Reproduction Dynamics in Three Genotype RNA Networks

By: Sanjay Ramprasad
Are We Alone?

- What is the distribution of life in the universe?
- How did life originate on Earth 4 billion years ago?
These results show that a mixture of a variety of organic compounds including amino acids could form spontaneously in the primitive atmosphere.
The RNA World

• Discoveries since the early 1980s have demonstrated that RNA can fold into complex structures, catalyze chemical reactions or bind to another molecule for either activation or deactivation in addition to carrying genetic information.
• The key step in the origin of life was the evolution of a molecule that could copy itself.
• RNA becomes the prime suspect for the earliest self-replicating molecule.
Azoarcus Ribozyme

- **Azoarcus** tRNA\textsubscript{Ile} group I intron
- Partitioned into W,X,Y,Z fragments
- Can self-assemble into covalently contiguous ribozymes via RNA-directed recombination events.
- Fully assembled ribozyme catalyzes its own reproduction through the recombination of fragments of itself.

Group I Intron Splicing

- Group I introns catalyze their own excision from nascent RNA transcripts.
- Splicing is processed by two sequential transesterification reactions.
- Two-metal-ion mechanism
Two-Piece Self-Assembly Reaction

Yeates et al. PNAS 2016
Experimental Self-Assembly Rate Constants

- Raw data for the self-assembly rates for 16 genotypes where the middle nucleotide (M,N) are varied.
- Rates were measured by doping 0 μM, 0.5 μM, 1.0 μM, or 2 μM full length WXYZ into reactions containing 1 μM WXY and 1 μM Z fragments.
- 10 genotypes selected for the purposes of this study: CG, GA, GG, AC, UU, AU, UC, GU, AA, CA

Table 1. Rate constants, $k_a (\text{min}^{-1})$, for the 16 genotype variations of WXY.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$k_a (\text{min}^{-1})$</th>
<th>Std. error</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>0.0415</td>
<td>0.0066</td>
<td>0.98</td>
</tr>
<tr>
<td>AU</td>
<td>0.0319</td>
<td>0.0011</td>
<td>1.00</td>
</tr>
<tr>
<td>UA</td>
<td>0.0197</td>
<td>0.0004</td>
<td>1.00</td>
</tr>
<tr>
<td>GC</td>
<td>0.0125</td>
<td>0.0021</td>
<td>0.97</td>
</tr>
<tr>
<td>GU</td>
<td>0.0091</td>
<td>0.0007</td>
<td>0.99</td>
</tr>
<tr>
<td>AC</td>
<td>0.0069</td>
<td>0.0002</td>
<td>1.00</td>
</tr>
<tr>
<td>UG</td>
<td>0.0049</td>
<td>0.0004</td>
<td>0.99</td>
</tr>
<tr>
<td>UC</td>
<td>0.0038</td>
<td>0.0002</td>
<td>1.00</td>
</tr>
<tr>
<td>UU</td>
<td>0.0022</td>
<td>0.0001</td>
<td>1.00</td>
</tr>
<tr>
<td>CA</td>
<td>0.0020</td>
<td>0.0000</td>
<td>1.00</td>
</tr>
<tr>
<td>CC</td>
<td>0.0016</td>
<td>0.0001</td>
<td>1.00</td>
</tr>
<tr>
<td>GG</td>
<td>0.0006</td>
<td>0.0001</td>
<td>0.99</td>
</tr>
<tr>
<td>GA</td>
<td>0.0005</td>
<td>0.0001</td>
<td>0.98</td>
</tr>
<tr>
<td>AA</td>
<td>0.0004</td>
<td>0.0001</td>
<td>0.92</td>
</tr>
<tr>
<td>CU</td>
<td>0.0004</td>
<td>0.0000</td>
<td>1.00</td>
</tr>
<tr>
<td>AG</td>
<td>0.0001</td>
<td>0.0000</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Yeates et al. PNAS 2016 SI
3 Player Networks

(26) All possible 3-player networks for the 10 genotypes being studied given no two intra-species interaction has an equivalent rate constant within a network.
Simple Graphs

- Graph theory is a branch of mathematics that studies the topological artifacts of pairwise interactions between elements in a discrete set.
- Small 3 genotype networks graphs can be simplified by concentrating on the 3 most dominant interactions in a given network. These dominant interactions give rise to equilibrium dynamics.
- Nodes represent a specific genotype and edges are vectors that represent the magnitude and direction of each inter-species interaction.
Graph Topologies for 3 Genotype Networks

17 possible topologies given the constraint that any composed graph that is superimposable upon another via a rotation or a flip is isomorphic.

Topologies represented among the 26 3-player networks:
2,3,4,6,7,8,9,11,12, and 13

4. GG/AC/CA
6. AC/GU/CG
9. UC/GU/CA
11. GG/AC/UU
12. CA/AC/GU
Serial Dilution Experiments

- **WXY** fragments prepared via *in-vitro* transcription from DNA plasmid templates, radiolabeled with γ-³²P-ATP, and gel purified. **Z** fragments are purchased from a biotechnologies vendor.

- Three **WXY** genotypes are mixed at equal molar ratio and provided equal molar **Z** fragments, and then reacted for 1 minute while incubating at 48°C. At this time, 10% is transferred to a second tube in which new raw materials are provided concomitant with the chemical quenching of the remaining reaction in tube 1. This procedure is repeated over 8 transfers at the following time points in minutes: 1, 5, 10, 15, 20, 25, 30, 40.

- Results are quantified by gel electrophoresis and phosforescence imaging.
Hypothesis and Expected Results

Proposed: Simple graphs can be used to predict dynamic equilibrium in small 3 genotype RNA networks.

Models will be verified through empirical demonstration involving *in vitro* experimental methods.
Network Experiments

GU Self-Assembly: 0.0091 μM/min
AC/GU Cross-Assembly: 0.0125 μM/min
GU/AC Cross-Assembly: 0.0319 μM/min
Acknowledgments

My mentor and PI, Dr. Niles Lehman

Dr. Jessica Yeates, Sr. Researcher in the Lehman Research Group

The Lehman Research Group

Department of Chemistry, Portland State University

Richard and Helen Philips and the Carl Wamser Student Summer Research Scholarship Committee

Special Thanks to the Oregon NASA Space Grant Consortium for funding this work