Reflections on mirrors

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The SELEX (systematic evolution of ligands by exponential enrichment) method allows rapid screening of, typically, 10^{14-15} oligonucleotide sequences to identify rare molecules with suitable binding or catalytic activities against target molecules[1,2]. SELEX-derived ligands bind with K_d values below 1 nM to many proteins, and often have K_d values in the range 10 pM to 100 pM (refs 3, 4 and many others). Although most scientists have been surprised by the robustness of the SELEX process, subsequent skepticism has focused on the presumed instability of SELEX-derived compounds in biological fluids, an instability that might limit the usefulness of SELEX in drug discovery.

One clever and thoughtful solution to the problem is presented in this issue by Fürste and colleagues[5,6]. They report mirror-image, unnatural, stable L-RNAs that bind either D-adenosine[5] or L-arginine[6]. In the first paper, an unnatural target molecule, L-adenosine, is used with the SELEX process to identify a 58-mer natural D-RNA that binds to the intended target with a 1.7 μM K_d. As predicted, the mirror-image L-RNA binds D-adenosine with an equivalent K_d. The ligands bind the targets of reciprocal chirality roughly 9000-fold more weakly. In the second paper, the unnatural target D-arginine yielded a natural D-RNA ligand with a K_d of roughly 130 μM, the same K_d shown by the unnatural L-RNA ligand for L-arginine, again as predicted. In this second case, the chiral specificity of the aptamers for their intended targets is rather low (1.7-fold), although SELEX methodologies are mentioned that might enhance chiral selectivity for even a target such as arginine. For example, the authors note that counter-SELEX could eliminate ligands that show substantial cross-reactivity toward the undesired chiral isomer.

The authors note that oligonucleotide combinatorial libraries yield high-affinity ligands for a "surprising variety" of molecular targets, that the affinities and specificities of aptamers rival those of large proteins (including antibodies), and thus that "nucleic acids are becoming attractive candidates for diagnostic and therapeutic applications." I agree. Their lovely work, including the preparation of "synthons" for L-RNA, may provide a solution to the "obvious limitation" (their phrase) for diagnostic and therapeutic uses of SELEX-derived ligands, namely degradation of aptamers in biological fluids. In these papers, the data for stability of L-RNA in human serum are powerful; for 60 hours these unnatural RNAs resisted degradation, and perhaps avoided even a nibble at either end. The mirror-image strategy works exactly as the authors desired.

Will mirror-image SELEX become a standard methodology for creating useful drugs and diagnostic reagents? First, the authors note that mirror-design is effectively a post-SELEX modification that provides stability against nucleases without altering the affinity and specificity for the target. Chemical synthesis is required because the enzymes presently used forbid direct L-RNA SELEX. Regarding this point, we routinely have used libraries that are modified with 2′-fluoro or 2′-amino pyrimidines (which are acceptable to the enzymes of the SELEX process and provide stability against the most abundant and active endonucleases in blood), followed by post-SELEX modifications that stabilize some idiosyncratic number of purines and simultaneously can enhance the affinity and specificity of the ligand[3,7]. Fürste and colleagues's supposition that mirror-image RNA may be more stable than RNA modified by other approaches remains to be proved.

Second, the authors note that mirror-design is "only applicable to targets that can be prepared by chemical synthesis." Regarding this point, chemists and enzymologists may couple unnatural peptide synthesis and ligation to prepare protein subdomains suitable for mirror-image SELEX. The authors note that small peptides have been used as SELEX targets[8], and thus one only would need to determine the minimum size peptide target for D-peptide synthesis and mirror-image SELEX. However, that minimum size might be substantial, analogous to the issues of antipeptide antibodies and the large number of such antibodies one must screen to find a ligand that binds to the entire protein with high affinity and specificity. Recently, Xu and Ellington[9] made aptamers against a peptide fragment of the HIV Rev protein and found cross-reactivity toward the intact protein, suggesting that mirror-image SELEX might be practical for protein targets. However, cross-reactivity toward the whole protein was only weak. Furthermore, the Rev/peptide experiments may not be widely applicable because the peptide target was very basic and may even be relatively unconstrained in the intact protein. It is likely that most high-affinity aptamers, like high-affinity antibodies, will recognize constrained protein conformations[4].

The issue of stability in biological fluids may be of minor significance for SELEX-derived drugs and diagnostics. Pharmacokinetic and efficacy data suggest that full-length (pre-SELEX and post-SELEX stabilized) oligonucleotides survive in animals long enough to be efficacious (B. Hicke et al., personal communication). Larger issues are oligonucleotide production costs and scale, toxicities (if any), and the impact of chirality on the pharmacokinetics and biodistribution of these drugs.

The power of the SELEX process centers on ligand affinities and specificities, as well as the speed with which the ligands can be identified and tried in suitable animal disease models. Mirror-image SELEX adds time at the front end for target (that is, protein subdomain) identification and preparation, a task that grows exponentially as the human genome project names potential targets based on a correlation between gene expression and some pathogenic condition. I hold a contrary notion: Combinatorial chemistries will allow drug identification to occur without genomic knowledge through methods that use complex targets with multiple epitopes (tumors, atherosclerotic plaques, brain lesions, and so on). That is, combinatorial chemistries will be aimed at undefined, mixed targets, allowing unique drug candidates and diagnostic agents to be selected from the ligands in a pool. Even if single protein targets become available as their D-amino acid mirrors, mirror-image SELEX cannot be done on tumor cells or other human cells enriched with proteins made out of D-amino acids (biology on this planet won't allow this). Thus, target preparation for mirror-image SELEX will be very tedious. For precise, known, molecular targets (especially small, bioactive peptides) and achiral targets, however, mirror-image SELEX adds a powerful weapon to the drug discovery armamentarium.

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