The Ribosome: Pumping Iron

Fe^{2+} substitutes for Mg^{2+} in ancient and modern ribosomal RNA

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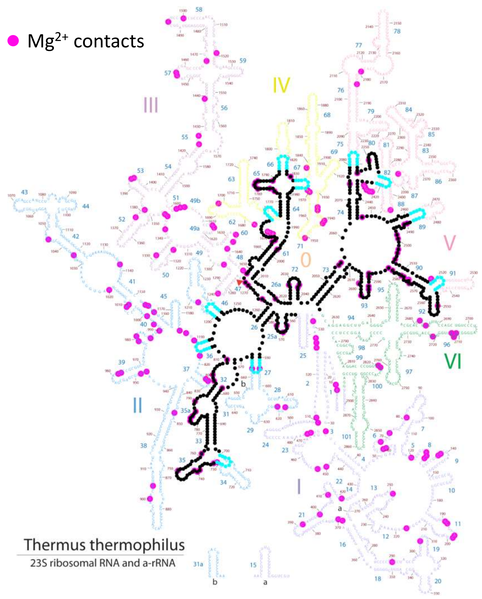
Background

Iron was abundant and soluble at the time of life's origin during the Archaean Eon. The anoxic atmosphere of the ancient earth sustained soluble Fe^{2+}. The central tenets of modern biology, including RNA structure and protein synthesis, emerged in the context of highly available, benign iron. Fe^{2+} can substitute structurally for Mg^{2+} in small RNA systems[1]. The ribosome, a biomolecular complex responsible for peptide synthesis in all living systems, is a "molecular fossil" that originated during this period of abundant soluble Fe^{2+}. The ribosomal large subunit (LSU) is the site of peptide bond formation, and an ancestor of its main RNA component (the 23S rRNA) is thought to have been the earliest functional unit of the ribosome. The modern 23S is held together by a scaffold of RNA-Mg^{2+} interactions. Here, we test whether Fe^{2+} can substitute for Mg^{2+} in rRNA systems under anoxic conditions, including a model ancestral LSU rRNA (a-rRNA)[2] and modern bacterial 23S rRNA.

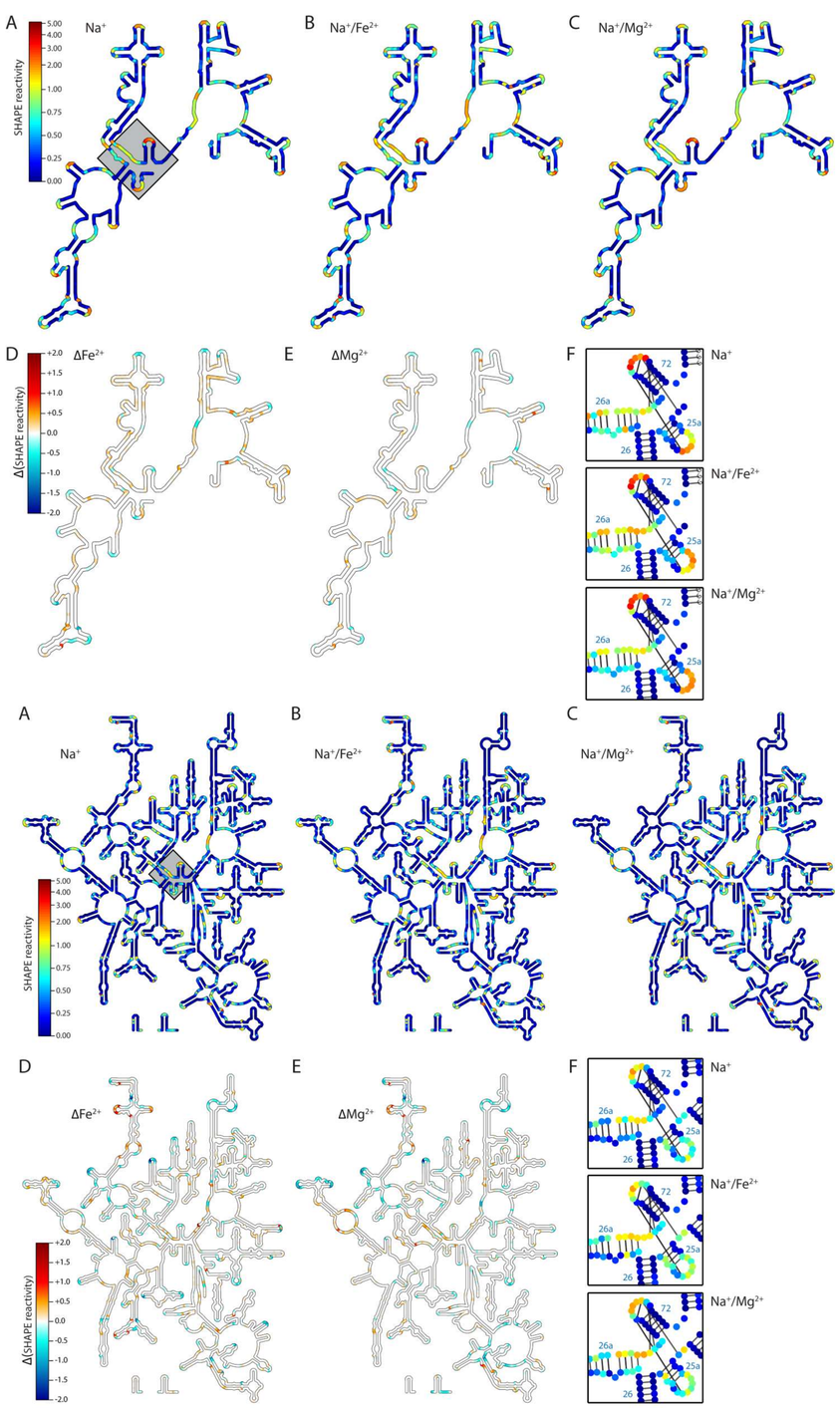


Above. Ancient 23S sequences (black) and stitching tetraloops (cyan) comprise a-rRNA, a single 615 nt RNA polymer. A direct sealing of 23S sequence is indicated by a red arrow. Secondary structure of the ~2,900 nt 23S RNA is underlaid for comparison. Magenta circles denote nucleotides observed to interact directly with Mg^{2+} atoms (first-shell interactions, 2.4 Å cut-off) in the *T. thermophilus* LSU crystal structure (PDB IDs: 2J00 and 2J01). Figure generated using RiboVision visualization suite: http://apollo.chemistry.gatech.edu/RiboVision/

Hypotheses

1. Secondary structure of rRNA systems will be retained with Na^+, Na^+/Fe^{2+}, or Na^+/Mg^{2+}
2. Tertiary structure of rRNA systems will be induced similarly by Fe^{2+} and Mg^{2+}
3. Overall structure of a-rRNA and 23S will be similar in analogous regions

Anoxic *in vitro* SHAPE footprinting of rRNA with Fe^{2+} and Mg^{2+}



SHAPE (Selective 2'-Hydroxyl Acylation analyzed by Primer Extension) is a single-nucleotide RNA footprinting method that provides quantitative information about nucleotide conformation, including base pairing. Nucleotides that are constrained by canonical cis Watson-Crick/Watson-Crick (cWW) base pairing exhibit very low reactivity toward SHAPE reagents, while nucleotides that participate in non-canonical or unusual RNA-RNA interactions exhibit variable reactivity, and flexible unpaired nucleotides are highly SHAPE reactive.

A-C. a-rRNA maintains expected secondary structure with Fe^{2+} and Mg^{2+}
SHAPE reactivities mapped onto expected a-rRNA secondary structure with 250 mM Na^+ and:
A. 1 mM trans-1,2-Diaminocyclohexane-N,N,N',N'-tetraacetic acid (DCTA, a chelator of divalent cations)
B. 2.5 mM Fe^{2+}
C. 10 mM Mg^{2+}
All data was collected under anoxic conditions.

D-E. Fe^{2+} substitutes for Mg^{2+} in a-rRNA folding, and induces larger/more numerous structural changes than Mg^{2+}
Changes in SHAPE reactivity for a-rRNA induced by:
D. 2.5 mM Fe^{2+}
E. 10 mM Mg^{2+}

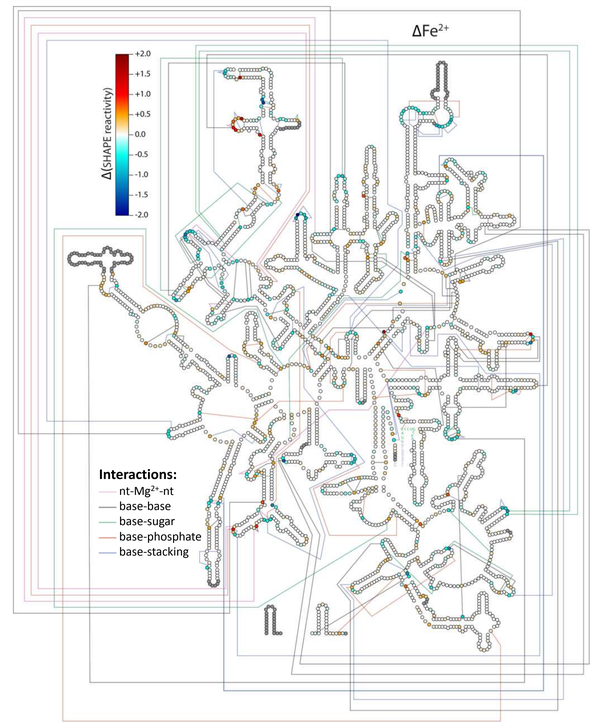
F. Non-canonical RNA helices exhibit anomalous SHAPE reactivity
Helices 25a and 26a are comprised of non-cWW base pairs, and exhibit unusual SHAPE patterns under all conditions tested.

A-C. 23S rRNA maintains expected secondary structure with Fe^{2+} and Mg^{2+}
SHAPE reactivities mapped onto expected 23S secondary structure with 250 mM Na^+ and:
A. 1 mM DCTA
B. 2.5 mM Fe^{2+}
C. 10 mM Mg^{2+}
All data was collected under anoxic conditions.

D-E. Fe^{2+} substitutes for Mg^{2+} in 23S folding, and induces larger/more numerous structural changes than Mg^{2+}
Changes in SHAPE reactivity for 23S induced by:
D. 2.5 mM Fe^{2+}
E. 10 mM Mg^{2+}

F. Non-canonical RNA helices exhibit anomalous SHAPE reactivity
Helices 25a and 26a are comprised of non-cWW base pairs, and exhibit unusual SHAPE patterns under all conditions tested.

Fe^{2+} induces native tertiary structure



Above. Fe^{2+}-induced SHAPE changes in the 23S rRNA are attributable to known LSU internucleotide tertiary interactions. Lines represent selected RNA-RNA tertiary contacts observed in the *T. thermophilus* LSU crystal structure (PDB 2J01). Only those interactions are displayed that occur at or near (within 1 nt) a site that exhibits a significant Fe^{2+}-induced change in SHAPE reactivity (ΔFe^{2+}>0.3). Nucleotides where crystal structure data is unavailable are shown as grey circles. Loops that do not participate in 23S interactions exhibit little to no Fe^{2+}-dependent changes in SHAPE reactivity.

Conclusions

All hypotheses are supported by SHAPE data:
1. Secondary structure is retained overall with Fe^{2+} and Mg^{2+}.
2. Fe^{2+} induces tertiary structural changes at similar positions to Mg^{2+} in rRNA systems. Fe^{2+} induces more numerous and greater changes than Mg^{2+}, even at 4-fold lower [Fe^{2+}], suggesting that Fe^{2+}-rRNA binding is generally 'tighter'.
3. Secondary structures and divalent-dependent changes of a-rRNA and 23S are similar in analogous regions.

References

1. Athavale, S.S. et al. (2012) RNA folding and catalysis mediated by iron(II). *PLoS One*, **7**, e38024.
2. Hisao, C., Lenz, T.K. et al. (2013) Molecular paleontology: a biochemical model of the ancestral ribosome. *Nucleic Acids Research*, **41**, 3373-3385.

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