Impact

1. Provide the field with in *vivo-like* MCM conditions to help understand how RNA folds *in vivo*

2. Show changes in global RNA secondary structure stability

Experiments

1. Model metabolome/metalome

2. Use ITC to determine exact Kds at pH 7 and relevant ionic strength - Temperature

3. Use Draper dye (8-hydroxyquinoline-5-sulfonic acid) to confirm free and chelated Mg concen trations

4. Use fluorescence Tms to show changes in global RNA secondary structure stability + configurational stability

Side notes for discussion

1. Rouskin Nat. Commun. ~2014 How much energy would the helicase secondary structure weakening cost?

2. Swap single stranded and double stranded state in fluorescence layout

3. Write abstract – Wait on the intro

4. Martin Grueblee JACS communications

5. Mathew’s JACS communication

6. Figure 1 Change A to C and Show MCM

7. Add a cut off line Table 1

8. Commas SE, sig figs, MCM into Table 1

9. Intro, Pilak and coworkers begin with cell extracts. However, we will take a bottom up approach to complexity, with the intention of understanding speciation and molecular contribution.

10. Try 1B as a van’t hoff plot

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The Bacterial Metabolome and Metalome Weakens Global RNA Secondary Structure Stability and Protects RNA from Degredation

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KEYWORDS

Magnesium ion, Chelated magnesium, RNA folding, secondary structure, metabolites

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ABSTRACT: Herein, we examine the complicated network of interactions among RNA, the metabolome, and the metalome in conditions that mimic the *E. coli* cytoplasm. First, we determined Mg2+ binding constants for the top 15 *E. coli* metabolites, comprising 80% of the total metabolome, at physiological pH and monovalent ion concentrations. Then, we used this information to inform creation of artificial cytoplasms that mimic *in vivo E. coli* conditions, termed Eco80. We empirically determined that the mixture of *E. coli* metabolites in Eco80 approximates single sit binding behavior towards Mg2+ in the biologically relevant free Mg2+ range of ~0.5 to 10 mM Mg2+, using a Mg2+ binding fluorescent dye (8-Hydroxy-5-quinolinesulfonic acid). Furthermore, we examined the effects of Eco80 conditions on the thermodynamic stability, chemical stability, catalysis, and compactness of RNA. We find that these Eco80conditions lead to opposing effects, wherein thermodynamic stability of RNA helices were weakened but chemical stability, compactness, and catalysis were enhanced. We propose a mechanism where increased RNA compactness and catalysis is facilitated in Eco80.

Summary of progress on *in vivo-*like conditions. Studies that consider cellular components one at a time. Studies that consider cellular components together in artificial cytoplasm. In contrast, we take a bottom up, “aufbau”, approach that builds up complexity, in artificial cytoplasms. This aufbau approach allows us to understand the effects of most of the metabolite and metal ions species that compose the network of interactions that RNA experiences in *E. coli* cells.

First, we considered metabolites. *E. coli* cells contain hundreds of metabolites for a total metabolite concentration of about 243 mM, too many metabolites to test systematically . However, 15 abundant metabolites comprise 80% (194 mM) of total metabolites, and 15 metabolites is an experimentally manageable number (Figure 1A).

Next, we considered the metalome. As a negatively charged polymer, RNA folding and function is highly dependent on positively charged metal ion concentrations, especially divalent magnesium ions (Mg2+), as thousands of studies show. *E. coli* cells contain about 240 mM monvalent sodium (Na+), 140 mM divalent potassium (K+), and 54 mM Mg2+. Most Mg2+ in the cell is chelated, were Mg2+ is coordinated with only an estimated 0.5 to 3.0 fully hydrated, so called “free”, Mg2+ in *E. coli*.

Lasly, we considered how metabolites effect the speciation of free and chelated Mg2+. All 15 of the most abundant metabolites have functional groups, carboxylates and phosphates, that drive chelating interactions with divalent Mg2+ ions (Table 1), and we have previously estimated thatthe metabolite pool in *E. coli* has potential to chelate 51 mM Mg2+, assuming 2 mM free Mg2+, an ionic strength of 0.15 M and a pH of 7.5.

While extensive liturature exists on chelating interactions between Mg2+ and small molecules, our   
previous estimates are putative at best because Mg2+ chelation strength is highly dependent on enviromental factors such as pH, ionic strength, composition of background ions, and temperature. Thus, we sought to better characterize the Mg2+ chelation strength of the top 15 most abundant *E. coli* metabolites at the physiological ionic strength of 390 mM, physiological pH of 7.0, and physiological temperature range of 25°C to 50 °C.

**FFigure 1.** The top 15 metabolites in *E. coli* bind to Mg2+. **(A)**

Isothermal titration calorimetry (ITC) was used to determine inverse molar association constants for 9 of the 15 most abundant *E. coli* metabolites (Figure 1B & C).

The 6 remaining metabolites were not amenable to isothermal titration calorimetry because their interactions with Mg2+ are very weak and require high, molar, concentrations of reagents making heat of dilution a large portion of the ITC signal.

**Figure 2.** Mg2+ speciation at cellular pH and ionic strength.

**Figure 2**. The temperature dependence of metabolites binding Mg at cellular pH and ionic strength.

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**Figure 5.** Cartoon depiction of the three way network of RNA, metabolite, and Mg2+ interactions.

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ABBREVIATIONS

CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; TLC, thin layer chromatography.

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