Beyond Mg²⁺: Functional Interactions between RNA and Transition Metals

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Abstract

It is well-known that RNA structure and function depend heavily on cations, and the ability of Mg^{2+} to stabilize RNA structures has been emphasized. Recent studies, however, highlight the importance of transition metals in RNA function. Riboswitches that selectively bind Ni^{2+} , Co^{2+} , and Mn^{2+} have been discovered with specific RNA-metal sites that influence metal-related gene expression. Exogenous metals such as Pt(II) from therapeutics also bind and may inhibit cellular RNA. Novel reports that RNA can host Fe(II) in catalytic sites are relevant to early life in pre-oxygenic atmospheres. These new observations emphasize the importance of transition metals in the field of RNA metallobiochemistry.

Introduction

By current estimates, ~80% of the human genome is transcribed into a vast amount of cellular RNA, both coding and noncoding, that regulates gene expression at many levels. RNA structure can rival proteins in terms of size and complexity of noncovalent interactions. RNA is a negatively charged biopolymer, and electrostatic forces drive a majority of cation-RNA interactions. Within this context, however, select sites are found wherein cations are coordinated by RNA functional groups. These 'metal' sites are often integral to function, supporting specific RNA structures or, in the case of ribozymes, catalysis.

Presumably due to high intracellular concentration and relative non-reactivity, Mg^{2+} is assumed to support the majority of RNA-'metal' interactions. Intracellular Mg^{2+} concentrations are not held to the very low numbers estimated for transition metal ions such as Zn^{2+} , Cu, Ni, Mn, and Fe. Moreover, hydrolysis of the RNA ribose 2'-OH is catalyzed by transition metal-hydroxo species formed near neutral pH. Diversity in RNA conformation and hydrogen bonding patterns creates great selectivity in binding non-metal 'guests,' but selectivity in cation binding is rare (reviewed in [1]). Metal ligands provided by RNA are limited to 'hard' and 'borderline' phosphate, ribose, and keto oxygens and imino nitrogens, respectively, whereas 'soft' cysteine thiolate ligands add diversity in metalloproteins. Overall, the biological importance of transition metal-RNA interactions has not been clear, and 'metals' have remained ' Mg^{2+} ' in the world of RNA metallobiochemistry.

In this context, recent studies indicating functional interactions between structured RNAs and non- Mg^{2+} transition metal ions are exciting. Here we review recent reports of: (a) transition metal-specific riboswitches that control metal efflux pumps; (b) Pt-RNA adduct formation from Pt therapeutics; and (c) RNA-catalyzed Fe(II) chemistry relevant to early life. Together, these studies make a clear case that expectations for biological RNA-metal interactions should be expanded beyond Mg^{2+} to include transition metals, with functional consequences.

Transition metal-binding riboswitches

Riboswitches are RNA domains that function as receptors for metabolites and regulate gene expression based on those interactions. Canonically, riboswitches reside in the 5'UTR of nascent mRNA transcripts. Upon binding specific metabolites, they adopt complex structures that control transcriptional elongation or translation initiation [2]. In bacteria, riboswitches are ubiquitous gene regulation elements, but only one class of riboswitch has also been characterized in archaea and eukaryotes [3]. Although prescient development of synthetic RNA aptamers demonstrated the potential to recognize small molecules with high selectivity [4,5], the discovery of riboswitches revealed the biological significance of this ability [6-9]. The discovery that the orphan (lacking a known ligand) "M-box" riboswitch specifically bound Mg^{2+} ions refined the potential selectivity of riboswitches down to the level of atoms [10]. However, the Mg^{2+} riboswitch exhibits broad specificity for divalent cations and its function relies on the relatively high cellular concentration of Mg^{2+} . In contrast, a more recently-described FI⁻ riboswitch is able to exclude similar anions, despite their higher abundance, making it a truly ion-specific aptamer [•11]. Although this demonstrated remarkable ability of RNA to specifically bind small ions, it remained doubtful that RNA possessed the capacity to recognize metal ions with a selectivity comparable to metal-sensing metalloregulatory proteins. However, the recent discovery of two new classes of transition metal-sensing riboswitches presents a new apogee of sophistication in the selectivity of RNA aptamers [••12,••13,••14].

Computational predictions by the Winkler and Breaker groups identified a potential riboswitch in 5'UTRs of *czcD* genes, which encode members of the Cation Diffusion Facilitator (CDF) family of metal export and resistance proteins [••14,•15]. In-line probing analysis [16] revealed that this *czcD* motif folds into an aptamer that selectively binds Ni^{2+} and Co^{2+} ions at low- μM affinity, even in the presence of excess competing Mg^{2+} ions. Strikingly, binding properties suggested that the Co^{2+} or Ni^{2+} ions cooperatively bind at multiple sites; such cooperativity, resembling that of allosteric proteins, had not been previously characterized in riboswitches [••17]. In vitro assays show that binding of Co^{2+} or Ni^{2+} to the interhelix junction supports transcription by

stabilizing the P1 helix relative to a competing Rho-independent terminator (Figure 1A). The NiCo riboswitch has unique architectural features that support this remarkable specificity and cooperative behavior. Crystallography of a Co^{2+} -bound NiCo riboswitch revealed coaxially-stacked helices that adopt a twisted 'H' conformation (Figure 1B). Atypically for riboswitches, neither the structure nor binding mechanism rely on long-range tertiary interactions or close helical packing. Instead, the twisted interhelix junction forms a binding pocket where four Co^{2+} ions were located by anomalous dispersion. An interaction network links three adjacent Co^{2+} ions, suggesting that cooperative binding behavior results from synergistic stabilization of the sites (Figure 1B). Whereas Mg^{2+} typically coordinates phosphoryl oxygens, the Co^{2+} ions are largely coordinated through the N7 of several strictly-conserved guanines, suggesting that selectivity arises in part through these ligands favoring 'borderline' Co^{2+} ions over 'hard' Mg^{2+} .

While over 600 distinct NiCo riboswitches are predicted within existing bacterial genomes and metagenomic data [6], their distribution is limited to the *Firmicutes* and related bacterial clades. A more widespread role for transition metal-sensing riboswitches emerges from the discovery that the *yybP-ykoY* motif, an orphan riboswitch distributed throughout bacteria, functions as a Mn^{2+} ion sensor. This riboswitch populates UTRs of diverse, mostly uncharacterized genes, leaving few clues to its function [18]. However, work from the Storz lab demonstrated that the *E. coli* *mntP* gene encodes a Mn^{2+} exporter and contains a *yybP-ykoY* motif in its 5'UTR, prompting investigation of *yybP-ykoY* as a Mn^{2+} -sensor [•19,20]. Now, two recent studies published simultaneously have elucidated mechanisms of Mn^{2+} binding and gene regulation by this riboswitch [••12,••13]. The Storz lab has shown that Mn^{2+} induces both transcriptional and translational expression of MntP [••13,•19], achieved in part by direct binding of Mn^{2+} to the *yybP-ykoY* aptamer domain. In the absence of Mn^{2+} , a stem-loop structure occludes the ribosomal binding site, whereas addition of Mn^{2+} induces a conformational change enabling translation [••13].

The Ke lab further reports a 2.85Å-resolution crystal structure of Mn^{2+} -bound *yybP-ykoY* riboswitch from *Lactococcus lactis*, and suggests a mechanism of Mn^{2+}

binding [••12]. Unlike the NiCo riboswitch, the *yybP-ykoY* aptamer domain creates docking sites for two metal ions through long-range tertiary interactions (Figure 1C). One site binds divalent cations such as Mg^{2+} with low specificity, possibly stabilizing tertiary structure and priming a second site to accept Mn^{2+} . Although both ions are coordinated octahedrally with five phosphoryl oxygens, the Mn^{2+} is also coordinated by the N7 of a conserved adenosine, whereas the Mg^{2+} site includes one water ligand. As with the NiCo riboswitches, imino N7 coordination appears to contribute specificity for transition metal ions by introducing borderline ligands. An earlier study characterized a Mn^{2+} -sensing riboswitch in enteric bacteria that, under elevated Mn^{2+} levels, suppressed transcription of the *mntH* gene encoding a Mn^{2+} importer [•21]. Further structural and comparative analysis may offer more insight into mechanisms of Mn^{2+} selectivity.

The NiCo and Mn^{2+} riboswitches establish precedence for selective transition metal sensing by RNA elements. The biological importance and potential toxicity of ions such as $Cr^{2+/3+}$, $Fe^{2+/3+}$, Zn^{2+} , Cu^{2+} , and Mo(III-V), raises the question of whether riboswitches that sense these and other transition metals might be more widespread. Bacteria also possess related export mechanisms for common toxic transition metals and metalloids Cd^{2+} , Ag^{2+} , Hg^{2+} , and Pb^{3+} , and RNA-metal sensing elements could provide an added layer of regulation. There are physical and chemical constraints to these possibilities; some ions, such as Zn^{2+} , $Cu^{1+/2+}$, and Pb^{2+} , naturally destabilize RNA by catalyzing hydrolysis or oxidative cleavage [22]. Another consideration is the oxidative state of the cell, which could alter reactivity and coordination chemistry of certain ions. For example, the concentration of NiCo riboswitches in the anaerobic *Clostridia* family could be related to a functional requirement for an anoxic environment. Nonetheless the diversity of metal export proteins suggests an expanded role for transition metal-sensing RNAs.

RNA-Pt adducts from Platinum therapeutics

The propensity for RNAs to coordinate metals suggests the relatively unexplored concept that metal-based therapeutics are sequestered in RNA sites. This concept has recently been pursued for Pt(II) therapeutics (Figure 2), used commonly in chemotherapy [23]. Square-planar Pt(II) has defined preferences for both ligand type

and geometry. In biological environments substitution of the more labile non-nitrogen ligands forms exchange-inert Pt(II)-biomolecule adducts. Unlike many transition metal interactions, binding of Pt(II) compounds to biomolecules is relatively slow and likely under kinetic rather than thermodynamic control. The best-studied target for Pt therapeutics is genomic DNA, where Pt-DNA adducts cause cell death by multiple pathways [24]. In the most common 5'GpG and 5'ApG sites, Pt(II) coordinates purine imino N7 ligands (Fig. 2). While early analyses also found Pt-RNA species [25, •26] and Pt drug inhibition of RNA processes [reviewed in •26], these were not pursued until recently. Growing recognition of the importance of RNA regulatory pathways [27] has heightened interest in the potential of RNA as a drug target. Pt(II) complexes also support robust RNA-RNA and RNA-protein crosslinking [28,29,30], providing a cell-permeable structural tool.

To establish the extent of RNA-Pt interactions *in vivo*, Hostetter et al. characterized RNA extracted from cisplatin-treated *S. cerevisiae* [31]. At 12 hr treatment, the *density* of Pt ions sequestered by DNA ($\sim 10^{-4}$ Pt per nucleotide) was ~ 3 -fold that of RNA. However, since the *amount* of RNA exceeds DNA in an average yeast cell, total Pt sequestered by cellular RNA exceeded that of DNA by 4-20- fold. The majority of Pt-RNA adducts occur in 25S and 18S rRNA. Although clearly detected, density and predicted total Pt were lower for extracted polyA-RNA following *in vivo* treatment with cisplatin.

The sequence preference of 5'GpG or 5'A-G for Pt(II)-DNA adducts is only one determinant for putative Pt(II)-RNA binding sites. As with other metal-RNA sites, accessibility, local structure, dynamics, and electrostatics are important factors, and are particularly relevant in large structured RNAs such as the ribosome. Using primer extension, Chow and coworkers identified Pt-RNA sites in helix 24 of *E. coli* ribosomes following cisplatin treatment *in vitro* and *in vivo* [••32]. As expected, results depended on the state of the RNA when exposed to cisplatin: sites observed from treatment of isolated (renatured) 16S rRNA were protected in context of protein-bound 30S ribosomes. A major adduct at G800, in helix 24 (Fig. 3), was observed in all samples, indicating Pt accessibility to this site in all states of the bacterial ribosome.

In an exciting demonstration of the power of secondary interactions for steering Pt(II) adducts, novel amino acid-linked Pt(II) compounds were found to re-direct the major Pt-RNA site to different sites in helix 24 [••33]. This important result shows the potential to exploit differences between bacterial and mammalian ribosomes for treatments beyond current antibiotics. Indeed, Osborn et al. probed this same helix in RNA extracted from cisplatin-treated *S. cerevisiae* and found different major Pt adducts ascribed to primary sequence variations between eukaryotes and *E. coli* (Fig. 3) [••34]. Direct evidence for tRNA-Pt adduct formation was also found using novel ‘clickable’ Pt reagents [35].

Although a seemingly minor modification, platination has the potential to alter RNA structure and function. Pt-crosslinked RNA [29,30] could be disruptive given the dynamic nature of cellular RNA. Dedduwa-Mudalige and Chow showed that Pt-rRNA adducts in the stem of *E. coli* helix 69, near a neomycin site, alter structure of a nearby loop [•36]. Few studies, however, pursue a direct link between RNA platination and downstream consequences. In one notable exception, Elmroth and coworkers demonstrated that siRNA platination could disrupt RNA silencing in mammalian cells [••37]. Silencing by siRNAs and their well-characterized platinated partners was measured using a Wnt-5a 5'-UTR-luciferase reporter system (HB2 cells). Platination resulted in measurable reduction of silencing efficiency, with a maximum of up to 7-fold change for an oxaliplatin-treated siRNA. One Pt-siRNA oligo showed statistically insignificant silencing differences, possibly because its major platination site was too distant to influence the seed region. Since even small expression changes induced by RNA silencing can have dramatic consequences, these studies show clear potential for RNA-Pt adducts to influence cellular processes.

Fe(II) and ancient RNA: The Mg^{2+} replacement theory

Since metals have the power to dramatically alter RNA structure and reactivity, evolution of complex RNA function would include pressure to preserve or avoid specific metal-RNA interactions. As the earth's ancient metallochemistry changed, RNA-metal interactions would have to respond. Dramatic changes include the availability of soluble Fe(II) in early anaerobic environments, and later adaptation to oxygen and insoluble

Fe(III). Hsaio and coworkers [••38] hypothesize that Fe(II) was integral to RNA function during the ~1B year period before evolution of photosynthesis, and adaptation to the resulting oxygenic atmosphere was accompanied by replacement of Fe(II) with 'benign' Mg^{2+} . Moreover, they hypothesize that early RNA-Fe(II) sites supported reactivity. Testing this using a peroxidase assay, they report that Fe(II) bound to T. *Thermophilus* 23S rRNA, tRNA^{Phe}, and the highly structured Group I intron P4-P6 subdomain, but not ATP or short oligonucleotides, catalyzes reduction of peroxide using a dye as electron donor (Figure 4). Earlier work demonstrated that Fe(II) supports the same complex P4-P6 tertiary structure achieved in Mg^{2+} , and also enhances ribozyme catalysis [••39]. Interestingly, a DFT study comparing Fe(II)- and Mg^{2+} - ligand interaction energies showed significantly more favorable Fe(II)-phosphate interactions, and larger potential activation for phosphoryl transfer.

In a separate study, Popovic et al. examined RNA populations selected for self-cleavage activity with either Mg^{2+} or Fe(II) [•40]. The two metals supported evolution of different sequence pools, indicating a difference in fitness landscape. In support of 'Fe-first' evolution, the extant, conserved hammerhead ribozyme sequence was more highly populated in the Fe(II)-dependent selection; Mg^{2+} could replace Fe in these sequences, but the reverse was not always true for Mg^{2+} -selected self-cleaving RNAs.

While the studies above support a hypothetical Fe-dominant RNA world replaced more recently by Mg^{2+} , there is at least one relatively modern example of a functional RNA-Fe(II) interaction. The Iron responsive Element (IRE) is an RNA regulatory domain found in iron metabolism mRNAs [•41]. In 5'-UTR IREs, low [Fe(II)] facilitates repressor binding, whereas high [Fe(II)] enhances translation. Multiple lines of evidence support a model wherein direct RNA-Fe(II) interactions govern this IRE structure/function switch. Highly conserved IRE sequences are found in organisms from sponges to mammals, appearing to set their origin at early metazoan [42] and after the Great Oxygenation Event.

Conclusions

RNA biology continues to yield surprising levels of sophistication. This review summarizes recent reports that together highlight the potential for RNA to control, and be controlled by, transition metal ions. The discovery of Mn^{2+} , Ni^{2+} , and Co^{+} -dependent riboswitches, characterization of Fe(II)-supported RNA structure and catalysis and its relevance to early life, and recognition of Pt(II)-RNA adducts following treatment with chemotherapeutics, all represent another frontier in RNA properties. These discoveries rely on comparative genomics married to careful biochemical and structural analyses, and extrapolation of the results to cellular environments. With exception of exchange-inert Pt(II), obtaining direct evidence for specific metal-RNA interactions by isolating cellular RNAs retaining 'native' metals remains a challenge. Given its propensity to acquire metals, however, it may be interesting to consider RNA in cellular budgets of transition metal ions.

Acknowledgements

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References:

- [1] Ward WL, Plakos K, DeRose VJ: **Nucleic Acid Catalysis: Metals, nucleobases, and other cofactors.** *Chem Rev* 2014, **114**:4318–4342.
- [2] Wakeman CA, Winkler WC, Dann CE III: **Structural features of metabolite-sensing riboswitches.** *Trends Biochem Sci* 2007, **32**:415–424.
- [3] Sudarsan N, Barrick JE, Breaker RR: **Metabolite-binding RNA domains are present in the genes of eukaryotes.** *RNA* 2003, **9**:644–647.
- [4] Ellington AD, Szostak JW: **In vitro selection of RNA molecules that bind specific ligands.** *Nature* 1990, **346**:818–822.
- [5] Tuerk C, Gold L: **Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase.** *Science* 1990, **249**:505–510.
- [6] Nahvi A, Sudarsan N, Ebert MS, Zou X, Brown KL, Breaker RR: **Genetic control by a metabolite binding mRNA.** *Chemistry & Biology* 2002, **9**:1043.
- [7] Winkler WC, Cohen-Chalamish S, Breaker RR: **An mRNA structure that controls gene expression by binding FMN.** *Proc Natl Acad Sci USA* 2002, **99**:15908–15913.
- [8] Winkler W, Nahvi A, Breaker RR: **Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression.** *Nature* 2002, **419**:952–956.
- [9] Mironov AS, Gusarov I, Rafikov R, Lopez LE, Shatalin K, Kreneva RA, Perumov DA, Nudler E: **Sensing small molecules by nascent RNA: a mechanism to control transcription in bacteria.** *Cell* 2002, **111**:747–756.
- [10] Dann CE, Wakeman CA, Sieling CL, Baker SC, Irnov I, Winkler WC: **Structure and mechanism of a metal-sensing regulatory RNA.** *Cell* 2007, **130**:878–892.
- [•11] Baker JL, Sudarsan N, Weinberg Z, Roth A, Stockbridge RB, Breaker RR: **Widespread genetic switches and toxicity resistance proteins for fluoride.** *Science* 2012, **335**:233–235.

Demonstrates that a Fl⁻ riboswitch can selectively bind target ions while excluding others with similar size and charge.

- [••12] Price IR, Gaballa A, Ding F, Helmann JD, Ke A: **Mn(2+)-Sensing Mechanisms of yybP-ykoY Orphan Riboswitches.** *Mol Cell* 2015, **57**:1110–1123.

The authors present a crystal structure of Mn²⁺-bound yybP-ykoY riboswitch showing a possible mechanism of Mn²⁺ selectivity.

- [••13] Dambach M, Sandoval M, Updegrove TB, Anantharaman V, Aravind L, Waters LS, Storz G: **The Ubiquitous yybP-ykoY Riboswitch Is a Manganese-Responsive Regulatory Element.** *Mol Cell* 2015, **57**:1099–1109.

The authors not only demonstrate Mn^{2+} binding activity of the yybP-ykoY riboswitch, but also analyze the function of this riboswitch in the context of the Fur/MntR metalloregulatory circuit.

- [••14] Furukawa K, Ramesh A, Zhou Z, Weinberg Z, Vallery T, Winkler WC, Breaker RR: **Bacterial riboswitches cooperatively bind $Ni(2+)$ or $Co(2+)$ ions and control expression of heavy metal transporters.** *Mol Cell* 2015, **57**:1088–1098.

The authors genetically, biochemically, and structurally characterize a new class of riboswitches that selectively bind Ni^{2+} and Co^{2+} ions with a level of cooperativity unprecedented for riboswitches. Crystal structure of Co^{2+} -bound riboswitch reveals a unique architecture and suggests mechanisms of selectivity and cooperativity.

- [•15] Weinberg Z, Wang JX, Bogue J, Yang J, Corbino K, Moy RH, Breaker RR: **Comparative genomics reveals 104 candidate structured RNAs from bacteria, archaea, and their metagenomes.** *Genome Biol* 2010, **11**:R31.

Compendium of predicted riboswitches from existing genomes and metagenomes, many of which remain orphan and regulate expression of yet uncharacterized genes.

- [16] Regulski EE, Breaker RR: **In-line probing analysis of riboswitches.** *Methods Mol Biol* 2008, **419**:53–67.

- [••17] Peselis A, Gao A, Serganov A: **Cooperativity, allostery and synergism in ligand binding to riboswitches.** *Biochimie* 2015, **117**:100–109.

Presents a thorough review of higher-order ligand binding mechanisms of riboswitches, including the NiCo riboswitches discussed in this review.

- [18] Meyer MM, Hammond MC, Salinas Y, Roth A, Sudarsan N, Breaker RR: **Challenges of ligand identification for riboswitch candidates.** *RNA Biol* 2011, **8**:5–10.

- [•19] Waters LS, Sandoval M, Storz G: **The Escherichia coli MntR Miniregulon Includes Genes Encoding a Small Protein and an Efflux Pump Required for Manganese Homeostasis.** *Journal of Bacteriology* 2011, **193**:5887–5897.

The authors demonstrate that the *mntP* gene encodes a Mn^{2+} efflux pump. Furthermore, the 5' UTR contains the previously orphan yybP-ykoY riboswitch.

- [20] Veyrier FJ, Boneca IG, Cellier MF, Taha M-K: **A novel metal transporter mediating manganese export (MntX) regulates the Mn to Fe intracellular**

ratio and *Neisseria meningitidis* virulence. *PLoS Pathog* 2011, **7**:e1002261.

- [•21] Shi Y, Zhao G, Kong W: **Genetic analysis of riboswitch-mediated transcriptional regulation responding to Mn²⁺ in *Salmonella*.** *Journal of Biological Chemistry* 2014, **289**:11353–11366.

The authors describe a Mn²⁺-sensing riboswitch that terminates transcription of the *mntH* gene upon binding to Mn²⁺. This provides yet another transition metal-sensing riboswitch, which will be interesting to compare mechanistically with the *yybP-ykoY* riboswitch.

- [22] Ciesiolka J, Michałowski D, Wrzesinski J, Krajewski J, Krzyzosiak WJ: **Patterns of cleavages induced by lead ions in defined RNA secondary structure motifs.** *J Mol Biol* 1998, **275**:211–220.
- [23] Kelland L: **The resurgence of platinum-based cancer chemotherapy.** *Nature Reviews: Cancer* 2007, **7**:573-584.
- [24] Jung Y, Lippard SJ: **Direct cellular responses to platinum-induced DNA damage.** *Chem Rev* 2007, **107**:1387-1407.
- [25] Akaboshi M, Kawai K, Maki H, Akuta K, Ujeno Y, Miyahara T: **The number of platinum atoms binding to DNA, RNA and protein molecules of HeLa-cells treated with cisplatin at its mean lethal concentration.** *Jpn J Cancer Res* 1992, **83**:522–526.
- [26] Chapman EG, Hostetter AA, Osborn MF, Miller AL, DeRose VJ: **Binding of Kinetically Inert Metal Ions to RNA: the Case of Pt(II).** In *Metal Ions in Life Sciences Volume 9: Structural and Catalytic Roles of Metal Ions in RNA*. Edited by Sigel H, Sigel R, Sigel RKO. RSC Publishing; 2011:347-377.
- [27] Morris KV, Mattick JS: **The rise of regulatory RNA.** *Nat Rev Genet* 2014, **15**: 423-437.
- [28] Yusupova G, Reinbolt J, Wakao H, Laalami S, Grunberg-Manago M, Romby P, Ehresmann B, Ehresmann C: **Topography of the *Escherichia coli* initiation factor 2/fMet-tRNA(f)(Met) complex as studied by cross-linking.** *Biochemistry* 1996, **35**, 2978-2984.
- [29] Hostetter A, Chapman EG, DeRose VJ: **Rapid crosslinking of an RNA internal loop by the anticancer drug Cisplatin.** *J Am Chem Soc* 2009, **131**:9250-9257.
- [30] Chapman, EG, DeRose VJ: **Site-specific platinum crosslinking at a ribozyme active site.** *J Am Chem Soc* 2012, **134**: 256-262.
- [31] Hostetter AA, Osborn MF, DeRose VJ: **RNA-Pt adducts following cisplatin treatment of *Saccharomyces cerevisiae*.** *ACS Chem Biol* 2011, **7**:218–225.
- [32] Rijal K, Chow CS: **A new role for cisplatin: probing ribosomal RNA structure.**

Chem Commun 2009, 1:107–109.

- [••33] Rijal K, Bao X, Chow CS: **Amino Acid-Linked Platinum(II) Analogues Have Altered Specificity for RNA Compared to Cisplatin.** *Chem Comm* 2014, 50:3918–3920.

Binding sites of new Pt(II) compounds with amino acid chelating ligands were determined in comparison with cisplatin after treating renatured 16S *E. coli* ribosomal RNA. Sites in helix 24 were found to vary with the ligand type. This demonstration of Pt-ligand influence on sites in a structured RNA suggests new therapeutics based on rRNA sequence differences between bacteria and mammals.

- [••34] Osborn MF, White JD, Haley MM, DeRose VJ: **Platinum-RNA modifications following drug treatment in *S. cerevisiae* identified by click chemistry and enzymatic mapping.** *ACS Chem Biol* 2014, 9:2404–2411.

Pt(II)-RNA sites in RNA extracted from *S. cerevisiae* treated with cisplatin were probed by primer extension. Sites in the peptidyl transferase center and sarcin-ricin loop are consistent with Pt(II) access to purine-rich exposed regions. A new modified Pt(II) compound based on picoplatin was used to demonstrate *in vivo* Pt interactions with both rRNA and tRNA using post-treatment fluorescent labeling via click chemistry.

- [35] White JD, Haley MM, DeRose VJ: **Multifunctional Pt(II) Reagents: Covalent Modifications of Pt Complexes Enable Diverse Structural Variation and In-Cell Detection.** *Accts Chem Res* 2015, *in press*.

- [•36] Dedduwa-Mudalige GNP, Chow CS: **Cisplatin Targeting of Bacterial Ribosomal RNA Hairpins.** *Int J Mol Sci* 2015, 16:21392–21409.

Using mass spectrometry and DMS footprinting, Pt sites on *E. coli* rRNA were identified and shown cause non-local structural changes.

- [••37] Hedman HK, Kirpekar F, Elmroth SKC: **Platinum interference with siRNA non-seed regions fine-tunes silencing capacity.** *J Am Chem Soc* 2011, 133:11977–11984.

Pre-platinated siRNAs were tested for silencing activity with a Wnt-5a 5'-UTR-luciferase reporter system in HB2 cells. Platination resulted in lowered silencing efficiency of up to 7-fold in the case of oxaliplatin-siRNA. Interestingly, Pt-induced changes were observed in sequences for which the predicted Pt-RNA adducts were outside of the siRNA seed region, but could cause structural changes.

- [••38] Hsiao C, Chou I-C, Okafor CD, Bowman JC, O'Neill EB, Athavale SS, Petrov AS, Hud NV, Wartell RM, Harvey SC, Williams LD: **Iron(II) Plus RNA Can Catalyze Electron Transfer**. *Nature Chemistry* 2013, **5**:525-528.

The ability of Fe(II) to catalyze electron transfer when embedded in an RNA complex was determined using a peroxidase assay. Electron transfer from peroxide to TMB (3,3',5,5'-tetramethyl benzidine) was observed for Fe(II)-treated 23S rRNA, P4-P6, tRNA^{Phe}, but not ATP or unstructured oligonucleotides. The results are interpreted as support for a hypothesis that RNA evolved in an early anoxic, Fe(II)-rich environment and had transition-metal mediated enzymatic properties.

- [••39] Athavale SS, Petrov AS, Hsiao C, Watkins D, Prickett CD, Gossett JJ, Lie L, Bowman JC, O'Neill E, Bernier CR, Hud NV, Wartell RM, Harvey SC, Williams LD: **RNA Folding and Catalysis Mediated by Iron (II)**. *PLoS ONE* 2012, **7**:e38024.

A collection of results regarding the properties of RNA with Fe(II) in place of Mg²⁺. A DFT study comparing Mg²⁺ and Fe²⁺ models of a 'phosphate clamp' RNA binding site shows higher stability and polarization in the case of Fe²⁺. The ability of Fe²⁺ to support intricate RNA tertiary interaction is demonstrated using SHAPE to probe RNA structure in the P4-P6 domain. Finally, ribozyme catalysis in the hammerhead ribozyme is enhanced with Fe²⁺ in comparison with Mg²⁺.

- [•40] Popovic M, Fliss PS, Ditzler MA: **In vitro evolution of distinct self-cleaving ribozymes in diverse environments**. *Nuc Acids Res* 2014, **43**:7070-7082.

An in vitro evolution selecting for self-cleaving RNAs supported by either Mg²⁺ or Fe²⁺ finds that 'winning' sequence pools depend on metal ion. More Fe-selected RNAs work with Mg²⁺ than the inverse, supporting the hypothesis of early evolution in an Fe-rich environment.

- [•41] Theil EC: **IRE mRNA riboregulators use metabolic iron (Fe²⁺) to control mRNA activity and iron chemistry in animals**. *Metallomics* 2015, **7**:15-24.

A recent review of this critical class of Fe-mediated RNA regulatory elements, found in organisms from sponges to mammals, but not earlier life forms.

- [42] Piccinelli P, Samuelsson T: **Evolution of the iron-responsive element**. *RNA* 2007, **13**:952-966.

- [43] Petrov AS, Bowman JC, Harvey SC, Williams JD: **Bidentate RNA-magnesium clamps: on the origin of the special role of magnesium in RNA folding**. *RNA* 2011, **17**:291-297.

Figure captions

Figure 1. Riboswitches that respond to transition metals with high-selectivity.

A) Cartoon of a representative NiCo riboswitch showing disruption of Rho-independent termination by metal binding, permitting transcription elongation. Red: terminator sequence. B) Crystal structure of NiCo riboswitch from *Erysipelthichaceae bacterium* bound to Co^{2+} . Tinted regions indicate different stem loops. Zoomed region shows a network of interactions linking the Co^{2+} binding sites 1-3. C) Crystal structure of *L. lactis* Mn^{2+} -sensing riboswitch and Mg^{2+} , Mn^{2+} binding site. Zoomed region shows coordination of the ion by N7 of a strictly-conserved A41. Cyan spheres: water molecules.

Figure 2. Pt compounds and their nucleic acid adducts.

(Top) FDA-approved Pt(II) therapeutics have exchange-inert *cis*-diamine ligands, and Cl^- or oxo ligands that are exchanged with biomolecules. (Lower left) *bis*-guanine $[(\text{NH}_3)_2\text{Pt(II)}]$ adduct in a DNA oligonucleotide (PDB 3LPV). (Lower right) Pt(II) adducts predicted in an RNA subdomain [29].

Figure 3. Pt-RNA adducts in ribosomes.

(a) Pt(II) adducts in helix 24 (*E. coli* numbering) rRNA observed after cisplatin treatment. A major adduct observed in *E. coli* [32] is not seen in *S. cerevisiae* [31], due to a 5'C-G to 5'G-C substitution (boxes). (b) *S. cerevisiae* ribosome showing helix 18 (*E. coli* helix 24) in orange (PDB 3O30, 3O5H). (c) New amino acid-modified Pt(II) compounds (O/K; ornithine/lysine) reposition major Pt(II) adduct sites in *E. coli* bacterial rRNA [••33].

Figure 4. Fe(II)-RNA predicted structures and observed reactivity.

(Left) Comparison of DFT-optimized structures for Mg^{2+} - and Fe^{2+} -bound versions of a 'phosphate clamp,' originally observed crystallographically in an L1 RNA ligase [••39,43]. (Right) Electron transfer catalysis reported in Fe(II)-bound 23S rRNA, tRNA^{Phe}, and P4-P6 RNA, but not ATP or unstructured oligonucleotides [••38].

Figure 2

Figure 3

Figure 4

a. L1 ligase RNA-Mg²⁺ clamp

Optimized structures

Mg²⁺ model

Fe²⁺ model

b.