Selection of a Ribozyme Capable of Exonuclease Activity

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Short Abstract — The protocell is the simplest theoretical cell and would consist of only genetic material encapsulated by a membrane. With just these tools, the protocell must perform genetic replication and cell growth/division; functions normally regulated/catalyzed by protein. The synthesis of a functional protocell is an important area of research for its relevance to the origins of life and to synthetic biology, however, several limitations are preventing its realization. A key limitation is the near inability of non-enzymatic primer extension to recover from mismatches. Thus, a ribozyme capable of mismatch recognition and repair is of critical importance for enabling the speed and fidelity of genetic replication demanded by a functional protocell. We will apply SELEX and a unique selection scheme towards discovering such a ribozyme. This ribozyme will be encapsulated in a fatty acid membrane and its ability to improve genetic replication in a protocell-like environment will be demonstrated.

I. Purpose

The RNA World hypothesis is the theory that RNA was the precursor to both protein and DNA [1]. The first protocell would theoretically consist of a self replicating ribozyme and a fatty acid membrane. The construction of such a protocell would both give insight into the origins of cellular life and lead to the development of new tools for synthetic biology. The development of a protocell from its most basic components represents a "bottom-up approach" to synthetic biology. Instead of trying to reduce the complexity of a cell, by removing nonessential genes [2], our aim is to increase complexity of a cellular system from the basic building blocks of life.

Formation of protocell-computable vesicles has been accomplished [3] and the evolution of a ribozyme that can perform short primer extension has been accomplished, but a protocell has not yet been produced due to various obstacles [4]. One such obstacle is that the basic self-replicating ribozyme will not have the error correction property that most DNA polymerases have. Therefore, if an incorrect nucleotide is added to the chain, the speed and fidelity of RNA copying will be significantly reduced, hindering the cell's growth and division. To address this limitation, we are performing the *in vitro* selection of an RNA catalyst capable of recognizing and cleaving mismatched base pairs in double stranded RNA. Such a ribozyme would be crucial for the development of a competent protocell.

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II. Proposed Methods

To select for ribozymes with mismatch recognition and repair activity, we have constructed RNA featuring a 60-nucleotide long semi-random sequence from a fused to an RNA sequence designed to overlas with template RNA featuring an engineered terminal mismatch. The semi-random region has patterned and random segments, such that the RNA is more likely to form secondary structures.

A. Selection Scheme

The selection scheme works as follows: The RNA construct is incubated with the RNA template and a short biotinylated RNA strand complementary to the remainder of the template strand. The construct recognizes the template and binds, but with a one base pair mismatch at the end. If the random sequence has exonuclease activity, the mismatch will be cleaved off. After the incubation, a ligase is added to the reaction, so that if the mismatched base pair was removed, the biotinylated RNA strand will be ligated to the RNA with exonuclease activity. The stringency of the selection will be gradually increased by decreasing in length of incubation each round. Repeated rounds of selections will ultimately afford a small group of RNAs capable of recognizing and removing mismatches in RNA duplexes.

B. Activity Assay

After several rounds of positive selection, we will test the activity of the enriched sequences. To accomplish this, we will incubate the ribozymes connected in *cis* with the complementary template sequence with a mismatch at the end with the RNA template, and a short RNA sequence that is fluorescently labeled. We expect to see an increase in activity after each round of selection, as an increase in the fluorescence intensity on a gel.

III. Conclusion

We will perform the *in vitro* selection of a ribozyme with exonuclease activity through preferential ligation and selection of RNAs that remove a mismatched base pair on a template sequence. This will not only be applicable in studying the Origin of Life as a possible method for speeding up primitive RNA copying, but it will also be instructive for future selections of ribozymes with other possible activities.

References

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