Smith and Franklin Briefs in Biosciences

Pramod Yadava

Aptamers: Emerging Applications

Smith & Franklin
Academic Publishing Corporation
www.smithandfranklin.com

Pramod Yadava

Applied Molecular Biology Laboratory,
School of Life Sciences,
Jawaharlal Nehru University,
New Delhi 110067,
INDIA

Contents

An Introduction

An operational definition of aptamers could be given as "relatively small oligonucleotides or peptides that bind their cognate targets based on their higher order structure rather than primary sequence". One can argue that the higher order structures of these molecules are engrailed in the primary sequence of their residues. There are two approaches to designing or finding a cognate partner for a given ligand also referred to as combinatorial or rational approaches. Combinatorial process involves affinity selection of the binding molecule from among a pool of diverse molecules as in whole extracts of plants and animals or in intermediate soups of un directed and unprotected synthesis. Nucleic acids offer the added convenience of chemical synthesis of libraries of over 10^{15} different sequences, and re-amplification of selected sequences in vitro. This has accelerated the discoveries on new aptamers at a pace difficult to cope with. Cognate molecules find their applications in all spheres of clinical activity including diagnosis, prophylaxis and therapy to mention a few. Many of these applications are inseparable and hence one may find them distributed in different sections. What is not being mentioned here is the quest for novel catalytic RNA.

Selecting for a Nucleic Acid Aptamer Binding a Chosen Ligand

The advent of selective amplification of ligand by exponential enrichment (SELEX) protocol (1, 2) marked the beginning of a phase in research where one could select for nucleic acid molecules assuming structure recognizing a chosen ligand even without prior information on the structure of the ligand (Please see Fig 1 for a flow diagram of SELEX protocol). The entire process being carried in aqueous media, did limit on the probability of selecting for aptamers against hydrophobic targets. This has also opened up the possibility to track patterns of conservation and diversity among cognate molecules selected for binding the same ligand. Such cognate molecules, which may include peptides and oligonucleotides, recognize their target ligands based on higher order structure rather than sequence of bases and are referred to as aptamers. It should be appreciated that a 25-mer oligonucleotides with complete degeneracy offer 4^{25} (1.12×10^{15}) different sequences and a pool with single molecule of each variant would re quire a mass of 15.8 microgram required to include one molecule of each type while a 30 mer oligonucleotides with similar degeneracy would have a variant pool size of 4^{30} (1.15×10^{18}) with a minimal theoretical mass of 19.5 milligrams for inclusion of each variant. The practical pool size therefore gets limited to ca. 10^{15}. Larger size molecules, if required, may be selected with limited degeneracy or limited pool size.

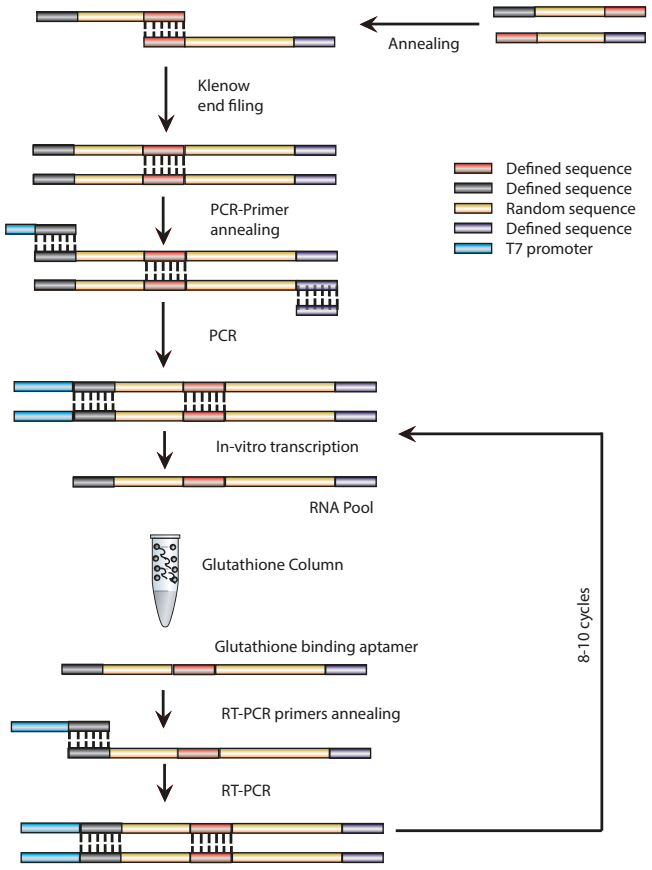

Figure 1: *A schematic representation of SELEX protocol for selecting RNA aptamers (after Baby Santosh and Pramod Yadava, Biomed Res International, in press).*

In terms of therapeutic potentials, antisense oligonucleotides (3) and ribozymes were established through the eighties and early nineties (4). Subsequently, interfering siRNA, miRNA and shRNA were adopted as targeted therapeutic molecules. It must be appreciated that RNA aptamers interact with their targets based on structure assumed by RNA while all other forms of therapeutic RNA recognize their targets based on sequence complementarity. As for other drugs pharmacokinetic parameters including water solubility, molecular size, target affinity, and metabolic disposal had to be comparatively assessed

vis a vis small molecular weight chemotherapeutics. The smallest among RNA drugs would minimally be 20 nucleotides long (ca. 6.6 kD) and hence should have a dissociation coefficient orders of magnitude favorable to compensate for the large molecular weights. Stability of RNA in serum has always been a concern. Hence modifications protecting them against a commonly ribonuclease-rich environment have been tested. Targeted delivery and sub-cellular co-localization with target molecules were yet other considerations made (5). With the spurt of activity in this area, a comprehensive database for ribozymes and aptamers was created by Thodima et al. in 2006. The database included370 artificial ribozymes and 3842 aptamer sequences and can be accessed at http://mfgn.usm.edu/ebl/riboapt/ (6).

Diagnostic Aptamers and Biosensors

Aptamers were projected as probable diagnostic and therapeutic molecules with the introduction of SELEX protocol and through the decades following (7). Protein and aptamer microarrays have been highlighted for their potential use in lab on a chip approach (8). Aptamers targeting extracellular markers offer new ways of targeted delivery of drugs for intracellular action (9). Aptamers selected directly for their binding Burkitt cell lymphoma were found to recognize immunoglobulin heavy mu chain and were projected as enhanced diagnostic tool (10, 11).

Table 1: *A summary of kinds of emerging applications of aptamers*

Diagnostics	Prophylaxis	Therapeutics
• Cell surface targeting	• Unlabeled aptamers	• Toxin
• Organellar targeting	• Labeled aptamers	• Inhibitors of crucial functions
• Molecular Targeting	• Biomimetics	• Differentiation factors

Direct SELEX on cells in combination with other biophysical approaches has been muted to capture cancer cells with enhanced sensitivity and specificity for counteracting metastatic relapse at an early stage (12). Cell-SELEX and *in silico* maturation have been used to select for aptamers targeting *Proteus mirabilis*, the pathogen for catheter associated urinary tract infection (13). Epithelial cell adhesion molecules (EpCAM) are over expressed in trans forming cells and RNA aptamers binding these molecules have been shown to have greater sensitivity of detection in formalin-fixed and paraffin-embedded breast cancer than the antibodies available (14). DNA aptamers with Kd in 10-50 nM have also been selected for EpCAM (15). Quantitative visualization of aptamer protein complexes employing piezoelectric biosensors for thrombin has been demonstrated experimentally (16). Aptamers binding with vaccinia virus hemagglutinin have been presented as promising diagnostic tools (17). Kim et al have recently reported diagnosing brain tumor initiating cells (TICs) based on markers of glioblastoma TIC for cell-SELEX (18). Cell SELEX has been used to select for aptamers specifically binding with colorectal cancer cells. It has been argued by the authors that such an approach is particularly important in case of cancers with a heterogeneous mass of surface displayed markers (19). A biosensor consisting of micro-arrayed anti-thrombin aptamers to assess the association/dissociation parameters has been developed by Daniel et al (20). The interactions could be monitored in subnanomolar range by surface Plasmon resonance imaging. This yielded distinct values for solution and surface-phase affinity. An aptamer-based sensor for continuous measurement of drug in cells was developed promising applications in personalized dose optimization. The results were verified by measuring doxorubicin and kanamycin in live rats and in human whole blood (21). Ultrasensitive cocaine biosensors employing DNA aptamer reversible in its conformation from triangular pyramid frustum to equilateral triangle was proposed. The conformational changes were associated with faradic impedance as a linear function of analyte concentration (22). Electrochemical aptasensors have been developed for differentially detecting viral and bacterial strains and assessment of viability (23). In electrochemical devices of this kind, the electrode-bound aptamer is specific for analyte. The ensuing signal leading to electrochemical sensing of the analyte were optimized to measure aminoglycoside and tobramycin antibiotics with high sensitivity (24). Table 1 presents a summary of various kinds of emerging applications of aptamers.

Aptamers for Targeted Drug Delivery

Aptamers binding with prostate specific membrane antigen (PSMA) with very high specificity and