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Supplementary Information for

Multiple prebiotic metals mediate translation

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Supplementary Information Text

Translation Reaction Buffer

The reaction buffer was based on Shimizu et al. (1), with HEPES-OH instead of phosphate buffer to avoid precipitation of metal phosphates. We found that rates of translation were consistently lower with Tris-HCl than HEPES-OH, and therefore HEPES-OH was used as the buffer for all experiments. Buffer consisted of 20 mM HEPES-OH (pH 7.3), 95 mM potassium glutamate, 5 mM NH_4Cl, 0.5 mM $CaCl_2$, 1 mM spermidine, 8 mM putrescine, 1 mM dithiothreitol (DTT), 2 mM adenosine triphosphate (ATP), 2 mM guanosine triphosphate (GTP), 1 mM uridine triphosphate (UTP), 1 mM cytidine triphosphate (CTP), 10 mM creatine phosphate (CP), and 53 μM 10-formyltetrahydrofolate. The reaction buffer was lyophilized and stored at -80°C until resuspension in anoxic nuclease-free water immediately before experiments in an anoxic chamber.

DNA and mRNA templates

A plasmid containing the gene encoding dihydrofolate reductase (DHFR) from *E. coli* was provided as a gift from New England Biolabs. The DHFR control plasmid was linearized using HindIII restriction enzyme, purified by phenol/chloroform extraction, precipitated in ethanol, and resuspended in nuclease-free water. Messenger RNA was generated using the HiScribe™ T7 High Yield RNA Synthesis Kit (New England Biolabs catalog # E2040S) and purified using the MEGAclear™ Transcription Clean-Up Kit (Thermo Fisher, Waltham MA, USA; catalog # AM1908).

Extended Details of Ribosome Metal Content Methods

In order to measure the Fe and Mn content of ribosomes, 300 μL reactions were prepared with *E. coli* ribosomes (New England Biolabs catalog # P0763S), 7 mM $FeCl_2$ or 7 mM $MnCl_2$, and reaction buffer. Blank samples without ribosomes were prepared with 7 mM $FeCl_2$ or 7 mM $MnCl_2$, and reaction buffer. All mixtures were incubated at 37°C for 2 hours. After incubation, samples were transferred to Vivaspin 500 centrifugal concentrators (Vivaproducts, Littleton, MA; catalog # VS0141) and spun at 15,000 x g until minimum retentate volume (~5 μL) was achieved. Retentate was transferred to a 1.5 mL microcentrifuge tube and suspended in 100-200 μL of 10 mM HEPES-OH (pH 7.3, 50 mM NaCl). Samples were spiked with 1 ppm Ga concentration standard, and 2 μL of each sample was dried onto siliconized quartz discs. Metal analysis was performed by total reflection x-ray fluorescence (TRXF) spectroscopy (S2 Picofox TRXF spectrometer; Bruker, Billerica, MA). Samples were run at 50 kV for 2000 s each. TRXF spectra were used to calculate the concentration of Fe or Mn in mg L^{-1}. Blank values were subtracted from ribosome samples and these corrected concentrations were converted to mM. The final ribosomal concentration (3 μM) was based on our dilution of NEB ribosomes supplied at 13.3 μM using RNA absorbance at 260 nm ($1A_{260}$ = 60 μg mL^{-1}). Values were reported as moles of Fe or Mn per mole ribosome ± standard error of the mean of triplicate experiments.

Extended Details of Quantum Mechanical Calculations

The binding of hexahydrated metal ions (M^{2+}) to an rRNA clamp fragment ($rRNAcl^{2-}$) was described by the following reaction:

$rRNAcl^{2-} + M^{2+}(H_2O)_6 \rightarrow rRNAcl^{2-}\text{-}M^{2+}\bullet(H_2O)_4$ complex + $2H_2O$, where M^{2+} = (Mg^{2+}, Fe^{2+}, Mn^{2+})

The reactants and products of this reaction were fully optimized using the density functional theory (DFT) with the hybrid B3LYP functional (2, 3), at the 6–311++G(d,p) basis set as implemented in

Gaussian 09 (4). The Fe^{2+}-rRNA and Mn^{2+}-rRNA clamps as well as their water hexahydrates were optimized at the unrestricted UB3LYP/6–31G(d,p) level of theory with spin = 2, multiplicity = 5 for iron and spin=2.5, multiplicity=6. Single point energies for these complexes were further obtained at the UB3LYP/6–311++G(d,p) level of theory using SCF options DIIS, NOVARACC, VTL, MaxCyc = 1000. Natural Bond Order (NBO) (5) and NBO charge calculations were performed on the optimized complexes at the (U)B3LYP/6–31G(d,p) level of theory using the GAMESS package (6) and NBO 5.0 routine.

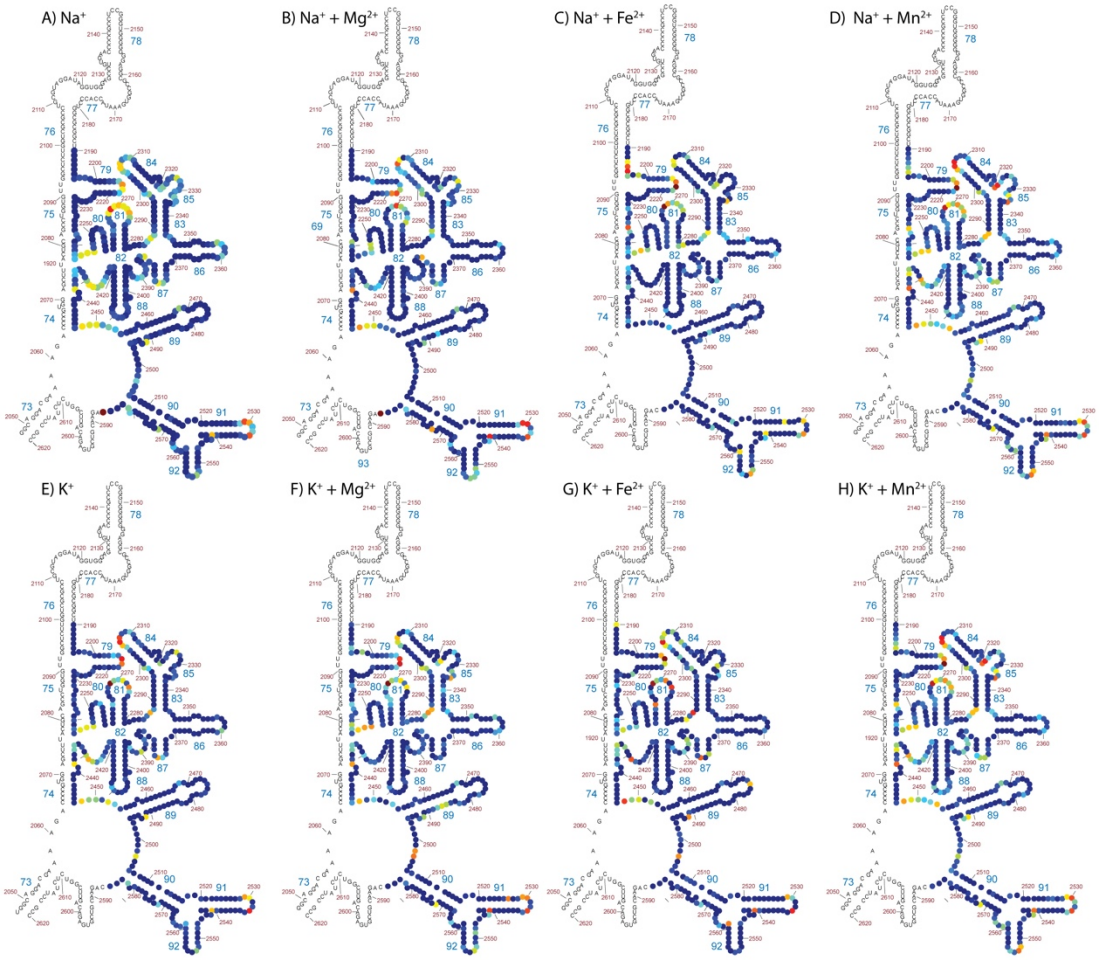

Fig. S1. SHAPE data mapped onto the PTC region of the *T. thermophilus* ribosome, obtained in the presence of A) Na^+ alone, B) Na^+ plus Mg^{2+}, C) Na^+ plus Fe^{2+}, D) Na^+ plus Mn^{2+}, E) K^+ alone, F) K^+ plus Mg^{2+}, G) K^+ plus Fe^{2+}, H) K^+ plus Mn^{2+}. K^+ or Na^+ is 250 mM. Mg^{2+} is 10 mM. Fe^{2+} or Mn^{2+} is 2.5 mM. Red indicates high reactivity and blue indicates low reactivity as shown in Figure 2.

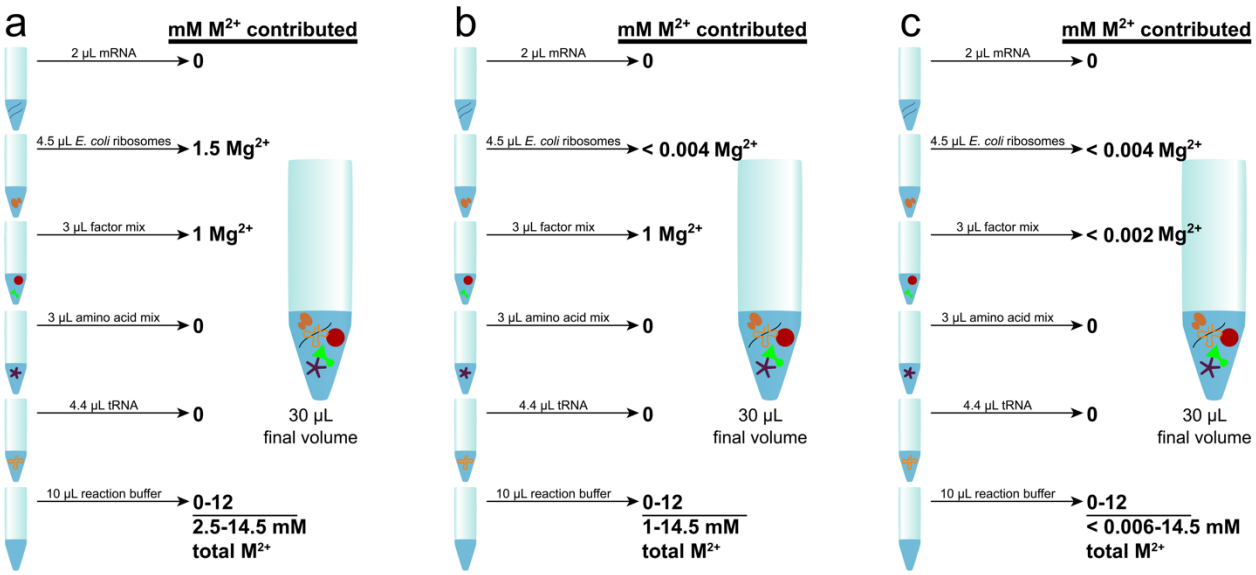

Fig. S2. Anoxic translation reaction experimental design. Reactants are shown in the order they were added to the reaction (top to bottom). Divalent cation (M^{2+}) concentrations were varied in the 3x HEPES-OH reaction buffer (pH 7.3). Anoxic nuclease-free water was added to a final reaction volume of 30 µL. Three different reactions were performed: **a)** Standard reaction set up. *E. coli* ribosomes and factor mix contributed 1.5 and 1 mM Mg^{2+}, respectively, for a total background of 2.5 mM Mg^{2+}. Data presented in **Figure 3, 4,** and **S2** are from reactions prepared this way; **b)** Reactions prepared using washed *E. coli* ribosomes, which reduced the background Mg^{2+} to 1 mM. Data presented in **Figure 5a** were from reactions prepared this way; **c)** Reactions prepared using washed ribosomes and washed factor mix, which reduced background Mg^{2+} to 4-6 µM range. Data presented in **Figure 5b** are from reactions prepared this way.

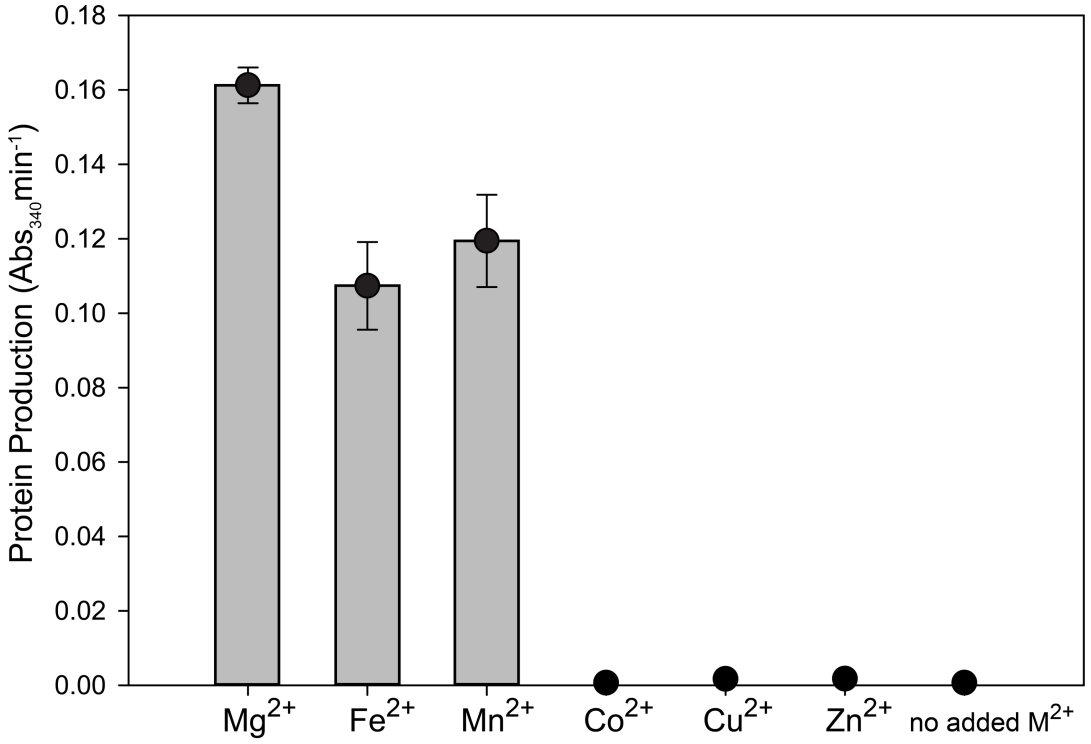

Fig. S3. *In vitro* translation of active protein in the presence of different divalent cations (M^{2+}). The activity of the translation product (dihydrofolate reductase, which catalyzes the oxidation of NADPH, with a maximum absorbance at 340 nm) was used as a proxy for protein production. Reactions received 7 mM M^{2+}, in addition to 2.5 mM background Mg^{2+}, summing to 9.5 mM total divalent cations. Translation reactions were run for 120 minutes. The error bars for triplicate experiments (n=3) are plotted as the standard error of the mean.

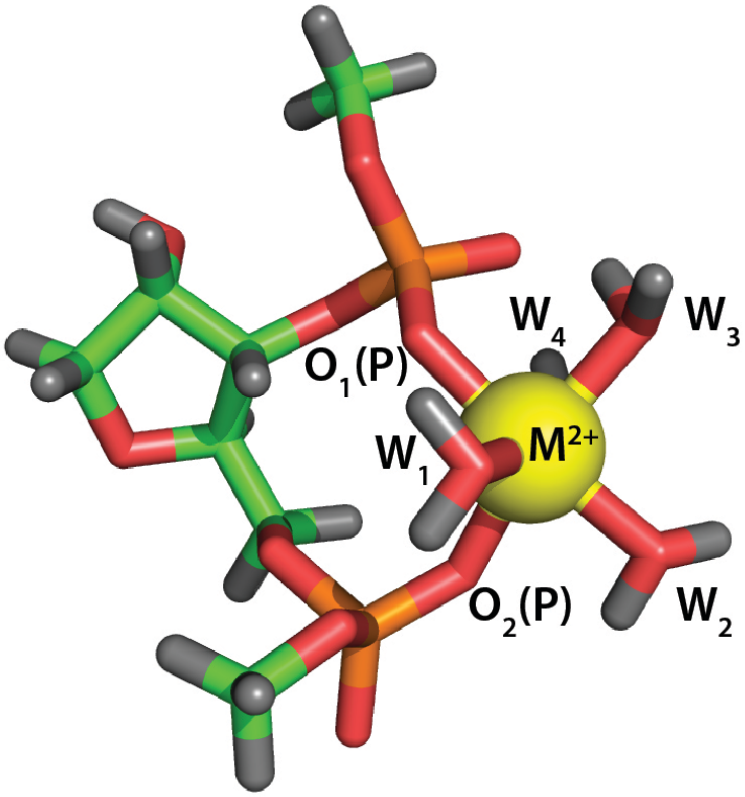

Fig. S4. The $rRNA^{2-}$–M^{2+}•$(H_2O)_4$ complex ("M^{2+}-rRNA clamp") used for quantum mechanical calculations. The base has been replaced by a hydrogen atom and the chain is terminated with methyl groups. **Abbreviations:** M^{2+}: metal ion (Mg^{2+}, Fe^{2+}, or Mn^{2+}); W: water (H_2O); O1 and O2: oxygen atoms from phosphate; P: phosphate (PO_4^{3-}).

Table S1. M-O distance (Å) in diphosphate-sugar-hydrated metal complexes ("M^{2+}-rRNA clamps", shown in **Fig. 1a** and **Fig. S3**) obtained from calculations performed at the B3LYP/6-311g**(d,p)//B3LYP/6-311g**(d,p) level of theory. **Abbreviations:** M: metal ion (Mg^{2+}, Fe^{2+}, or Mn^{2+}); w: water (H_2O); O: oxygen; P: phosphate (PO_4^{3-}).

Complex	**M-O Distance, [Å]**					
	M-O1 (P)	M-O2 (P)	M-O3 (w1)	M-O4 (w2)	M-O3 (w3)	M-O4 (w4)
$rRNAcl^{2-}$-$Mg(H_2O)_4^{2+}$	2.0040	2.0046	2.1392	2.1613	2.1404	2.1819
$rRNAcl^{2-}$-$Mn(H_2O)_4^{2+}$	2.1369	2.0832	2.2524	2.3004	2.2644	2.3100
$rRNAcl^{2-}$-$Fe(H_2O)_4^{2+}$	2.0267	2.0771	2.0464	2.0274	2.2650	2.2458

Table S2. Natural Bond Order (NBO) charges calculated for diphosphate-sugar-hydrated metal complexes ("M^{2+}-rRNA clamps", shown in **Fig. 1a** and **Fig. S3**) at the HF/6-31d(d,p) level of theory.

Complex	**NBO Charges**		
	Backbone	Cation	Waters
$rRNAcl^{2-}$-$Mg(H_2O)_4^{2+}$	-1.82959	1.77630	0.05329
$rRNAcl^{2-}$-$Mn(H_2O)_4^{2+}$	-1.81532	1.76526	0.05006
$rRNAcl^{2-}$-$Fe(H_2O)_4^{2+}$	-1.77911	1.69864	0.08047

Table S3. Interaction energies for diphosphate-sugar-hydrated metal complexes ("M^{2+}-rRNA clamps", shown in **Fig. 1a** and **Fig. S3**) calculated in the gas phase and water calculated at the B3LYP/6-311g**(d,p)//B3LYP/6-311g**(d,p) and B3LYP/6-311g**(d,p)//B3LYP/6-311g**(d,p)[cpcm].

Complex	**Interaction energy^{gas}, kcal mol^{-1}**	**Interaction energy^{cpcm}, kcal mol^{-1}**
rRNAcl^{2-}-Mg$(H_2O)_4^{2+}$	-387.5	-31.9
rRNAcl^{2-}-Mn$(H_2O)_4^{2+}$	-385.1	-35.7
rRNAcl^{2-}-Fe$(H_2O)_4^{2+}$	-391.3	-43.7

Dataset S1 (separate file)

SHAPE reactivity and Fe^{2+}/Mg^{2+}-dependent SHAPE changes for the LSU rRNA of *Thermus thermophilus.*

Dataset S2 (separate file)

SHAPE reactivity and Mg^{2+}/Fe^{2+}/Mn^{2+}-dependent SHAPE changes for the peptidyl transferase center of the LSU rRNA of *Thermus thermophilus.*

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