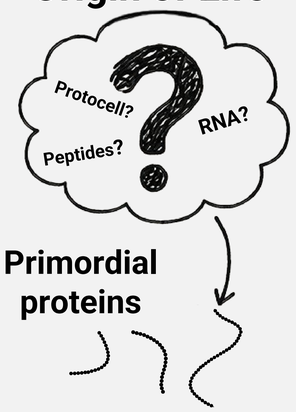
Did Early Functional Proteins Use Metal Ions to Bind ATP?

Alexander D. Soltau, Eli Blascyk, Peter J. Winslow, and Burckhard Seelig

Department of Biochemistry, Molecular Biology and Biophysics & Biotechnology Institute, University of Minnesota, St. Paul, MN 55108, USA

Introduction

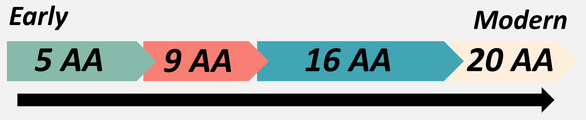
Origin of Life



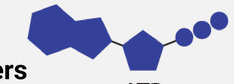
Primordial proteins

Proteins -> Chains of amino acids

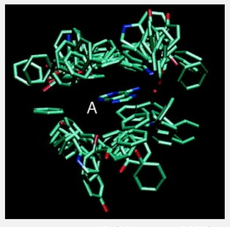
- Proteins are building blocks of life
- **20** canonical amino acids
- Trifonov et al.[1] **order of amino acids**
- Four libraries of **random** proteins[2]
  - Only **5, 9, 16, or all 20** amino acids



mRNA display selection:
- Stick to ATP → **ATP Binders**

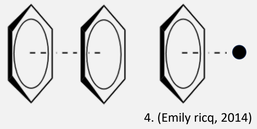


- **How** do they bind ATP?
- **Effect** of divalent metals?



3. (Mao et al., 2004)

- 5AA library lacks aromatic and (+) amino acids
- No π-π and cation−π interactions

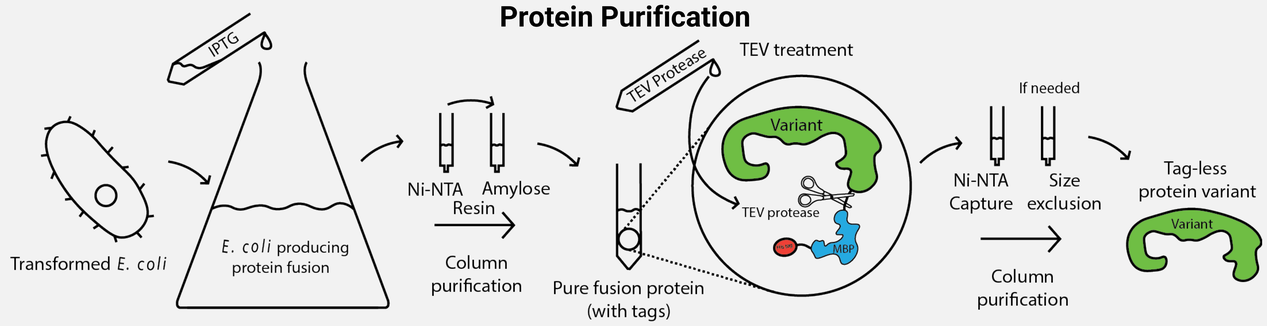


4. (Emily ricq, 2014)

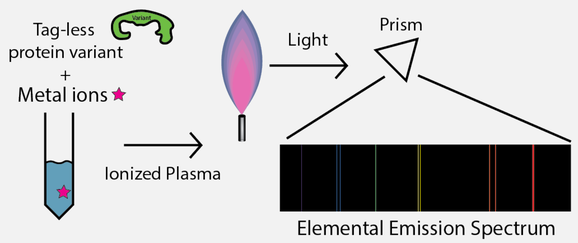
We hypothesized that magnesium ions, commonly used in nature to coordinate the phosphate groups of ATP, may also be utilized by our primordial-like model proteins.

Methods

Protein Purification

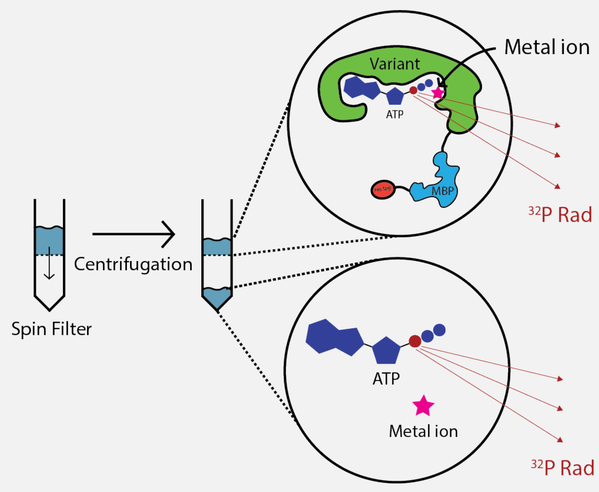


Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)



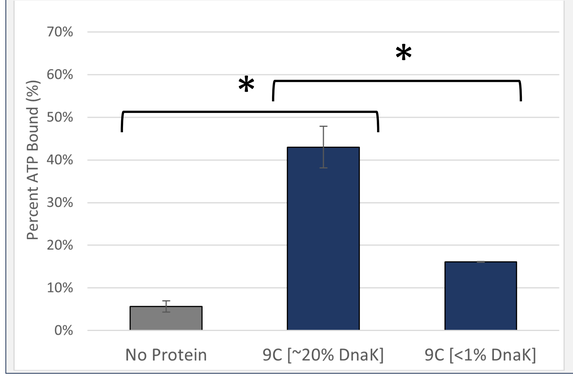
- **8 divalent metal ions** (Ni^{2+}, Ca^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Mo^{6+}, and Mg^{2+}) tested
- Triplicate samples with +/- SEM
- Metals at 50 uM except Mg2+ at 400uM
  - 7.5uM protein and 1 nM 32P-ATP

^{32}P ATP-Binding Assay

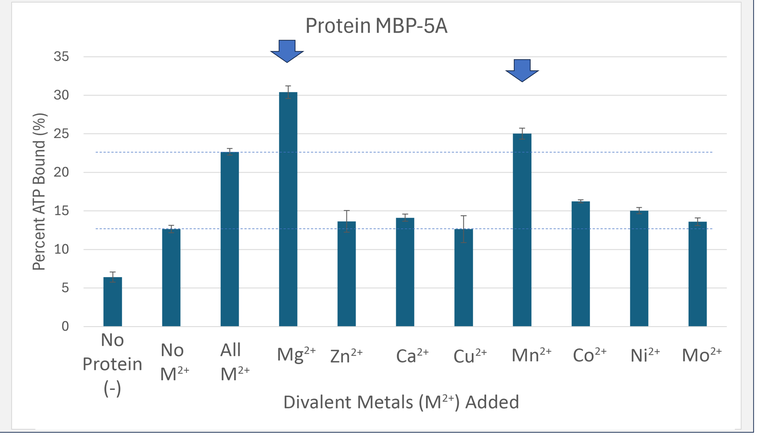


Results

- 9C's ATP-binding was 27% higher with ~20% DnaK
  - Pairwise student's t-tests:
  - 9C [~20% DnaK] vs no protein: **p=0.007**
  - 9C [~20% DnaK] vs 9C: **p=0.002**
  - No protein vs. 9C: p=0.059



- **Magnesium** and **manganese** both recovered ATP-binding in 5A and 5D
- Minimal effect from other divalent metals
- Intermediate ATP-binding of no M^{2+} control



Conclusions

- **Magnesium** and **manganese** both recovered or improved ATP-binding in 5A and 5D
  - Also utilized by DnaK, could be the result of contamination
  - ATP-binding of **no M^{2+}** condition could be due to trace metals in sample
- Preliminary **ICP-OES** results were **inconclusive**
  - Dialysis was incomplete
- Strange proteins had strange behavior
  - **Highly negatively charged** peptides may have had an early evolutionary role in coacervates

Future Directions

- **Validate** metal dependent ^{32}P ATP-binding affinity with a **DnaK KO** strain (EN2)
- **Measure** ^{32}P ATP-binding affinity of **dialyzed protein control**
- **Repeat** ICP-OES trace metal testing with new metals stock solutions, multiple sample replicates, and multiple rounds of dialysis for **more stringent removal of metal ions**
- **Measure** ^{32}P ATP-binding affinity of protein fragments to determine **minimal binding domains**

Acknowledgements

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References:

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