Selection of a Ribozyme Capable of 2',3' Cyclic Phosphatase 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. A functional protocell must perform several key tasks including genetic replication, cell growth, and cell division. Although the relative simplicity of a protocell prohibits protein catalysts, ribonucleic acid (RNA) catalysts represent attractive alternatives. 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 challenge for the replication of protocells is that the thermodynamically favored mechanism of base pair mismatch cleavage leads to the formation of a 2',3' cyclic phosphate which blocks any further extension of the RNA strand. Thus, a ribozyme capable of recognizing and converting a 2',3' cyclic phosphate into a free 3' OH group capable of extension is of critical importance for enabling 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 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. The favored mechanism of RNA base pair mismatch cleavage leads to the formation of a 2',3' cyclic phosphate which blocks any further extension, compromising the replication of the genetic material To address this limitation, we are performing the *in vitro* selection of an RNA catalyst capable of recognizing and cleaving a 2',3' cyclic phosphate into a free 3' OH group. 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 76-nucleotide long semi-random sequence from a fused to an RNA sequence designed to overlaps with template RNA. The construct features on the 3' end the sequence for an HDV ribozyme which will cleave itself off leaving a 2',3' cyclic phosphate at the end of the construct. 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 library is incubated so that the random region, if active will replace the 2',3' cyclic phosphate with a free 3' OH. A complimentary RNA template and a short biotinylated RNA substrate complementary to the remainder of the template are then incubated with the construct. RNAs with 2',3' cyclic phosphate recognition will convert the 2',3' cyclic phosphate into a 3' OH. A ligase is added to the reaction after an incubation period. If the 2',3' cyclic phosphate was converted into a 3' OH, the ligase can covalently link the active RNA with the biotinylated substrate. However, if the 2',3' cyclic phosphate was not recognized and converted by the RNA, then the ligation cannot occur. The biotinylated substrate is then pulled out the solution and moved into another round of selection. Repeated rounds of selection will result in the enrichment of RNAs with the desired 2',3' cyclic phosphate recognition activity. The stringency of the selection will be gradually increased by decreasing the length of incubation each round. After several rounds, we will test the enriched pool for the desired activity.

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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