DYNAMIC EQUILIBRIA MAY PROVIDE A PREBIOTIC ROUTE TO INCREASING COMPLEXITY

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Introduction: To understand the extent to which complexity can emerge in an RNA World and how it might be effected by peptides or amino acids, we are pursuing a novel experimental approach based on dynamic combinatorial chemistry (DCC)[1,2]. It is hypothesized that when subject to a persistent equilibrium of ligation and cleavage, RNAs will naturally increase in complexity while gaining resistance to degradation over time. It will be of immense interest to see if this equilibrium or the pathways towards increasing complexity are strongly affected by the presence of amino acids or peptides. To obtain such equilibrium, we are using a two enzyme system. The cleavage enzyme is Benzonase [3], which is the commercial name for an extracellular endonuclease secreted by *Serratia marcescens*. This enzyme cleaves RNA, including circular forms, to produce products with a 3' hydroxyl and 5' phosphate. This is ideal for ligation by T4 RNA ligase [4], which requires these exact ends and utilizes ATP as a source of energy. In order to monitor population changes, samples are extracted from the reactions mix and millions of individual RNAs sequences are determined using RNA-seq technology on an Illumina NextSeq 500 system [5].

Experimental setup and preliminary results: Initial experiments verified the behavior of both enzymes and established a mutually compatible buffer system. An initial DCC experiment was then conducted. It was expected that initially the sequence pool should be dominated by ligations of the original RNA. However, as the DCC churns, these products should be replaced by a greater mixture of sequences. A 180 minute equilibrium experiment was conducted using a defined sequence 20-mer as the starting RNA. Gel analysis suggested that the RNA was initially ligated, as expected as large products were seen. These persisted for 120 minutes after which their quantity began to diminish, presumably because the Benzonase was still active while the ligase likely began to run out of ATP. Consistent with the DCC interpretation, sequence analysis revealed that initially the dominant products were ligations of the 20-mer to itself. However, as time passed these sequences began to be replaced by others. Detailed analysis of the sequencing data is ongoing and will further clarify what is happening in the equilibrium. In the immediate future, we will scale up the reaction and extend the life of the equilibrium by adding ATP at regular intervals. In addition a random 20-mer will be used as the initial starting RNA.

References:

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