of Alu ($-23+54$) on tRNA$^{Asp}$7. Autoradiogram of a 12% denaturing gel showing probing patterns of the 5′-labeled tRNA$^{Asp}$7 in the absence (–) or in the presence (+) of Alu ($-23+54$). Experiments were performed with RNases T1 (0.25 U), S1 ($7.5\times10^{-3}$ U), and V1 ($6\times10^{-3}$ U); c stands for the control experiment without nucleases; HA and T1 stand for alkaline and denaturing RNase T1 ladders, respectively. Numbering defines the position of G residues specifically cleaved by RNase T1. Dashed boxes emphasize the footprint of Alu ($-23+54$) on tRNA$^{Asp}$7. (*B*) Lead probing supports the presence of a E-loop structure in tRNA$^{Asp}$7. Autoradiogram of 12% denaturing gels displaying lead probing experiments performed on 5′-labeled tRNA$^{Asp}$69 (*Left*) and tRNA$^{Asp}$7 (*Right*). tRNA$^{Asp}$69 was probed in the presence of 1 mM $Pb^{2+}$ and 5 mM Mg acetate. Folding of tRNA$^{Asp}$7 was tested with 0, 5, 10, and 20 mM of Mg acetate and 1 mM $Pb^{2+}$. Dashed boxes emphasize significant changes in the cleavage profile when Mg Acetate varies. HA and T1 stand for alkaline and denaturing RNase T1 ladders, respectively. Numbering defines the position of G residues specifically cleaved by RNase T1. (*C*) The complex formation between tRNA$^{Asp}$7 and the Alu insertion is strongly dependent on the concentration of $Mg^{2+}$ ions. Binding experiments were performed between tRNA$^{Asp}$7 and the 3′ UTR ($-23+279$) fragment in the presence of decreasing concentration of $MgCl_2$.