6.4 Role of Cations in RNA Folding and Function

Jessica C. Bowman, Anton S. Petrov, and Loren Dean Williams

CONTENTS

6.4.1 Cations in Water: Relevance to Origin of Life and Evolution on Earth ........................................ 421
6.4.2 Hydrated Cations ................................................................................................................................. 422
6.4.2.1 Coordination by Water ................................................................................................................... 422
6.4.2.2 Dehydration by Phosphate Oxyanions ......................................................................................... 423
6.4.3 Cations as RNA Ligands .................................................................................................................... 425
6.4.3.1 Conceptual Frameworks for Binding ........................................................................................... 425
6.4.3.2 A Quantum Mechanical Perspective on First-Shell RNA-Cation Interactions........................ 426
6.4.4 Structural Roles in the Ribosome ...................................................................................................... 427
6.4.4.1 A Mg2+ Scaffold for the Ribosome Peptidyl Transferase Center ............................................. 427
6.4.4.2 Ribosomal RNA Folding in the Absence of Proteins ................................................................. 429
6.4.4.3 Ribosomal RNA Folding in the Presence of Proteins ................................................................. 430
6.4.5 Ribozyme Function ........................................................................................................................... 431
6.4.5.1 Ribozymes with Mg2+, Fe2+, and Other Cations ........................................................................ 431
6.4.6 Conclusions ...................................................................................................................................... 432
Acknowledgments .................................................................................................................................... 433
References ................................................................................................................................................. 433

6.4.1 CATIONS IN WATER: RELEVANCE TO ORIGIN OF LIFE AND EVOLUTION ON EARTH

Inorganic metal cations are abundant in cells and are essential to life on Earth. Metal cations are vital for a variety of biological processes. Fundamental biological processes such as electron transfer, photosynthesis, and respiration require these ions (Bertini et al. 2007). Cations stabilize transition states in the replication of DNA, transcription of DNA to RNA, and ligation and cleavage of the nucleic acids by both proteins and ribozymes (Lykke-Andersen and Christiansen 1998; Steitz 1999; Doherty and Dafforn 2000; Lee et al. 2000; Yin and Steitz 2004; Ellenberger and Tomkinson 2008). Protein sequences encoded in messenger RNA are translated by amino-acid-processing steps, due to its similarity in position and structure through all three major evolutionary branches of life, suggesting that they preceded, or at least coincided with, the last universal common ancestor (LUCA).

The availability of biologically useful inorganic ions is dependent on the existence of certain minerals and on their solubility in water. The composition of the atmosphere affects cation solubility, especially partial pressures of O2(g) and CO2(g). An influx of O2(g) to an aqueous system diminishes the solubility of redox-sensitive ions such as Cu2+, Fe2+, and Mn2+. Similarly, CO2(g) decreases pH, mobilizing ions such as Ca2+ and Na+ from soils and precipitants. Fe2+ has been proposed and demonstrated to be a functional early Earth analogue for Mg2+ in nucleic-acid-processing steps, due to its similarity in size and charge density (Athavale et al. 2012; Okafor et al. 2017). Yet, in the pH range that sustains most life, Fe2+ is precipitated as Fe(OH)2(s) (Morgan and Lahav 2007) or Fe(III)ox species (Derry 2015) in a complex mechanism and at a rate that is dependent on the concentration of dissolved O2(g). Together with well-documented substitutions of Cu2+, Zn2+, and Mn2+ for Fe2+ in catalytic proteins (Torrents et al. 2002;
Wolfe-Simon et al. 2006; Anjem et al. 2009; Cotruvo and Stubbe 2011; Martin and Imlay 2011; Ushizaka et al. 2011; Aguirre and Culotta 2012; Harel et al. 2014), these observations suggest that changes in Earth’s atmosphere over evolution may have altered the ions of key ion-dependent biological processes.

Water’s interactions with ions are as important to biology as the ions themselves. Cations observed at the atomic level in three-dimensional molecular structures are almost always at least partially hydrated. Water molecules are coordinated, oriented, polarized, and acidified by ions in a manner that depends on the properties of the ion—mainly size and charge density (Hribar et al. 2002; Collins et al. 2007). Hydrated cations mitigate the negative charge on nucleic acids by long-range electrostatic and shorter-range hydrogen-bonding interactions with backbone phosphates. Partial dehydration allows direct coordination of the cation by phosphates (up to four for Mg$^{2+}$) of the nucleic acid backbone, while remaining cation-coordinated water molecules are available to participate in hydrogen bonding. Cation hydration mitigates the thermodynamic favorability of direct coordination by phosphodiester by requiring unfavorable dehydration. Together, these interactions provide access to a range of complex and finely tunable conformational states of the RNA. The functional roles of cations in extant biology are interdependent with the unique properties of water.

### 6.4.2 HYDRATED CATIONS

#### 6.4.2.1 COORDINATION BY WATER

Water molecules are oriented and polarized by cations, forming acidic metal hydrates (Me(H$_2$O)$_n$)$^{m+}$ with elevated potential for participation in hydrogen bonding and function in enzymatic activity and proton donation. The extent of attraction between a cation and water molecules is given by the cation’s hydration enthalpy. A large, negative hydration enthalpy indicates a stronger attraction than a small, negative one. The hydration enthalpies of cations vary in magnitude by cation identity, roughly based on size and charge. Hydration enthalpies of Zn$^{2+}$ and Mg$^{2+}$, for example, are large in magnitude (−450 kcal/mol) compared with those of Na$^{+}$ and K$^{+}$ (−100 kcal/mol) (Rashin and Honig 1985).

Biologically relevant metal hydrates include those of Ca$^{2+}$, Fe$^{3+}$, K$^+$, Mg$^{2+}$, Mn$^{2+}$, Na$^+$, and Zn$^{2+}$. These cations are coordinated by multiple water molecules in their hydrate form. The inherent polarity of each water molecule is increased by coordination with a cation, increasing the acidity of the water hydrogens. Experimentally derived pK$_a$s (Table 6.4.1) indicate a relative hydrate acidity of Zn$^{2+}$ > Fe$^{3+}$ > Mn$^{2+}$ > Mg$^{2+}$ > Ca$^{2+}$ > Na$^+$ > K$^+$ (Baes and Mesmer 1976; Stumm and Morgan 1996; Mähler and Persson 2012; Jackson et al. 2015), where the most acidic hydrate [Zn(H$_2$O)$_6$]$^{2+}$ has, by definition, the largest relative abundance of potentially reactive oxygen ions in water near physiological pH (~7.4). All of these hydrates are more acidic than bulk water (pK$_a$ 15.7) (Baes and Mesmer 1976). The standard free energy for ionization of an uncoordinated water molecule (H$_2$O $\rightarrow$ OH$^-$ + H$^+$) is large and positive (382.8 kcal/mol); coordination in a hexahydrate of Mg$^{2+}$, Mn$^{2+}$, or Zn$^{2+}$ reduces the energy of ionization by more than half (Bock et al. 1999).

Cations constrain water molecules to specific geometries. Though the number of water ligands for a hydrated cation is not fixed, especially when considering the dynamics of water molecules in solution, crystallographic structures reveal dominant coordination numbers (Table 6.4.1). Coordination by water ligands compresses metal ions: hydration decreases the effective ionic radius by more than half (Bock et al. 1999). The effective ionic radius of a hexahydrated cation is also related to spin state and ion identity. Comparison of ionic radii, assuming hexahydrate coordination and low spin, yields relative atomic sizes Fe$^{3+}$ < Mn$^{2+}$ < Mg$^{2+}$ ≈ Zn$^{2+}$ < Ca$^{2+}$ ≈ Na$^+$ < K$^+$ (Shannon 1976). The radius of a hexahydrated Fe$^{3+}$ ion is less than half the radius of a hexahydrated K$^+$ ion. With the exception of Zn$^{2+}$, the radii of cation hydrates mentioned here correlate inversely with hydrate acidity. The availability of d-orbital electrons in the transition metals (Fe$^{3+}$, Mn$^{2+}$, and Zn$^{2+}$) is known to affect hydrate pKa (Roychowdhury-Saha and Burke 2006).

#### Table 6.4.1

<table>
<thead>
<tr>
<th>Cation</th>
<th>Effective Ionic Radii (Å) in a Hexahydrate (Shannon 1976)</th>
<th>Common Hydrate Coordination (Baes and Mesmer 1976; Jackson et al. 2015)</th>
<th>Experimentally Derived pK$_a$s (Baes and Mesmer 1976; Jackson et al. 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{3+}$</td>
<td>0.61, 0.78</td>
<td>[Fe(H$_2$O)$_6$]$^{3+}$</td>
<td>9.3–9.5</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>0.67, 0.83</td>
<td>[Mn(H$_2$O)$_6$]$^{2+}$</td>
<td>10.6–11.0</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>0.72</td>
<td>[Mg(H$_2$O)$_6$]$^{2+}$</td>
<td>11.2–11.4</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>0.74</td>
<td>[Zn(H$_2$O)$_6$]$^{2+}$</td>
<td>9.0</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>1.00</td>
<td>[Ca(H$_2$O)$_6$]$^{2+}$</td>
<td>12.7–12.9</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>1.02</td>
<td>[Na(H$<em>2$O)$</em>{10}$]$^+$</td>
<td>14.2</td>
</tr>
<tr>
<td>K$^+$</td>
<td>1.38</td>
<td>[K(H$<em>2$O)$</em>{10}$]$^+$</td>
<td>14.5</td>
</tr>
</tbody>
</table>

* Low spin (estimate);

* High spin.
Water molecule dynamics are suppressed by cations. The lifetime of a water molecule in the first coordination shell of a cation is generally longest for ions with the greatest charge density (e.g., highly charged with small ionic radius) (Diebler et al. 1969). It may be expected, based on ionic radii alone, that the relative exchange rate for the cations of Table 6.4.1 is Fe$^{2+} < $Mn$^{2+} < $Mg$^{2+} \leq $Zn$^{2+} < $Ca$^{2+} \equiv $Na$^+ \equiv $K$^+$. Exchange rates have been estimated experimentally as Mg$^{2+} <$ Fe$^{2+} \leq $Mn$^{2+} <$ Zn$^{2+} <$ Ca$^{2+} \equiv $Na$^+ \equiv $K$^+ $ (Diebler et al. 1969), where rates within one order of magnitude are considered within the range of uncertainty. The rate of exchange of water from the first coordination shell of K$^+$ (on the order of ~10$^9$ waters per second) occurs four orders of magnitude faster than from the first shell of Mg$^{2+}$ (Diebler et al. 1969).

Ions affect water enthalpically and entropically. In pure water, intramolecular interactions are dominated by hydrogen bonds. Hydrogens and electron lone pairs on each water participate in hydrogen-bonding interactions with surrounding water molecules. The high electronegativity of the oxygen makes each water molecule a dipole, with partial negative and positive charges. The partially negative oxygen of a water dipole interacts electrostatically with adjacent cations. Conversely, the partially positive charge of the dipole (bearing two hydrogens) interacts electrostatically with anions. Ions organize first-shell water molecules, affecting surrounding water molecules. The geometric perturbation of waters around an ion in aqueous solution reflects the outcome of competition between the ion and surrounding water molecules (or between electrostatic and hydrogen-bonding interactions) for the ion-constrained water. Theoretical studies suggest that the outcome of this competition strongly favors electrostatic interactions when a cation is small and densely charged, which disrupts canonical water-water hydrogen bonds (Hribar et al. 2002). Large, less-charge-dense cations have a greater tendency to share their most proximal water molecules with other water molecules and even promote canonical hydrogen bonding among surrounding waters (Hribar et al. 2002). The combined result is a difference in the distance of propagation of ion-induced organization of water. One report suggests that small, charge-dense cations reorganize up to two layers of water molecules around the ion (<5 Å), whereas large, less-charge-dense cations perturb only one layer (Collins et al. 2007). Differences in the organization of water around ions may explain, if only in part, the lack of interchangeability of ions in some biochemical processes.

### 6.4.2.2 Dehydration by Phosphate Oxyanions

Biology employs partially hydrated cations that constrain ligands other than water in the first coordination shell. Ligand binding to hexahydrated Mg$^{2+}$, for example, displaces a water molecule in the reaction (Mayaan et al. 2004):

$$\text{L}^{3-} + [\text{Mg(OH)}_2]^2+ \rightarrow [\text{Mg(H}_2\text{O})_5(L)]^{2-q} + \text{H}_2\text{O} \quad (6.4.1)$$

Single substitution of a water molecule for an alternate first-shell ligand type alters the structure of the Mg$^{2+}$ hydrate (Mayaan et al. 2004). If the ligand (L) is the oxyanion OH$, for example, the interatomic distance between Mg$^{2+}$ and the oxygen atom of L is slightly less than the distance between Mg$^{2+}$ and each of its remaining five waters oxygens in $[\text{Mg(H}_2\text{O})_5(\text{OH})]^4$. In addition, the interatomic distance between Mg$^{2+}$ and the remaining five water oxygens increases slightly relative to $[\text{Mg(H}_2\text{O})_5]^4$. That is, the oxyanion ligand OH$^-$ is held more tightly by Mg$^{2+}$ than the neutral waters in $[\text{Mg(H}_2\text{O})_5(\text{OH})]^4$, and the neutral waters are less constrained (and possibly less acidic) than those in hexahydrated Mg$^{2+}$ (Figure 6.4.1).

![Figure 6.4.1](image-url) Small oxyanion ligands of cations are held more tightly than neutral water. A conceptual model of the difference in interatomic distances between a charge-dense cation and a water ligand or oxyanion ligand. (a) A hexahydrated cation with octahedral coordination geometry and six roughly equal cation-water oxygen interatomic distances. (b) The interatomic distance between the cation and an oxyanion ligand, in this case OH$, is less than remaining cation-water distances. The magenta sphere is a generic charge-dense cation. Neutral water ligand and oxyanion ligand are shown in red font. Remaining cation-water distances are increased slightly in panel B, relative to panel A. The change in bond length is exaggerated for clarity.
Phosphates and phosphate esters are common first-shell ligands of cations in biology. Like water, they are oriented, polarized, and constrained by cations. Most biological phosphate is covalently bound in adenosine mono-, di-, or tri-phosphate (AMP, ADP, or ATP); deoxyribonucleic acid (DNA); or ribonucleic acid (RNA) (Figure 6.4.2), and these molecules are well-documented partners of cations, especially Mg$^{2+}$. The transition states in condensation of the nucleic acid phosphodiester backbone by polymerases, ligases, and ribozymes, for example, are directly stabilized by divalent cations (Lykke-Andersen and Christiansen 1998; Steitz 1999; Doherty and Dafforn 2000; Lee et al. 2000; Yin and Steitz 2004; Ellenberger and Tomkinson 2008; Butcher 2011; Johnson-Buck et al. 2011).

Computation suggests that Mg$^{2+}$ binds phosphate or phosphate esters more tightly than water molecules. In work by

![Common biological ligands of cations](image)

**FIGURE 6.4.2** Common biological ligands of cations. (a) Two monomers of RNA connected by the phosphodiester backbone (yellow highlight), with conventional ribose atom designations in parentheses. (b) Adenosine triphosphate (ATP) with conventional phosphate designations. (c) Dimethyl phosphate. Dimethyl phosphate, as discussed, is a molecular model representing the phosphodiester connecting unit of the nucleic acid backbone. Hydrogens on the ribose sugars of panels (a) and (b) have been omitted for clarity.
Mayaan et al. (2004), substitution of a single water molecule in Mg$^{2+}$ hexahydrate for a biologically derived ligand resulted in ligand-dependent changes in Mg$^{2+}$-ligand interatomic distances and remaining cation-water interatomic distances, consistent with Figure 6.4.1. Mg$^{2+}$ coordinated all evaluated small oxygen- and phosphate-bearing ligands with shorter cation-ligand oxygen interatomic distances than cation-water oxygen interatomic distances in single-substitution reactions (Mayaan et al. 2004). These ligands included free phosphate species hydrogen phosphate (HPO$_4^{2-}$) and dihydrogen phosphate (H$_2$PO$_4^-$), which dominate at physiological pH of 7.4, and dimethyl phosphate. Since the electronic structure of dihydrogen phosphate is somewhat different than phosphodiester, the dimethyl-phosphate anion (CH$_3$—O—PO$_2$—O—CH$_3$) (Figure 6.4.2) is often used as a model system representing the phosphodiester connection between the base-bearing sugars of nucleic acids. In dimethyl phosphate, one methyl group is an analogue for the 3’ ribose carbon and the other an analogue for the 5’ carbon of the adjacent ribose sugar. Reported relative coordination distances for Mg$^{2+}$ are OH- < dimethyl phosphate < free phosphate species < water (Mayaan et al. 2004). In other words, Mg$^{2+}$ binds to dimethyl phosphate more tightly than to free phosphate or water. The geometry of the nucleic acid backbone is cation-dependent. Work by Panteva et al. aiming, in part, to computationally replicate experimentally derived thermodynamic parameters for the interactions of Mg$^{2+}$, Mn$^{2+}$, or Zn$^{2+}$ with RNA, derived parameters for interactions of these cations with the nucleic acid analogue dimethyl phosphate. Their results suggest that contact and minimum energy distances between cation and phosphate follow a trend in which dimethyl phosphate oxanion-Mg$^{2+}$ and -Zn$^{2+}$ distances are approximately equal and less than that of Mn$^{2+}$ (Panteva et al. 2015), suggesting that dimethyl phosphate binds more tightly to Mg$^{2+}$ and Zn$^{2+}$ than to Mn$^{2+}$. Fujimoto et al. (1994) reported cation-dependent variations in the nucleic acid hydrodynamic radius, in which Na$^+$ slightly decreased while divalent cations Mg$^{2+}$ and Mn$^{2+}$ substantially increased the hydrodynamic radius of DNA. These observations suggest cation-specific structural changes in nucleic acids, perhaps phosphodiester bond stabilization and/or changes in hydration. Mg$^{2+}$ polarizes first-shell phosphate ligands, shortening phosphodiester bonds and leaving phosphorus more susceptible to nucleophilic attack. Upon substitution of a Mg$^{2+}$-constrained first-shell water with dimethyl phosphate in computational analyses, Mayaan et al. (2004) observed corresponding changes in the phosphate ligand. While the interatomic bond distance between phosphorus and the phosphate oxygen interacting with Mg$^{2+}$ increased, bond distances between the phosphorus atom and phosphate oxygens covalently bound to the methyl groups decreased, suggesting that Mg$^{2+}$ polarizes phosphate, drawing negative charge away from phosphate oxygens not directly coordinated by the cation. The magnitude of decrease in the length of the phosphodiester bonds of dimethyl phosphate has been calculated to be cation-specific, where the change induced by Ni$^{2+}$ > Mg$^{2+}$ > Na$^+$ (Zhang et al. 2015). Application of this observation to nucleic acids suggests that first-shell coordination of a backbone phosphate with cations may increase the reactivity of phosphorus while rigidifying the backbone by shortening the phosphodiester bonds in a cation-dependent manner. The expected change in bond lengths based on these calculations are within the range of precision of most nucleic acid structures but are believed to be corroborated by a few very-high-resolution structures.

6.4.3 CATIONS AS RNA LIGANDS

6.4.3.1 CONCEPTUAL FRAMEWORKS FOR BINDING

Cations stabilize RNA through a continuum of associations, not just first-shell interactions. Williams and co-workers classified cation interactions with RNA as chelated, condensed, glassy, or diffuse, based on six criteria: relative population, number of first-shell RNA ligands, extent of mobility, dimensionality of mobility, contribution to RNA thermodynamic stability, and contribution to non-canonical RNA structure (Bowman et al. 2012).

The most populous ions in solutions of RNA are diffuse ions, which are fully hydrated without first-shell or first-shell water interactions with RNA and are no more restricted in mobility than bulk water. Diffuse cations are much more abundant than partially hydrated cations that directly coordinate RNA. The phosphodiester backbone of RNA is negatively charged and therefore self-repellent; diffuse cations electrostatically neutralize the RNA backbone. They interact with RNA by numerous weak, long-range electrostatic interactions (Manning 1969), contributing to the overall thermodynamic stability of nucleic acids and their complexes. These ions are undetectable in crystallographic structures in part due to their positional disorder. Diffuse ions do not stabilize specific non-canonical RNA structures.

Condensed ions (Manning 1969) have slightly attenuated mobility due to electrostatic or hydrogen-bonding interactions of one or more ion-coordinated waters with RNA or an RNA ligand. Their relative population and rate and dimensionality of diffusion are less than that of diffuse ions but are greater than glassy ions. Condensed ions neutralize charge that is more localized to the RNA than diffuse ions. Their population is sensitive to a local base pairing of nucleic acids and affects the geometry and conformational dynamics of the nucleic acid grooves (Lavery and Pullman 1981). Condensed ions can be visualized as a shell or coating on the surface of a three-dimensional RNA, extending beyond the van der Waals radius of the RNA.

Glassy cations have at least one first-shell RNA ligand and restricted mobility. They are typically found in the interior of the RNA, encased within the external three-dimensional surface of the native structure, such that the dimensionality of diffusion is reduced. The mobility of glassy cations is less than that of condensed cations. Glassy cations may contribute to stabilization of specific RNA structural states that are inaccessible in their absence. Divalent ions, especially Mg$^{2+}$, play a special role in the stability of RNA, due to their ability...
to mediate strong tertiary interactions. Charge-dense Mg\(^{2+}\) is glassy, with one first-shell RNA ligand, while less-charge-dense cations such as Na\(^{+}\) and K\(^{+}\) require four to five first-shell RNA ligands to similarly restrict diffusion.

Chelated cations, bearing more than one first-shell RNA ligand in the case of Mg\(^{2+}\), are the least frequent ions in RNA structures but often have the most significance for the structure-function relationship. Chelated cations are typically at the interior of the three-dimensional surface of the native RNA. Their rate of diffusion is dependent on the number of first-shell RNA ligands. These cations are the least mobile of the four classes and, being rigidly bound by the RNA, are difficult to remove in vitro without disturbing the RNA structure.

The mode of Mg\(^{2+}\)-RNA interaction is influenced by local RNA structure. Experimental work suggests that sites of Mg\(^{2+}\) chelation in RNAs are not random. Linear RNAs are less likely than folded RNAs to form first-shell interactions with Mg\(^{2+}\), suggesting secondary structure and even sequence dependence (Porschke 1979). However, Mg\(^{2+}\) forms first-shell interactions less readily with double-stranded RNA than with compact single-stranded RNA (Kanka 2003, 2004). In many cases, site-specific Mg\(^{2+}\) binding and RNA conformation are interdependent: the conformational space in which an RNA achieves its native structure, including chelation of Mg\(^{2+}\), is not available without Mg\(^{2+}\), even with high concentration of monovalent cations (Draper et al. 2005; Grilley et al. 2006).

### 6.4.3.2 A QUANTUM MECHANICAL PERSPECTIVE ON FIRST-SHELL RNA-CATION INTERACTIONS

First-shell RNA-Mg\(^{2+}\) complexes occur so frequently in ribosome and ribozyme x-ray structures that they appear to be fundamental units of RNA folding (Petrov et al. 2011). These complexes often appear in a singular and defined geometry, referred to here as the bidentate chelation complex (or bidentate clamp, Figure 6.4.3). Bidentate clamps are characterized by a 10-membered ring system (Mg\(^{2+}\)–O'–P–O5'–C5'–C4'–C3'–O3'–P–O'), which includes a single Mg\(^{2+}\) chelated by phosphates of adjacent nucleotides. (From Petrov et al. 2011; Hsiao, C. et al., Complexes of nucleic acids with group I and II cations, in Nucleic Acid Metal Ion Interactions, edited by N. Hud, pp. 1–35, The Royal Society of Chemistry, London, UK, 2008; Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3142, 2009) Bock et al. 1999) and to the resulting non-canonical RNA backbone confirmation (Klein et al. 2004; Hsiao et al. 2008; Hsiao and Williams 2009). Using DFT, Petrov et al. explored the reaction:

\[
\text{RNA}^{2+} + \left[\text{Mg} (\text{H}_2\text{O})_6\right]^{2+} \rightarrow \left[\text{RNA-Mg} (\text{H}_2\text{O})_4\right]_{\text{complex}} + 2\text{H}_2\text{O}
\] (6.4.2)

In this reaction, two waters of hexahydrated magnesium are displaced as the cation is chelated by phosphate oxyanions on consecutive RNA nucleotides. Petrov et al. modeled the reaction by computationally optimizing the RNA structure in the absence and presence of [Mg(H\(_2\)O)\(_6\)]\(^{2+}\), followed by optimization of the bidentate RNA-Mg(H\(_2\)O)\(_4\) complex. Optimization was repeated with [Na(H\(_2\)O)\(_6\)]\(^{+}\) or [Ca(H\(_2\)O)\(_6\)]\(^{2+}\) in place of the Mg\(^{2+}\) hexahydrate, with the objective of understanding the uniqueness of the role of Mg\(^{2+}\) in complex stability.

RNA forms a more stable bidentate chelation complex with Mg\(^{2+}\) than with Na\(^{+}\) or Ca\(^{2+}\) (Petrov et al. 2011). The RNA-Mg(H\(_2\)O)\(_4\) complex is ~10 kcal/mol more stable than the RNA-Ca(H\(_2\)O)\(_4\) complex. The [RNA-Na(H\(_2\)O)\(_6\)]\(^{−}\) complex approaches neutral interaction energy and is unstable. The ionic radius of hexahydrated Mg\(^{2+}\) < Ca\(^{2+}\) < Na\(^{+}\), therefore Mg\(^{2+}\) forces first-shell oxyanion RNA ligands closer together than Ca\(^{2+}\) or Na\(^{+}\). The close proximity of the RNA oxyanions coordinated by Mg\(^{2+}\) incurs an electrostatic penalty of ~10 kcal/mol from the opposing negative charges on the two oxygens; this is mitigated by other favorable energy components.

The octahedral coordination geometry of hexahydrate metals is distorted by RNA phosphate oxyanion ligands.
Distances from metal to oxygen are smaller for RNA phosphates than for water in Mg\(^{2+}\) and Ca\(^{2+}\) RNA chelation complexes. The metal-to-RNA phosphate oxyanion interatomic distances in the Na\(^+\) RNA chelation complex are polymorphic. Optimization with Na\(^+\) yields a monodentate structure with only one RNA phosphate oxyanion ligand in the first coordination shell of Na\(^+\).

Energies of binding are substantial for small, charge-dense Mg\(^{2+}\) complexes but are less so for Ca\(^{2+}\) due to its larger size and for Na\(^+\) due to its smaller charge. Decomposition of the interaction energy of bidentate clamps reveals that, for all cations modeled, electrostatic components make the greatest contributions to complex stability, followed by polarization (of the RNA phosphates) and charge transfer. There is a ~20 kcal/mol difference in the electrostatic contribution to bidentate clamp stability by Mg\(^{2+}\) compared with Ca\(^{2+}\); and a ~220 kcal/mol difference by Na\(^+\) compared with Mg\(^{2+}\). The polarization component is less than half of the electrostatic contribution for all cations. Charge transfer components include a net reduction of positive charge on each cation (Mg\(^{2+}\): 0.2 e\(^{-}\) > Ca\(^{2+}\): 0.1 e\(^{-}\) > Na\(^+\): 0.1 e\(^{-}\)) (Petrov et al. 2011).

Magnesium’s high charge density brings phosphate oxyanions of adjacent RNA nucleotides into closer proximity than Ca\(^{2+}\) or Na\(^+\), inducing non-canonical RNA backbone conformations found especially in catalytic RNAs. Complex formation may be driven, in part, by magnesium’s preference for RNA’s negatively charged and polarizable phosphate oxyanions over water. Though electrostatic energy components dominate stability, polarization and charge transfer components are not negligible. The small size of Mg\(^{2+}\) creates a symmetrical distortion of the metal’s octahedral coordination geometry, in which phosphate oxyanions are bound more tightly than water oxygens.

### 6.4.4 STRUCTURAL ROLES IN THE RIBOSOME

#### 6.4.4.1 A Mg\(^{2+}\) Scaffold for the Ribosome Peptidyl Transferase Center

All proteins of living organisms are synthesized by the ribosome, a highly conserved and ancient RNA-protein enzyme. The ribosome is the site of execution of the genetic code. With the help of aminoacyl tRNA synthetases and transfer RNAs, messenger RNA is decoded at the small subunit (SSU), while peptide bonds are made in the ribosomal LSU at the catalytic center, the peptidyl transferase center (PTC). The bacterial LSU contains two ribosomal RNA (rRNA) polymers, the 23S rRNA and the 5S rRNA, as well as numerous ribosomal proteins. The LSU rRNA of *Thermus thermophilus* is made up of more than 2,900 nucleotides, subdivided into seven domains (Domains I, II, III, IV, V, and VI; Figure 6.4.4), based on the native structure common to all ribosomes (Petrov et al. 2014). Bokov, Steitz, and Williams, and others observed that the occurrence of Mg\(^{2+}\) in the LSU is greatest in Domain V near the PTC (Hansen et al. 2001; Klein et al. 2004; Bokov and Steinberg 2009; Hsiao et al. 2009).

Hsiao and Williams (2009) reported a framework of highly conserved Mg\(^{2+}\) microclusters in the LSU. These microclusters include the 10-membered Mg\(^{2+}\)-RNA ring structure (Figure 6.4.3), modeled using DFT by Petrov et al. (2011), but in microclusters, the Mg\(^{2+}\) ion is one of a pair of Mg\(^{2+}\) ions chelated by a common bridging phosphate. The bridging phosphate, which has the form Mg\(^{2+}\)−O−P−O−Mg\(^{2+}\)′, is part of the 10-membered chelation ring (Mg\(^{2+}\)−O−P−O−C\(^{5}\)′−C\(^{4}\)−C\(^{6}\)−O\(^{3}\)−P−O−) (Hsiao and Williams 2009). As in Petrov et al. (2011), the phosphates of the chelation ring are contributed by adjacent nucleotides, but in a microcluster, at least one additional phosphate oxyanion interacts with at least one of the Mg\(^{2+}\) ions.

Mg\(^{2+}\)-RNA microclusters support long-range intra- and inter-domain interactions relevant to the global structure of the ribosome. Hsiao and Williams (2009) found three ancient Mg\(^{2+}\)-RNA microclusters supporting the PTC and a fourth located near the LSU exit tunnel (Figure 6.4.4). These microclusters are conserved among archaea (represented by *Haloarcula marismortui*), bacteria (represented by *T. thermophilus*), eukarya, and mitochondria. Two of the ancient microclusters make intra-domain nucleotide connections, while the other two connect very remote rRNA nucleotides of multiple domains of the LSU. References to specific nucleotides are given as *T. thermophilus* residues in the *Escherichia coli* numbering convention.

Microcluster D2 connects Domain V of the LSU, near the PTC, to Domain II of the LSU (Figure 6.4.5). Here, the 10-membered ring is made up of the phosphate groups and ribose atoms of A783 and A784 in Domain II. One Mg\(^{2+}\) of this microcluster interconnects these nucleotides to the phosphate of A2589 in Domain V near the PTC. The other Mg\(^{2+}\) links the phosphate of A784 to the phosphate of G2588. Five phosphate oxygens of four nucleotides representing two disparate locations in the rRNA sequence are bridged by these two Mg\(^{2+}\). Microcluster D4 connects Domain IV of the LSU to Domain II (Figure 6.4.6). There are two 10-membered rings in this microcluster. One ring is made up of the phosphate groups and ribose atoms of C1782 and A1783; the other is made up of the phosphate groups and ribose atoms of A1783 and A1784. All of these nucleotides are in LSU Domain IV. The phosphate of A1780, a non-consecutive nucleotide of Domain IV, chelates one of these Mg\(^{2+}\). One Mg\(^{2+}\) of this microcluster is also chelated by the phosphate of U740 in Domain II.

Microclusters D1 and D3 make long-range intra-domain connections of the rRNA (Figure 6.4.4). Microcluster D1 links RNA nucleotides of Domain V that are not adjacent in the linear sequence or secondary structure; microcluster D3 links non-adjacent nucleotides of Domain III. In microcluster D1, the 10-membered ring is made up of the phosphate groups and ribose atoms of C2498 and C2499. The phosphate of A2448 (50 nucleotides away in the primary sequence) bridges both of the microcluster magnesiums. In microcluster D3, located near the exit tunnel, the 10-membered ring is made up of the phosphate groups and ribose atoms of
FIGURE 6.4.4 Ancient Mg²⁺ microclusters interconnect domains of 23S rRNA in the LSU. (a) Divisions in rRNA secondary structure Domains 0, I, II, III, IV, V, and VI are represented by changes in nucleotide color. (b) Microclusters D1, D2, D3, and D4 are shown as thick gray lines connecting nucleotide phosphates sharing a common first-shell Mg²⁺. Other first-shell Mg²⁺ interactions are shown as thin gray or black lines (some have been omitted for clarity). Nucleotides are colored spheres. Mg²⁺ ions are magenta spheres. (c) Mg²⁺ microcluster D3 of Domain III. (d) Mg²⁺ microcluster D1 of Domain V. Secondary structure data generated with RiboVision (Bernier et al. 2014); microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3142, 2009.)

A1603, along with the phosphate group of C1604. Phosphates of U1394 and U1395 chelate the Mg²⁺ of this microcluster.

Mg²⁺ microclusters cause bases to unstack, priming them for short- and long-range rRNA interactions. Microcluster D2 unstacks base A784 of Domain II, which forms a pocket for ribosomal protein L2, and base A2587 of Domain V, which intercalates with unstacked bases C1782 and A1783 from microcluster D4 of Domain IV (Figure 6.4.5). Microcluster D4 induces a large array of unstacked bases in Domain IV, some of which intercalate with A2587 (unstacked in D2) of Domain V (Figure 6.4.6). Microcluster D1 unstacks G2447 from A2448 and A2497 from C2498 in Domain V. Microcluster D3 unstacks base U1602 from A1603 and base A1393 from U1394 in Domain III.

The Mg²⁺-RNA microcluster appears to be an ancient motif, with roles in rRNA folding and function, though it does not participate directly in peptide bond formation. The microclusters and rRNA elements linked by microclusters are conserved over evolution among archaea, bacteria, eukarya, and mitochondrial rRNA. Mg²⁺-RNA microclusters have been identified in other rRNAs, including the bacterial SSU rRNA (Wimberly et al. 2000), and the P4-P6 domain of the Tetrahymena thermophila Group I intron ribozyme (Cate et al. 1997).
Acids Res. structure. (b) The D2 microcluster mediates short- and long-range interactions in the 23S rRNA. The two Mg ions are shown as magenta spheres. D2 microcluster nucleotides A783 and A784 are in Domain II of the 23S rRNA secondary structure. Magnesium ions are shown in magenta. The area of the 10-membered ring is shown in gray. RNA carbons are light gray, oxygens red, phosphorus orange, and nitrogen blue. Mg ion bridged by the common phosphate of A784. The area of the 10-membered ring is shown gray. RNA carbons are light gray, oxygens red, phosphorus orange, and nitrogen blue. Magnesium ions are magenta spheres. D2 microcluster nucleotides A783 and A784 are in Domain II of the 23S rRNA secondary structure. Magnesium ions are shown in magenta. The area of the 10-membered ring is shown in gray. RNA carbons are light gray, oxygens red, phosphorus orange, and nitrogen blue. Mg ion bridged by the common phosphate of A784.

FIGURE 6.4.6 Inter-domain Mg$^{2+}$-RNA microcluster D2 of 23S rRNA. (a) The D2 microcluster is a 10-membered ring (composed of a single Mg$^{2+}$, the phosphate and ribose backbone atoms of nucleotide A783, and the phosphate of A784) and a second Mg$^{2+}$ ion bridged by the common phosphate of A784. The area of the 10-membered ring is shown gray. RNA carbons are light gray, oxygens red, phosphorus orange, and nitrogen blue. Mg ions are magenta spheres. D2 microcluster nucleotides A783 and A784 are in Domain II of the 23S rRNA secondary structure. (b) The D2 microcluster mediates short- and long-range interactions in the 23S rRNA. The two Mg$^{2+}$ of D2 are chelated by nucleotide phosphates of Domain II (atoms colored as in panel [a]) and Domain V RNA (pink). Microcluster formation induces a non-canonical RNA backbone conformation, unstacking A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

FIGURE 6.4.6 Inter-domain Mg$^{2+}$-RNA microcluster D4 of 23S rRNA. (a) The D4 microcluster has two consecutive 10-membered rings composed of two Mg$^{2+}$ and the phosphate and ribose backbone atoms of nucleotides C1782 and A1783, and A1783 and A1784. The phosphate of A1780 also chelates one of the Mg$^{2+}$. (b) The D4 microcluster mediates short- and long-range interactions in the 23S rRNA. One Mg$^{2+}$ of D4 is chelated by the phosphate of U740 of Domain II (blue). C1782 and A1783 intercalate with A2587 of Domain V (pink). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

6.4.4.2 Ribosomal RNA Folding in the Absence of Proteins

The 23S rRNA of the ribosome LSU is a single polymer, thousands of nucleotides in length. Folding of the native ribosome to its catalytic state in vivo occurs with cations, including Mg$^{2+}$; many ribosomal proteins; and post-transcriptional modifications.

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. A2587 of Domain V, which intercalates with C1782 and A1783 of Domain IV (yellow). Structure of T. thermophilus [PDB 2J01 (obsolete). Superseded by 4V51]; microcluster data from Hsiao. (From Hsiao, C. and Williams, L.D., Nucleic Acids Res., 37, 3134–3140, 2009.)

The 23S rRNA of the ribosome LSU is a single polymer, thousands of nucleotides in length. Folding of the native ribosome to its catalytic state in vivo occurs with cations, including Mg$^{2+}$; many ribosomal proteins; and post-transcriptional modifications.

It has been shown that the 23S rRNA, without ribosomal proteins or post-transcriptional modifications, can fold to form RNA-RNA interactions resembling those of the native ribosome. Lenz et al. (2017). Lenz et al. used a chemical footprinting technique called Selective 2′ Hydroxyl Acylation Analyzed by Primer Extension (SHAPE) (Mortimer and Weeks 2007) to assess the reactivity of each ribose 2′-OH in the 23S rRNA sequence to an electrophile. SHAPE reactivity can be viewed as a measure of a nucleotide’s mobility or tendency to adopt a reactive conformation, meaning that the least-reactive nucleotides are the most likely participants in canonical hydrogen-bonding interactions.

Lenz et al. (2017) found that Na$^+$ alone supports formation of native-like rRNA secondary structure in the 23S rRNA. In other words, regions of rRNA such as helices, which are base-paired in the x-ray crystallographic structure, show minimal reactivity by SHAPE, whereas bases in or near the bulges and loops of rRNA, which should be more mobile and/or unpaired, are reactive.

Structural collapse of 23S rRNA to form the tertiary interactions of the native ribosome LSU requires Mg$^{2+}$ (Lenz et al. 2017). Addition of Mg$^{2+}$ alters the pattern of reactivity, observed with Na$^+$ alone. Some bases or regions of bases at or near bulges and loops become more, and others less, reactive in the presence of Mg$^{2+}$ and Na$^+$ than in the presence of Na$^+$ alone. The reactivities in helical regions, unreactive in the presence of Na$^+$ alone, are largely unaltered in the presence of Mg$^{2+}$. However, non-helical regions showed changes in reactivity upon addition of Mg$^{2+}$. Inspection of regions of crystallographic structures of native ribosomes showing altered reactivity by SHAPE suggests that these regions are largely associated with or are proximal to (1) known RNA-RNA tertiary interactions or (2) known RNA-protein interactions. Regions of altered reactivity are not limited to nucleotides expected to form first-shell tertiary interactions with Mg$^{2+}$, as observed by Hsiao and Williams (2009).
Magnesium-induced tertiary interactions include formation of an intricate inter-domain rRNA interaction network. Nucleotides within secondary structure domains come into close proximity in three-dimensional space on folding and formation of the native 23S rRNA structure, interacting, in part, via base-pairing, base stacking, and phosphate-Mg\(^{2+}\) interactions. Of the 21 possible combinations of domain-to-domain interactions, the native 23S rRNA (as represented by bacterium *T. thermophilus*) has 14 interactions. Lenz et al. (2017) found that 12 of the 14 domain-domain interactions are detectable as Mg\(^{2+}\)-induced by SHAPE in the absence of ribosomal proteins. Here, one domain-domain interaction may be represented by a series of nucleotide-nucleotide or nucleotide-Mg\(^{2+}\) interactions.

Mg\(^{2+}\) appears necessary to position rRNA nucleotides for native interactions with ribosomal proteins and form a catalytic structure. Folding of the LSU 23S rRNA in the presence of native interactions with ribosomal proteins and form a catalytic Mg\(^{2+}\) structure. Folding of the LSU 23S rRNA in the presence of native interactions with ribosomal proteins and form a catalytic Mg\(^{2+}\) structure. Folding of the LSU 23S rRNA in the presence of native interactions with ribosomal proteins and form a catalytic Mg\(^{2+}\) structure. Folding of the LSU 23S rRNA in the presence of native interactions with ribosomal proteins and form a catalytic Mg\(^{2+}\) structure. Folding of the LSU 23S rRNA in the presence of native interactions with ribosomal proteins and form a catalytic Mg\(^{2+}\) structure. Folding of the LSU 23S rRNA in the presence of native interactions with ribosomal proteins and form a catalytic Mg\(^{2+}\) structure. Folding of the LSU 23S rRNA in the presence of native interactions with ribosomal proteins and form a catalytic Mg\(^{2+}\) structure.

### 6.4.4.3 Ribosomal RNA Folding in the Presence of Proteins

Divalent cation-rRNA backbone interactions induce conformational changes that accommodate other ligands. Magnesium-microcluster D2 forms a pocket in the ribosome for ribosomal protein L2 (now known as uL2; Ban et al., 2014). Hsiao et al. found that Mg\(^{2+}\)-microcluster D2 connects Domain V of the 23S rRNA, near the PTC, to Domain II of the 23S rRNA (Hsiao and Williams 2009). The 10-membered ring of this microcluster is made of up A783 and A784 in Domain II. One Mg\(^{2+}\) forms a phosphate-mediated tertiary interaction with A2589 in Domain V near the PTC. The other Mg\(^{2+}\) forms a phosphate-mediated tertiary interaction with G2588 of Domain V. The D2 microcluster unstacks base A784 of Domain II, forming a pocket for ribosomal protein uL2 (Figure 6.4.5).

Ribosomal protein uL2 is unique in that its interaction with 23S rRNA appears to be more extensively mediated by Mg\(^{2+}\) than the interactions of other LSU proteins. The two Mg\(^{2+}\) ions of the D2 microcluster have (in sum) 12 first-shell ligands: five RNA phosphate oxyanions and seven waters (Petrov et al., 2012). Protein uL2 interacts directly with four of these waters through two highly conserved amino acids, alanine (or valine) and asparagine, interposed by a conserved methionine. The conserved uL2 sequence is most frequently alanine-methionine-asparagine (AMN) (Petrov et al., 2012). In *T. thermophilus*, these are residues Ala225, Met226, and Asn227 (Figure 6.4.7).

The observed interaction at the D2 microcluster is protein-water-Mg\(^{2+}\)-RNA, where the protein-water components interact via hydrogen bonds between a protein carbonyl and an acidic Mg\(^{2+}\)-bound water. In addition to directly coordinating the phosphates of A783, G784, G2588 and A2589 of the D2 microcluster, the Mg\(^{2+}\) ions, through their water ligands, interact with lone pairs of electrons on the backbone carbonyl of Ala225 and the side-chain carbonyl of Asn227 (Figure 6.4.7). Petrov et al. (2012) dissected and examined the stability of each component of the D2 microcluster protein-water-Mg\(^{2+}\)-RNA interaction by using quantum methods.

In Mg\(^{2+}\) microcluster D2, the Mg\(^{2+}\) ions stabilize amino acid-water interactions, while the RNA ligands of the Mg\(^{2+}\) attenuate them slightly. Though Ala225 and Asn227 each have favorable and roughly equal interaction energies with water alone, interaction energies with water bound to Mg\(^{2+}\) in a hexahydrate (e.g., [Mg(H\(_2\)O)\(_6\)]\(^{2+}\)) are four-fold more favorable. The charge transfer component of the interaction energies implies that the charge on the amino acid carbonyl is transferred to the Mg\(^{2+}\) by polarization of the mediating water. To understand the effect of the RNA phosphates on the stability of the complex, two dimethyl phosphate molecules (Figure 6.4.2) were used to simulate chelation of [Mg(H\(_2\)O)\(_4\)]\(^{2+}\) by the RNA bidentate clamp. Chelation of the metal by the RNA analog decreases the overall stability of the amino acid-water-Mg\(^{2+}\)-RNA complex by roughly one third, though interaction energies remained favorable. In other words, chelation of the Mg\(^{2+}\) by the phosphates of the RNA analog decreases polarization of the remaining water ligands of the Mg\(^{2+}\) and thus the acidity of the protein-mediating water, slightly attenuating the strength of the water-protein interaction. Even so, the amino acid-water-Mg\(^{2+}\)-RNA complex remains more than two-fold more stable than the amino acid-water complex, and the charge transfer component of the interaction energy remains strong enough to
infer that some charge from the amino acid carbonyl remains with the Mg\(^{2+}\).

The D2 microcluster stabilizes a segment of protein uL2 through orientation and polarization of water molecules. The AMN segment of protein uL2 interacts with the D2 microcluster through six hydrogen bonds. Four of these are with Mg\(^{2+}\)-polarized water molecules. The backbone carbonyl oxygen of A1225 hydrogen bonds (H-bonds) with two Mg\(^{2+}\)-bound waters of the same D2 Mg\(^{2+}\) and one 2′OH of RNA A782. The side-chain carbonyl of Asn227 H-bonds with one water of each D2 Mg\(^{2+}\), while the side-chain amine of Asn227 shares a hydrogen with a phosphate oxyanion of A784. To determine the role of the Mg\(^{2+}\) in supporting this interaction, Petrov et al. (2012) used DFT to examine the stability of the hydrated L2 segment, AMN-water, with and without the Mg\(^{2+}\) and RNA of the D2 microcluster. The AMN-water-Mg\(^{2+}\)-RNA complex is four-fold more stable than AMN-water, even though AMN does not interact directly with the Mg\(^{2+}\). In the case of uL2, neither Mg\(^{2+}\) nor RNA alone stabilizes the interaction with ribosomal protein; it is rather the orientation and polarization of the water molecules by Mg\(^{2+}\) that enhance stability.

### 6.4.5 RIBOZYME FUNCTION

#### 6.4.5.1 RIBOZYMES WITH Mg\(^{2+}\), Fe\(^{2+}\), AND OTHER CATIONS

Cations in the LSU play, to current knowledge, strictly structural roles in their support of the peptidyl transfer reaction. Although Mg\(^{2+}\) is necessary for folding and function of the LSU, there is not a direct role for any Mg\(^{2+}\) ion in the mechanism of peptidyl transferase (Noller et al. 1992). This is not the case for all catalytic RNAs.

The transition states of many ribozymes (see also Chapters 6.4 and 6.6) are directly stabilized by divalent metals (Butcher 2011; Johnson-Buck et al. 2011). In the activity of the in vitro evolved L1 ligase ribozyme (Robertson and Scott 2007), for example, the 5′ α-phosphate of a nucleotide triphosphate (Figure 6.4.2) is polarized by a Mg\(^{2+}\) ion, priming the α-phosphorus for nucleophilic attack by an acidic 3′-OH of the adjacent nucleotide. Nucleophilic attack of the 3′-OH on the 5′ α-phosphate completes the phosphodiester bond necessary for backbone ligation, generating a pyrophosphate leaving group (Robertson and Scott 2007). In the hammerhead ribozyme, an acidic metal-coordinated water at the active site is believed to donate a hydrogen, stabilizing the oxyanion leaving group during cleavage (Scott et al. 1995; Murray et al. 1998; Scott 2007). Unlike the L1 ligase ribozyme, which requires the divalent metal for activity, the hammerhead ribozyme retains some activity in the absence of divalent metals in high (e.g., >1.0 M) Na\(^{+}\) concentration (Nakano et al. 2014) or low Na\(^{+}\) concentration (100 mM) at freezing temperature (Lie and Wartell 2015).

The hammerhead ribozyme, in particular, has a promiscuous relationship with cations. Computed barriers in the free-energy landscapes of “metal migration” within the active site of the self-cleaving hammerhead ribozyme are generally larger for Mg\(^{2+}\) and Zn\(^{2+}\) than for Mn\(^{2+}\) (Panteva et al. 2015). This observation is consistent with experimental work showing that at pH 7, in the presence of 0.1 M NaCl, the catalytic rate of self-cleavage by the hammerhead is greater with Mn\(^{2+}\) than with Mg\(^{2+}\) (Hunsicker and DeRose 2000).

Compaction and formation of secondary structure in the Group I intron P4-P6 domain ribozyme of *T. thermophila* are supported by Na\(^{+}\) alone, but divalent cations are needed to induce tertiary structure (Cate et al. 1997; Deras et al. 2000). Athavale et al. (2012) used SHAPE to probe the P4-P6 domain in the presence of Na\(^{+}\) and observed low SHAPE reactivity, indicative of canonical base pairing, in regions observed in the crystallographic structure to be helical, while loop regions (unpaired in the structure) tended to be much more reactive. Addition of mM concentrations of Mg\(^{2+}\) caused distinct changes in SHAPE reactivity, suggestive of specific magnesium binding and formation of tertiary RNA structure (Figure 6.4.8).
Most of the residues having an increase in SHAPE reactivity in the presence of Mg\(^{2+}\) (compared with Na\(^+\) alone) bear a C2’-endo sugar pucker in structure PDB 1GID (Cate et al. 1996). This geometry of the ribose, typical of DNA, but rare and restricted to non-helical regions of RNA structures (Auffinger and Westhof 1997), was previously demonstrated to have enhanced reactivity by SHAPE (Cate et al. 1996; Vicens et al. 2007). The region with a decrease in SHAPE reactivity in the presence of Mg\(^{2+}\) is consistent with packing of phosphates by Mg\(^{2+}\) in a core inaccessible to solvent (Cate et al. 1997). Mg\(^{2+}\) microclusters are present in this region of the P4-P6 RNA (Cate et al. 1997; Hsiao and Williams 2009).

Fe\(^{2+}\) oxidizes and precipitates rapidly in the presence of atmospheric oxygen, but under anoxic conditions, it induces folding of P4-P6 domain RNA to a near-native state. Athavale et al. (2012) demonstrated that the pattern of SHAPE reactivity in P4-P6 domain RNA in the presence of Fe\(^{2+}\) under anoxic conditions closely resembles that in the presence of Mg\(^{2+}\). Regions of RNA with pronounced increases in SHAPE reactivity upon the addition of Mg\(^{2+}\) (compared with Na\(^+\) alone) also increase in reactivity upon addition of Fe\(^{2+}\). Parallel decreases in SHAPE reactivity were also observed in other regions of the RNA, suggesting that, in anoxia, Fe\(^{2+}\) replaces Mg\(^{2+}\), without perturbation of the native structure of the P4-P6 ribozyme.

Catalysis by the L1 ligase or hammerhead ribozyme occurs at an enhanced rate in the presence of Fe\(^{2+}\). Athavale et al. (2012) compared the catalytic rate of the L1 ligase ribozyme in Na\(^+\), Na\(^+\)/Mg\(^{2+}\), and Na\(^+\)/Fe\(^{2+}\). As expected, the L1 ligase was inactive in Na\(^+\) alone. Micromolar concentrations of Mg\(^{2+}\) or Fe\(^{2+}\) induced catalytic activity. Surprisingly, the rate of Fe\(^{2+}\)-induced catalysis was 25 times that of Mg\(^{2+}\)-induced catalysis. A similar effect was observed for the hammerhead ribozyme: catalysis in the presence of Fe\(^{2+}\) initiated at a rate three times higher than in the presence of Mg\(^{2+}\). The interchangeability of Mg\(^{2+}\) and Fe\(^{2+}\) in RNA folding and function is supported by theoretical computational analyses that revealed only subtle differences in the coordination geometries of these RNA-divalent metal (RNA-M\(^{2+}\)) complexes. Furthermore, the calculations demonstrated that stability of RNA-M\(^{2+}\) complexes is enhanced when Mg\(^{2+}\) is replaced with Fe\(^{2+}\) (Petrov et al. 2011; Athavale et al. 2012), possibly due to the availability of d-orbitals in Fe\(^{2+}\).

6.5.6 CONCLUSIONS

Fundamental biochemical processes of life as we know it depend on inorganic cations. Inorganic cations in association with RNA are always hydrated to some extent, and the importance of water in their interactions cannot be overstated. The unique properties of each cation—principally size and charge—alter the mobility and physicochemical properties of first-shell waters, which in turn affect surrounding water and non-water molecules. The energetic penalties associated with cation dehydration are a part of the net energy of reaction for every first-shell interaction with a biological ligand.

Inorganic cations have a special relationship with the phosphodiester backbone of nucleic acids. Backbone phosphates are anionic and highly polarizable, coordinating some cations with more compact geometry than others. The intricacies of a cation’s individual properties can be viewed as magnified by interactions with phosphate esters, due to phosphate’s enhanced polarizability relative to water. Magnesium stabilizes RNA through a continuum of associations, from neutralization of bulk negative charge in solution by mobile hexahydrates to compact first-shell coordination of up to four RNA backbone phosphates, while the range of associations of Na\(^+\) and other large, monovalent cations is less diverse (Bowman et al. 2012).

The role of inorganic cations in protein translation provides an essential perspective on their importance in biology and relevance to the origin of life. Multiple high-resolution crystal structures of the ribosome reveal that the core structure of the LSU is essentially unchanged throughout extant life, including the locations of cations and their binding sites’ geometries (Hsiao and Williams 2009). When compared with other functional RNAs (ribozymes), the rRNA of the LSU is immense in length and complexity. An understanding of the principles underlying folding of such a large RNA to the universal conformation of peptide bond synthesis informs our understanding of LUCA and the constraints under which it might have evolved.

The LSU provides every indication that it has been dependent on Mg\(^{2+}\) or Mg\(^{2+}\)-like divalent cations for folding and function since, and likely before, LUCA. Reoccurring motifs of rRNA in first-shell interactions with Mg\(^{2+}\) help to organize the three-dimensional structure of the LSU, though Mg\(^{2+}\) does not participate in catalysis directly. A recent study suggests that Mg\(^{2+}\) may also play an indirect role in stabilization of pre- and post-peptidyl transfer states (Polikanov et al. 2014). For perspective, Mg\(^{2+}\) ions observed in a crystal structure of the ribosome are shown in Figure 6.4.9.

These motifs include the bidentate RNA-Mg\(^{2+}\) clamps and Mg\(^{2+}\) microclusters (Hsiao and Williams 2009; Petrov et al. 2011). In an RNA-Mg\(^{2+}\) clamp, the phosphates of two consecutive nucleotides chelate a single Mg\(^{2+}\), altering the geometry of the metal hydrate and in turn the local properties of the RNA. Computation suggests that Na\(^+\) is incapable of stabilizing this type of RNA geometry. In a Mg\(^{2+}\) microcluster, one phosphate of an RNA-Mg\(^{2+}\) clamp serves as a bridge to a second Mg\(^{2+}\) ion, which, in three-dimensional structures, coordinates and restrains the phosphate of nucleotide(s) that are hundreds to more than a thousand waters coordinating the Mg\(^{2+}\) also stabilize the LSU. In one case, waters coordinating the Mg\(^{2+}\) of the D2 microcluster participate in hydrogen-bonding interactions with side-chain and backbone carbonyls of two highly conserved residues.
exceptions, these enzymes generally tolerate partial to complete substitutions of Mg\(^{2+}\) for Mn\(^{2+}\) and occasionally other cations. One analysis ties the early evolution of eukaryotes to an oxic Earth and prokaryotes to an anoxic one, based on trends in metal substitutions in metallo-proteins (Dupont et al. 2006). Another result of this work is that the overall abundance of metallo-proteins was found to scale with proteome size, suggesting that biology’s dependence on inorganic cations is not vestigial.

**ACKNOWLEDGMENTS**

We thank Roger M. Wartell for critical review of an early version of the manuscript and Afrah Ghauri and Chieri Ito for careful editorial review. This work was supported by the National Aeronautics and Space Administration (NNX16AJ29G and NNX16AJ28G) and the National Science Foundation (1724274).

**REFERENCES**


