The Decorated RNA Supermolecular World and A Method to Develop Novel Ligands and Catalysts

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In recent years, continuous discoveries of catalytic RNA and DNA by the *in vitro* selection method greatly support the RNA World theory[1], which assumes that the chemical process leading to the appearance of life was carried out by RNA. Trying to overcome the structural limitation of the four bases, ingenious strategies such as introduction of coenzymes and unnatural bases [2] have been developed. However, it seems that the ultimate catalytic potential of nucleic acid has yet to be fully exploited. Does this imply that the structural types of these ribozymes generated by *in vitro* selection are only a small portion of the vast existing ribozymes in the RNA (DNA) World? In the RNA library for *in vitro* selection, the RNAs are generally single-strained "pure" nucleic acid in similar length. This is unlikely to occur in the pristine world. These postulations raise an interesting question:

Must the ribozyme in the RNA World be a single-strained "pure" RNA? Could it be a supermolecular system consisting of several partially complementary RNA strands, among which, some would be covalently modified by other molecules?

Orgel's report on the *genetic take over* [3] of nucleic acid shows that many short complementary nucleic acids of different length have been generated by a long nucleic acid template during its self replication. This experiment suggests that in the primitive soup of nucleic acids, there were not only many long nucleic acids but also more short nucleic acids which are complementary to these long nucleic acids since at that time the fully developed RNA polymerase had not evolved yet. Thus the generation of nucleic acid

supermolecular systems (Fig.1) should be a general phenomenon. Therefore in the primitive world, some enzyme-like nucleic acids might be single stranded RNA while other such nucleic acids might be supermolecular systems which are consisted of several partially complementary RNA strands, just as some protein enzymes are consisted of several subunits.

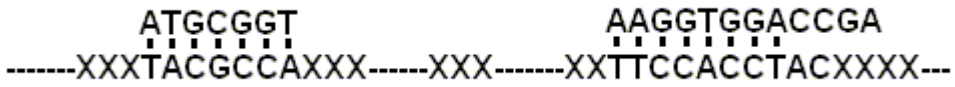

Figure 1. Self-assemble RNA Supermolecular System (x: varied nucleotides)

The advantage of the self-assemble strategy is that sophisticate structures and higher diversity can be generated from fewer and smaller building blocks, a strategy similar to that antibody employs for more functions. The tradeoff is that lower rigidity of the structure may cause lower efficacy, therefore building blocks other than nucleotides are necessary to compensate the efficacy.

Since the primitive soup contains both amino acids and nucleic acids, there should be some chemical connections between these two main kinds of bio-molecules. First, research [4] shows that phosphoryl amino acids can generate short peptide decorated nucleic acids in mild environment, indicating that some chemical activators might generate the hybridization of peptides and nucleic acids in primitive world. Secondly, the discovery of amino acid transfer ribozyme[5] reveals the possibility of the existence of nucleic acid-amino acid conjugation (Fig.2). Since more and more ribozymes that catalyze alkylation, acylation, cycloaddition reactions on the RNA substrate [6] producing RNA-other molecule conjugation have been discovered, we expect that some other small molecules decorated RNAs (Fig.3) could also exist in the primitive world. Some long RNAs from these conjugations may have new catalytic functions, because introducing new function groups would overcome nucleotides' structural limitation and give propensity of generating novel activity.

Fig.2 RNA -amino acid conjugation

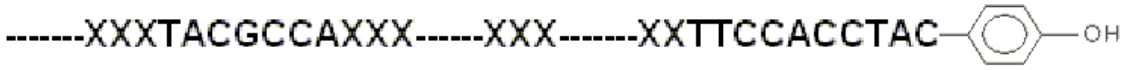

Fig.3 RNA-small organic molecule conjugation

Currently most artificial ribozymes' catalytic activities are self-modification. An enzyme which can self modify and generate other catalytic function thereafter is rare. In the primitive world, a probable strategy might be that: one group of ribozymes modify short, partially complementary nucleic acids with amino acid (Fig.2) or other small organic molecules. The modified short nucleic acids then self assemble with other long nucleic acids to form supermolecular systems (Fig.4). Some of these supermolecular systems would have ability to catalyze special reactions. This postulation could be supported by (or explain) the fact that most current catalytic RNAs need a RNA fragment as substrate.

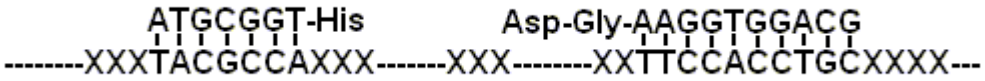

Figure 4 An amino acid decorated RNA system, which can be regarded as a RNA that can utilize RNA coded amino acids cofactors

If life did originate from the RNA world, a mixed and modified RNA world would be more plausible than a single strand, pure RNA world. In fact, the first discovered ribozyme is also a supermolecular system consisting of not only RNA, but also Mg^{2+} and guanosine. Recent discovery that the RNA-RNA interaction can be an activator for RNA synthesis shows us again the power of complementary base pairing [7]. It is reasonable to assume that nature would use such supermolecule to build more sophisticated ribozymes, since it is such a wonderful tool for biofunctions.

Along the evolution course, the amino acids and peptides on the nucleic acid backbone would become longer and longer while the nucleic acid parts would become shorter and shorter and eventually vanish completely. Then the evolution from ribozyme to protein enzyme would complete. Therefore, we may postulate that some of the modern intron could be the relic of these complementary nucleic acids fragments, and that the peptide modified RNA (DNA) would be the missed link between the RNA (DNA) enzyme and protein enzyme.

A catalytic DNA using amino acid as cofactor has been discovered recently [2]. It is more convenient that ribozymes utilize DNA coded cofactors (Fig. 5) rather than a cofactor alone to expand their catalytic potential. Today, there are still many nucleotide-based coenzymes such as NADH and other conjugation such as tRNA, which may be the relic of these kinds of original RNA (DNA) cofactor conjugations.

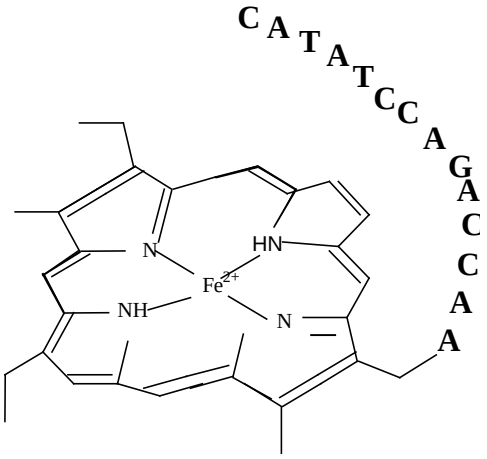

Fig.5 A DNA-porphyrin conjugation as a DNA coded cofactor

In *in vitro selection* for new catalysts and useful ligands, DNA coded cofactor may also help. For a RNA (DNA) library such as:
----XXXXXXXXATTGGCTTCGXXXXXXXXTATACCCXXX---, we can add chemically synthesized complementary RNA, DNA, or PNA (peptide nucleic acid) fragments such as **TAAXXXXCCGAA-Gly, ATATGGG-Ala**, and **ATATGGG-His**

into the system to form a supermolecular library. The supermolecular structure may have superior affinity or catalytic activity due to the amino acid parts.

There are infinite new structures such as unnatural small molecules, chelate-metallic ions and coenzymes, and large systems such as C_{60} and nanoparticles even molecular devices. They can be linked to nucleic acid fragments by organic synthesis easily. By introducing these new building blocks into *in vitro selection* system (Fig.6), we may be able to do better than nature to create more novel functions.

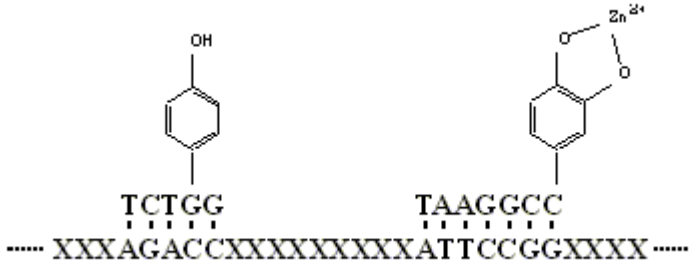

Figure 6 A decorated DNA supermolecular library containing novel functional groups for new activities

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