



Supplemental Material to:

Sylvie Auxilien, Vincent Guérineau, Zofia Szweykowska-Kulińska and Béatrice Golinelli-Pimpaneau

The Human tRNA: m5C methyltransferase Misu is multisite-specific.

2012; 9(11)

<http://dx.doi.org/10.4161/rna.22180>

www.landesbioscience.com/journals/rnabiology/article/22180

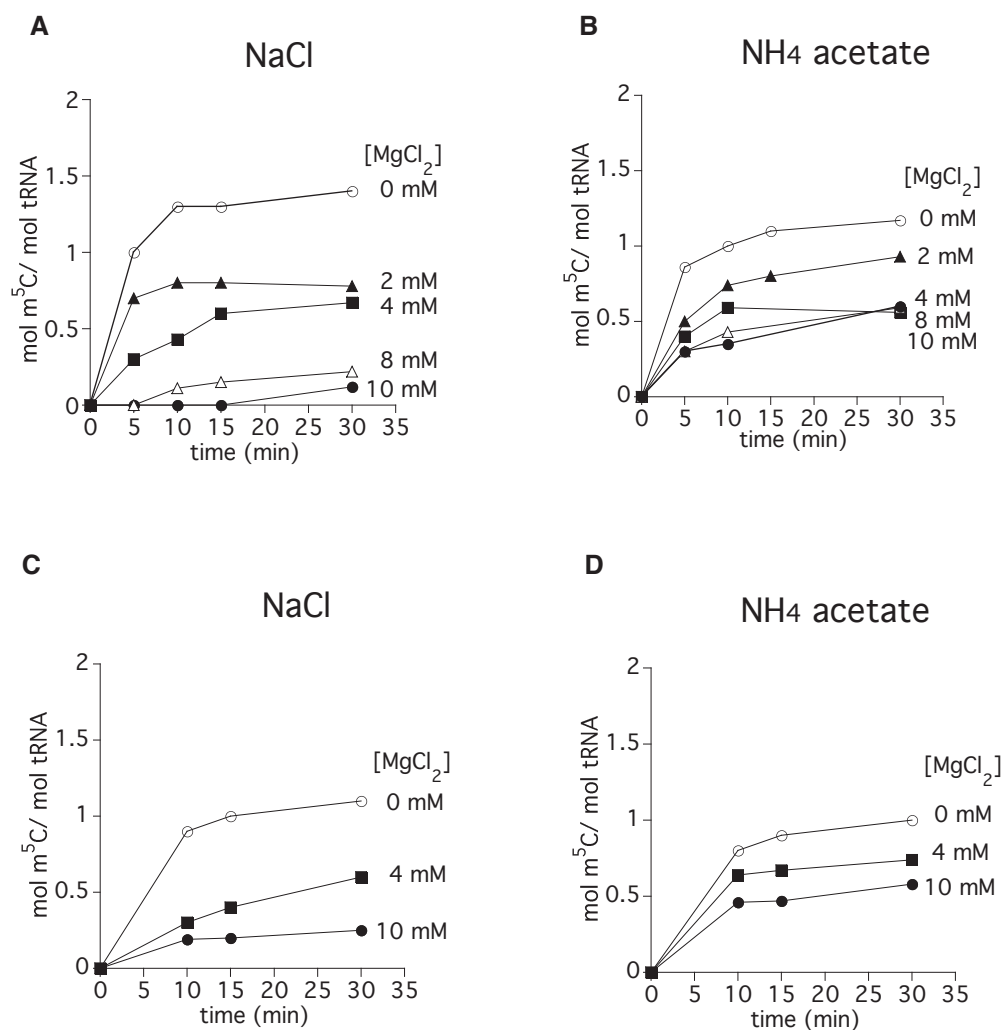


FIGURE S1: Inhibition by magnesium of the m⁵C formation in tRNA^{Leu} (CAA) catalyzed by hMisu. Methylation kinetics have been performed in 20 mM Tris-HCl pH 8.0, 2 mM DTT, 160 μM SAM, 50 mM NaCl (A and C) or 50 mM NH₄ acetate (B and D) in the absence (open circles) or presence of MgCl₂ at various concentrations: 2 mM (filled triangles), 4 mM (filled squares), 8 mM (open triangles) and 10 mM (filled circles). The tRNA^{Leu} (CAA) and hMisu concentrations were as follows: for (A) and (B), 1 nM of radiolabelled tRNA and 46 nM of hMisu; for (C) and (D), a mix of 1 nM of radiolabelled tRNA and 1.5 μM of unlabelled tRNA and 1.5 μM of hMisu.

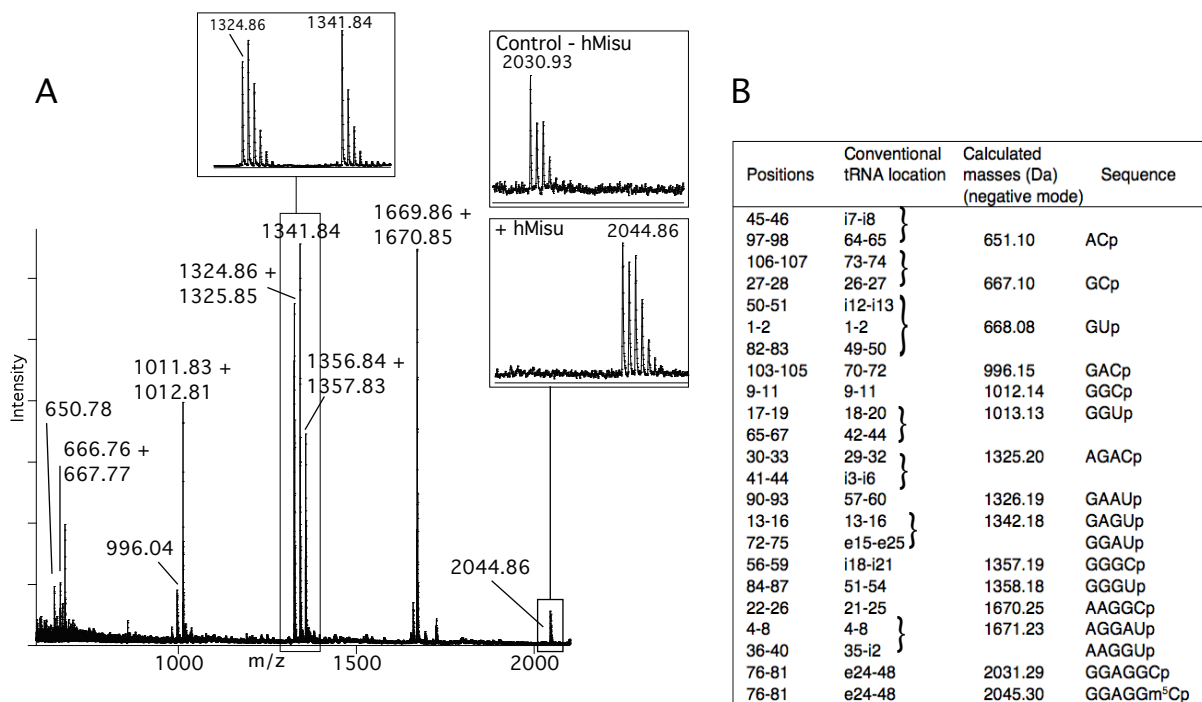


FIGURE S2: MALDI mass spectrometry analysis of intron containing human pre-tRNA^{Leu}(CAA) for methylation by hMisu at position 48. (A) MALDI mass spectrum of pre-tRNA^{Leu} methylated by hMisu and digested by RNase A. The spectral region around the fragment GGAGGCp containing C48 is enlarged to show the peak of the nonmethylated ion (m/z 2031.18) from the control without enzyme and the ion methylated by hMisu (m/z 2045.62). The spectral region around the fragment AGAC(32)p is also enlarged to show that AGAC(32)p is not methylated into a fragment with a mass increment of 14 Da (m/z 1339.20). (B) List of theoretical masses of RNase A fragments of pre-tRNA^{Leu}.

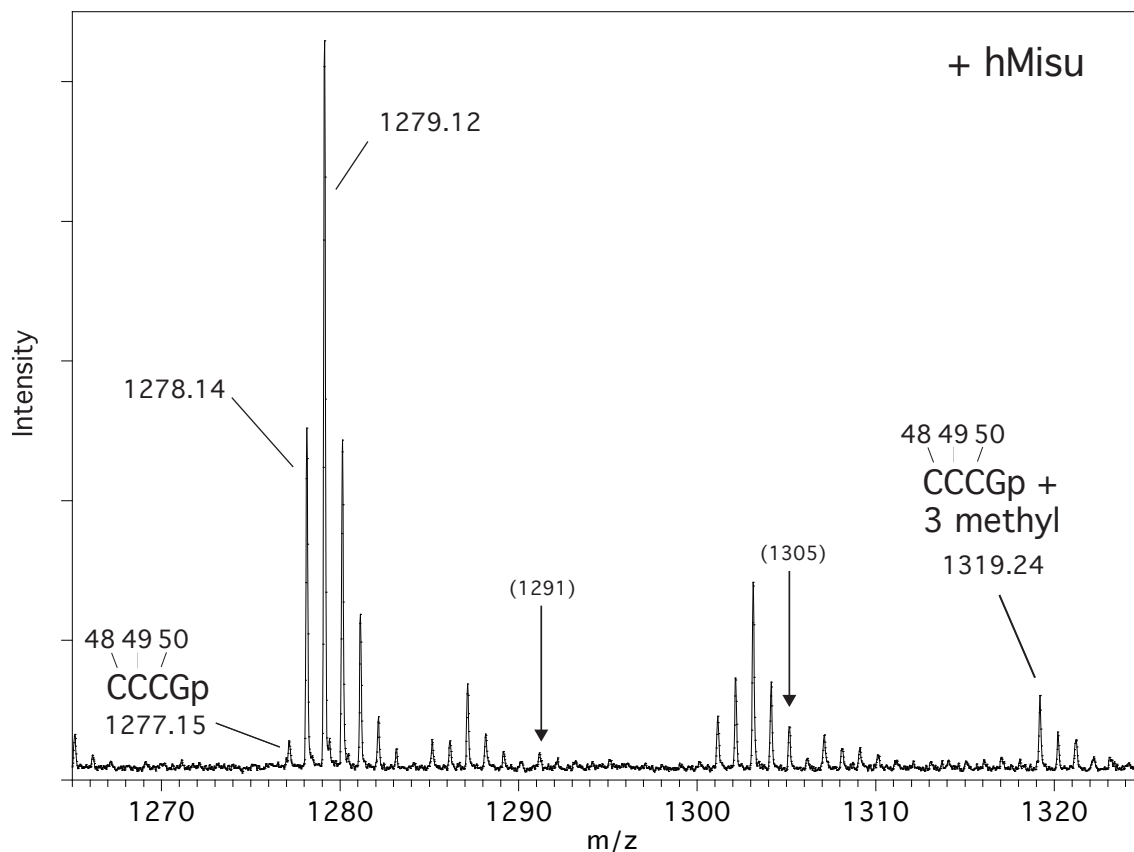
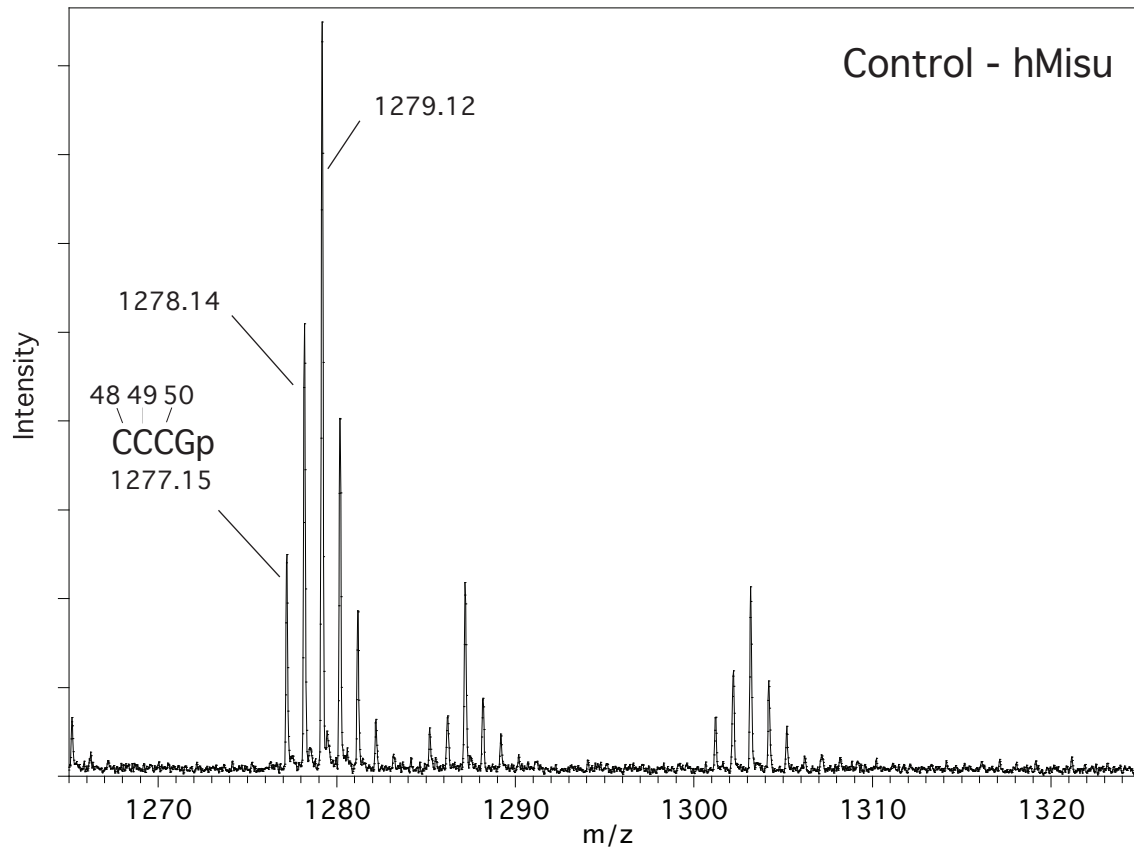


FIGURE S3: Close-up of the insert of Fig4B. The fragment C48C49C50Gp (m/z 1277.15) is present in the control without hMisu and disappears almost completely upon incubation with hMisu while a new peak at m/z 1319.24 appears, corresponding to the fragment C48C49C50Gp containing three methyl groups. The unidentified peaks around m/z 1303.17, present both in the absence and presence of Misu, do not correspond to the mono and dimethylated fragment (theoretical m/z 1291.19 and 1305.20, respectively, at the position indicated by the arrows).