SMGroup

Drug Design Targeting RNA

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The large numbers of steps involved in DNA replication and gene expression which involve RNA molecules are playing crucial important roles that might reflect the descendence of the present day organisms from an inherited RNA world in which both replication and storage of the genetic information were accomplished only by RNA [1]. Whether such a nucleic acid based prelude to extent life existed or not, the wealth of different contemporary RNA structure folds justifier anther notion of a world of RNA molecules. RNA plays key roles in biological process [2] and has many advantages as an attractive drug targeting because its accessibility in ribonucleo protein complexes the diversity of 3D structure folds and the absence of celluer RNA repair mechanism[3]to allow a selective binding of active molecules. Technical progress of both RNA synthesis and structure determination has growing rapidly in recent years and this in turn expanding the knowledge about the 3D structure of RNA and this paving the way for rational design of therapeutic compounds which binds specifically to RNA folds .

ADVANTAGES OF RNA-BASED THERAPIES

The main advantages of RNA as an Active Pharmaceutical Ingredient **(API)** are listed to be: 1- its production and conversation are easy biological process 2- its half-life can be exactly determined through chemical modification, 3- RNA is not immunogenic as opposed to DNA or proteins, 4- RNA can be, theoretically, an active ingredient against any diseases : for correction of pathological genetic defects , prevention or cure of infections , treatment of tumors , therapy against degenerative disease and control of allergies .

Here in this chapter, there will be attempts to introduce a survey about current efforts to benefit from RNA as a drug target. In section 2, overview of the many roles RNA play in biological process with emphasis on RNA molecules as a potential target in pathological process. Finally, current knowledge on RNA recognition by small molecules may foster the development of techniques for the stricter based design of drug target at RNAs.

ROLES OF RNAS IN BIOLOGICAL PROCESS

Genomic replication and gene expression are one of the most ancient cellular processes that involving many different RNAs structures (Figure 1) while following the flow of genetic dogma from DNA to messenger RNA (mRNA) to protein. So that the accurate synthesis of proteins is critical to the proper functioning of cells and organisms. We will see in this chapter that the linear order of amino acids in each protein determines its three- dimensional structure and activity. For this reasons, assembly of amino acids in their correct order, as encoded in DNA, is the key to produce a functional proteins.

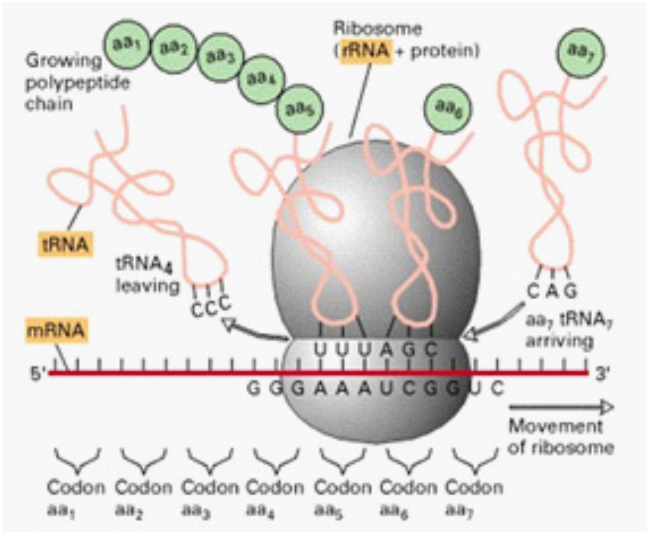


Figure 1: The three functions of RNA in protein synthesis. Messenger RNA (mRNA) is translated into protein by the combined of transfer RNA (tRNA) and the ribosome, which is formed from numerous proteins and two major ribosomal RNA (rRNA) molecules [46].

Firstly, mRNA carries the genetic information copied from DNA in the form of series of three-base code each of which formed specifies particular amino acid. Secondly, Transfer RNA (tRNA) is the key component to decrypt the code words in m RNA. Each type of amino acid has its own type of tRNA, which bind and carries it to growing end of a polypeptide chain if the next code on mRNA needs it. The correct tRNA with its attached amino acid is selected at each step because each specific tRNA molecule contains three-base sequences that can base–pair with its complementary code in the mRNA. Thirdly, Ribosomal RNA (rRNA) which associated with a set of proteins to form ribosomes. These complex structures catalyze the assembly of amino acids into protein chains when moving along an mRNA molecule. In addition they bind tRNA and many accessory molecules necessary for protein synthesis. Ribosomes are composed of a large and small subunit, each of which contains its own rRNA molecules. Finally, Translation is the whole process by which the base sequence of an mRNA is used to bind and order the amino acids in a protein. The three types of RNA participate in this essential protein-synthesizing pathway in all cells, in fact, the development of the three distinct functions of RNA was probably the molecular key to the origin of life.

REPLICATION

At their termini, eucryotic chromosomes contain stretches of tandemly repeated species specific DNA sequences, called telomeres, which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes and are compulsory for continued maintenance of chromosome length during replication [4]. Ribonucleoprotein enzyme telomerase is used to synthesize the telomeric DNA which uses its RNA component as an internal template for terminal addition of deoxynucleotides [5]. In the early stage embryo of vertebrates and in germ cells, telomerase is active but it is shut off in somatic cells. In the majority of human cancers, telomerase activity is up regulated, implying that malignancy may be linked to telomerase dependent immortalization. So that inhibition of telomerase has been suggested as a target for anticancer [6]. The RNA component of human telomerase comprising approximately about 450 nucleotides has been targeted with RNA cleaving ribozymes [7] and antisense nucleic acids [8]. Either modified nucleic acid [9] or highly selective binding of small molecule to the telomerase RNA -protein complex will be needed to overcome the limitation of antisense approach in vivo and ribozyme and exploit the ribonucleoprotein as a target for cancer treatment [10]. In addition telomerase activity in kinetoplastid parasitic protozoa such as trypanosome and leishmania has lead to suggestion that inhibition of telomerase activity will lead to anti parasitic activity [11].

The pathogen encoded enzyme Reverse Transcriptase **(RT)** converts the genome viral strand RNA into double–strand DNA for insertion into the eucaryal host chromosome in reterovirus infection [12]. Initiation of reverse transcriptase is set by cellular tRNA which hybridizes to the Primer Binding Set **(PBS)** of the viral RNA. In several retroviruses, such as Human Immunodeficiency Virus type 1 **(HIV-1)**, additional between the viral genomic RNA and the primer tRNA enhance the specificity of the initiation of reverse transcription [13] based on the data formed from probing

and mutational experiments, a model of the initiation complex , formed between primer tRNA 3Lys and HIV-1RNA bound to the RT, has been constructed which suggests an intricate three dimension [14] which serves as a putative target for RNA-binding drugs. Structural details of the contracts between nucleic acid substrates and HIV-1 RT in the transcription complex have also been elucidated by crystal structure analyses [15].

Another intermolecular RNA-RNA interaction playing an important role in the HIV-1 retroviral cycle is the dimer formation between two copies of the viral genome. [16] Dimerization is begun by an RNA loop-loop interaction [17], which is important for both replication and encapsulation of the virus [18]. Models for the 3D structure of the hairprin dimer initiation complex have been obtained by solution techniques. [19] Both a crystal and a solution structure of the mature complex are available [20]. For a mechanism of plasmid copy number control widespread in eubacteriaan RNA loop-loop interaction is also responsible. Plasmids are not important for the survival of bacteria, but they encode a wide variety of genetic determinants of medical importance, including specific virulence traits and antibiotic resistance. [21] In antisense control of plasmid replication, two plasmid- encoded complementary RNAs form an initial contact through loop-loop interaction complex [22]. This could be targeted by small molecules. In some eubacterial plasmids, including Escherichia coli CoIE1, sense-antisense RNA pairing is dependent on an RNA binding protein, Rop in CoIE1, which alleviate the annealing process [23].

The 1700 nt genome of Hepatitis Delta Virus **(HDV)**, a small pathogenic RNA follow virus of the hepatitis B virus, is replicated in a rolling–circle mechanism which gives rise to multimeric genomic copies. Catalytic RNA motifs with in both the antigenomic and genomic RNAs, the HDV ribozymes, process the multimers to generate linear monomers of the viral genome [24]. Aminoglycoside can be used to inhibit HDV ribozymes [25] which bind specifically to RNA fold. The crystal structure of HDV has been used to determine the 3D stricter of HDV ribozyme [26].

BINDING OF AMINO GLYCOSIDE TO RNA

In addition also inhibit the self–cleavage of the hammerhead ribozymes, [27,28] small catalytic RNAs involved in the replication of plant pathogenic viroid [29] RNA self- cleavage in both hammerhead ribozymes and HDV proceed by metal ion dependent mechanism [30]. Displacement of catalytic metal ions by electrostatically complementary cationic compounds has been suggested as the general principle of aminoglycoside actions on these ribozymes [31,32].

TRANSCRIPTION

During transcription, the first step in gene expression, RNA polymerase synthesizes an RNA copy of the DNA template. Transcription initiation, RNA chain elongation, and termination are tightly controlled both in eubacteria and eukaryotes [33,34]. RNA structure mediate several of the regulatory mechanisms, [35] such as transcription termination by the eubacterial mRNA-binding Rho factor, [36] the tRNA- dependent transcription anti-termination in eubacteria [37] and importantly, the transactivation of viral genome transcription in HIV and related retroviruses [38].

The Trans-Activation Region (TAR) near the 5, terminus of HIV and mRNA forms a hairpin structure that interact the viral Tat protein. The interaction between the Tat protein and cellular cycline-dependent kinases resulting to Phosphyorylation of the C terminal domain of RNA polymerase II and enhancing both initiation and processive of viral mRNA synthesis [39]. Since Tat-TAR interaction is important for viral replication, in the search for anti-HIV drugs, considerable effort has concentrated on identifying and developing compounds which interfere with formation of this protein RNA complex [38-40] Small molecule inhibitor of the Tat has been obtained by combinatorial synthesis [41], rational drug design [42], High-Throughput Screening **(HTS)** and a combination of these approaches [43]. Structure based design of legends specific for TAR [42,44] will be greatly facilated by the availability of 3D structure of the free RNA , [45] the RNA binded with argininamide, [46] and peptides derived from the Tat protein [47]. Interestingly, it has been found that in the Red Clover Necrotic Mosaic Virus **(RCNMV)**, transcription transactivation is mediated not by a protein but by an RNA specifically binding to ATAR-like element [48]. The RCNMV RNA-mediated transactivation provides another example in which RNA accomplishes a function which, in many cases, depends on protein. It has been reported that the HIV TAR structure is also required for another crucial process in the viral replication cycle [49] namely the RNA encapsulation into viral particles [50]. RNA encapsulation required addition RNA interaction namely those between the viral Neuclocapaside protein **(NC)** and the ψ signal sequence, which consist of viral m RNA hairpin [51]. Structure based drug design could be used to prepare inhibitors that are capable to target NC-RNA interaction because of the viability of 3D structure of HIV-NC bound to the SL3 hairpin of ψ RNA which has been determine by NMR spectroscopy [52].

PROCESSING, TRANSPORTATION AND LOCALIZATION

Firstly, RNA transcription undergoes a variety of different processing and localization places, depending on the organism and the type of RNA (mRNA, rRNA, tRNA … et al.). In all contemporary cells, functional tRNA is tailored by the ribonucleoprotein enzyme RNase P cleaving the 5'terminus from longer precursor sequences [52]. RNase P is composed from one protein subunit (Eubacteria) or several protein subunits (Eucarya and Archaea) and one RNA component of 300-400 nt which, in all cases contains the catalytic center of the endonuclease activity. The RNA component of eubacterial RNase P can be catalyze the cleavage reaction in the absence of the protein subunit, thus may be different enough to allow specific binding of compounds to eubacterial enzyme.

Nucloside derivatives puromycin and aminoglycosides have been reported to inhibit RNase P of Eschericuia coli [53,54] in addition to protozoan Teteahymena thermophila ,[55] this can be done by the interaction with the RNA component . Analogues of vitamin A (retinoids) act as inhibitors of RNase P Dictyostelium discoideum. [56] The 3D model for the RNA component of eubacterial RNase P was obtained from compartaive phylogenetic analysis and biochemical date [57]. mRNA is generated by processig machine involving small nuclear ribonuclear proteins (snRNPs) in the nucleoplasm, ribosomal rRNAs (rRNAs) are maturated and assembled by different set of snRNP in the nucleolus [53]. Vertebrate cells contain around 200 different sn RNPs

which consist of one or several protein subunits and an RNA component (sn RNA) of 60-300nt. The large function of sn RNA is splicing, which is the process of assembled snRNAs into arg " spliceosomes" which remove interons from primary transcripts to yield mature mRNA .[58] The splicing part of parasites has been suggested as a target for drugs to treatment fungal infection [59] and trypanosomes [60]. More information has become accessible because of the viability of the 3D structure of snRNP protein and protein-RNA complexes obtained from crystallography and NMR spectroscopy [58]. A number of viruses encoded the small RNAs that associate with protein component of host cell snRNPs and so from viral snRNPs [60] which would plays role in the infection cycle by manipulating the host RNA processing machinery. Such snnRNP-forming RNAs have been found in infection of human B lymphocytes with Epstein- Barr Virus **(EBV)**. [61] In infections with kaposi sarcoma- associated herpes virus (KSHV) and as component of the viral replication complexes of Simian Hemorrhagic Fever Virus **(SHFV)** [62,63].

Splicing and transport of HIV mRNA is controlled by an interaction between the viral Rev Response Element **(RRE)** and the viral Rev protein [64]. Rev-RRE interaction is required for nuclear export for partially spliced and unsplicied viral mRNA encoding viral structures proteins. Similar splicing control systems are found in Human T- Cell Leukemia Virus **(HTLV)** [65]. While the primary structures of HTLV regulatory protein, Rex ,and its cognate RNA element, RxRE ,are disinct from HIV Rev/RPE . The mechanisms are similar for both systems [66]. So that aminoglycoside antibiotics such as neomycin B can selectively bund to RRE RNA and prevent Rev-RRE interaction in vivo [67,68] has intitied an intense search for RRE- binding compounds by rational design and combinatorial synthesis [69-71] .Development of compounds that can be targeted Rev-RRE system will be available because of the viability of 3D structure data for complexes between the RNA-binding domain of Rev and both RRE and a ptamer RNAs [72,73] A particular case of mRNA processing is the RNA catalyzed excision of introns independent from the cellular splicing machinery. Among this family of catalytic RNAs are self splicing group I introns [74]. Group I interons are very important target since this target since they occur in revelent genes of pathogenic microorganisms but both in mammalian cells [75-77]. Group I introns from large RNA folds with conserved core containing the splice site and the binding pocket for the guanosine cofactor [78]. The 3D crystal structure of group I interon has been modeled using phylogenetic data [79, 80]. Aminoglycoside [77, 81], tuberactinomycin family [82], and L- arganine derivatives [74] have been reported to inhibited interon self splicing .HTS assays have been developed in order to find other inhibitors [77,83]. The infrequent posttranscriptional modification process is Uridine **(U)** deletion/insertions editions of mitochondrial mRNA in trypanosomes such as leishmania and Trypanosoma [84]. The site specificity of U insertions and deletions is determined by short guide RNA (g RNA) molecules which hybridize in proximity to the editing region and provide structural elements recognized by the proteins of the editing machinery. [85] Because similar processes are absent in humans, mitochondrial mRNA editing provides an attractive target for RNA- directed drugs in therapy of trypanosome-caused diseases.

Localizations and stability control of mRNA mediated by specific interactions mechanism with protein are widely used to target the translation of messages in distinct regions of eucaryotic cells [86,87]. A large number of mRNA are tightly attached to the cytoskeleton by RNA – binding microtubule and actin-associated proteins [88]. mRNA localization has two complementary functions, either to cause high local concentration of the encoded protein or to prevent high level of the protein from being omnipresent in all cells. A common theme in RNA localization is interaction between a cis-acting element, usually located within the 3, Untranslated Regions **(3, -UTR)** of the mRNA, and a trans acting protein factor.

Defect in the recognition of mRNA regulatory elements by protein have been thought to be involved in the human autoimmune disease paraneoplastic Opsclonus-Myoclonus Ataxia **(POMA)** and the fragile X mentel retardation syndrome FMR. In POMA , a disorder associated with breast cancer and motor dysfunctions, specific binding of the neuronal protein Nova-1 to RNA targets, including the mRNAs coding for Nova-1 to RNA targets , including the mRNAs coding for Nova-1 itself and the inhibitory Glycin Receptor α-2 [GlyRα2] [88] is disrupted by antibodies . [89] The 3D structure of the RNA- binding K Homology **(KH)** domains of Nova protein has been determined by X-ray crystallography [90] revealing similarities to the RNA- binding regions of FMR1 [91] , an mRNA-recognizing protein involved in FMR [92].

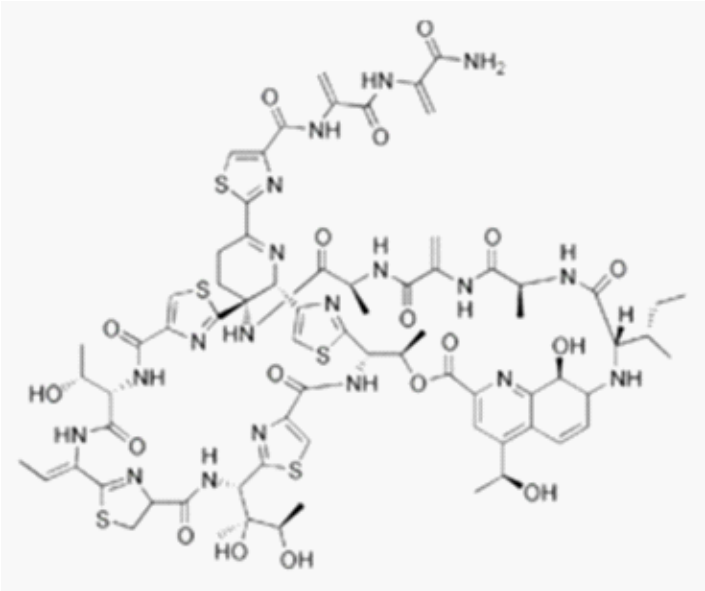
Cis-ccting sequence motifs located in the 3, -UTR of mRNA, are responsible for the binding of localization and stability control factors [93]. In β acting mRNA, a conserved 45 nt motif in the 3, -UTR is recognized by a Zipcode-Binding Protein **(ZBP-1)** which set the mRNA to the cell periphery of fibroblasts [94] . ZBP-1 shares sequence similarity with the other known protein firstly the Coding Region Determinant Binding Protein **(CRD-BP)** in mice [95], secondly the m RNA localization factor Vera in Xenopus , thirdly the KH domain protein (KOC) which is over expressed in human cancer cells [96], fourthly , the p62 Consenus Sequences RNA Binding Domain protein **(CS-RBD)** in human Hepatocellular Carcinoma **(HCC cells)** [97]. A highly conserved component of the mRNA binds specifically to an RNA motif in the 3-UTR of Vgl mRNA (vegetal mRNA) [98]. CRD-BP, a KH domain RNA-binding protein are involved in increasing the RNA half life by binding within the coding region of the oncogene c-myc mRNA, thereby preventing endonucleolytic degradation [96]. Similarly mRNA stabilization has been reported for Urokinase Receptor **(uPAR)** mRNA which is a target of a stability-decreasing regulatory protein [99].For Vascular Endothelial Growth Factor **(VEGF)** mRNA which at its 3'-UTR .binds Heterogenous Nuclear Ribonucleoprotein L **(hnRNPL)** [100] and for the tumer-specific c-fos and MYCN, mRNA which have 3'-UTR recognition sites for regulatory RNA- binding protein which are possible malignant factors [101]. Expression of human GLUT1 is responsible for shutting out the the glucose over the Blood Brain Barrier **(BBB)** which is responsible for regulating by a 10nt RNA element in the 3'-UTR which is responsible for enhancing the stability of GLUT1 mRNA [102]. Brain derived protein have been shown to exert the stabilization activity probably by the interaction with mRNA [103]. In addition, the RNA- controlled regulation of mRNA stability is involved in the overproduction of cytokines in

cancer and Amyloid Precursor Protein **(APP)** in cancer and Amyloid Precursor Protein **(APP)** in Alzheimer's disease [104]. Over production of APP can be caused by accumulation of APP mRNA which is stabilized by protein binding to the 3'-UTR. A 29 nt AU-rich RNA motif in the 3'-UTR of APP mRNA has been demonstrated to be a specific recognition site for several cytoplasmic RNA-binding protein (104) including Heterogenous Nuclear Ribonucleoprotein C **(hnRNP C)** which upon binding, increase APP mRNA stability [105,106].

TRANSLATION

Aminoglycosides have been founded to be used to target the ribosomes which were found to specifically recognize the eubacterial 16S ribosomal RNA component (rRNA) [107]. Its bind to the A site of decoding region of rRNA [108]. Which result to miscoding during translation [109]? The aminoglycosides have been founded to be interacted specifically with eubacterial A site which are different from euocaryotic A site because at poision 1408, a guanine is found in all eucayotes in contrast adenine is found in eubacteria [110]. The high identification specificity of aminoglycoside is further highlighted by the finding that a genetically determined point mutation in human mitocondrial rRNA which lead to increasing binding affinity to this drugs [111]. In order to know more about the molecular origin of binding specificity a biochemical studied has been done to known aminoglycoside-rRNA interaction [112] and biophysical methods [113-116]. The 3D structure of a 27nt model RNA containing the eubacterial a site complexed with the aminoglycoside paromomycin and gentamycin have been determined by NMR specteroscopy [117]. More than one techniques have been used to study the interaction sites of aminoglycosides on ribosomal A site RNA constructs [118]. Synthetic analogues of aminoglycosides [117] and mimetics [119] have been used to tested the ability to target the ribosomal decoding region in addition a high-throughput screening has been used to identify molecules with high affinity for the 16S rRNA A site [120].

A number of antibiotics known to inhibit the peptidyl transferase reaction in the ribosome by different mechanisms have been looked into for their binding regions on 23S rRNA [121]. For example, thiostrepton which is a large thiazole-containing cyclic peptide, which bind to the conserved 58nt GTPase center of eubacterial 23s rRNA [122], and so that stablizes the tertiary interaction within this RNA [122] and contrast with the conformational change within the ribosomal L1 protein [123]. Crystallography analyses of complex between L11 binding site RNAs and the L11 protein provided a molecular basis for the interaction of thiostrepton with the ribosome [124].



Thiostrepton

RECOGNITION OF RNA BY PEPTIDE AND PROTEIN

Most functions of RNAs require interaction with RNA-binding proteins and this interaction are required for understanding RNA recognition by small molecules because protein may use similar strategies to bind to RNA in addition, protein are important site for drug action, for example a non- Watson-Crick G-G base pair within the HIV RRE RNA which is critical for the binding of theneomycin B binding and the viral Rev protein [125]. RNA can be recognize by peptide and protein [126]. An exponential growth of structural data on a wealth of novel protein-RNA interaction because of the viability of electron density maps of the ribosome with resolution around $8A^0$, 5 A^0 for the complete ribosome [127] and for the separated large and small subunits respectively [128].

GENERAL PRINCIPLES OF PROTEIN-RNA INTERACTIONS

Although the structural variety of DNA –targeted proteins mulls over strategies for binding double-strand nucleic acid helices , which is almost variable for the cellular DNA. RNA-binding protein has been involved to recognize the integrate three-dimensional RNA folds, comparable their complexity to protein structures. The major role of RNAs participating as functional players in cellular processes, rather than being simply an intermediate storage of genetic information, is attested by the occurrence of most RNAs participating as functional players in cellular process, rather than being simply an intermediate storage of genetic information, is attested by the occurrence of most RNA as least partially folded single–stranded molecules. Double-strand RNA in regular A-form helices is only marginally suite for specific intermolecular interaction. The distinguished base edges are placed in narrow deep groove [129] and the readily accessible

minor groove contain the less information for recognition. Distortion of the regular A-form by non-Watson-Crick base pairs, interspersed looped-out residues, and platform of consecutive nucleotides lead, however, to an accessible widened deep groove [130]. Protein not only recognizes but may also enhance such distortions of the RNA structure. Insertion of α helix (Figure 2A) or a flexible protein loop into a widened deep groove is a constant theme in several protein-RNA complexes [130]. Exposed β-strands in proteins provide an interaction surfaces for unpaired RNA regions which can bind by stacking of splayed-out bases on aromatic acid chain (Figure 2B) [131]. Flexible protein loops participate predominantly in interaction with the RNA backbone (Figure 2C) which often leading to reduced loop mobility [131].

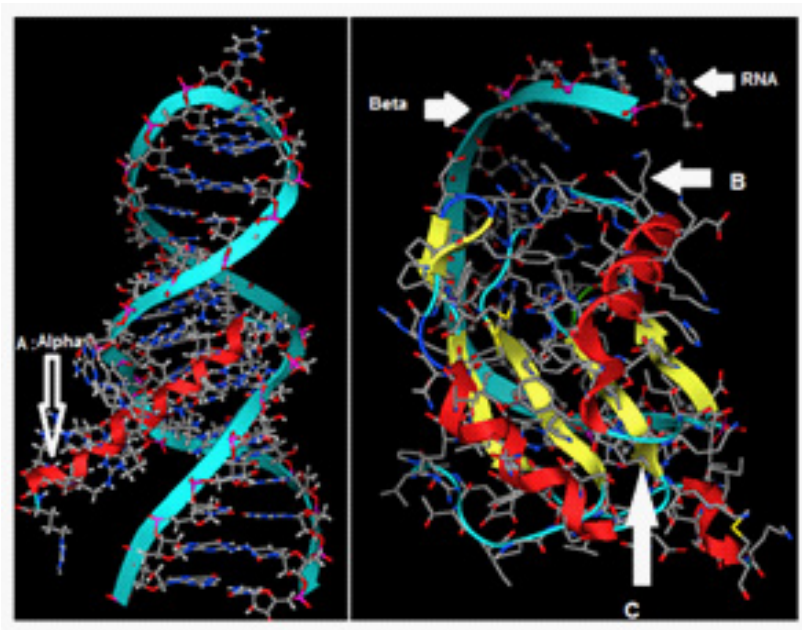


Figure 2: strategies for RNA recognition by protein. Explained by schematic views of a UIA protein-RNA complex (right) [132] and a Rev peptide-RRE complex (left) [72]. RNA is shown in dark gray in color. A: is an α helics can be inserted into the RNA deep groove which has been loaded by the looped out residues .this residue can be contact with the base pair edge and sugar phosphate backbone on the RNA. B: aromatic residue on the surface of β strands are accessible for stacking with unpaired base in RNA loop. C: flexible protein loop can penetrate into RNA folds. (PDB: 1EFT Left and PDB: 1URN right) this picture is made by Molecular Operating Environment (MOE program).

A remarkable difference is observed in RNA complexes of small peptides versus RNA bound to larger proteins [130]. In peptide–RNA complexes, adaptive conformational transitions are frequently observed for peptide upon binding to a largely changed RNA scaffold. On the contrast, larger proteins predominantly bind RNA as rigidly folded domains which provide exposed surfaces, cavities, and dissect for the RNA substrate which adapts by conformational changes.

MOLECULAR DETAILS OF PROTEIN-RNA INTERACTIONS

Protein-RNA interaction mostly involves the protein side chain (approximately 90% of the interactions in all complexes of known structure [133], which act predominantly as H-bond donors. In the RNA negatively charged phosphate groups of the backbone are the most important H-bond acceptor. In protein-RNA complexes of known structure about 20% of the intermolecular interaction involve the RNA 2' OH group, equally often as a hydrogen bond donor and as a hydrogen bond acceptor [133]. RNA-binding surfaces of peptides and protein are rich in the basic amino acids arginine and lysine which account for about 60% of intermolecular H-bonds in the RNA complexes [133]. The important function of arganine in protein-RNA interactions is due to both its flat guanidinium side chain which allows additional favorable stacking interaction with RNA bases and it is potency as a versatile basic H-bond donor.

Intermolecular stacking interactions in protein-RNA complexes can be extensive, involving residues of both components in an alternating fashion, termed interdigitation [130]. Hydrophobic parts of RNA bases accessible in triples, platforms, and non-canonical base pairs can act as surfaces for aligning non-polar side chains of proteins [130]. Important contact sites for H-bonds are provided by the Watson- Crick edges of RNA bases [130], namely the 6 and 7 positions of purines and the 4 position of pyrimidines which, in helical regions, are all located in the deep groove. Only one example of recognition by a peptide in the RNA shallow groove is shallow groove is known in which aten-residue peptide specifically binds to an RNA helix when the central base pair is G-U [134]. This system highlights a remarkable specificity potential in protein –RNA interactions which allows for discrimination of a shallow- groove asymmetry as subtle as a single amino group of a G-U pair. Non Watson-Crick base pairs can generally provide excellent recognition sites for protein in RNA folds [135]. An example is sheared G-A pairs for which a characteristic "in-plane breathing "motion has been observed in molecular dynamics (MD) simulation of RNA [136]. This facilitates potential interactions of functional group on the Watson-Crick edges of the bases with protein side chain.

Finally, there are ions and water which are intrinsic parts of the RNA three-dimensional structure and play important roles in protein-RNA interactions [137]. In 3D structures of protein–nucleic acid complexes, well orientated water molecules are often found at the binding interferences where they extend shape complementarily by filling cavities [133]. As polyanions, RNAs are inevitably surrounded by an important number of counter ions competing with proteins for binding site [138]. Proteins can bind to RNAs at low ionic strength by an entropically favored displacement of cations from electronegative pocket on the RNA. At higher salt concentration, however, this process is reversed due to salt induces destabliziation of polar intermolecular H-bonds and the competition between hydration equilibria of charged groups and salt ions [139].

RNA RECOGNITION BY SMALL MOLECULES

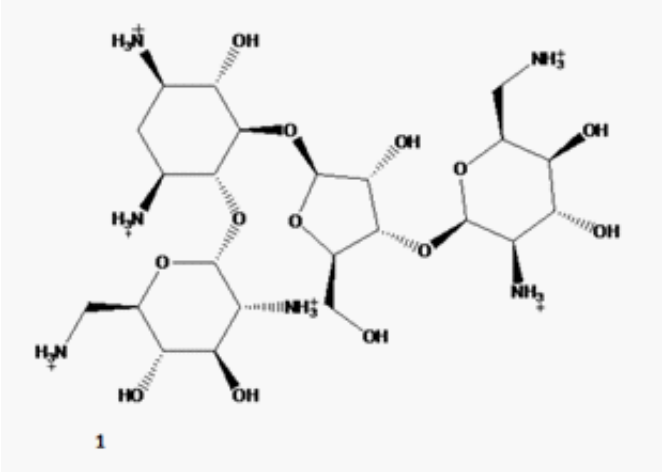
Lead design by making a new small synthesitic compounds or derivatives of natural low molecular weight compounds are become an important area of research. Aptamers are example of naturally occurring RNA target by small molecules. Apatemer-nucleic acid molecules are molecules which are obtained by in vitro selection (SELEX) of random sequence libraries and bind substrate molecule with high affinity and specificity. In addition, in vitro selected aptamar RNAs have been shown to recognize their cognate ligands in vivo [140]. So that, knowledge of principle of adaptive molecular recognition in nucleic acid aptamer complexes [141] is promise to accelerate rational drug design strategies for natural RNA targets. The are many reviewed that discuses the selection of aptamers [142] and their three dimensional structures [143] and their uses as potential diagnostic and therapeutic agents [144].

PHILOSOPHY OF TARGETING RNA FOLDS

In the early days of searching on small molecule- RNA interaction, May concepts of molecular recognition had been adapted from knowledge on DNA complexes. So that, intercalators and groove binders were among the first compounds systematically analyzed for their RNA- binding capacity [145]. The application of recognition strategies derived from DNA targets on RNA is largely limited by the very different three dimensional structures of functional RNA molecules [3] which are not limited to regular double helices. The goal of achieving special RNA recognition is factually closer to targeting protein than DNA, but with different weights on the distinct energetic contribution to molecular interaction. Such contribution result from electrostatic interaction, H-bond information, and non bonded interaction such as van der Waals force, Stacking forces, and hydrophobic forces, and molecular shape complementarily.

ELECTROSTATIC FORCES AND METAL ION BINDING

Electrostatic forces play an important role in the interaction with RNA because of the polyanionic nature of RNA. The majority of RNA- binding molecules both natural such as aminoglycoside [146] 1 and tuberactinomycin [82] 2,3 antibiotics and designed, including synthetic aminoglycoside derivatives and mimetics [28] 4,5,6 macrocycles [71], cyclophananes [43], diphenylfuran [69], polyamine-acridine conjugates [42] 7, argnine derivatives [147], and peptoid /peptide oligomer [41] are cationic molecules carrying several positively charged groups.



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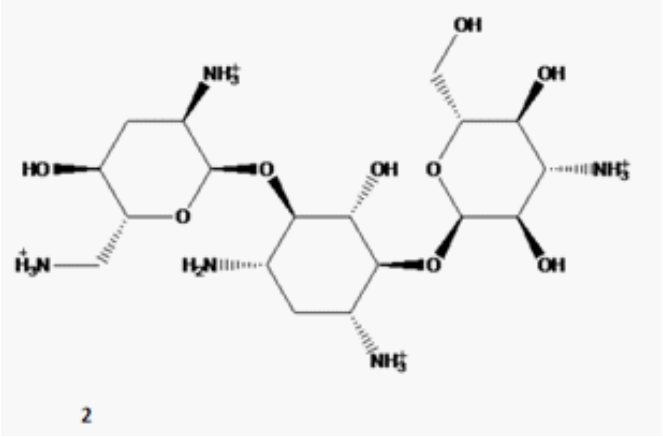

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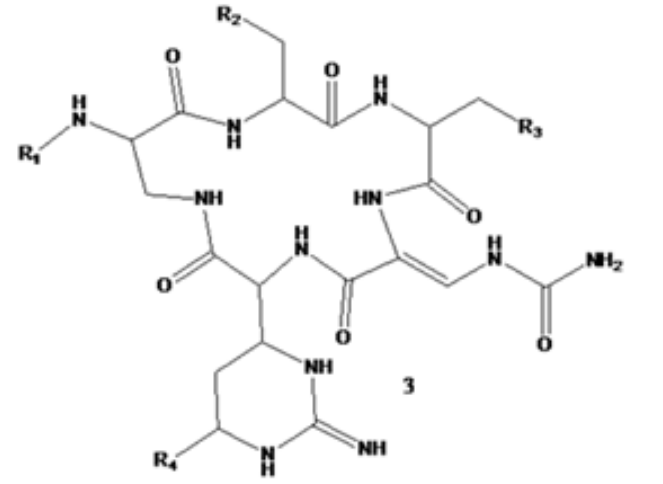

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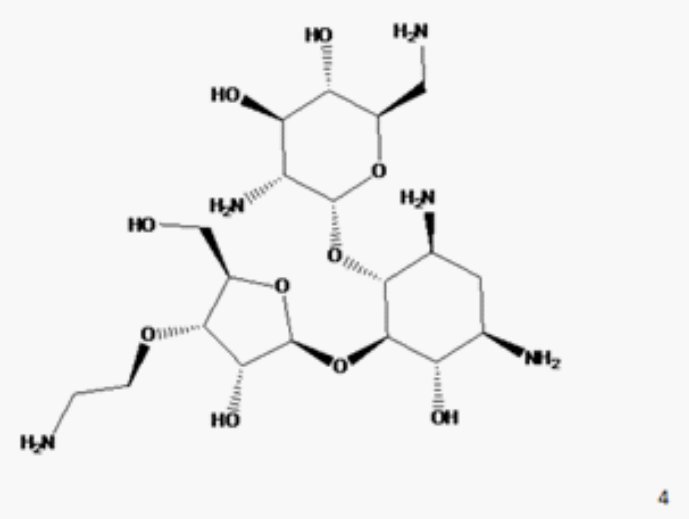

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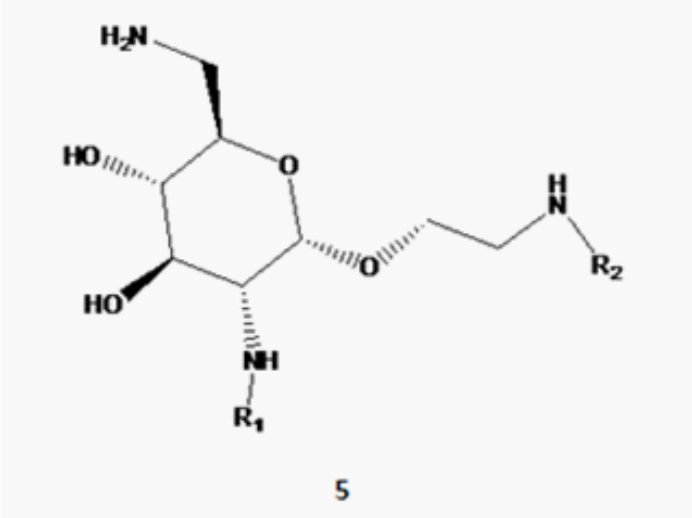

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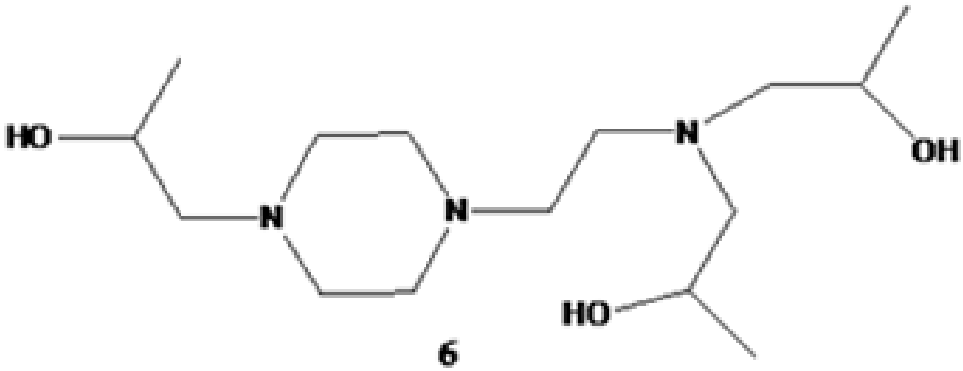

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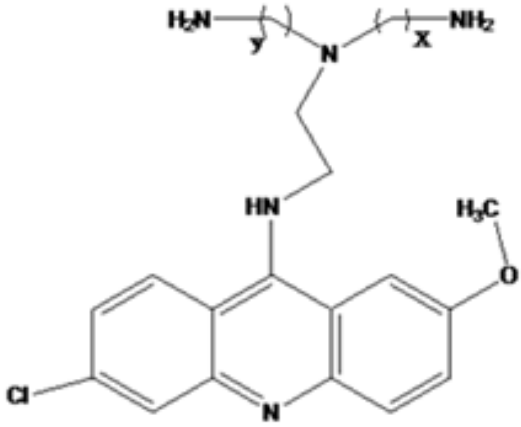

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Electrostatic interaction will be done between the cationic groups and the negatively charged RNA backbone and this lead to enhancing the binding affinity between drug-RNA complexes. But the problem that they lack the selectivity. However, specific recognition in the binding of conformationally constrained polycationic molecule to RNA folds as brought out by investigation on the interaction of aminoglycosides with different RNA targets. [32]. In quantitative approach using surface plasmon resonance, they are founded that by adding salt to RNA-aminoglycoside complexes decreased both the specific and non specific binding to about the same extent [117]. Two mechanism confer the specificity to electrostatic interactions in RNA-drug recognition. Firstly, electrostatic interaction with RNA are often part of polar H-bonds between anionic phosphoate oxagens as acceptors and positively charged H-donor groups in the bound molecule (Figure 3) while electroastatic force between the charged heavy atoms contribute to the binding energy , the oriented nature of the H- bond provides directional specificity in the interaction. Examples for this type of polar H-bond are the contacts between RNA phosphate oxagens and ammonium groups of aminoglycodide observed in three dimensional solution structures of RNA-aminoglycoside complexes [113].

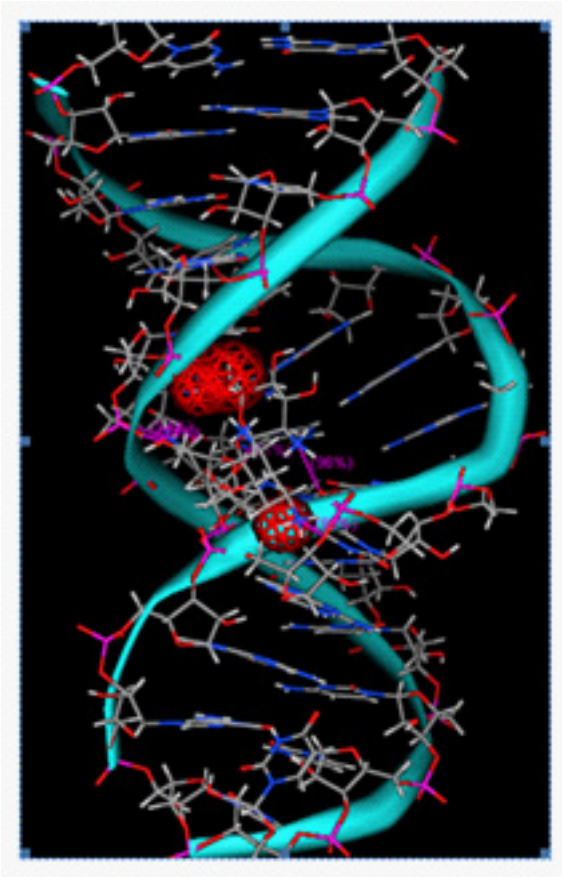


Figure 3: This figure illustrated the RNA recognition by small molecules; it's showed the schematic views of a 16S a site RNA-paromomycin complex [113]. RNA is shown as blue back bone tube. The drug is shown in light grey color. Hydrogen bonds can form between the polar donor and acceptor groups in the drug and the RNA. Frequently, negatively charged phosphate groups of RNA backbone participate as hydrogen bond acceptors with cationic substituents in the ammonium group (small molecule). This picture was made by Molecular Operating Environment (MOE program) PDB: 1A3M [31].

Secondly, the 3D folding of an RNA chain into a scaffold of spatially placed anionic groups which creates electronegative pocket where anionic group is focused [148]. The spatial distribution of negatively charge pockets in an RNA folds provide a 3D pattern that can selectivity bonded by compounds that exhibited structural electrostatic complementarily [31]. The principle is exploited by aminoglycosides binding to RNA targets which have been used to investigate the hammerhead ribozyme. This catalytic RNA is largely used for the studying of RNA- drug interaction since, in addition to the binding affinity, the specificity of RNA recognition can be determine by monitoring inhibition of the ribozyme. Following on the experimental observation that neomycin inhibits the self cleavage of the ribozyme by binding competitively with the

binding of catalytic metal ions to the RNA [149]. They are founded that solution conformers of aminoglycosides provide conformationally constrained scaffolds carrying cationic ammonium groups in the proper orientations so that it displaced several Mg II ions from their binding sites in electronegative pockets of the hammerhead ribozyme fold [31]. From molecular dynamic simulation of hammerhead RNA-aminoglycoside complexes they suggested that aminoglycosides can mimic the interaction of Mg II ions with the RNA down to an atomic level live [31].

Experimental studies has been done on the hammerhead RNA-drug complexes containing designed aminoglycoside derivatives in which the basicity, the number, the flexibiltiy of the attachment of ammonium groups has been systematically changed [28] support the model of structural electrostatic complementarily [32]. When both the charge density and the number of ammonium groups in aminoglycosides are climbed the inhibitory activity of the compounds is enhanced [28]. The binding selectivity of aminoglycosides to the target is largely diminished if the conformational constrains on the aminoglycoside scaffold is slightly changed by replacing one of the ring substructures with more flexiable alphatic chain carrying one or more amine group [117]. The conception of structural electrostatic complementarily has been greatly used to determine the structure basis of aminoglycoside inhibition of self-splicing group I interon [150]. The Mg II ions needed for the catalysis could be used to displace from the active site of a group I intron by docking aneomycin B to RNA.

While the direct participation of metal ions in RNA function may be limited to ribozymes, the role captions in stabilizing the three dimension structure of probably all RNAs offers a general strategy for targeting metal ion binding pockets with positively charged groups, in order to specifically anchor small molecules to RNA folds. Firstly: calculate electronegative pockets in RNA FOLDS [148], secondly, anticipate on the fundament of structural electrostatic complementarily, docking sites for the cationic compounds [43]. The anticipation method was used to construct complex of aminoglycosides bound to HIV-1 TAR which explain, on a molecular basis, the experimentally established allosteric mechanism [151] by which neomycin causes dissociation of Tat-TAR complexes [43].

H-BONDS AND WATER MEDIATED CONTACTS

For the H-bond forming capacity of single atoms in RNA- small molecule complexes. It has been discussed in protein- RNA complexes that the polar hydrogen bonds, predominantly between the phosphate groups of the RNA backbone as acceptors and hydrogen atoms of the small molecule, can contribute significantly to RNA-drug binding. there are a large date on H-bonding in RNA-drug complex resulted from studying on RNA- aminoglycoside complexes [152] such as oligonucleotides derived from the eubacterial A site rRNA [113] and NMR studies on aminoglycoside complex of apatamer RNAs[153]. From the experimental studies of the 3D structures of RNA- aminoglycoside complex it has been found that the ammonium and hydroxyl group of the drugs are H-Bond donor who interacts with phosphate oxygen atom and with the

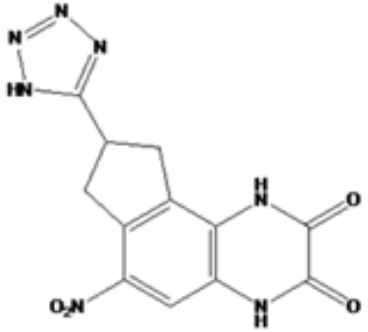
N7and O4 atoms of purines and uredines respectively of the RNA backbone [153]. Similarly observation has been seen on docking of aminoglycoside to RNA targets [31]. The variety of substituents for both ammonium and hydroxyl groups in aminoglycosides along with their conformational flexibility; provide a versatile tool for orientating H-bond donors in space so as to provide a tight H-bonding network for recognizing RNA folds. The versatility of aminoglycosides in RNA recognition is attested by the wide range of targets which bind binds compounds of these families of drugs with high affinity. So that the sugar building block can be used as synthons for the design of RNA –targeted drug [31]. There are many methods for example conventional synthetic [117] or combinatorial synthesis [155] that has used to simplify aminoglycoside building block and to obtain synthetically readily accessible carbohydrate mimetic [156]. H bond formation involving the 2- amino group of guanine is more likely to be responsible for the shallow groove recognition of the G-U wobble pairs by a small peptide [134]. Similarly, a specific h-bond between the 2-aminogroup of the isolloxazine moiety has been proposed in model for the shallow –groove recognition of G-U pairs by photo cleaving flavin derivatives [157]. Water may include in h- bond formation in RNA-small molecule complexes complex as has been suggested for atobramycin-RNA aptamer complex in this complex a water mediated H-bond between the hydroxyl group of the drug and an adenine N7 atom can occur. Such water mediated H-bond contact between the RNA and the small molecule, again preferably to phosphate oxygen atoms and N7 atoms of purines have frequently been observed in MD simulation of RNA-aminoglycoside complexes [31].

STACKING AND INTERCALATION

The bases for RNA provided a surface for stacking interaction driven by hydrophobic effects, that is, the exclusion of polar solvent from the proximity of the flat unipolar surfaces, van der Waals interaction and interactions between aromatic Π electron systems [158]. The full range of this interaction is contributed in this chelation of planer aromatic moieties between nucleic acid base pairs which is the binding mode of the variety of DNA targeted antitumor and antibiotic agents [159]. Different classes of DNA intercalators have been systemically studied for their affinity for RNA [160] were found to intercalate as well in A- form RNA duplexes [145]. For either partial intercalator such as copper phenanthroline [161] the interactions with RNA have been analyzed in modeled complexes of HIV TAR and tRNA respectively and classical intercalators such as ethidium [160].

Due to the physical nature of the interactions involved, classical intercalation is relatively non-specific. in an effort at rational design of drugs that capable to make inhibitors that capable to target the HIV Tat-TAR complex, an aromatic acridine moiety for the interaction and flexible polycationic anchor for electrostatic interaction with the RNA backbone have been done through an aliphatic linker to give rise to the inhibitor of Protein-Ribonuclotide Sequence (in-**PRiNts**;7). While this approach yielded a highly number of promising Tat-TAR inhibitors, large activity differences, not easily linked to compound structure were found [42]. This suggests that additional restriction, such as constrained conformational flexibility, might increase the inhibitors selectivity. For the in

PRiNts types of RNA binders, a stacking interaction different from classical interaction has been proposed which involve additional H-bonding between the subsisted acridine moiety and the RNA base pair edges [42]. Nonclassical threading interaction has been explained as the binding mode of synthetic diphenylfuran cations 8 which selectively inhibit the HIV resa binding to RRE RNA [69]. Specific inhibitions of the HIV Tat-TAR interaction has been reported for the dervitives of quinoxaline 2,3 dione and 2,4 diaminoquinozaline 9,10 which selectivity determine an internal bulge and a loop in TAR RNA respectively [154].



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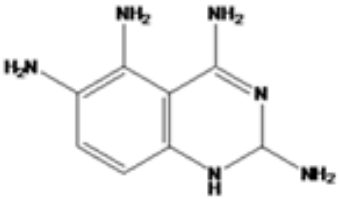

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The presence of several potential H-bond donar and H- bond acceptors suggested that this compounds (quinoxalinediones and diaminoquinozalines) bind similar to the in PRiNts in addition this compounds showed increasing the specificity of the intercalation attested by the frequent use of combination of stacking and H bonding as the recognition principle in RNA aptamer [141]. Stuffiest planer, unipolar molecules fragments can be involved in more general fashion in stacking interaction driven by van der Waals force and hydrophobic effects. spectacular examples are seen in aminoglycosides which stack with the less polar side of six-membered sugar rings on purine bases in the 3D structure of tobramycin bound to aptamer RNA [162] and gentamycin bound to an A site oligonucleotide [116]. In contrast, when a flat aromatic molecules stacking on the ribose moiety of the RNA backbone, this clear when flavin depravities 8 bound to the shallow groove of an RNA helix with a central G-U wobble pair [163]. Stacking of the isoalloxazine core of the flavin on a ribose orients the small molecules to direct a carbonyl group into H-bonding with the free 2-amino group of guanine in the shallow -groove asymmetry created by the G-U pair.

SHAPE COMPLEMENTARITY AND CONFORMATIONAL ADAPTION

The complementarity of molecular shape can add a major force in the formation of molecules with both high affinity and specify. This is mostly founded on many RNA aptamer [141]. For example, in atobramycin-RNA aptmer complex, about 75% of surface area of aminoglycoside is place inside the RNA fold [162]. Legend binding to an aptemer RNA is typically associated with the conformational changes as the nucleic acid adapt to their substrate [141]. For natural RNA targets, which on the other side of aptamers didn't capable to bind specifically and tightly

to small molecules so that shape complementarity might be more difficult to achieve? In the complexes between eubacterial ribosomal A site oligonucleotides and aminoglycosides, however. A reasonably good complementarity between the binding surface of the RNA and the drugs [113]. Shape complementarity can be accounted for remarkable site discrimination as was reported for photocleaving phenanthroline rhodium complex that selectivity recognizes G-U base pair within double–helical regions of folded RNAs such as tRNAs [164].

Conformational changes in RNA upon binding of other molecules can be large as for the HIV TAR RNA interaction with argininamide [46] or a smaller as for the A site oligonuclotide-aminoglycoside system [116]. The development of compounds of small molecule that upon binding to an RNA fold, prevent a conformational changes necessary for the biological function of the target is an important way to produce a promising target in the searching for therapeutic compounds. The thiazole-containing peptide antibiotic thiostrepton recognizes a complex structure in the conversed GTPase center of eubacterial 23S rRNA and stabilizes a tertiary interaction which might required to be flexible for a functional interaction with the ribosomal L11 protein [123] rational design of effector with RNA conformational flexibility requires a detailed picture of the 3Dstrucuture and functional of the RNA target . Extensive studies have led to the suggestion that the allosteric mechanism by which neomycin B induces dissociation of HIV Tat –TAR complexes [165] is based on a conformational arrest in which aminoglycoside locks TAR in a conformer with low affinity for Tat [44].

Lastly, shape complementarity in RNA- ligand interactions is enhanced by structure water molecules at the complex interfance, as they are commonly found in protein –drug complexes [166]. By reorienting, water molecules can provide certain plasticity in H-bond information and fill interfacial cavities. In order to include information about the positions of tightly-bound water molecules in rational drug design, preferred hydration sites have to be determined by crystal structure analyses [167] and MD simulations [168].

LEAD COMPANY

Eyetech Pharmaceuticals, Inc. is a biopharmaceutical company that specialized in the development and commercialization of novel therapeutics to treat diseases of the eye (see www.eyetech.com). Eyetech is traded on the NASDAQ National Market System under the symbol EYET. Its initial public offering of 6,500,000 shares at a price of $21.00 per share was in January 2004. Today Eyetech is a company with over 150 employees with locations in New York, New Jersey and Massachusetts.

Macugen TM (Pegaptanib sodium injection), the anti-VEGF pegylated aptamer, Eyetech's lead product, is BEING developed for diseases of the eye together with Pfizer. By docking to and blocking the function of VEGF, it interferes with the development of abnormal vessel growth and leakage seen in neovascular (wet) Age-Related Macular Degeneration **(AMD)** and diabetic retinopathy.

In the United States, it is estimated that as many as 15 million people suffer from some form of AMD and that there are more than 1.6 million cases of wet AMD. Approximately 200,000 new cases of wet AMD arise each year in the United States. Although wet AMD represents approximately 10 per cent of all AMD cases, it is responsible for 90% of the vision loss associated with AMD. A majority of wet AMD patients experience severe vision loss in the affected eye within months to two years after diagnosis of the disease. Because AMD generally affects adults over 50 years of age, it is expected that the incidence of AMD will increase significantly as the baby boom generation ages and overall life expectancy increases.

Macugen TM has also been studied in patients with DME (diabetic macular edema). In the United States, there are approximately 500,000 people suffering from DME, with approximately 75,000 new cases each year. It is expected that the incidence of DME in the United States will increase as the number of people with diabetes increases. Because the existing treatments for both wet AMD and DME have significant limitations, there is a significant unmet medical need for a new therapy for these diseases.

In 2001, Eyetech initiated two Phase II/III pivotal clinical trials of Macugen for the treatment of wet AMD. It involved 117 medical centers and enrolled patients with subfoveal wet AMD: 578 patients in the North American trial and 612 patients in the international trial. Patients received one intravitreal injection of macugen EVERY SIX WEEKS. The primary efficacy endpoint in these trials was the proportion of patients losing less than 15 letters, or three lines, of visual acuity on the eye chart from baseline after 54 weeks. Based on the analysis of the data from the two trials, the primary efficacy endpoint was met. Macugen was approved as a drug against AMD in 2004. Meanwhile, a phase II placebo controlled trial was done in the context of DME. Three doses were tested: 0.3mg, 1mg and 3mg every six weeks for at least twelve weeks and endpoints were measured after 30 weeks. Results showed statistical significance for all doses, with the highest efficacy for the 0.3mg dose, similarily to the AMD trial. IN ADDITION TO AMD and DME, MacugenTM may prove to be efficient in the treatment of Retinal Vein Occlusion **(RVO)**, a condition that is also characterized by high VEGF levels, abnormal blood vessel growth and blood vessel leakage. RVO occurs when the circulation of a retinal vein becomes obstructed, causing blood vessel bleeding and leakage in the retina. Laser therapy is sometimes used to treat this condition, but with limited efficacy. A phase II trial with Macugen TM started in May 2004.

CONCLUSION

Aptamers, ribozymes, siRNA, immunostimulating RNA, and mRNA are as many tools that have proven in pre clinical and clinical studies to be effective against a wide variety of diseases. Production of these molecules is available at large scale and in GMP quality. The first RNA oligonucleotide that reached the market (Macugen, Eyetech) has proven the pharmaceutical potential of RNA and has paved the way for qualification and large-scale production of RNA.

Most diseases with unmet medical needs can be addressed by one or several therapies based on RNA. Cancer-related diseases, for example, may be treated by one or a combination of RNA therapies. It could consist in an antitumor vaccine (based on mRNA together with immunostimulating RNA) and/or specific inhibitors of oncogen production ribozymes or siRNA specifically blocking the expression of growth factors) and/or aptamers that would recognize.

References

1. RF Gesteland, TR Cech, JF Atkins. The RNA world, 2 edn. New York: Cold Spring Harbor Laboratory Press. 1999.
2. (a) Pearson ND, Prescott CD. RNA as a drug target. Chem Biol. 1997; 4: 409-414. (b) Hermann T, Westhof E. RNA as a drug target: chemical, modelling, and evolutionary tools. Curr. Opin. Biotechnol. 1998; 9: 66-73. (c) Michael K, Tor Y. Designing novel RNA binders. Chem Eur J. 1998; 4: 2091-2098. (d) Afshar M, Prescott CD, Varani G. Structure-based and combinatorial search for new RNA-binding drugs. Curr Opin Biotechnol. 1999; 1: 59-63. (e) Tor Y. RNA and the small molecule world. Angew Chem Int Ed. 1999; 38: 1579-1582.
3. (a) Ferré-D'Amaré AR, Doudna JA. RNA folds: insights from recent crystal structures. Annu Rev Biophys Biomol Struct. 1999; 28:57-73. (b) Moore PB. Structural motifs in RNA. Annu Rev Biochem. 1999; 68: 287-300. (c) Batey RT, Rambo RP, Doudna JA. Tertiäre Motive bei Struktur und Faltung von RNA. Angew. Chem. 1999; 111: 2472-2491. (d) Hermann T, Patel DJ. Stitching together RNA tertiary architectures. J Mol Biol. 1999; 294: 829-849.
4. Zakian VA. Telomeres: beginning to understand the end. Science. 1995; 270: 1601-1607.
5. EH Blackburn. Telomerase in Lit. 609-635.
6. (a) Raymond E, Sun D, Chen SF, Windle B, Von Hoff DD. Agents that target telomerase and telomeres. Curr Opin Biotechnol. 1996; 7: 583-591. (b) Sharma HW, Maltese JY, Zhu X, Kaiser HE, Narayanan R. Telomeres, telomerase and cancer: is the magic bullet real?, Anticancer Res. 1996; 1511-1515. (c) Arthur J Lustig. Crisis intervention: The role of telomerase Proc.Natl.Acad.Sci. USA. 1999; 96: 3339-3341.
7. Kanazawa Y, Ohkawa K, Ueda K, Mita E, Takehara T, Sasaki Y, Kasahara A. Hammerhead ribozyme-mediated inhibition of telomerase activity in extracts of human hepatocellular carcinoma cells. Biochem Biophys Res Commun. 1996; 225: 570-576.
8. Glukhov AI, Zimnik OV, Gordeev SA, Severin SE. Inhibition of telomerase activity of melanoma cells *in vitro* by antisense oligonucleotides. Biochem Biophys Res Commun. 1998; 248: 368-371.
9. (a) Norton JC, Piatyszek MA, Wright WE, Shay JW, Corey DR. Inhibition of human telomerase activity by peptide nucleic acids. Nat Biotech. 1996; 5: 14615- 619. (b) Pitts AE, Corey DR. Inhibition of human telomerase by 2'-O-methyl-RNA. Proc Natl Acad Sci U S A. 1998; 20: 11549-11554.
10. Hamilton SE, Corey DR. Telomerase: anti-cancer target or just a fascinating enzyme? Chem Biol. 1996; 3: 863-867.
11. Cano MI, Dungan JM, Agabian N, Blackburn EH. Telomerase in kinetoplastid parasitic protozoa. Proc Natl Acad Sci U S A. 1999; 96: 3616-3621.
12. AM Skalka, SP Goff. Reverse transcriptase. New york: Cold spring Harbor laboratory press. 1993.
13. Isel C, Ehresmann C, Keith G, Ehresmann B, Marquet R. Initiation of reverse transcription of HIV-1: secondary structure of the HIV-1 RNA/tRNA (3Lys) (template/primer). J Mol Biol. 1995; 247: 236-250.
14. Isel C, Westhof E, Massire C, Le Grice SF, Ehresmann B, Ehresmann C, Marquet R. Structural basis for the specificity of the initiation of HIV-1 reverse transcription. EMBO J. 1999; 18: 1038-1048.
15. Ding J, Das K, Hsiou Y, Sarafianos SG, Clark AD, Jacobo-Molina A, Tantillo C. Structure and functional implications of the polymerase active site region in a complex of HIV-1 RT with a double-stranded DNA template-primer and an antibody Fab fragment at 2.8 A resolution. J Mol Biol. 1998; 284: 1095-1111.
16. Paillart JC, Marquet R, Skripkin E, Ehresmann C, Ehresmann B. Dimerization of retroviral genomic RNAs: structural and functional implications. Biochimie. 1996; 78: 639-653.
17. Skripkin E, Paillart JC, Marquet R, Ehresmann B, Ehresmann C. Identification of the primary site of the human immunodeficiency virus type 1 RNA dimerization *in vitro*. Proc Natl Acad Sci U S A. 1994; 91: 4945-4949.
18. (a) Paillart JC, Berthoux L, Ottmann.M, Darlix L, Marquet R, et al. A Dual Role of the Putative RNA Dimerization Initiation Site of Human Immunodeficiency Virus Type 1 in Genomic RNA Packaging and Proviral DNA Synthesis, J. Virol. 1996; 70: 8348-8354, (b) Harrison GP, Miele G, Hunter E, Lever AM. Functional analysis of the core human immunodeficiency virus type 1 packaging signal in a permissive cell line. J. Virol. 1998; 72: 5886-5896.

19. Jossinet F, Paillart JC, Westhof E, Hermann T, Skripkin E, Lodmell JS, Ehresmann C. Dimerization of HIV-1 genomic RNA of subtypes A and B: RNA loop structure and magnesium binding. RNA. 1999; 5: 1222-1234.

20. (a) Mujeeb A, Parslow TG, Zarrinpar A, Das C, James TL. NMR structure of the mature dimer initiation complex of HIV-1 genomic RNA.FEBS Lett. 1999; 458: 387-392. (b) Ennifar E, Yusupov M, Walter P, Marquet R, Ehresmann B, et al. The crystal structure of the dimerization initiation site of genomic HIV-1 RNA reveals an extended duplex with two adenine bulges. Structure. 1999; 7: 1439-1449.

21. Actis LA, Tolmasky ME, Crosa JH. Bacterial plasmids: replication of extrachromosomal genetic elements encoding resistance to antimicrobial compounds. Front Biosci. 1999; 4: D43-62.

22. Wagner EG, Brantl S. Kissing and RNA stability in antisense control of plasmid replication. Trends Biochem Sci. 1998; 23: 451-454.

23. Predki PF, Nayak LM, Gottlieb MB, Regan L. Dissecting RNA-protein interactions: RNA-RNA recognition by Rop. Cell. 1995; 80: 41-50.

24. Been MD, Wickham GS. Self-cleaving ribozymes of hepatitis delta virus RNA. Eur J Biochem. 1997; 247: 741-753.

25. (a) Rogers J, Chang AH, von Ahsen U, Schroeder R, Davies J. Inhibition of the self-cleavage reaction of the human hepatitis delta virus ribozyme by antibiotics. J.Mol.Biol. 1996; 259: 916-925. (b) Chia JS, Wu HL, Wang HW, Chen DS, Chen PJ. Inhibition of Hepatitis Delta Virus Genomic Ribozyme Self-Cleavage by Aminoglycosides. J.Biomed.Sci. 1997; 4: 208-216.

26. Ferré-D'Amaré AR, Zhou K, Doudna JA. Crystal structure of a hepatitis delta virus ribozyme. Nature. 1998; 395: 567-574.

27. (a) Stage TK, Hertel KJ, Uhlenbeck OC. Inhibition of the hammerhead ribozyme by neomycin. RNA. 1995; 1: 95-101. (b) Clouet-d'Orval B, Stage TK, Uhlenbeck OC. Neomycin inhibition of the hammerhead ribozyme involves ionic interactions. Biochemistry. 1995; 34: 11186-11190.

28. Wang H, Tor Y. Electrostatic Interactions in RNA Aminoglycosides Binding. J.Am.Chem.Soc. 1997; 119: 8734-8735.

29. Symons RH. Plant pathogenic RNAs and RNA catalysis. Nucleic Acids Res. 1997; 25: 2683-2689.

30. Kuimelis RG, McLaughlin LW. Mechanisms of Ribozyme-Mediated RNA Cleavage. Chem Rev. 1998; 98: 1027-1044.

31. Hermann T, Westhof E. Aminoglycoside binding to the hammerhead ribozyme: a general model for the interaction of cationic antibiotics with RNA. J Mol Biol. 1998; 276: 903-912.

32. Tor Y, Hermann T, Westhof E. Deciphering RNA recognition: aminoglycoside binding to the hammerhead ribozyme. Chem Biol. 1998; 5: R277-283.

33. Uptain SM, Kane CM, Chamberlin MJ. Basic mechanisms of transcript elongation and its regulation. Annu Rev Biochem. 1997; 66: 117-172.

34. Greenblatt J. RNA polymerase II holoenzyme and transcriptional regulation. Curr Opin Cell Biol. 1997; 9: 310-319.

35. Von Hippel PH, Rees WA, Wilson KS. RNA interactions in the regulation of transcription. Nucleic Acids Symp Ser. 1995: 1-4.

36. Platt T. Rho and RNA: models for recognition and response. Mol Microbiol. 1994; 11: 983-990.

37. Henkin TM. tRNA-directed transcription antitermination. Mol Microbiol. 1994; 13: 381-387.

38. (a) Gait M J, Karn J. RNA recognition by the human immuno-deficiency virus Tat and Rev proteins, Trends Biochem Sci. 1993; 18: 225-259. (b) Frankel AD, Young AT. HIV-1: Fifteen Proteins and an RNA Annu.Rev. Biochem. 1998; 67: 1-25.

39. Yankulov K, Bentley D. Transcriptional control: Tat cofactors and transcriptional elongation. Curr Biol. 1998; 8: R447-449.

40. (a) Gait MJ, Karn J. Progress in anti-HIV structure-based drug design.Trends Biotechnol. 1995; 13: 430-438. (b) Inoue R, Watanabe K, Katou T, Ikezawa Y, Hamasaki K. Nucleobase modified neamines with a lysine as a linker, their inhibition specificity for TAR-Tat derived from HIV-1. Bioorg. Med. Chem. Lett. 1995; 5: 2755-2760. (c) Wang J, Huang SY, Choudhury I, Leibowitz MJ, Stein S. Use of a polyethylene glycol-peptide conjugate in a competition gel shift assay for screening potential antagonists of HIV-1 Tat protein binding to TAR RNA. Anal. Biochem. 1995; 232: 238-242. (d) Bailly C, Colson P, Houssier C, Hamy F. The binding mode of drugs to the TAR RNA of HIV-1 studied by electric linear dichroism. Nucleic Acids Res. 1996; 24: 1460-1464. (e) Lim AC, Barton JK. Targeting the Tat-binding site of bovine immunodeficiency virus TAR RNA with a shape-selective rhodium complex. Bioorg. Med.Chem, 1997; 5: 1131-1136.

41. Hamy F, Felder ER, Heizmann G, Lazdins J, Aboul-ela F, Varani G, Karn J. An inhibitor of the Tat/TAR RNA interaction that effectively suppresses HIV-1 replication. Proc Natl Acad Sci U S A. 1997; 94: 3548-3553.

42. Hamy F, Brondani V, Flörsheimer A, Stark W, Blommers MJ, Klimkait T. A new class of HIV-1 Tat antagonist acting through Tat-TAR inhibition. Biochemistry. 1998; 37: 5086-5095.

43. An H, Haly BD, Cook PD. New piperazinyl polyazacyclophane scaffolds, libraries and biological activities. Bioorg Med Chem Lett. 1998; 8: 2345-2350.

44. Hermann T, Westhof E. Docking of cationic antibiotics to negatively charged pockets in RNA folds. J Med Chem. 1999; 42: 1250-1261.

45. (a) Aboul-ela F, Karn J, Varani G. Structure of HIV-1 TAR RNA in the absence of ligands reveals a novel conformation of the trinucleotide bulge. Nucleic Acids Res. 1996; 24: 3974-3981. (b) Ippolito JA, Steitz TA. A 1.3-A resolution crystal structure of the HIV-1 trans-activation response region RNA stem reveals a metal ion-dependent bulge conformation. Proc.Natl Acad.Sci. USA. 1996; 95: 9819-9824.

46. (a) Puglisi JD, Tan R, Calnan BJ, Frankel AD, Williamson JR. Conformation of the TAR RNA-arginine complex by NMR spectroscopy. Science. 1992; 257: 76-80. (b) Puglisi JD, Chen L, Frankel AD, Williamson JR. Role of RNA structure in arginine recognition of TAR RNA. Proc.Natl.Acad.Sci. USA. 1993; 90: 3680-3684. (c) Brodsky AS, Williamson JR. Solution structure of the HIV-2 TAR-argininamide complex. J Mol. Biol. 1997; 267: 624-639.

47. Puglisi JD, Chen L, Blanchard S, Frankel AD. Solution structure of a bovine immunodeficiency virus Tat-TAR peptide-RNA complex. Science. 1995; 270: 1200-1203.

48. Sit TL, Vaewhongs AA, Lommel SA. RNA-mediated trans-activation of transcription from a viral RNA. Science. 1998; 281: 829-832.

49. Das AT, Klaver B, Berkhout B. The 5' and 3' TAR elements of human immunodeficiency virus exert effects at several points in the virus life cycle. J Virol. 1998; 72: 9217-9223.

50. Clever JL, Eckstein DA, Parslow TG. Genetic dissociation of the encapsidation and reverse transcription functions in the 5' R region of human immunodeficiency virus type 1. J Virol. 1999; 73: 101-109.

51. Clever JL, Parslow TG. Mutant human immunodeficiency virus type 1 genomes with defects in RNA dimerization or encapsidation. J Virol. 1997; 71: 3407-3414.

52. Altman S, Kireseoborn L. Ribonuclease P, in Lit. [1]: 351-380.

53. Y-T.Yu.E.C. Scharl,CA Smith, Steliz JA. The Growing World of Small Nuclear Ribonucleoproteins in Lit [1]: 487-524.

54. Chopra S, Reader J. tRNAs as antibiotic targets. Int J Mol Sci. 2014; 16: 321-349.

55. True HL, Celander DW. Ribonuclease P of Tetrahymena thermophila. J Biol Chem. 1996; 271: 16559-16566.

56. Papadimou E, Georgiou S, Tsambaos D, Drainas D. Inhibition of ribonuclease P activity by retinoids. J Biol Chem. 1998; 273: 24375-24378.

57. Massire C, Jaeger L, Westhof E. Derivation of the three-dimensional architecture of bacterial ribonuclease P RNAs from comparative sequence analysis. J Mol Biol. 1998; 279: 773-793.

58. CB Burge, T Tuschi PA Sharp. Splicing of Precursors to mRNAs by the Spliceosomes, in Lit [1], 525-560.

59. Silver L, Bostian K. Screening of natural products for antimicrobial agents. Eur J Clin Microbiol Infect Dis. 1990; 9: 455-461.

60. Wang CC. Molecular mechanisms and therapeutic approaches to the treatment of African trypanosomiasis. Annu Rev Pharmacol Toxicol. 1995; 35: 93-127.

61. Toczyski DP, Matera AG, Ward DC, Steitz JA. The Epstein-Barr virus (EBV) small RNA EBER1 binds and relocalizes ribosomal protein L22 in EBV-infected human B lymphocytes. Proc Natl Acad Sci U S A. 1994; 91: 3463-3467.

62. Zhong W D, Ganem D. Characterization of ribonucleoprotein complexes containing an abundant polyadenylated nuclear RNA encoded by Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) J Virol. 1997; 71:1207-1212.

63. Hwang YK, Brinton MA. A 68-nucleotide sequence within the 3' noncoding region of simian hemorrhagic fever virus negative-strand RNA binds to four MA104 cell proteins. J Virol. 1998; 72: 4341-4351.

64. Pollard VW, Malim MH. The HIV-1 Rev protein. Annu Rev Microbiol. 1998; 52: 491-532.

65. Bogerd HP, Huckaby GL, Ahmed YF, Hanly SM, Greene WC. The type I human T-cell leukemia virus (HTLV-I) Rex trans-activator binds directly to the HTLV-I Rex and the type 1 human immunodeficiency virus Rev RNA response elements. Proc Natl Acad Sci U S A. 1991; 88: 5704-5708.

66. Askjaer P, Kjems J. Mapping of multiple RNA binding sites of human T-cell lymphotropic virus type I rex protein within 5'- and 3'-Rex response elements. J Biol Chem. 1998; 273: 11463-11471.

67. Zapp ML, Stern S, Green MR. Small molecules that selectively block RNA binding of HIV-1 Rev protein inhibit Rev function and viral production. Cell. 1993; 74: 969-978.

68. Werstuck G, Zapp ML, Green MR. A non-canonical base pair within the human immunodeficiency virus rev-responsive element is involved in both rev and small molecule recognition. Chem Biol. 1996; 3: 129-137.

69. Ratmeyer L, Zapp ML, Green MR, Vinayak R, Kumar A, Boykin DW, Wilson WD. Inhibition of HIV-1 Rev-RRE interaction by diphenylfuran derivatives. Biochemistry. 1996; 35: 13689-13696.

70. Zapp ML, Young DW, Kumar A, Singh R, Boykin DW, Wilson WD, Green MR. Modulation of the Rev-RRE interaction by aromatic heterocyclic compounds. Bioorg Med Chem. 1997; 5: 1149-1155.

71. Li K, Fernandez-Saiz M, Rigl CT, Kumar A, Ragunathan KG, McConnaughie AW, Boykin DW. Design and analysis of molecular motifs for specific recognition of RNA. Bioorg Med Chem. 1997; 5: 1157-1172.

72. Battiste JL, Mao H, Rao NS, Tan R, Muhandiram DR, Kay LE, Frankel AD. Alpha helix-RNA major groove recognition in an HIV-1 rev peptide-RRE RNA complex. Science. 1996; 273: 1547-1551.

73. Ye X, Gorin A, Ellington AD, Patel DJ. Deep penetration of an alpha-helix into a widened RNA major groove in the HIV-1 rev peptide-RNA aptamer complex. Nat Struct Biol. 1996; 3: 1026-1033.

74. TR Cech, BL Golden. Building a Catalytic Active Site Using Only RNA, in Lit [1]: 321-349.

75. Von Ahsen U, Davies J, Schroeder R. Antibiotic inhibition of group I ribozyme function. Nature. 1991; 353: 368-370.

76. Liu Y, Leibowitz MJ. Bidirectional effectors of a group I intron ribozyme. Nucleic Acids Res. 1995; 23: 1284-1291.

77. Mei HY, Cui M, Sutton ST, Truong HN, Chung FZ, Czarnik AW. Inhibition of self-splicing group I intron RNA: high-throughput screening assays. Nucleic Acids Res. 1996; 24: 5051-5053.

78. Lehnert V, Jaeger L, Michel F, Westhof E. New loop-loop tertiary interactions in self-splicing introns of subgroup IC and ID: a complete 3D model of the Tetrahymena thermophila ribozyme. Chem Biol. 1996; 3: 993-1009.

79. Michel F, Westhof E. Modelling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. J Mol Biol. 1990; 216: 585-610.

80. Cate JH, Gooding AR, Podell E, Zhou K, Golden BL, Kundrot CE, Cech TR. Crystal structure of a group I ribozyme domain: principles of RNA packing. Science. 1996; 273: 1678-1685.

81. von Ahsen U, Davies J, Schroeder R. Non-competitive inhibition of group I intron RNA self-splicing by aminoglycoside antibiotics. J Mol Biol. 1992; 226: 935-941.

82. Wank H, Rogers J, Davies J, Schroeder R. Peptide antibiotics of the tuberactinomycin family as inhibitors of group I intron RNA splicing. J Mol Biol. 1994; 236: 1001-1010.

83. Mei HY, Cui M, Lemrow SM, Czarnik AW. Discovery of selective, small-molecule inhibitors of RNA complexes--II. Self-splicing group I intron ribozyme. Bioorg Med Chem. 1997; 5: 1185-1195.

84. Simpson L. RNA Editing- An Evolutionary Perspective, in Lit [1]: 585-608.

85. (a) Hermann T, Schmid B, Heumann H, Göringer HU. A three-dimensional working model for a guide RNA from Trypanosoma brucei. Nucleic Acids Res. 1997; 12: 2311-2318. (b) Köller J, Müller UF, Schmid B, Missel A, Kruft V, et al. Trypanosoma brucei gBP21. An arginine-rich mitochondrial protein that binds to guide RNA with high affinity. J Biol Chem. 1997; 6: 3749-3757.

86. (a) Etkin LD, Lipshitz HD. RNA localization. FASEB J. 1999; 3: 419-420. (b) Lasko P. RNA sorting in Drosophila oocytes and embryos. FASEB J. 1999; 13: 421-433. (c) Mowry KL, Cote CA. RNA sorting in Xenopus oocytes and embryos. FASEB J. 1999; 13: 435-445.

87. Jansen RP. RNA-cytoskeletal associations. FASEB J. 1999; 13: 455-466.

88. Buckanovich RJ, Darnell RB. The neuronal RNA binding protein Nova-1 recognizes specific RNA targets *in vitro* and *in vivo*. Mol Cell Biol. 1997; 17: 3194-3201.

89. Buckanovich RJ, Yang YY, Darnell RB. The onconeural antigen Nova-1 is a neuron-specific RNA-binding protein, the activity of which is inhibited by paraneoplastic antibodies. J Neurosci. 1996; 3: 1114-1122.

90. Lewis HA, Chen H, Edo C, Buckanovich RJ, Yang YY, Musunuru K, Zhong R. Crystal structures of Nova-1 and Nova-2 K-homology RNA-binding domains. Structure. 1999; 7: 191-203.

91. Musco G, Stier G, Joseph C, Castiglione Morelli MA, Nilges M, Gibson TJ, Pastore A. Three-dimensional structure and stability of the KH domain: molecular insights into the fragile X syndrome. Cell. 1996; 85: 237-245.

92. Brown V, Small K, Lakkis L, Feng Y, Gunter C, Wilkinson KD, Warren ST. Purified recombinant Fmrp exhibits selective RNA binding as an intrinsic property of the fragile X mental retardation protein. J Biol Chem. 1998; 273: 15521-15527.

93. Kislauskis EH, Singer RH. Determinants of mRNA localization. Curr Opin Cell Biol. 1992; 4: 975-978.

94. Ross AF, Oleynikov Y, Kislauskis EH, Taneja KL, Singer RH. Characterization of a beta-actin mRNA zipcode-binding protein. Mol Cell Biol. 1997; 17: 2158-2165.

95. Doyle GA, Betz NA, Leeds PF, Fleisig AJ, Prokipcak RD, Ross J. The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins. Nucleic Acids Res. 1998; 26: 5036-5044.

96. Müeller-Pillasch F, Lacher U, Wallrapp C, Micha A, Zimmerhackl F, Hameister H, Varga G. Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein. Oncogene. 1997; 14: 2729-2733.

97. Zhang JY, Chan EK, Peng XX, Tan EM. A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. J Exp Med. 1999; 189: 1101-1110.

98. Deshler JO, Highett MI, Abramson T, Schnapp BJ. A highly conserved RNA-binding protein for cytoplasmic mRNA localization in vertebrates. Curr Biol. 1998; 8: 489-496.

99. Shetty S, Idell S. A urokinase receptor mRNA binding protein from rabbit lung fibroblasts and mesothelial cells. Am J Physiol. 1998; 274: L871-882.

100. Shih SC, Claffey KP. Regulation of human vascular endothelial growth factor mRNA stability in hypoxia by heterogeneous nuclear ribonucleoprotein L. J Biol Chem. 1999; 274: 1359-1365.

101. Chagnovich D, Cohn SL. Activity of a 40 kDa RNA-binding protein correlates with MYCN and c-fos mRNA stability in human neuroblastoma. Eur J Cancer. 1997; 33: 2064-2067.

102. Boado RJ, Pardridge WM. Ten nucleotide cis element in the 3'-untranslated region of the GLUT1 glucose transporter mRNA increases gene expression via mRNA stabilization. Brain Res Mol Brain Res. 1998; 1: 109-113.

103. Boado RJ. Brain-derived peptides increase blood-brain barrier GLUT1 glucose transporter gene expression via mRNA stabilization. Neurosci Lett. 1998; 255: 147-150.

104. Zaidi SH, Denman R, Malter JS. Multiple proteins interact at a unique cis-element in the 3'-untranslated region of amyloid precursor protein mRNA. J Biol Chem. 1994; 39: 24000-24006. (b) Zaidi SH, Malter JS. Amyloid precursor protein mRNA stability is controlled by a 29-base element in the 3'-untranslated region. J Biol Chem. 1994; 39: 24007-24013.

105. Zaidi SH, Malter JS. Nucleolin and heterogeneous nuclear ribonucleoprotein C proteins specifically interact with the 3'-untranslated region of amyloid protein precursor mRNA. J Biol Chem. 1995; 270: 17292-17298.

106. Rajagopalan LE, Westmark CJ, Jarzembowski JA, Malter JS. hnRNP C increases amyloid precursor protein (APP) production by stabilizing APP mRNA. Nucleic Acids Res. 1998; 26: 3418-3423.

107. Moazed D, Noller HF. Interaction of antibiotics with functional sites in 16S ribosomal RNA. Nature. 1987; 327: 389-394.

108. Purohit P, Stern S. Interactions of a small RNA with antibiotic and RNA ligands of the 30S subunit. Nature. 1994; 370: 659-662.

109. HF Chambers, Sande MA. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gliman, editors. The pharmaceutical Basis of therapeutics, 9th edn. New York: McGraw-Hill.1996; 1103-1121.

110. Van de Peer Y, Robbrecht E, de Hoog S, Caers A, De Rijk P, De Wachter R. Database on the structure of small subunit ribosomal RNA. Nucleic Acids Res. 1999; 27: 179-183.

111. Hutchin T, Haworth I, Higashi K, Fischel-Ghodsian N, Stoneking M, Saha N, Arnos C. A molecular basis for human hypersensitivity to aminoglycoside antibiotics. Nucleic Acids Res. 1993; 21: 4174-4179.

112. (a) Woodcock J, Moazed D, Cannon M, Davies J, Noller HF. Interaction of antibiotics with A- and P-site-specific bases in 16S ribosomal RNA. EMBO J. 1991; 10: 3099-3103. (b) Recht MI, Fourmy D, Blanchard SC, Dahlquist KD, Puglisi JD. RNA sequence determinants for aminoglycoside binding to an A-site rRNA model oligonucleotide.J Mol Biol. 1996; 262: 421-436.

113. Fourmy D, Recht MI, Blanchard SC, Puglisi JD. Structure of the A site of Escherichia coli 16S ribosomal RNA complexed with an aminoglycoside antibiotic. Science. 1996; 274: 1367-1371.

114. Fourmy D, Yoshizawa S, Puglisi JD. Paromomycin binding induces a local conformational change in the A-site of 16 S rRNA. J Mol Biol. 1998; 277: 333-345.

115. Fourmy D, Recht MI, Puglisi JD. Binding of neomycin-class aminoglycoside antibiotics to the A-site of 16 S rRNA. J Mol Biol. 1998; 277: 347-362.

116. Yoshizawa S, Fourmy D, Puglisi JD. Structural origins of gentamicin antibiotic action. EMBO J. 1998; 17: 6437-6448.

117. Alper PB, Hendrix M, Sears P, Wong, CH. Design and Synthesis of New Aminoglycoside Antibiotics Containing Neamine as an Optimal Core Structure: Correlation of Antibiotic Activity with *in Vitro* Inhibition of Translation. J.Am.Chem. Soc. 1999; 121: 6527-6541.

118. Griffey RH, Hofstadler SA, Sannes-Lowery KA, Ecker DJ, Crooke ST. Determinants of aminoglycoside-binding specificity for rRNA by using mass spectrometry. Proc Natl Acad Sci U S A. 1999; 96: 10129-10133.

119. Hai Wang, Yitzhak Tor. RNA-Aminoglycosid-Wechselwirkungen: Design und Synthese von, Amino-aminoglycosiden" und deren Bindung an RNA. Angew.chem.int. 1997; 36: 95-98.

120. Hamasaki K, Rando RR. A high-throughput fluorescence screen to monitor the specific binding of antagonists to RNA targets. Anal Biochem. 1998; 261: 183-190.

121. Egebjerg J, Douthwaite S, Garrett RA. Antibiotic interactions at the GTPase-associated centre within Escherichia coli 23S rRNA. EMBO J. 1989; 8: 607-611.

122. Ryan PC, Lu M, Draper DE. Recognition of the highly conserved GTPase center of 23 S ribosomal RNA by ribosomal protein L11 and the antibiotic thiostrepton. J Mol Biol. 1991; 221: 1257-1268.

123. Porse BT, Leviev I, Mankin AS, Garrett RA. The antibiotic thiostrepton inhibits a functional transition within protein L11 at the ribosomal GTPase centre. J Mol Biol. 1998; 276: 391-404.

124. Wimberly BT, Guymon R, McCutcheon JP, White SW, Ramakrishnan V. A detailed view of a ribosomal active site: the structure of the L11-RNA complex. Cell. 1999; 97: 491-502.

125. Cho J, Rando RR. Specificity in the binding of aminoglycosides to HIV-RRE RNA. Biochemistry. 1999; 38: 8548-8554.

126. K Nagai, IW Mattaj. RNA –Protein interactions. Oxford: Oxford University Press. 1994.

127. Cate JH, Yusupov MM, Yusupova GZ, Earnest TN, Noller HF. X-ray crystal structures of 70S ribosome functional complexes. Science. 1999; 5436: 2095-2104.

128. Clemons WM, May JL, Wimberly BT, McCutcheon JP, Capel M S, et al. Structure of a bacterial 30S ribosomal subunit at 5.5 A resolution. Nature. 1991; 400: 833-840.

129. Weeks KM, Crothers DM. Major groove accessibility of RNA. Science. 1993; 17: 1574-1577.

130. Patel DJ. Adaptive recognition in RNA complexes with peptides and protein modules. Curr Opin Struct Biol. 1999; 9: 74-87.

131. Cusack S. RNA-protein complexes. Curr Opin Struct Biol. 1999; 1: 66-73.

132. Oubridge C, Ito N, Evans PR, Teo CH, Nagai K. Crystal structure at 1.92 A resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin. Nature. 1994; 372: 432-438.

133. Nadassy K, Wodak SJ, Janin J. Structural features of protein-nucleic acid recognition sites. Biochemistry. 1999; 38: 1999-2017.

134. Frugier M, Schimmel P. Subtle atomic group discrimination in the RNA minor groove. Proc Natl Acad Sci U S A. 1997; 94: 11291-11294.

135. Hermann T, Westhof E. Non-Watson-Crick base pairs in RNA-protein recognition. Chem Biol. 1999; 6: R335-343.

136. Hermann T, Auffinger P, Westhof E. Molecular dynamics investigations of hammerhead ribozyme RNA. Eur Biophys. 1998; J2:153-165.

137. Feig AL, Uhlenbech OC. The Role of Metal Ions in RNA Biochemistry in Lit [1]. 287-319.

138. Anderson CF, Record MT. Salt-nucleic acid interactions. Annu Rev Phys Chem. 1995; 46: 657-700.

139. Hermann T, Westhof E. Simulations of the dynamics at an RNA-protein interface. Nat Struct Biol. 1999; 6: 540-544.

140. Werstuck G, Green MR. Controlling gene expression in living cells through small molecule-RNA interactions. Science. 1998; 5387: 296-298.

141. Hermann T, Patel DJ. Adaptive recognition by nucleic acid aptamers. Science. 2000; 287: 820-825.

142. Li Y, Breaker RR. Deoxyribozymes: new players in the ancient game of biocatalysis. Curr Opin Struct Biol. 1999; 9: 315-323.

143. Hermann T, Patel DJ. Adaptive recognition by nucleic acid aptamers. Science. 2000; 287: 820-825.

144. Osborne SE, Matsumura I, Ellington AD. Aptamers as therapeutic and diagnostic reagents: problems and prospects. Curr Opin Chem Biol. 1997; 1: 5-9.

145. Wilson WD, Ratmeyer L, Zhao M, Strekowski L, Boykin D. The search for structure-specific nucleic acid-interactive drugs: effects of compound structure on RNA versus DNA interaction strength. Biochemistry. 1993; 15: 4098-4104.

146. Michael Conn. Progress in Nucleic Acid Research and Molecular Biology. 2008; 82: 1-263.

147. Litovchick A, Evdokimov AG, Lapidot A. Arginine-aminoglycoside conjugates that bind to HIV transactivation responsive element RNA *in vitro*. FEBS Lett. 1999; 445: 73-79.

148. Hermann T, Westhof E. Exploration of metal ion binding sites in RNA folds by Brownian-dynamics simulations. Structure. 1998; 6: 1303-1314.

149. Clouet-d'Orval B, Stage TK, Uhlenbeck OC. Neomycin inhibition of the hammerhead ribozyme involves ionic interactions. Biochemistry. 1995; 35: 11186-11190.

150. Hoch I, Berens C, Westhof E, Schroeder R. Antibiotic inhibition of RNA catalysis: neomycin B binds to the catalytic core of the td group I intron displacing essential metal ions. J Mol Biol. 1998; 3: 557-569.

151. T Hermanm, E Westhof. In: MG Wallis, RWallis, R Schroeder, editors. RNA binding Antibiotics. Austin: Landes Bioscience. 2000; 48-158.

152. Aimoto S. Polypeptide synthesis by the thioester method. Biopolymers. 1999; 51: 247-265.

153. Jiang L, Majumdar A, Hu W, Jaishree TJ, Xu W, Patel DJ. Saccharide-RNA recognition in a complex formed between neomycin B and an RNA aptamer. Structure. 1999; 7: 817-827.

154. Mei HY, Cui M, Heldsinger A, Lemrow SM, Loo JA, Sannes-Lowery KA, Sharmeen L. Inhibitors of protein-RNA complexation that target the RNA: specific recognition of human immunodeficiency virus type 1 TAR RNA by small organic molecules. Biochemistry. 1998; 37: 14204-14212.

155. William K, Park C, Manfred A, Herbert J, Chi-Huey W. Rapid Combinatorial Synthesis of Aminoglycoside Antibiotic Mimetics:? Use of a Polyethylene Glycol-Linked Amine and a Neamine-Derived Aldehyde in Multiple Component Condensation as a Strategy for the Discovery of New Inhibitors of the HIV RNA Rev Responsive Element J.Am.Chem.Soc. 1996; 118: 10150-10155.

156. Sears P, Wong C-H. Mechanism-based inhibition of carbohydrate-mediated biological recognitions, Chem. Commum. 1998; 1161-1169.

157. Krishnamohan CV Sharma, GA Broke, J G. Huddleston, JW Baldwin RM, Robin D Rogers. Design Strategies for Solid-State Supramolecular Arrays Containing Both Mixed-Metalated and Freebase Porphyrins J.Am.Chem.Soc. 1997; 119: 1127-1137.

158. Saenger W. Principle of Nucleic Acid Strucuture. New York: Springer. 1984.

159. Baguley BC. DNA intercalating anti-tumour agents. Anticancer Drug Des. 1991; 6: 1-35.

160. Chow CS, Bogdan FM. A Structural Basis for RNAminus signLigand Interactions. Chem Rev. 1997; 97: 1489-1514.

161. Hermann T, Heumann H. Determination of nucleotide distances in RNA by means of copper phenanthroline-generated hydroxyl radical cleavage pattern. RNA. 1995; 10:1009-1017.

162. Jiang L, Patel DJ. Solution structure of the tobramycin-RNA aptamer complex. Nat Struct Biol. 1998; 5: 769-774.

163. Burgstaller P, Hermann T, Huber C, Westhof E, Famulok M. Isoalloxazine derivatives promote photocleavage of natural RNAs at G.U base pairs embedded within helices. Nucleic Acids Res. 1997; 20: 4018-4027.

164. Chow CS, Barton JK. Recognition of G-U mismatches by tris (4,7-diphenyl-1,10-phenanthroline)rhodium (III). Biochemistry. 1992; 31: 5423-5429.

165. Wang S, Huber PW, Cui M, Czarnik AW, Mei HY. Binding of neomycin to the TAR element of HIV-1 RNA induces dissociation of Tat protein by an allosteric mechanism. Biochemistry. 1998; 37: 5549-5557.

166. Poornima CS, Dean PM. Hydration in drug design. 3. Conserved water molecules at the ligand-binding sites of homologous proteins. J Comput Aided Mol Des. 1995; 9: 521-531.

167. Jenison RD, Jennings SD, Walker DW, Bargatze RF, Parma D. Oligonucleotide inhibitors of P-selectin-dependent neutrophil-platelet adhesion. Antisense Nucleic Acid Drug Dev. 1998; 8: 265-279.

168. Burge CB, Karlin S. Finding the genes in genomic DNA. Curr Opin Struct Biol. 1998; 8: 346-354.