Sequence Selective Recognition of Biologically Relevant RNA Duplexes

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Compared to DNA, molecular recognition of double helical RNA has received relatively little attention. Until early 90’s, RNA was viewed mostly as a passive messenger in the transfer of genetic information from DNA to proteins. However, since the discovery that RNA can catalyze chemical reactions, the number and variety of non-coding RNAs and the important roles they play in biology has been growing steadily. Most notable recent examples of important regulators of gene expression are short interfering RNAs, microRNAs, riboswitches and the RNA motifs involved in splicing machinery [1-3]. It is clear now, that RNA plays important regulatory roles in living cells. The ability to selectively recognize, detect and inhibit the function of a regulatory RNA molecules, most of which are folded in a well-organized double helical forms, would be highly useful for both fundamental biological studies and practical applications in biotechnology and medicine. However, discovery of small molecules that bind double helical RNA sequence selectively has proven to be a challenging and involved process [4,5].

RNA double helix has a relatively uniform and polar surface that presents little opportunity for hydrophobic shape selective recognition. The sequence selectivity of RNA binding intercalators, which rely on hydrophobic stacking between nucleobases of helix, is inherently low. On the other hand, binding of small molecules to RNA bulges and internal loops, which are the most common targets for naturally occurring RNA binders, is frustrated by conformational flexibility of non-helical RNA. Moreover, most of the RNA binding ligands are positively charged and relay on electrostatic interactions with the negatively charged phosphate backbone to boost the affinity at the expense of selectivity. For example, the cationic aminoglycosides, naturally occurring antibiotics, are very strong RNA binders, however, their therapeutic applications are limited by high toxicity due to indiscriminate binding to a variety of RNA species. Designing compounds that bind specific RNA sequences is a highly desirable and formidable challenge [4,5].

Despite the inherent difficulties several groups have made significant progress in targeting RNA using small molecules; the details are discussed in comprehensive recent reviews by Hergenrother [4] and Disney [5]. Somewhat outside the realm of “small molecules” stands a group of less explored compounds: oligonucleotides, peptides and various hybrid oligomers, such as peptide nucleic acids (PNA). Several recent studies demonstrate the potential of targeting biologically significant RNAs using peptide analogues. Beal and co-workers [6] designed helix-threading peptides that had high selectivity for 5'–CG–3' and 5'–UG–3' flanked by bulged nucleosides. The helix-threading peptides bound pre-microRNAs and pre-mRNAs having such motifs with low micromolar affinity and good selectivity [7,8]. In the latter case, binding of helix-threading peptide to pre-mRNA inhibited RNA editing by adenosine deaminase [8], Varani and co-workers [9] developed structurally constrained β-hairpin cyclic peptides that recognized HIV-1 TAR RNA with nanomolar affinity and excellent selectivity. Santos and co-workers [10] used branched peptides to bind TAR RNA with low micromolar to high nanomolar affinity and good sequence selectivity. It is likely that binding of peptides to TAR RNA involve extended binding sites including both the bulge and the apical loop [9]. The peptides bind RNA similarly to small molecules by using a combination of shape selective and charge mediated hydrophobic and electrostatic interactions. The main advantages that increase the selectivity and affinity of peptides over small molecules are the larger interaction area and multivalent display of functional groups.

Hydrogen bonding to nucleobases is an inherently effective way of sequence selective recognition of nucleic acids. Dervan and co-workers pioneered DNA recognition using minor groove-binding polyamides [11] and major groove-binding oligonucleotides (triple helix) [12]. Although binding to Watson-Crick faces has recently been used to recognize RNA bulges [13-15], hydrogen bonding to nucleobases is under utilized in the current design of RNA binders. Compared to DNA, the minor groove of RNA is wide and shallow and less suited for molecular recognition. The major groove of RNA is deep and narrow, which complicates triple helical binding. Modestly stable RNA triple helices are formed via parallel binding of a pyrimidine rich third strand to a purine rich strand of the double helix at mildly acidic conditions [16-18]. The sequence selectivity derives from uridine recognition of adenosine-uridine base pairs (U* A-U triplet) and protonated cytidine recognition of guanosine-cytidine base pairs (C* G-C triplet) via the Hoogsteen hydrogen-bonding scheme (Figure 1).

Figure 1: Triple helical binding of peptide nucleic acids to bacterial A-site RNAs.

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We recently discovered that PNA, a nucleic acid analogue that has the entire sugar-phosphate backbone replaced by a neutral N-(2-aminoethyl) glycine moiety (Figure 1) [19], forms unexpectedly strong and sequence selective triple helix with double helical RNA [20]. Interestingly, the binding of PNA to RNA duplex was an order of magnitude stronger than binding of PNA to DNA duplex of the same sequence. Furthermore, nucleobase modifications allowed recognition of isolated pyrimidine inversions in short polyuridine tracts, thus, expanding the potential of recognition to biologically relevant double helical RNA [21]. Intriguingly, the sequences of ribosomal A-site conserved among several pathogenic bacteria, such as E. coli, P. aeruginosa and S. aureus (HRP1) and M. Tuberculosis (HRP2) features a stretch of eight purines (blue in Figure 1) interrupted by single uridine or cytidine (bold in Figure 1). Nucleobase-modified PNA1 and PNA2 recognized their respective targets with low micromolar affinity and excellent selectivity, as virtually no binding was observed to the human A-site RNA (HRP3) [21,22]. The high selectivity was not unexpected because the purine-rich strands of human and bacterial A-site sequences had only four out of nine nucleosides common (red in HRP3). This is in contrast to the A-rich loop, the target of aminoglycoside antibiotics, which is remarkably similar for different organisms. Thus, triple helical recognition of RNA has a unique potential of unprecedented selectivity because of the stringent Hoogsteen hydrogen bonding rules and diversity of the recognition sequences.

As more cellular processes regulated by RNA are discovered, the sequence selective recognition of such species will become more important and urgent goal. Recent studies briefly reviewed above show that peptide and oligonucleotide analogues may be uniquely fit to bind RNA. For the triple helical recognition, review of secondary structure databases of non-coding RNAs reveals that it is relatively common to find short homopurine tracts of eight and more contiguous purines, sometimes interrupted by one or two pyrimidines, in bacterial ribosomal RNAs (http://www.rna.ccbb.utexas.edu/) and micro RNAs (http://www.mirbase.org/). It is conceivable that further development of chemical modifications will allow in vivo triple helical recognition of such RNAs, which would open the doors for novel ways to interfere with a broad range of RNA mediated biological processes.

References