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The Dual Life of RNA

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Abstract. Molecular biology techniques have enabled us to prepare and select RNA aptamers that can bind specifically to small targets. RNA oligonucleotides can also be used as fluorescent probes. We have combined the two approaches to obtain Aptamer Beacons, in which molecular recognition is linked to the emission of an optical signal. These RNA biosensors could be used to detect directly the signatures of life in samples of mineral and extra-terrestrial material.

1. Introduction

Life as we know it can be viewed as a complex system based on the cooperation of two kind of biopolymers: nucleic acids and proteins. Protein enzymes catalyze the biochemical reactions of cellular metabolism. DNA, which contains the information encoding the sequences of RNAs and proteins, is the template for their replication and transcription. RNA directs protein synthesis and some RNA molecules, the ribozymes, can, like proteins, have catalytic activity. RNA is involved in many key cellular functions in all living cells and it has been argued that RNA played a predominant role in early life (Meli et al. 2001). There are several lines of evidence in support of this. First, ribonucleotides are the metabolic precursors of 2'-deoxyribonucleotides, uracil is the metabolic precursor of thymine, and DNA replication needs RNA primers. Second, the enzymes involved in protein metabolic often need ribonucleotide cofactors (AMP, FAD, NAD, CoA, etc.), and ever increasing numbers of cell RNAs (including ribosomal RNA) are being found to be catalytic *per se*. Hence, the metabolic reactions in modern cells are catalyzed not only protein enzymes but also ribonucleic acid ones, the ribozymes. This picture suggests that RNA might have predated DNA and proteins in evolution, and that our modern ribozymes are the remnants of an ancient RNA world (Gilbert 1986; Maurel 1992; Gesteland et al 1999; Jeffares, Poole, & Penny 1998). This RNA world hypothesis assumes that modern life forms arose from a molecular ancestor in which RNA both stored genetic information and catalyzed chemical reactions. This hypothesis requires three assumptions about the early periods of evolution. First, genetic continuity was ensured by RNA replication; second, replication was based on weak links (Watson-Crick type pairings); and third,catalysis was performed by small non-genetically-coded peptides and by RNA (Joyce & Orgel 1999).

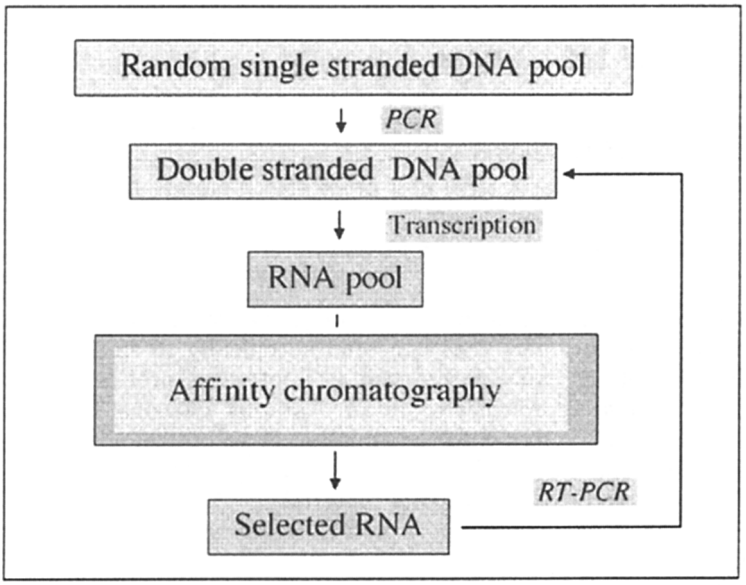

Figure 1. Typical selection scheme (from Wilson & Szostak 1999). Legend: A random single stranded DNA pool was amplified by PCR (polymerase chain reaction) to obtain a pool of double stranded DNA. Transcription of this DNA led to the RNA pool, which was submitted to selection by affinity chromotography. The selected RNA was then back-transcribed into DNA, and the whole cycle begun all over again. The low specificity of the reverse-transcriptase enzyme meant that several mutations could occured during this step. The system went through several rounds of selection, amplification and mutation. Lastly, we cloned and sequenced selected molecules.

2. RNA Selectivity and Adaptability

In vitro selection (SELEX: Systematic Evolution of Ligands by Exponential Enrichment) can be used to test the validity of the *RNA world* hypothesis. This technology exploits the Darwinian behavior of randomized, replicable populations of RNA considered as populations of species. The populations of molecules are made to go through several rounds of selection, amplification and mutation, which causes them to evolve interesting properties. Appropriate selection criteria can be used to select molecules having the best aptitudes that will enrich the following generations (Tuerk & Gold 1990; Joyce 1994).

It is thus possible to generate a few selected molecules, aptamers, from a starting population of at least 10^{15}-10^{16} variants. Many RNA aptamers, including catalytic ones, have already been isolated using diverse SELEX protocols.

Aptamers with high, specific affinities for diverse targets, ranging from small molecules such as nucleotide bases or aminoacids, to bigger structures such as proteins and cells have been selected.

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Adenine is a prebiotic compound that is easily synthesized by HCN tetramerisation (Orò 1961). It has been detected in the Murchison meteorite (Stocks & Schwartz 1981) and adenylated compounds are ubiquitous in the metabolism of living cells (AMP, FAD, NAD, CoA, etc.). We have shown that adenine reactivity is equivalent to histidine, an amino acid well represented at the active site of numerous enzymes (Maurel & Ninio 1987; Maurel & Convert 1990).

RNA aptamers capable of complexing free adenine have been isolated by a SELEX procedure : the adenine binding site is composed of two interdependent secondary structural elements forming a bipartite binding site that interacts with adenine in a new mode of purine recognition (Meli et al. 2002).

This binding is of great interest, since the imidazole moiety is not trapped in the binding site but remains readily available for catalytic activity. More recent studies have selected hairpin ribozyme aptamers that are dependent on adenine for catalyzing their own reversible cleavage. Such adenine-binding aptamers may well mimic the substrate binding employed by early "ribo-organisms" that lived on abiotically synthesized adenine "food" (Meli et al. 2003).

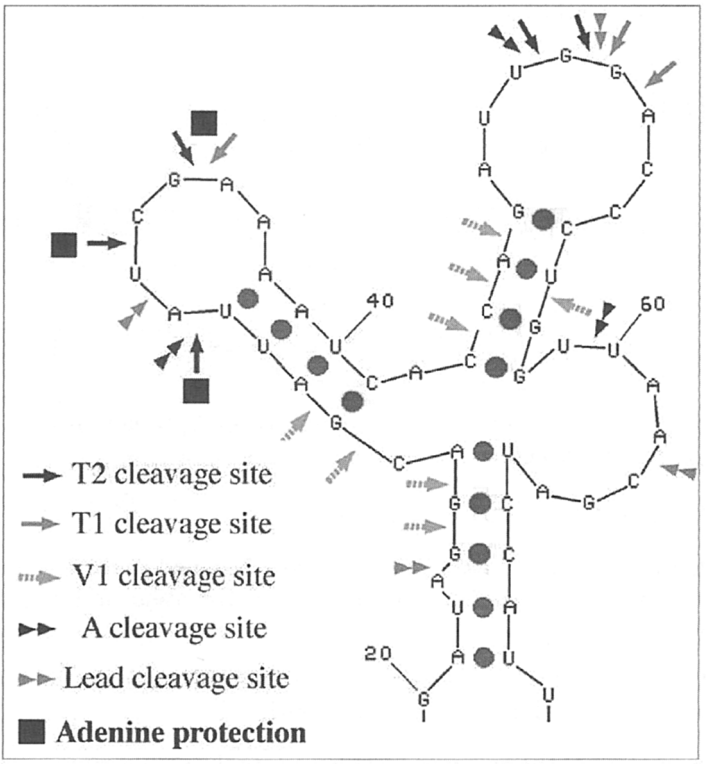

Figure 2. Aptamer adenine binding site.
Legend: Rnase mapping of the apatmer. Cleavage sites were experimentally obtained with Rnases T1, T2 and V1 in the presence and absence of adenine, showing that adenine can protect the aptamer against cleavage by Rnase.

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3. Aptamer Beacons as Biosensors for Detecting Biosignatures

Aptamers can discriminate between targets on the basis of subtle differences such as the presence or absence of a methyl (CH_3), or a hydroxyl (OH) group; they can also distinguish between D/L enantiomers targets (Nolte et al. 1996; Klussmann et al. 1996). They display a very high degree of specificity, sometimes even better than that of antibodies, and they can adopt two or more conformations, one of which allows ligand binding. Thus aptamers are biological tools which can be used as biosensors to detect organic molecules in meteorites. For instance, if a meteorite is believed to contain 1 ppm of purines (i.e., 0.25 μg adenine/g of meteorite or 2 nanomoles of adenine/g of meteorite) , it is now possible to detect them by a selection procedure that employs aptamers that recognize adenine trapped in minerals. A fluorophore can be bound to the selected aptamer to provide a beacon. Aptamer beacons are hairpin-oligonucleotide probes derived from target binding aptamers. They are flexible biosensors that can directly transduce molecular recognition into an optical signal (Potyrailo et al. 1998; Frauendorf & Jaschke 2001).

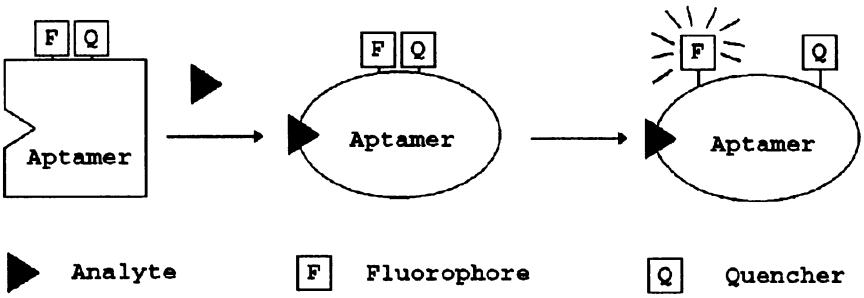

Figure 3. Transduction of a signal by an Aptamer Beacon (Analyte is a specific target).

The transduction occurs as follows:

a. In the absence of target, aptamers are designed to form a stem-loop structure with a fluorophore on one end of the stem and a quencher on the other.

b. The stem is broken, separating the fluorophore from the quencher. Consequently, the fluorescence is restored.

c. Formation of the aptamer complex alters the equilibrium between quenched and unquenched structures, generating a change in fluorescence intensity.

These aptamer beacons can generate a specific fluorescent signal when they detect their own target. RNA beacons can be produced from aptamers that are specific to aminoacids, nucleotide bases, or sugars so as to obtain powerful

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biosensors for detecting very small amounts of a range of organic molecules in any sample.

Our aim is now to prepare a library of aptamer-beacons that are capable of recognizing a number of target molecules. The optical signals emitted by an aptamer beacons when it is placed in contact with a rock-extract or a meteorite containing the target would reveal its presence. Such sensitive tools could help us to detect the signatures of life.

References

Frauendorf, C., & Jaschke, A. 2001, Bioorg. Med. Chem., 9, 2521

Gesteland, R. F., Cech, T. R., & Atkins, J. F. 1999, in The RNA world, (N.Y.: Cold Spring Harbor Laboratory Press), 37

Gilbert, W. 1986, Nature, 319, 618

Jeffares, D. C, Poole, A. M, & Penny, D. 1998, J. Mol. Evol., 46, 18

Joyce, G. F. 1994, Curr. Opin. Struct. Biol., 4, 331

Joyce, G. F., & Orgel, L. E. 1999, in The RNA world, (N.Y.: Cold Spring Harbor Laboratory Press), 49

Klussmann, S., Nolte, A., Bald, R., Erdmann, V. A. , & Furste, J. P. 1996, Nat. Biotechnol., 14, 1112

Maurel, M-C., & Ninio, J. 1987, Biochimie, 69, 551

Maurel, M-C., & Convert, O. 1990, Origins of Life Evol. Biosphere, 20, 43

Maurel, M-C. 1992, J. Evol. Biol., 2, 173

Meli, M., Albert-Fournier, B., & Maurel, M-C. 2001, Int Microbiol., 4, 5

Meli, M., Vergne J., Decout, J. L., & Maurel, M-C. 2002, J. Biol. Chem., 277, 2104

Meli, M., Vergne J., & Maurel, M-C. 2003, J. Biol. Chem. 278, 9835

Nolte, A., Klussmann, S., Bald, R., Erdmann, V. A., & Furste, J. P. 1996, Nat. Biotechnol., 14, 1116

Orò, J. 1961, Nature, 191, 1193

Potyrailo, R. A., Conrad, R. C., Ellington, A. D., & Hieftje, G. M. 1998, Anal. Chem., 70, 3419

Stocks, P. G., & Schwartz, A. W. 1981, Geochimica et Cosmochimica Acta, 45, 563

Tuerk, C., & Gold, L. 1990, Science, 249, 505

Wilson, D. S., & Szostak, J. W. 1999, Annu. Rev. Biochem., 68, 611

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