

THE SELECTION OF APTAMERS SPECIFIC FOR MEMBRANE MOLECULAR TARGETS

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Abstract: A growing number of RNA aptamers have been selected experimentally using the SELEX combinatorial approach, and these aptamers have several advantages over monoclonal protein antibodies or peptides with respect to their applications in medicine and nanobiotechnology. Relatively few successful selections have been reported for membrane molecular targets, in contrast to the situation with non-membrane molecular targets. This review compares the procedures and techniques used in selections against membrane proteins and membrane lipids. In the case of membrane proteins, the selections were performed against soluble protein fragments, detergent-membrane protein mixed micelles, whole cells, vesicles derived from cellular membranes, and enveloped viruses. Liposomes were used as an experimental system for the selection of aptamers against membrane lipids. RNA structure-dependent aptamer binding for rafts in lipid vesicles was reported. Based on the selected aptamers against DOPC and the amino acid tryptophan, a specific passive membrane transporter composed of RNA was constructed. The determination of the selectivity of aptamers appears to be a crucial step in a selection, but has rarely been fully investigated. The selections, which use whole cells or vesicles derived from membranes, can yield aptamers not only against proteins but also against membrane lipids.

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Abbreviations used: AChR – acetylcholine receptor; CHO cells – Chinese hamster ovary cells; CT – cytoplasmic tail; DOPC – dioleoylphosphatidylcholine; DOPS – dioleoylphosphatidylserine; ECD – extracellular domain; GPCR – G-protein-coupled receptor; HER3 – human epidermal growth factor receptor-3; IgM – immunoglobulin M; mAbs – monoclonal protein antibodies; MBP – maltose binding protein; NT – neurotensin; PSMA – prostate-specific membrane antigen; SELEX – systematic evolution of ligands by exponential enrichment

Key words: RNA, SELEX, Aptamers, Membranes, Membrane proteins, Lipids, Liposomes, Rafts, Membrane transporters

INTRODUCTION

RNA molecules exhibit remarkable conformational flexibility and functional versatility [1]. RNA aptamers are single-stranded oligonucleotides composed of ca. 20 to 100 nucleotides. Their unique three-dimensional structures confer specificity for binding to the target: the Latin “aptus” means “to fit”. The possible size of their targets ranges from small organic molecules, such as amino acids [2], through large protein complexes (although only a small part of such a protein is the actual binding site for the aptamer), to nanometer-size supramolecular structures such as liposomes [3]. In addition to cellular RNA aptamers, functioning for example as binding sites for free amino acids on the *Tetrahymena* self-splicing rRNA intron [4] or as the 34-nts binding domain (in the form of a deep pocket) of a riboswitch [5, 6], a growing number of RNA aptamers have been selected experimentally through the SELEX (systematic evolution of ligands by exponential enrichment) combinatorial approach [7, 8]. The initial library for SELEX consists of ca. 10^{14} different RNA sequences. The smallest size of the random region used successfully in a selection is 17 nts (the arginine RNA aptamer) [9], and very short aptamers can be engineered through a truncation of the aptamers obtained from the SELEX procedure: down to 15 nts (thrombin DNA-aptamer) [10] or 13 nts (theophylline RNA-aptamer) [11]. The RNA aptamers possess several advantages over monoclonal protein antibodies (mAbs) or peptides with respect to their application in medicine and nanobiotechnology [12-15]. Both aptamers and mAbs bind to targets with high affinity (K_D in pico- to nanomolar range) and specificity. However, the target range for mAbs is narrow (only immunogenic compounds), in contrast to the wide range of possible aptamer targets. Unlike mAbs and peptides, aptamers are non-immunogenic. Aptamers are usually better for *in vivo* applications since 2'-modified RNA aptamers are nuclease resistant, whereas mAbs or peptides are sensitive to hydrolyzing enzymes. Aptamers can be chemically synthesized quickly and cheaply, with minimal batch-to-batch variability when the production is scaled up. Expensive and labor-intensive biological systems are needed to produce mAbs; in addition, the large-scale biological production of mAbs is prone to significant batch-to-batch variability. Peptides and mAbs are more labile when compared to aptamers: aptamers are stable over a wide range of pH, storage conditions, and temperature; their thermal denaturation is reversible, and they are not as sensitive to organic solvents. The chemical modifications of aptamers (e.g. 5' conjugation to PEG, which prolongs their circulatory half-life and enables connection to drug-containing nanoparticles) is straightforward, whereas chemical modifications of mAbs and peptides are limited. Finally, aptamers often undergo significant conformational changes

upon target binding, which offers a great flexibility in the design of novel biosensors.

The ssDNA sequences were also used during some selections. Both unmodified DNA aptamers and unmodified RNA aptamers are susceptible to nuclease activity *in vivo* [16]. Relatively few successful selections have been reported for membrane molecular targets, due to various challenges, while there are hundreds of reports of selections for non-membrane molecular targets. This resembles the situation with the solving of the 3D structures of membrane proteins versus soluble proteins using crystallography and X-ray diffraction. Aptamers for membrane targets are worth the extra effort because of their potential important applications, e.g. in targeted drug delivery. Recent papers have contained some fragmentary review information on aptamers with membrane molecular targets [15, 17-20].

APTAMERS AGAINST MEMBRANE PROTEINS

Selections against membrane proteins should include at least two negative controls: first, a non-binding sequence or pool should be used as a control for nonspecific binding; and second, a cell line that is not expected to bind a given aptamer (i.e. not expressing the target protein) should be shown to be incapable of binding that aptamer [20]. It is also important to quantify the binding relative to the negative controls.

Aptamers selected using soluble fragments of membrane proteins

The first successful selection of aptamers against a membrane protein using a soluble protein fragment, and demonstrating the cell-specific activity of the selected aptamers, was performed against the prostate-specific membrane antigen (PSMA) [21], a plasma membrane glycoprotein overexpressed on human prostate cancer cells. The extracellular 706 amino acids of PSMA, termed xPSM, were expressed in Sf-9 insect cells using a recombinant Baculovirus, since this system is capable of mammalian cell-like post-translational modifications. The size of the purified xPSM suggested glycosylation of the product, and the xPSM fusion protein was tested for enzymatic activity to ensure native protein conformation. The xPSM, bound to magnetic beads, was incubated with a pool of RNA sequences with a 40-nts random region flanked by constant regions, and then the protein-bound RNA was partitioned by magnetic separation, reverse transcribed, and amplified using PCR. The products of PCR amplification were then used as templates for *in vitro* transcription. To increase the nuclease stability of the aptamer in the serum, 2'-fluoro-pyrimidines were incorporated during the transcription step of the SELEX cycle. After 6 cycles, the RNA sequences were cloned and sequenced. The aptamer pool inhibited xPSM enzyme activity with a K_i in the range of 2 to 10 nM. One aptamer was truncated by 15 nucleotides while retaining xPSM binding ability. This truncated aptamer specifically bound LNCaP human

prostate cancer cells expressing PSMA but not PSMA-devoid PC-3 human prostate cancer cells, as shown using fluorescence-labeled aptamers and fluorescence microscopy.

RNA aptamers were selected against the soluble extracellular domain (ECD) of human epidermal growth factor receptor-3 (HER3) [22]. HER3 is a heavily glycosylated plasma membrane protein, and its overexpression is associated with a variety of solid tumor malignancies. The recombinant domain (HER3ECD) was produced in S2 insect cells. The starting pool of RNA sequences contained a 49-nts random region. Over the course of selection, the unbound aptamers were separated from protein-bound aptamers using a filter-binding assay (for the first eight cycles) and a gel-shift assay (for the last seven cycles). The selected aptamers chosen for specificity tests had K_D of 2-8 nM for binding to HER3ECD. The selectivity tests were performed using only proteins as targets, but not the whole cell surface. The binding of one of these aptamers was much smaller for HER2ECD and heregulin (although the K_D for these bindings was not calculated) than for HER3ECD. Later study of the specificity of this aptamer for several cell lines using flow cytometry [20] showed the best affinity for MDA-MB-435 cells, and a lower affinity for MCF7 and LNCaP cells.

A selection of RNA aptamers was also performed for the cytoplasmic soluble domain (cytoplasmic tail, CT) of β -secretase (BACE1) [23]. BACE1 is a transmembrane aspartyl protease involved in the initial cleavage of the β -amyloid precursor protein to generate β -amyloid peptides. The CT of BACE (B1-CT) is responsible for subcellular localization and trafficking. The 24-amino acid tail was chemically synthesized and coupled to cyanogen bromide-activated Sepharose beads. The RNA sequences with a 40-nts random region were used for selection. After incubation of the RNA with the beads, the mixture was poured into a column. Using this affinity chromatography technique, the unbound RNA sequences were eluted first, and the bound sequences next, by using different buffers. The negative selection step was performed before the selection step with Sepharose beads coupled to Tris molecules. The best K_D s of the selected aptamers for binding to B1-CT were ca. 300 nM. These aptamers bound to recombinant GST-B1-CT with reduced activity. The aptamers were tested for selectivity using only protein targets: full-length BACE1 coupled to agarose and an antibody-derivatized agarose.

Other selections of RNA aptamers against soluble extracellular fragments of membrane proteins, albeit without any testing for selectivity, were reported for: L-selectin (a cell adhesion molecule) [24], β -catenin (a component of the Wnt signaling pathway) [25] and KRAS protein (a molecular switch in the propagation of receptor signals related to cell proliferation, division and mortality) [26]. There were two reports on selections of DNA aptamers against soluble extracellular fragments of the following membrane proteins: hemagglutinin (located in the influenza virus envelope) [27] and MUC1 (located in the membrane of epithelial cells and overexpressed on some cancer cells) [28].

Aptamers selected using detergent-membrane protein mixed micelles

RNA aptamers were generated against the recombinant G-protein-coupled receptor (GPCR) for a tripeptide neurotensin (NT) [29]. This receptor (termed NTS-1) was expressed as a maltose-binding protein (MBP) fusion in *Escherichia coli*. Since this protein is glycosylated in its native form, the recombinant protein from *E. coli* probably lacks the proper glyco-domain. The neurotensin receptor fusion protein, MBP-NTS, contained the protease sensitive sites delineating the receptor for the cleavage and production of NTS-1 without both the N-terminal MBP moiety and the C-terminal affinity tag. The purified NTS-1 protein was obtained in the form of detergent-protein mixed micelles, where the detergent was a mixture of CHAPS (a derivative of propanesulfonate, a zwitterionic detergent), CHS (cholesteryl hemisuccinate, an anionic detergent), and LM (dodecyl maltoside, a non-ionic detergent). The protein was allowed to hydrophobically adsorb to paramagnetic polystyrene beads, and the beads were incubated with a 2'F-modified RNA library with a random region of 40 nts. NT was used for elution to obtain RNA aptamers, which had bound to or near the ligand-binding pocket of NTS-1 on its extracellular side. The negative selection was performed by using another protein (histidine-tagged osteoponin) as the target for non-specific RNA-protein interactions. A magnetic rack was used wherever separation of the paramagnetic beads from the supernatant was required. The aptamers from the seventh cycles were cloned and sequenced. The K_D of the selected aptamers for binding to *E. coli* membrane-inserted MBP-NTS was .4-20 nM. Only a background binding was noted with the membrane-inserted MBP-A2a receptor. The aptamers were also tested for their ability to bind to intact Chinese hamster ovary (CHO) cells expressing NTS-1 (CHO-NTR cells) as well as intact wild-type CHO cells. The results indicated that one of the aptamers was able to bind specifically to NTS-1 on the surface of intact CHO-NTS cells.

DNA aptamers were selected for a mixture of outer membrane proteins from *Salmonella enterica* [30]. The incubation mixture contained a ssDNA pool and mixed micelles of the proteins with Tween-20 (PEG20-sorbitan monolaurate, a non-ionic detergent). Although membrane proteins can be reconstituted into liposomal membranes using mixed micelles, we could not identify any reports on selections of aptamers against membrane proteins reconstituted into liposomes (proteoliposomes).

Aptamers selected using vesicles derived from cellular membranes

The SELEX procedure was applied for the selection of RNA molecules against the nicotinic acetylcholine receptor (AChR) [31]. AChR is a glycosylated integral protein in a plasma membrane of nerve and muscle cells, forming a transient transmembrane channel upon acetylcholine binding. AChR-rich membrane vesicles were prepared from *Torpedo californica* electric organs and incubated with a pool of RNA sequences each consisting of a 40-nt randomized region flanked by two constant regions. To separate the membrane vesicle-

bound RNA sequences from the unbound ones, the filter-binding and gel-shift methods were used. After nine selection cycles, RNA aptamers were yielded that bind with nanomolar affinities (K_D in the range of 2-12 nM) to the AChR in the electroplax membrane, and that could be displaced from the receptor by phencyclidine and cocaine (which bind to the receptor). Although some of the aptamers showed an inhibitor activity on AChR in muscle cells, no selectivity data (in respect to proteins or cells) was reported.

Aptamers selected using whole cells or enveloped viruses

The cell surface selection technique has been designated as “Complex Target SELEX” [32] or “Cell-SELEX” [33]. It is important to identify the surface molecular target of the aptamers from Cell-SELEX. This is possible through mass spectrometry of fragments of the target surface molecule, which can be a transmembrane protein, a protein with a membrane anchor, an extracellular protein associated with the membrane components, or a lipid molecule.

There was a gradual development in the Cell-SELEX methodology. The selection of aptamers to targets on cells was pioneered in 1998 [32] when human red blood cell membranes were used for the selection of ssDNA aptamers, and deconvolution-SELEX was introduced in order to identify which DNA sequences are ligands for components of interest within complex targets. The photo-cross-linked DNA-target products were detected by SDS/PAGE and autoradiography, and the ssDNA molecules were isolated and their nucleotide sequences were determined. A similar approach was used for the selection of RNA aptamers against surface components of African trypanosomes (protozoan parasites), and aptamers were isolated that crosslink to a single 42-kDa surface protein [34].

The first successful identification of the Cell-SELEX target by using mass spectrometry was performed with transformed endothelial cells [35]. Both the selection procedure and the subsequent deconvolution-SELEX were based on the fluorescence method: flow cytometry and fluorescence microscopy. The molecular target of one of the ssDNA aptamers was isolated from endothelial cells by ligand-mediated magnetic DNA affinity purification, and this target was identified by mass spectrometry as an endothelial protein, specifically a rat homologue of mouse pigpen. During the ligand-mediated protein purification, the biotin/fluorescein double-labeled aptamer immobilized to streptavidin magnetic beads was incubated with lysed endothelial cells. The magnetic separation of the protein-aptamer-magnetic bead complex was applied, and then the protein was removed from the aptamer-coated beads, analyzed by gel electrophoresis, and trypsin digested within the gel. The mass fingerprinting and sequencing of the tryptic peptide fragments of the protein respectively via mass spectrometry and tandem mass spectrometry were used to identify the aptamer-specific protein. The protein database searches were done using the MASCOT software.

Cell-SELEX without a negative selection step and with identification of the target protein was performed for the B-cell Burkitt's lymphoma cell line (Ramos cells), and the target of one of the selected aptamers was identified as a membrane protein: the immunoglobulin heavy mu chain (IGHM) [36]. The membrane-bound form of IGHM has a transmembrane domain at its carboxyterminal region. A modified procedure from [35] was applied to identify the target protein, with the following steps.

- The preparation of a modified ssDNA aptamer containing photo-active 5-iodo-deoxyuridine within the sequence (the first four and final four nucleotide regions), and a biotin at the 3' end and a fluorescence probe at the 5' end. The binding of the modified aptamer to the Ramos cells was confirmed using cell cytometry and different fluorescent signals from the aptamer and the cells).
- Covalent binding of the aptamer with the target via photocross-linking, cell lysis-biotin-streptavidin conjugation with magnetic beads and protein-aptamer extraction by magnetic separation.
- The release of the captured complex, its protein gel electrophoresis, then excision of the protein band, and digestion *in situ*.
- Analysis of the protein fragments via mass spectrometry (QSTAR LC-MS/MS) and a MASCOT database search.
- A confirmation analysis by testing both the Ramos cells and a cell line (Toledo cells) that does not express the surface immunoglobulin M (IgM), with the fluorescence-labelled aptamer and fluorescence-labeled anti-IGHM antibody. The excellent selectivity of this aptamer for Ramos cells was confirmed using 14 different cell lines [20].

The other reported Cell-SELEX was performed using the cultured precursor T-cell acute lymphoblastic leukemia cell line (CCRF-CEM) [33] with a negative selection step using another hematopoietic tumor cell line (Ramos cells). The subsequent identification procedure for one of the selected ssDNA aptamers, showed a transmembrane receptor, tyrosine kinase 7 (PTK7), as the target [37]. However, selectivity tests using the cell-sorting technique indicated that it is possible that the aptamer instead identifies a propensity for adherence [20].

A technology for biomarker discovery was developed in which DNA aptamers to biomarkers that are differentially expressed on the surfaces of cells in different states were selected [38]. The isolated biomarkers (mostly plasma membrane proteins) were identified by means of mass spectrometry. The affinities of the aptamers to the cells were estimated using flow cytometry. The two *in vitro* selected DNA aptamer pools specifically bound to mature and immature dendritic cells with an approximately 100-fold difference in strength.

Reports on Cell-SELEX against a molecular surface target, which can be a protein, a glycoprotein, a lipid or a glycolipid, but without identification of the target using mass spectrometry, include selections against: cell-adhesion molecules of *Trypanosoma cruzi* (a protozoan causing Chagas' disease) [39], RET receptor tyrosine kinase (2'F-modified ssRNA aptamers) [40],

hemeagglutinin on the surface of human influenza viruses (ssRNA aptamers) [41], and growth factor- β type III receptor (2'F-modified ssRNA aptamers) [42].

RNA APTAMERS AGAINST MEMBRANE LIPIDS

Membrane lipids consist of a hydrophilic and polar head group, usually containing charged groups (e.g. zwitterionic choline, negatively charged phosphate, negatively charged sialic acid), an intermediate part that is less polar and not charged, usually containing dipole groups (e.g. carbonyl, hydroxyl), and a hydrophobic, apolar domain, usually containing hydrocarbon chains or hydrocarbon rings. While RNA aptamers for charged polar molecules (e.g. positively charged amino acid arginine [9] or negatively charged ATP [43]) and for dipole polar molecules (e.g. theophylline [44]) have been selected, the apolar, hydrophobic domain might be thought of as an improbable RNA ligand. However, RNA aptamers can fold to form specifically shaped, hydrophobic motifs that interact favorably with hydrophobic domains, e.g. valine or isoleucine side chains [45-46], isoprenoid chains within farnesylated peptide [47], vitamin B₁₂ tetrapyrrole macrocycle [48], phenylalanine hydrocarbon rings [49], the isoprenoid side chain of the antibiotic moenomycin A [50], or the two-ringed aromatic carcinogen methylene [51]. In the crystallographic structure of the RNA aptamer for vitamin B₁₂, a large surface complementary to the tetrapyrrole face of the vitamin is in a cleft at the junction of a triplex and a duplex domain [48].

Similarly to the membrane protein targets, the selections against membrane lipids should include at least two negative controls including quantitative comparison of the binding: first, a pool of non-binding sequences (e.g. random sequences) should be used as a control for nonspecific binding; and second, some liposomes that are not expected to bind a given aptamer (i.e. liposomes composed of different lipids) should be shown to be incapable of binding that aptamer [52].

Aptamers that bind DOPC liposomes

The selection of RNA aptamers was performed in low ionic-strength buffer (with a low concentration of monovalents, and a physiological concentration of divalents) against DOPC molecules forming 100-nm liposomes [53]. During the selection, the liposome-bound RNAs were separated from the unbound ones using gel chromatography. After 11 cycles, starting from a pool of RNA with an 80-nts random region, the selected aptamers formed functional complexes, which bound stably to the liposomal surface, caused the release of liposome-encapsulated solutes, and disrupted planar lipid bilayers. Characterizing of the RNA complexes, which were composed of RNA 9 and RNA 10 sequences, revealed small internal loops with partially complementary sequences, leading to the formation of a "kissing-loop" complex. These complexes were further visualized via atomic force microscopy (AFM) and fluorescence microscopy [54].

The observed aggregates had a tendency to concentrate at the bends, membrane junctions, and edges of altered lipid patches.

Aptamers that bind DOPC/cholesterol liposomes

Two-component (DOPC and cholesterol) liposomes were applied for the selection of membrane RNAs in a high-ionic strength buffer (physiological concentration of sodium ions, a high concentration of potassium ions and divalents) [3]. The selection-amplification procedure involved a pool of RNA with a 50-nts random region, 8 cycles, and selection based on gel filtration, and yielded monomeric aptamers that bound DOPC:cholesterol liposomes. The affinity of these aptamers for choline was reported. Truncation of one of these aptamers showed that an irregular hairpin structure was the active membrane-binding domain. Bound aptamers increased membrane ionic permeability for both the liposomes and the plasma membrane of cultured human cells.

A membrane transporter composed of RNA that binds DOPC liposomes and the amino acid tryptophan

A modular, bifunctional RNA aptamer designated RNA 10_{Trp} was constructed. In it, the hypothetically dispensable center of the RNA 10 aptamer was substituted by a stereo-specific binding site for the amino acid tryptophan [55]. The 2:1 complex of RNA 9 and 10_{Trp}, RNA(9:10_{Trp}), created a selective pathway through the DOPC bilayer for the amino acid. Both binding and enhanced tryptophan transport were non-linear in the RNA concentration, suggesting that RNA aggregation was required. The RNA(9:10_{Trp}) complex caused a two-fold reduction in the activation energy for tryptophan transport and specifically increased the amino acid permeation with little or no accompanying increase in the general permeability. Thus, a specific, passive membrane transporter the properties of which overlapped those of the transporter proteins, could be made of RNA. The selectivity of this transporter was tested using both different RNA complexes and different amino acids.

Aptamers that bind DOPC/DOPS/peptide liposomes

RNA aptamers for membrane-bound amyloid-42 (a 42-amino acid long amyloid peptide) were selected using the SELEX procedure [56]. Selections were performed using DOPC liposomes containing DOPS, which was shown to stabilize the monomeric form of amyloid peptides in lipid bilayers due to the interaction of the head-group of DOPS with the positively-charged lysine-28 located at the membrane-buffer interface. The liposomes were incubated with a pool of RNA sequences with a 40-nts random region flanked by constant regions. The negative selection steps with DOPC liposomes were performed before the selection step. After eight rounds of the selection, an increase in the radioactivity of RNA co-eluted with liposomes was observed, indicating the binding of some RNA sequences to DOPC/DOPS liposomes with incorporated amyloid peptide.

Aptamers that bind lipid rafts

The RNA structure-dependent aptamer binding for rafted (liquid-ordered) domains in sphingomyelin-cholesterol-DOPC vesicles was studied [52]. The binding to the more ordered gel phase lipid bilayers was stronger, but much less RNA structure dependent. The binding to the less ordered fluid (liquid disordered) was weaker but much more RNA structure dependent. All of the modes of RNA-bilayer associations seemed to be electrostatic and lipid head group directed. The RNA preference for lipid rafts was visualized and confirmed using FRET microscopy of giant lipid vesicles. The highest level of binding to rafts (17%) was observed for the RNA 10 aptamer and RNA 67-2, a derivative of RNA 10 having 8 nts changed within the left-hand hairpin loop. However, changing the central part of RNA 10 by replacing 40 nts for the amino acid tryptophan-binding motif, or by replacing 60 nts for the amino acid arginine-binding motif, reduced raft binding. For the random pool of RNA sequences, binding did not exceed 1%. The selectivity tests were performed using DOPC liposomes: no detectable binding of these aptamers was observed.

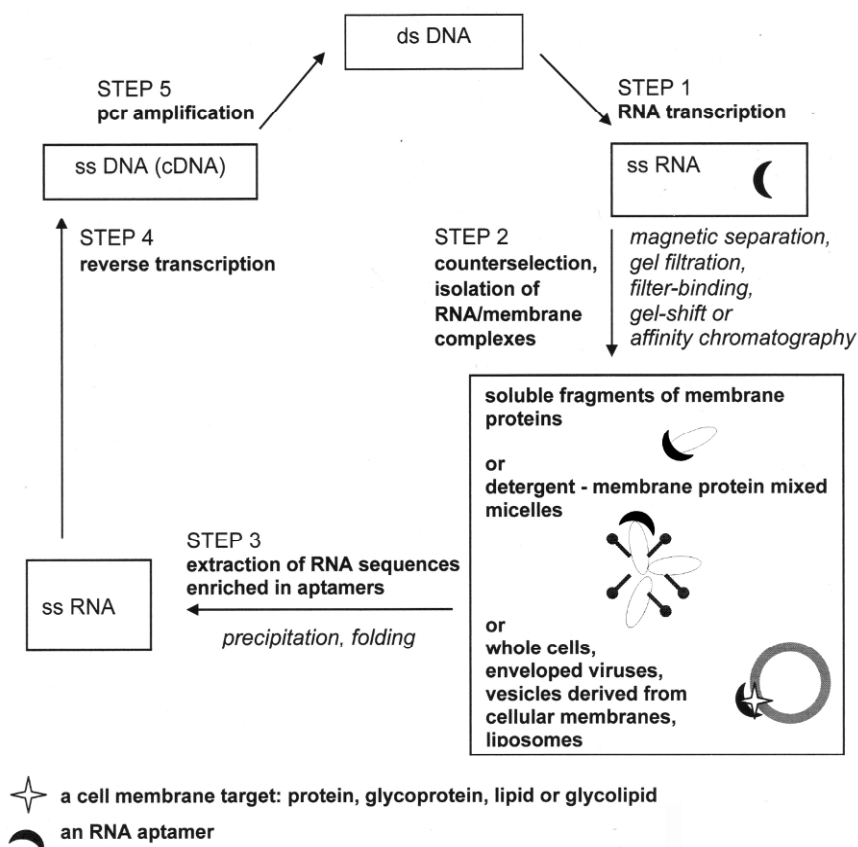


Fig. 1. The steps (during one cycle) of the selection-amplification (SELEX) procedure for aptamers against membrane molecular targets.

CONCLUSIONS

Fig. 1 shows a schematic overview of one cycle of the selections of RNA aptamers performed against membrane molecular targets. The selection begins with a chemically synthesized library of approximately 10^{14} single-stranded DNA sequences, containing a random region flanked by constant regions. In step 1, T7 RNA polymerase is used to transcribe the dsDNA library into a single-stranded RNA library. The isolation of RNA/membrane (or RNA/membrane protein) complexes and counterselection are performed during step 2. Next, RNA sequences enriched in aptamers are extracted (step 3) and reverse-transcribed (step 4), and the resulting cDNA sequences are amplified using PCR (step 5). In the case of selection of DNA aptamers, the reverse transcription step is not needed. Choosing an appropriate technique for the isolation of the RNA-membrane target complexes appears to be the critical step for a successful selection. The other factors, i.e. the length of the random region, the number of cycles, the negative steps (counterselections), and the selection buffer, seem to be less critical. The selections, which use whole cells or vesicles derived from cellular membranes, can yield aptamers not only against proteins but also for membrane lipids. Since a lot of cell surface proteins and lipids are glycosylated, the usually unsolved question is whether an aptamer binds to the polypeptide, lipid, or glycol domain of the membrane target. It is likely that many aptamers selected to be aptamers against membrane proteins without mass spectrometric identification of the target are in fact aptamers against a membrane lipid or the glyco domain of a membrane lipid. Researchers looking for aptamers against cellular membrane lipids may also take into account the complexity of the biological membrane structure. Selected aptamers can also be subject to drawbacks, such as unspecific binding; therefore the determination of the selectivity of aptamers should be fully investigated. The selection of aptamers specific to membrane molecular targets remains a state-of-the art procedure, and the arising applications of these aptamers in medicine and nanobiotechnology will certainly bring new developments in these areas.

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