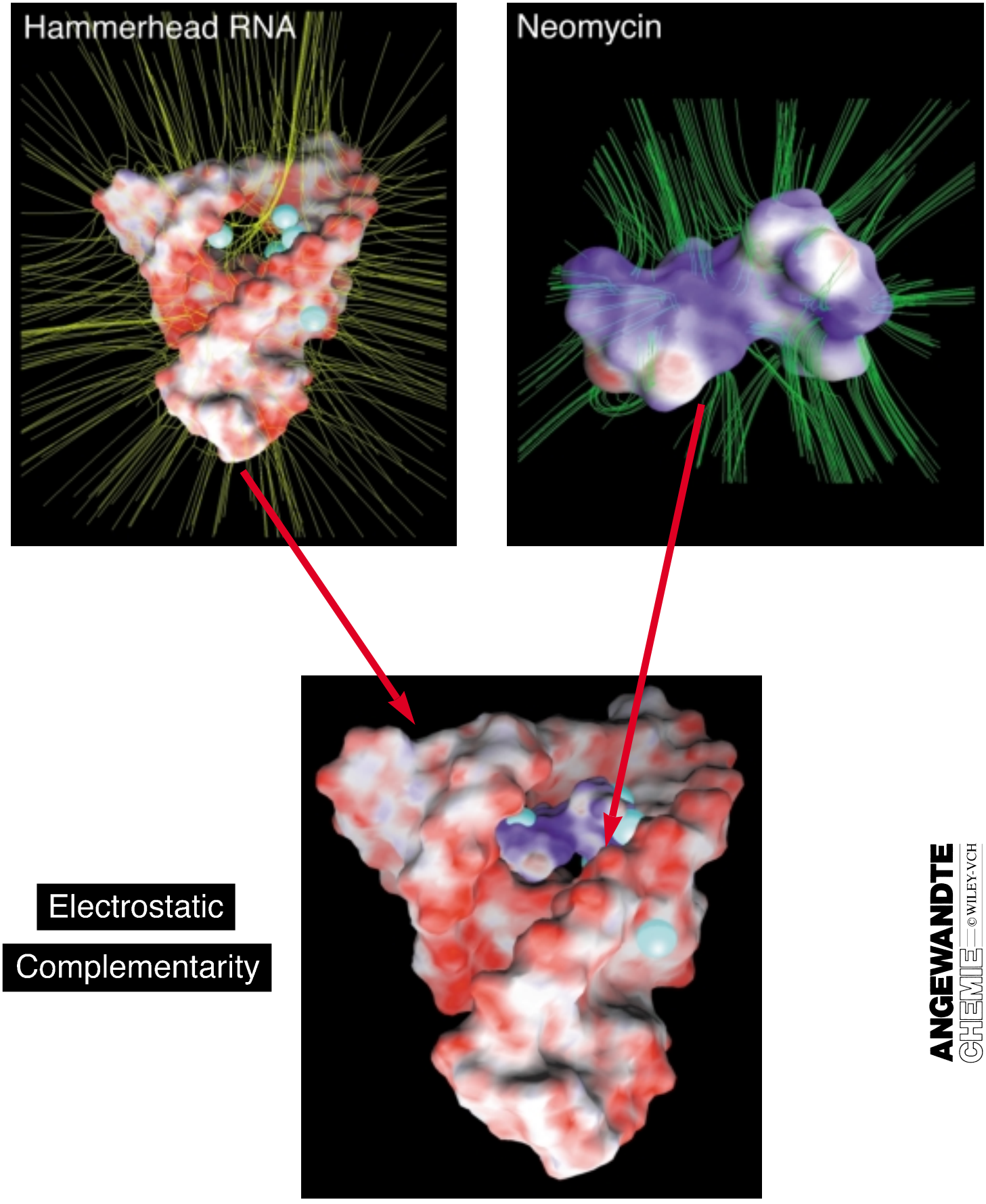

Strategies for the Design of Drugs Targeting RNA and RNA – Protein Complexes

Thomas Hermann*

In many steps of gene replication and expression, RNA molecules participate as key players, which renders them attractive targets for therapeutic intervention. While the function of nucleic acids as carriers of genetic material is based on their sequence, a number of important RNAs are involved in processes that depend on the defined three-dimensional structures of these molecules. As for proteins, numerous complex folds of RNA exist. The development of drugs that bind specifically to RNA folds opens exciting new ways to expand greatly the existing repertoire of protein-targeted therapeutics. Most functions of RNAs involve interactions with proteins that contain RNA-binding domains. Effector molecules targeted at RNA may either alter the functional three-dimensional structure of the nucleic acid, so the interaction with proteins is thereby inhibited or enhanced, or, as interface inhibitors, they may directly prevent the formation of competent RNA – protein complexes. While the same tools used for the design of protein-targeted drugs may be considered for studying effectors binding to nucleic acids, the differences between proteins and RNAs in the forces which dominate their three-dimensional folding call for novel drug design strategies. In the present review, I will outline how our rapidly expanding knowledge of RNA three-dimensional structure and function facilitates rational approaches to develop RNA-binding compounds. Putative RNA targets for therapeutic intervention will be discussed along with recent advances in understanding RNA – small molecule and RNA – protein interactions.

Keywords: aminoglycosides · antibiotics · drug research · RNA recognition · RNA structures

1. Introduction

The large number of steps in gene replication and expression which involve RNA molecules in pivotal roles might reflect the descendence of the present-day organisms from an ancestral "RNA world" in which both storage and replication of the genetic information were accomplished solely by RNA.[1] Whether such a nucleic acid based prelude to extant life existed or not, the wealth of different contemporary RNA folds justifies another notion of a "world of RNA molecules". Despite the key roles it plays in biological processes, RNA has been considered a drug target only recently.[2] In addition to its functional importance, RNA offers further advantages of an attractive drug target, namely its accessibility in ribonucleoprotein complexes, the absence of cellular RNA repair mechanisms, and the diversity of three-dimensional folds[3] to allow the selective binding of effector molecules. Technical progress in RNA synthesis and structure determination is fueling a rapidly expanding knowledge about RNA three-dimensional structure and its roles for biological functions and is paving the way for rational design of therapeutic compounds which bind specifically to RNA folds.

Here, I will attempt a survey of current efforts to exploit RNA as a drug target. In Section 2, I will give an overview of the many roles RNAs play in biological reactions. Emphasis will be on identifying RNA molecules as potential targets in pathological processes. Basic principles of RNA recognition by peptides and proteins will be discussed in Section 3. Finally, in Section 4 I will describe how our current knowledge on RNA recognition by small molecules may foster the development of techniques for the structure-based design of drugs targeted at RNAs.

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2. Roles of RNAs in Biological Processes

Gene replication and expression are probably the most ancient cellular processes involving many different RNAs (Figure 1). While following the flow of genetic information down from DNA through messenger RNA (mRNA) to proteins, I will briefly describe the role of RNA molecules

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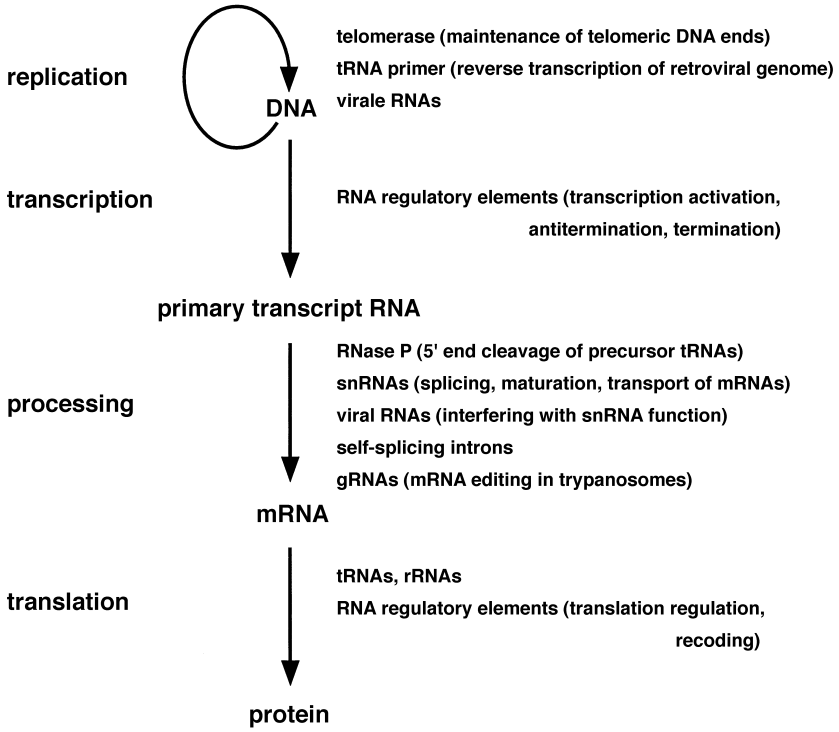

Figure 1. Schematic overview of the participation of RNAs in some of the most important processes of gene replication and expression. No distinction is made between normal and pathological processes. For further details, see text. gRNA = guide RNA, rRNA = ribosomal RNA, snRNA = small nuclear RNA, tRNA = transfer RNA.

in steps which are either obligatory for viability or specific to pathological conditions. The rapidly expanding and extremely exciting field of epigenetic mechanisms of gene regulation[4], which involves RNA molecules as central triggers of gene silencing and chromosomal modification, has been omitted for space limits. This overview is not meant to be an exhaustive or rigorous summary of the roles RNA molecules play in cells but merely a selected collection of highlights.

2.1. Replication

At their termini, eucaryotic chromosomes contain stretches of tandemly repeated species-specific DNA sequences, termed telomeres, which are required for continued maintenance of chromosome length during replication.[5] Telomeric DNA is synthesized by the ribonucleoprotein enzyme telomerase which uses its RNA component as an internal template for terminal addition of deoxynucleotides.[6] In germ cells and the early-stage embryo of vertebrates, telomerase is active but it is shut off in somatic cells. In the majority of human cancers, telomerase activity is upregulated, implying that malignancy may be linked to telomerase-dependent immortalization. Inhibition of telomerase has, thus, been suggested as a strategy for anticancer drug therapy.[7] The RNA component of human telomerase comprising approximately 450 nucleotides (nt) has been targeted with RNA-cleaving ribozymes[8] and antisense nucleic acids.[9] Either modified nucleic acids[10] or highly selective small molecule inhibitors binding to the telomerase RNA – protein complex will be needed to overcome the limitations of ribozyme and antisense approaches in vivo and exploit the ribonucleoprotein as a target for chemotherapy.[11] Recent finding of telomerase activity in kinetoplastid parasitic protozoa such as *Trypanosoma* and *Leishmania* has led to the suggestion that drugs which interfere with telomerase might be considered as a new therapeutic strategy for the treatment of parasitic diseases.[12]

In retrovirus infection, the pathogen-encoded enzyme reverse transcriptase (RT) converts the viral single-stranded RNA genome into double-stranded DNA for insertion into the eucaryal host chromosome.[13] Initiation of reverse transcription is primed by a cellular transfer RNA (tRNA) which hybridizes to the primer binding site (PBS) of the viral RNA.[14] In several retroviruses, such as the type 1 human immunodeficiency virus (HIV-1), additional interactions between the primer tRNA and the viral genomic RNA enhance the specificity of the initiation of reverse transcription.[15] Based on data from mutational and probing experiments, a model of the initiation complex, formed between primer $tRNA_3^{Lys}$ and HIV-1 RNA bound to the RT, has been constructed which suggests an intricate three-

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dimensional fold[16] which serves as a putative target for RNA-binding drugs. Structural details of the contacts between nucleic acid substrates and HIV-1 RT in the transcription complex have also been elucidated by crystal structure analyses.[17]

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.[18] Dimerization is initiated by an RNA loop–loop "kissing" interaction[19] which, in vivo, is required for both efficient replication and encapsulation of the virus.[20] Models for the three-dimensional structure of the hairpin dimer initiation complex have been obtained by solution techniques.[21] Both a solution and a crystal structure of the mature complex are available.[22]

A kissing interaction between two RNAs is also responsible for a mechanism of plasmid copy number control widespread in eubacteria. Plasmids are not essential for the survival of bacteria, but they encode a wide variety of genetic determinants of medical importance, including antibiotic resistance and specific virulence traits.[23] In antisense control of plasmid replication, two plasmid-encoded complementary RNAs form an initial contact through a loop–loop kissing complex[24] which could be targeted by small molecules. In some eubacterial plasmids, including *Escherichia coli* ColE1, sense–antisense RNA pairing is dependent on an RNA-binding protein, Rop in ColE1, which facilitates the annealing process.[25]

The 1700 nt RNA genome of hepatitis delta virus (HDV), a small pathogenic RNA satellite virus of the hepatitis B virus, is replicated in a rolling-circle mechanism which gives rise to multimeric genome copies. Catalytic RNA motifs within both the genomic and antigenomic RNAs, the HDV ribozymes, process the multimers to generate linear monomers of the viral genome.[26] HDV ribozymes are inhibited by aminoglycoside antibiotics[27] which bind specifically to the RNA fold. While the three-dimensional structure of an HDV ribozyme has been determined by crystal structure analysis,[28] the exact binding position of aminoglycosides is, as yet, unknown. Aminoglycosides also inhibit the self-cleavage of the hammerhead ribozymes,[29, 30] small catalytic RNAs involved in the replication of plant pathogenic viroids.[31] RNA self-cleavage in both HDV and hammerhead ribozymes proceeds by metal ion dependent mechanisms.[32] Displacement of catalytic metal ions by electrostatically complementary cationic compounds has been suggested as the general principle of aminoglycoside action on these ribozymes.[33, 34]

2.2. Transcription

During transcription, the first step in gene expression, RNA polymerase synthesizes an RNA copy of the DNA template. Transcriptional initiation, RNA chain elongation, and termination are tightly controlled both in eubacteria[35] and eucaryotes.[36] RNA structures mediate several of the regulatory mechanisms,[37] such as transcription termination by the eubacterial mRNA-binding Rho factor,[38] the tRNA-dependent transcription antitermination in eubacteria[39] and, importantly, the *trans* activation of viral genome transcription in HIV and related retroviruses.[40]

The *trans*-activating region (TAR) near the 5′ terminus of HIV mRNA forms a hairpin structure that binds the viral Tat protein. The contact between the Tat protein and the cellular cyclin-dependent kinases, which phosphorylate the C terminal domain of RNA-polymerase II, enhances both the initiation and the processivity of viral mRNA synthesis.[41] Since Tat–TAR interaction is absolutely essential for viral replication, in the search for anti-HIV drugs, considerable effort has concentrated on identifying and developing compounds which interfere with the formation and function of this protein–RNA complex.[42–50] Small molecule inhibitors of the Tat–TAR interaction have been obtained by rational design,[45] combinatorial synthesis,[43] high-throughput screening (HTS) assays,[44, 46] and a combination of these approaches.[47] Structure-based design of ligands specific for TAR[45, 50] will be greatly facilitated by the availability of three-dimensional structures of the free RNA,[51] the RNA complexed with argininamide,[52] and peptides derived from the Tat protein.[53]

Interestingly, it has been found that in the red clover necrotic mosaic virus (RCNMV), transcriptional *trans* activation is mediated not by a protein but by an RNA specifically binding to a TAR-like element.[54] While it is as yet unknown whether similar systems exist in other organisms, the RCNMV RNA-mediated *trans* activation provides another example in which an RNA accomplishes a function which, in many cases, depends on a protein.

Recently it has been reported that the HIV TAR structure is also required for another crucial process in the viral replication cycle[55] namely the RNA encapsulation into viral particles.[56] The protein partner interacting with TAR for the packaging activity has not yet been identified. Encapsulation requires additional protein–RNA interactions, namely those between the viral nucleocapsid protein (NC) and the ψ signal sequence, which consists of three viral mRNA hairpins.[57] Structure-based design of inhibitors of the NC–RNA interaction could be facilitated by the availability of the three-dimensional solution structure of HIV-1 NC bound to the SL3 hairpin of ψ RNA which has been determined by NMR spectroscopy.[58]

2.3. Processing, Transport, and Localization

Primary RNA transcripts undergo a variety of different processing and localization events, depending on the type of RNA (mRNA, rRNA, tRNA, etc.) and the organism. In all contemporary cells, functional tRNAs are tailored by the ribonucleoprotein enzyme RNase P cleaving the 5′ terminus from longer precursor sequences.[59] RNase P is composed of one (*Eubacteria*) or several (*Eucarya* and *Archaea*) protein subunits 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 catalyze the cleavage reaction in the absence of the protein subunit. The RNAs of eucaryotic and archaeal RNase P, however, require the protein constituents for activity. RNase P from eubacteria and eucaryotes, thus, may be

different enough to allow the development of compounds which specifically interfere with the eubacterial enzyme. Aminoglycosides and the nucleoside derivative puromycin have been reported to inhibit RNase P of *Escherichia coli*[60] and the ciliate protozoan *Tetrahymena thermophila*,[61] by interaction with the RNA component. Analogues of vitamin A (retinoids) act as inhibitors of RNase P of *Dictyostelium discoideum*, however, the binding site of the compounds is not known.[62] Refined three-dimensional models for the RNA component of eubacterial RNase P are available[63] which were derived from comparative phylogenetic analyses and biochemical data.

mRNA for export to the cytoplasm is generated by a processing machinery involving small nuclear ribonucleoproteins (snRNPs) in the nucleoplasm, whereas ribosomal rRNAs (rRNAs) are maturated and assembled by a different set of snRNPs, the snoRNPs (small nucleolar ribonucleoproteins), in the nucleolus.[64] Vertebrate cells contain around 200 different snRNPs consisting of one or several protein subunits and an RNA component (snRNA) of 60–300 nt. The most prominent function of snRNPs is splicing, a process which involves many snRNAs assembled into large "spliceosomes" which remove introns from primary transcripts to yield mature mRNA.[65] While information about the extent to which snRNAs contribute to catalytic steps in processing is currently scarce, they participate in many specific protein–RNA and RNA–RNA interactions. The splicing apparatus of parasites has been suggested as a target for drugs against fungal infections[66] and trypanosomes.[67] Information on molecular details of protein–RNA interactions in snRNPs is becoming accessible as several three-dimensional structures of snRNP proteins and protein–RNA complexes have been recently solved by crystallography and NMR spectroscopy.[68]

A number of viruses encode the small RNAs that associate with protein components of host cell snRNPs and, so, form viral snRNPs[64] which might play roles in the infection cycle by manipulating the host RNA processing machinery. Such snRNP-forming RNAs have been found, among others, in infection of human B lymphocytes with Epstein–Barr virus (EBV),[69] in infections of tumor and lymphoma cells with Kaposi sarcoma-associated herpes virus (KSHV),[70] and as components of the viral replication complexes of simian hemorrhagic fever virus (SHFV).[71]

Splicing and transport of HIV mRNA is controlled by an interaction between the viral Rev protein and the Rev response element (RRE),[72] an internal loop RNA structure located in the *env* gene of HIV. The exact role of Rev binding to RRE is not fully clear, but Rev–RRE interaction is required for nuclear export of unspliced and partially spliced viral mRNA encoding viral structural proteins. Similar splicing control systems are found in other retroviruses including the human T-cell leukemia virus (HTLV).[73] While the primary structures of the HTLV regulatory protein, Rex, and its cognate RNA element, RxRE, are distinct from HIV Rev/RRE, the mechanism of action is similar for both systems.[74] The finding that aminoglycoside antibiotics such as neomycin B can selectively bind to RRE RNA and prevent the Rev–RRE interaction in vivo[75, 76] has initiated an intense search for RRE-binding compounds by rational design and combinatorial synthesis.[77–80] Development of compounds targeted at the Rev–RRE system will benefit from three-dimensional structure data now available for complexes between the RNA-binding domain of Rev and both RRE[81] and aptamer RNAs.[82]

A peculiar 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, the first ribozymes found.[83] Group I introns are potential therapeutic targets since they occur in relevant genes of pathogenic microorganisms but not in mammalian cells.[84–86] Group I introns form large RNA folds with a conserved core containing the splice site and the binding pocket for the guanosine cofactor.[87] The three-dimensional structure of group I introns has been modeled using phylogenetic data.[88] Crystal structures of core domains have been obtained recently.[89] Aminoglycosides,[84, 90] peptide antibiotics of the tuberactinomycin family,[91] and L-arginine derivatives[85] have been reported to inhibit group I intron self-splicing. HTS assays have been developed in order to find other inhibitors.[86, 92]

A rare posttranscriptional modification process is uridine (U) insertion/deletion editing of mitochondrial mRNA in trypanosomes such as *Trypanosoma* and *Leishmania*.[93] The site specificity of U insertions and deletions is determined by short guide RNA (gRNA) molecules which hybridize in proximity to the editing region and provide structural elements recognized by the proteins of the editing machinery.[94] Since similar processes are absent in humans, mitochondrial mRNA editing provides an attractive target for RNA-directed drugs in therapy of trypansome-caused diseases.

Besides covalent processing and modification, localization and stability control of mRNA mediated by specific interactions with proteins, are widespread mechanisms for directing the translation of messages in distinct regions of eucaryotic cells.[95, 96] It has only recently become evident that a large number of mRNAs are tightly attached to the cytoskeleton by RNA-binding microtubule- and actin-associated proteins.[96] mRNA localization has two complementary functions, either to cause a high local concentration of the encoded protein or to prevent high levels of the protein from being ubiquitous in all cells. A common theme in RNA localization is interaction between a *cis*-acting element, usually located within the 3′ untranslated region (3′-UTR) of the mRNA, and a *trans*-acting protein factor. The number of discovered protein–RNA interactions controlling mRNA localization and stability in developmental regulation and pathological processes is rapidly increasing, pointing to a wide variety of mechanisms and only few examples are discussed here.

Defects in the recognition of mRNA regulatory elements by proteins have been thought to be involved in the human autoimmune disease paraneoplastic opsoclonus-myoclonus ataxia (POMA) and the fragile X mental retardation syndrome (FMR), the most common form of hereditary mental retardation. In POMA, a disorder associated with breast cancer and motor dysfunction, specific binding of the neuronal protein Nova-1 to RNA targets, including the mRNAs coding for Nova-1 itself and the inhibitory glycine receptor

alpha2 (GlyRα2),[97] is disrupted by antibodies.[98] The three-dimensional structure of the RNA-binding K homology (KH) domains of Nova protein has been determined by X-ray crystallography[99] revealing similarities to the RNA-binding regions of FMR1,[100] an mRNA-recognizing protein involved in FMR.[101]

cis-Acting sequence motifs, dubbed "zipcode RNA", located in the 3′-UTR of mRNAs, are responsible for the binding of localization and stability control factors.[102] In β-actin mRNA, a conserved 54 nt motif in the 3′-UTR is recognized by a zipcode binding protein (ZBP-1) which localizes the mRNA to the cell periphery of fibroblasts.[103] ZBP-1 shares sequence similarity with four other known proteins: 1) the general mRNA localization factor Vera in *Xenopus*, 2) the coding region determinant-binding protein (CRD-BP) in mice,[104] 3) the KH domain protein (KOC) which is overexpressed in human cancer cells,[105] and 4) the p62 consensus sequence RNA-binding domain protein (CS-RBD) in human hepato-cellular carcinoma (HCC) cells.[106] Vera, a highly conserved component of the mRNA localization machinery, binds specifically to an RNA motif in the 3′-UTR of Vg1 mRNA (vegetal mRNA 1).[107] CRD-BP, a KH domain RNA-binding protein, is not involved in localization but stabilization of mRNA. It increases the RNA half-life by binding to a motif within the coding region of the oncogene c-myc mRNA, thereby preventing endonucleolytic degradation.[105]

Similar mechanisms of mRNA stabilization have been reported for urokinase receptor (uPAR) mRNA which is the target of a stability-decreasing regulatory protein,[108] for vascular endothelial growth factor (VEGF) mRNA which, at its 3′-UTR, binds heterogeneous nuclear ribonucleoprotein L (hnRNP L),[109] and for the tumor-specific c-fos and MYCN mRNAs which have 3′-UTR recognition sites for regulatory RNA-binding proteins which are possible malignancy-promoting factors.[110]

Expression of the human GLUT1 transporter, responsible for shuttling of glucose over the blood–brain barrier, is regulated by a 10 nt RNA element in the 3′-UTR which enhances the stability of GLUT1 mRNA.[111] While the protein recognizing the GLUT1 stabilization motif is as yet unknown, brain-derived peptides have been shown to exert stabilizing activity, probably by interaction with the mRNA.[112] Interestingly, the RNA-controlled regulation of mRNA stability is involved in the overproduction of cytokines and amyloid precursor protein (APP) in cancer and Alzheimer's disease, respectively.[113] Overproduction of APP can be caused by accumulation of APP mRNA which is stabilized by proteins 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 proteins,[113] including heterogenous nuclear ribonucleoprotein C (hnRNP C) which, upon binding, increases APP mRNA stability.[114, 115] Other factors, yet unknown, might recognize the same 29 nt motif and accelerate APP mRNA degradation in normal cells, since mutant mRNAs lacking the 29 nt element have been shown, in an in vitro translation system in the absence of stabilizing proteins, to be more stable than wild-type sequences.[115]

2.4. Translation

The ribosome, which synthesizes the proteins encoded on mRNA, has been identified as the target of some of the oldest antibiotics, the aminoglycosides, which were later found to specifically recognize the eubacterial 16S ribosomal RNA component (rRNA).[116] Aminoglycosides bind to the A site of the decoding region of rRNA[117] which leads to miscoding during translation.[118] The aminoglycoside–rRNA interaction is remarkable as the drugs are able to specifically target the eubacterial A site fold which differs from the eucaryotic A site by essentially a single base exchange (at position 1408, a guanine is found in all eucaryotes while it is always an adenine in eubacteria[119]). The high recognition specificity of aminoglycosides is further highlighted by the finding that a genetically determined point mutation in human mitochondrial rRNA leads to increased binding affinity for aminoglycosides which causes hypersensitivity to these drugs.[120] In order to elucidate the molecular origin of binding specificity, considerable effort has been concentrated on studying the aminoglycoside–rRNA interaction by biochemical[121] and biophysical methods.[122–127] The three-dimensional solution structures of a 27 nt model RNA containing the eubacterial A site complexed with the aminoglycosides paromomycin and gentamicin have been determined by NMR spectroscopy.[122, 125] A promising technique based on the analysis of fragmentation patterns obtained in mass spectroscopy has been applied for studying the interaction sites of aminoglycosides on ribosomal A site RNA constructs.[127] Synthetic analogues[128] and mimetics[129–132] of aminoglycosides were tested for their ability to target the ribosomal decoding region and a high-throughput screen has been developed to identify molecules with high affinity for the 16S rRNA A site.[133]

A number of antibiotics known to inhibit the peptidyl transferase reaction in the ribosome by different mechanisms have been investigated for their binding regions on 23S rRNA.[134] A remarkable inhibition mechanism has been uncovered for thiostrepton, a large thiazole-containing cyclic peptide, which binds to the conserved 58 nt GTPase center of eubacterial 23S rRNA[135] and, thereby, stabilizes a tertiary interaction within this RNA[136] and interferes with a conformational change within the ribosomal L11 protein.[137] Recent crystallographic analyses of complexes between L11 binding site RNAs and the L11 protein[138] provide a molecular basis for detailed investigations of thiostrepton action on the ribosome.

Interestingly, thiostrepton also interacts specifically with 28S rRNAs encoded on extrachromosomal DNA in the plastide-like organelles of *Plasmodium falciparum*.[139, 140] Similar to its action on 23S rRNA, thiostrepton stabilizes a tertiary interaction in the plastid rRNA,[141] which ultimately leads to growth inhibition of the parasite.[140]

The licosamides, pyrrolidine–saccharide antibiotics, may exhibit a striking case of inhibition by substrate mimicry at the peptidyl transferase center of rRNA. It has been suggested that the licosamide clindamycin has a three-dimensional structure resembling the first adenine of tRNA linked to an L-proline–methionine dipeptide.[142]

The pyrrolidine antibiotic anisomycin provides a beautiful example of a small molecule triggering a complex process upon specific binding to RNA. In eucaryotic ribosomes, interaction of the antibiotic with the 28S rRNA initiates the stress-activated protein kinase JNK1, probably by inducing a conformational change in the rRNA.[143] Mechanistic studies on compounds which target rRNA and, thus, interfere with ribosome function will, in future, be greatly facilitated by current efforts to elucidate high-resolution structures of the ribosome.[144, 145]

Structural elements within mRNA play an important role in a number of recoding mechanisms, in which decoding alternatives of the message are exploited during translation.[146] A prominent example is programmed ribosomal frameshifting to read through a stop codon in retroviral Gag–Pol polyprotein synthesis; this occurs in response to three conserved RNA elements consisting of a frameshift site, a spacer region, and a downstream enhancer motif.[147] The downstream enhancer provides a stable RNA structure that causes the ribosome to pause during elongation so that kinetically unfavorable alternative decoding events can occur. In the RNA of some retroviruses, the enhancer sequence forms a pseudoknot[148] while others, such as HIV, contain a stable hairpin motif.[149] As replication of the retrovirus depends on production of a balanced ratio of the Gag protein and the Gag–Pol polyprotein, both artificial stabilization and destabilization of the frameshift enhancer motif might provide strategies for RNA-targeted therapy in retrovirus infection. The three-dimensional structures of frameshifting RNA pseudoknots are known from NMR spectroscopy and crystallography.[150] Interestingly, RNA pseudoknots have been demonstrated to form specific binding sites for small molecules such as the peptide antibiotic viomycin[151] and biotin.[152]

A peculiar frameshifting signal that does not depend on a downstream enhancer has been found in the mRNA coding for a thymidine kinase in a herpes virus mutant.[153] The frameshift site itself consists of a G-rich sequence which probably forms a unique three-dimensional structure recognized either by the ribosome or an additional factor.

Alternative decoding of a stop codon occurs in cotranslational selenocysteine incorporation during synthesis of selenoproteins.[154] In eucaryotes, the recoding event is triggered by the selenocysteine insertion sequence (SECIS) which forms a conserved structural motif in the 3′-UTR of selenoprotein mRNAs.[155] The SECIS element provides the binding site for SECIS-binding proteins (SBP)[155] which act as elongation factors to confer alternative decoding. Based on sequence analyses, it has been suggested that SECIS elements may occur in the genome of pathogenic viruses.[156]

Expression of several proteins involved in iron transport and uptake is regulated on the translational level by the interaction between an iron-responsive element (IRE) in the 3′-UTR of the mRNAs and iron regulatory proteins (IRP).[157] The IREs are a family of conserved, approximately 30 nt RNA hairpin motifs with defined three-dimensional structures[158] which are recognition sites for different IRPs, thereby providing finely tuned mRNA-specific responses to external iron signals.[159] Mutations in an IRP, which perhaps interfere with RNA binding, have been suggested to be the cause for the abnormal distribution of iron in the brain in Alzheimer's disease.[160]

Autoregulation of translation by binding of the protein to its own mRNA has been reported for human thymidylate synthase (TS),[161] which is an important target in cancer chemotherapy.[162] TS also binds specifically to other cellular mRNAs coding for the nuclear oncogene c-myc,[163] interferon-induced proteins,[164] and the tumor suppressor p53.[165] Two distinct domains of TS mRNA, one in the 5′-UTR and one within the coding message, are involved in TS protein recognition.[166] For the binding site in the 5′-UTR, a hairpin structure has been predicted which, when isolated in a smaller RNA construct, was also shown to specifically bind aminoglycoside antibiotics.[167] Based on these findings, a strategy for anticancer therapy has been suggested which requires small molecules targeted at the 5′-UTR TS binding site in the mRNA to inhibit translation of TS message.[167]

3. Recognition of RNA by Peptides and Proteins

Most functions of RNAs require interaction with RNA-binding proteins. Protein–RNA complexes are instructive for understanding RNA recognition by small molecules as, firstly, proteins may use similar strategies for binding to RNA and, secondly, protein-binding sites are potential targets for drug action. A prominent example for a structural element recognized in common by a protein and a small molecule is a non-Watson–Crick G–G base pair within the HIV RRE RNA which is critical for binding of the viral Rev protein as well as for neomycin B binding.[75, 168]

RNA recognition strategies of peptides and proteins will only be briefly surveyed here, as several excellent recent reviews on this topic are available.[169–173] An exponential increase of structural data on a wealth of novel protein–RNA interactions is expected soon from further refinement of currently available electron density maps of the ribosome, with a resolution of around 8 Å for the complete ribosome,[144] and around 5 Å for the separate small and large subunits.[145]

3.1. General Principles of Protein–RNA Interactions

While the structural variety of DNA-targeted proteins reflects strategies for binding double-stranded nucleic acid helices, which is almost invariably the form of cellular DNA, RNA-binding proteins have evolved to recognize intricate three-dimensional RNA folds, comparable in 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 as at least partially folded single-stranded molecules. Double-stranded RNA in regular A-form helices is only marginally suited for specific intermolecular interactions. The discriminatory base edges are buried in a narrow deep ("major") groove[174] and the readily accessible shallow ("minor") groove contains less information for recognition. Yet, large parts of the RNA folds participate in double-stranded base-paired regions. Distor-

tions of the regular A-form by non-Watson–Crick base pairs, interspersed looped-out residues, and platforms of consecutive nucleotides lead, however, to an accessible widened deep groove.[171, 172, 175] Proteins not only recognize but may also enhance such distortions of the RNA structure. Insertion of an α helix (Figure 2A) or a flexible protein loop into a

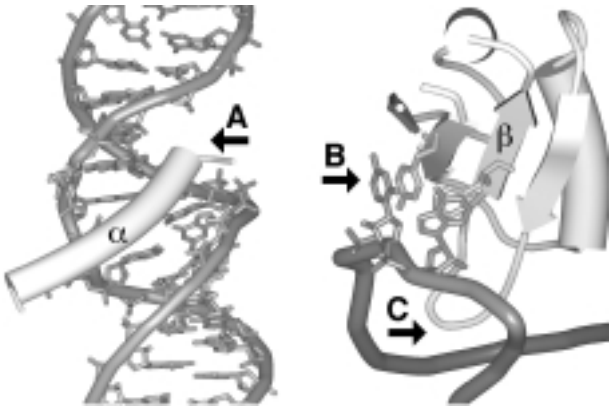

Figure 2. Strategies for RNA recognition by proteins, illustrated by schematic views of a Rev peptide–RRE complex (left)[81] and a U1A protein–RNA complex (right).[216] RNA is shown in dark grey, protein in light grey. In the right-hand panel, only the RNA backbone is shown as a tube. A: An α helix can be inserted into the RNA deep groove which has been widened by looped-out residues, noncanonical base pairs, or base triples. Side chains of residues in the α helix can form contacts with the base-pair edges and with the sugar phosphate backbone in the RNA. B: Aromatic residues on the surface of β-strands are accessible for stacking with unpaired bases in RNA loops. C: Flexible protein loops can penetrate into RNA folds and form H-bonds and ionic interactions, preferably with the RNA backbone. The figure was prepared from coordinates deposited in the protein data bank (PDB), Brookhaven (PDB codes: 1ETF, left panel; 1URN, right panel).

widened deep groove is a common theme in several protein–RNA complexes.[172, 173] Exposed β-strands in proteins provide interaction surfaces for unpaired RNA regions which can bind by stacking of splayed-out bases on aromatic amino acid side chains (Figure 2B).[170, 171] Flexible protein loops participate predominantly in interactions with the RNA backbone (Figure 2C), often leading to reduced loop mobility.[171, 173]

A remarkable difference is observed in RNA complexes of small peptides versus RNAs bound to larger proteins.[172, 176, 177] In peptide–RNA complexes, adaptive conformational transitions are frequently observed for the peptide upon binding to a largely unaltered RNA scaffold. On the contrary, larger proteins predominantly bind RNA as rigidly folded domains which provide exposed surfaces, cavities, and clefts for the RNA substrate which adapts by conformational changes.

3.2. Molecular Details of Protein–RNA Interactions

Protein–RNA interactions mostly involve the protein side chains (approximately 90% of the interactions in all complexes of known structure[178]), which act predominantly as H-bond donors. In the RNA, negatively charged phosphate groups of the backbone are the most important H-bond acceptors. In protein–RNA complexes of known structure, about 20% of the intermolecular interactions involve the RNA 2′-OH group, equally often as a hydrogen donor and as an acceptor.[178] RNA-binding surfaces of peptides and proteins are rich in the basic amino acids arginine and lysine which account for about 60% of intermolecular H-bonds in the RNA complexes.[178] Obviously, acidic residues are seldom found at the interface of proteins and RNA. The important role of arginine in protein–RNA interactions is due to both its potency as a versatile basic H-bond donor and its flat guanidinium side chain which allows for additional favorable stacking interactions with RNA bases.

Intermolecular stacking interactions in protein–RNA complexes can be extensive, involving residues of both components in an alternating fashion, termed interdigitation.[172] Hydrophobic parts of RNA bases, accessible in triples, platforms, and noncanonical base pairs can act as surfaces for aligning nonpolar side chains of proteins.[172] Important contact sites for H-bonds are provided by the Watson–Crick edges of RNA bases[172, 175], 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 known in which a ten-residue peptide specifically binds to an RNA helix when the central base pair is G–U but not when it is replaced by G–C.[179] 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 proteins in RNA folds. [175] A prominent example is sheared G–A pairs for which a characteristic "in-plane breathing" motion has been observed in molecular dynamics (MD) simulations of RNA;[180] this facilitates potential interactions of functional groups on the Watson–Crick edges of the bases with protein side chains.

Finally, there are ions and water which are intrinsic parts of the RNA three-dimensional structure[181] and play important roles in protein–RNA interactions. In three-dimensional structures of protein–nucleic acid complexes, well-oriented water molecules are often found at the binding interfaces where they extend shape complementarity by filling cavities.[178] As polyanions, RNAs are inevitably surrounded by an important number of counterions competing with proteins for binding sites.[182] Proteins can bind to RNAs at low ionic strength by an entropically favored displacement of cations from electronegative pockets on the RNA. At higher salt concentrations, however, this process is reversed due to salt-induced destabilization of polar intermolecular H-bonds and the competition between hydration equilibria of charged groups and salt ions.[183]

4. RNA Recognition by Small Molecules

In future, therapeutic strategies relying on complex biomolecular effectors such as antisense and ribozyme RNA may be facilitated by emerging techniques for the delivery of large,

often highly charged molecules and by safe systems for gene therapy and engineered viral vectors. For the time being, however, lead design, independently of whether proteins or nucleic acids are the targets, will continue to concentrate on small synthetic molecules and derivatives of natural low molecular weight compounds. In the following discussion, the focus will mainly be on the recognition of naturally occurring RNA targets by small molecules. Aptamers—nucleic acid molecules which are obtained by in vitro selection (SELEX) of random sequence libraries and bind a substrate molecule with both high affinity and specificity—will be discussed only in few selected cases. Recently, in vitro selected aptamer RNAs have been shown to also recognize their cognate ligands in vivo.[184] Thus, knowledge of principles of adaptive molecular recognition in nucleic acid aptamer complexes[177] is likely to influence rational drug design strategies for natural RNA targets. Concise overviews on the selection of aptamers,[185] their three-dimensional structures,[177, 186, 187] and their uses as diagnostic and therapeutic agents[188] and in conventional drug design[189] are given by recent reviews.

4.1. Philosophy of Targeting RNA Folds

In the early days of investigating small molecule – RNA interactions, many concepts of molecular recognition had been adapted from knowledge on DNA complexes. Thus, intercalators and groove binders were among the first compounds systematically analyzed for their RNA-binding capacity.[190] The application of recognition strategies derived from DNA targets on RNA is severely limited by the very different three-dimensional structures of functional RNA molecules[3] which are not restricted to regular double helices. In the light of RNA structure – function relations, regular undistorted A-form helices of double-stranded RNA are unlikely to be efficient targets for effector molecules. Consequently, in the search for compounds targeted at RNA molecules, the notion of sequence specificity is not a useful concept and is perhaps limited to the partially understood connection between the sequence of an RNA and its three-dimensional structure.

The goal of achieving specific RNA recognition is conceptually closer to targeting proteins than DNA, but with different weights on the distinct energetic contributions to molecular interactions. Such contributions come from electrostatic forces, H-bond formation, and different nonbonded interactions, including van der Waals, stacking, and hydrophobic forces, which are maximized by molecular shape complementarity. In the following, I will discuss how these forces direct the binding of small molecules to RNA folds and how drug-design strategies might benefit from our current understanding of RNA – small molecule interactions.

4.2. Electrostatic Forces and Metal Ion Binding

Electrostatic forces play a major role in interactions with RNA because of the polyanionic nature of RNA. The majority of RNA-binding molecules (Figure 3) both natural, such as the aminoglycoside[191] and tuberactinomycin[91] antibiotics, and designed, including synthetic aminoglycoside derivatives and mimetics,[30, 49, 79, 128–132, 192] diphenylfurans,[77] cyclophanes,[47, 193] macrocycles,[80] polyamine – acridine conjugates,[45] arginine derivatives,[48, 49] and peptoid/peptid oligom-

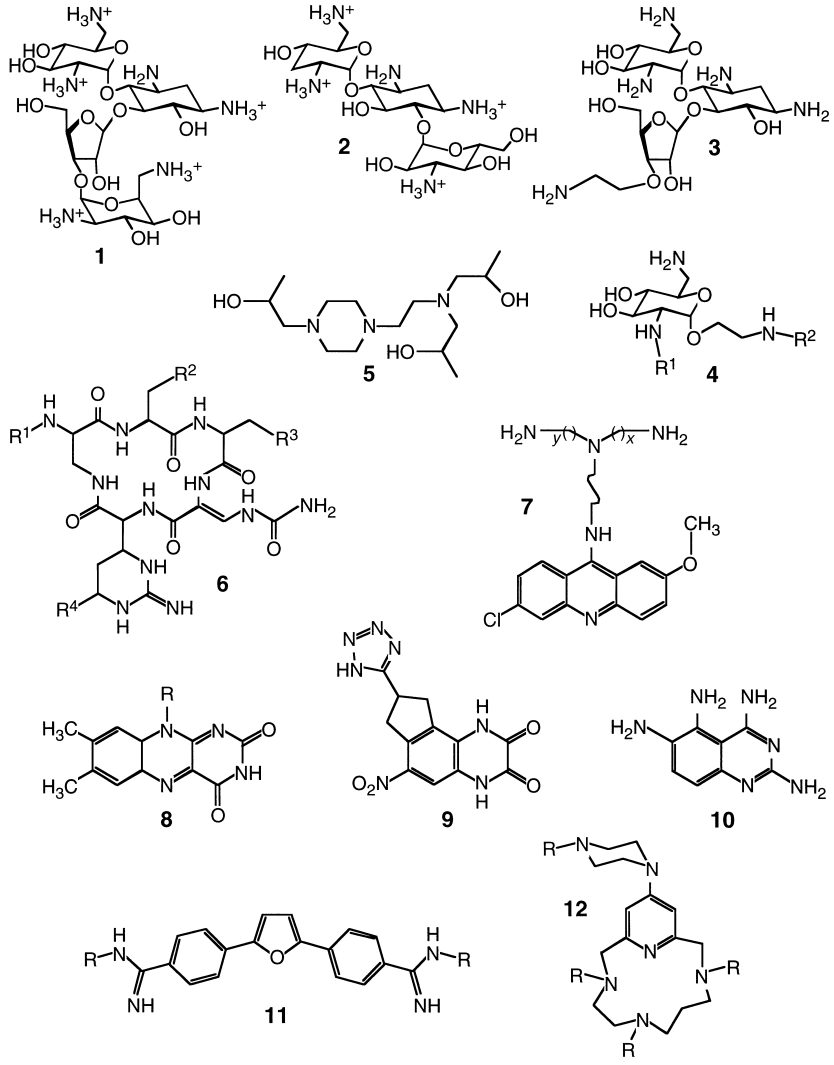

Figure 3. A collection of RNA-binding compounds which specifically recognize different RNA folds (see text for further details): natural aminoglycoside antibiotics, neomycin B (**1**) and tobramycin (**2**);[191] simplified semisynthetic aminoglycoside mimetics, **3** and **4**;[128, 130] an aminol aminoglycoside surrogate **5**;[131] natural tuberactinomycin antibiotics **6** (R^1 and/or R^3 are usually cationic substituents);[91] In-PRiNts **7**;[45] photocleaving flavin derivatives **8**;[204] a quinoxaline-2,3-dione derivative **9**;[46] a 2,4-diaminoquinozaline derivative **10**;[46] substituted diphenyl furans **11**;[77] and piperazinyl polyazacyclophanes **12**.[47] Most of the compounds carry positive charges at physiological pH, however, protonation is indicated here only for the natural aminoglycosides, for which exact pK_a values are available.[217]

ers,[43] are cationic compounds carrying several positively charged groups. Electrostatic interactions between the negatively charged RNA backbone and cationic groups enhance the binding affinity in RNA – drug complexes. But what about their role in mediating binding specificity? Lacking spatial directionality, electrostatic forces are considered to contribute to nonspecific binding. They can govern, however, specific recognition in the binding of conformationally constrained polycationic molecules to RNA folds as revealed by investigations on the interaction of aminoglycosides with different RNA targets.[30, 34, 128, 129, 192] In a quantitative approach using surface plasmon resonance, it has been demonstrated that increasing the ionic competition by adding salt to RNA – aminoglycoside complexes decreased both the specific and nonspecific drug binding to about the same extent.[128] In other words, the electrostatic component of the binding energy contributed, as expected, to the unspecific binding but also, to a remarkable extent, to the specific binding. Two mechanisms confer the specificity to electrostatic interactions in RNA – drug recognition.

Firstly, electrostatic interactions with RNA are often part of polar H-bonds between anionic phosphate oxygens as acceptors and positively charged H-donor groups in the bound molecule (Figure 4A). While electrostatic forces between the

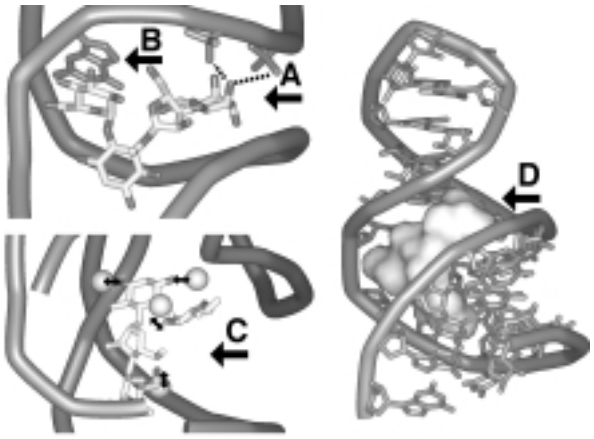

Figure 4. Some principles of RNA recognition by small molecules, illustrated by schematic views of a 16S A site RNA – paromomycin complex (top left and right)[122] and a hammerhead RNA – neomycin complex (bottom left).[33] RNA is shown in dark grey, in the left panels as the backbone tube only. The drug is shown in light grey, in the left panels as a stick representation, on the right as a space-filling surface representation. A: H-bonds can form between polar donor and acceptor groups in the drug and the RNA. Frequently, negatively charged phosphate groups of the RNA backbone participate as acceptors in H-bonds with cationic substituents in the small molecule (here, an ammonium group). B: Flat, but not necessarily planar hydrophobic molecule fragments, such as the sugar ring shown here, provide stacking surfaces for RNA bases. C: By exploiting structural electrostatic complementarity, small molecules can provide frameworks to orient cationic groups properly in space, so as to complement the electronegative metal ion binding pockets of the RNA fold. In the figure, a solution conformer of neomycin has been docked to the crystal structure of the hammerhead RNA in such a way that the positively charged ammonium groups of the drug point (arrows) towards the crystallographic Mg^{II} binding sites (spheres). D: Shape complementarity between the binding surfaces of the small molecule and the RNA maximizes intermolecular van der Waals interactions and contributes to binding specificity. The top left and right panels were prepared from coordinates deposited in the protein data bank (PDB), Brookhaven (file 1A3M), the bottom left panel is adapted from Ref. [33].

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 oxygens and ammonium groups of aminoglycosides observed in three-dimensional solution structures of RNA – aminoglycoside complexes.[122, 125, 194, 195]

Secondly, the three-dimensional folding of an RNA chain into a scaffold of spatially oriented anionic groups creates electronegative pockets where negative charge is focused.[196] The spatial distribution of negatively charged pockets in an RNA fold provides a three-dimensional pattern that can be specifically targeted by compounds exhibiting structural electrostatic complementarity.[33, 34, 50] This principle is exploited by aminoglycosides binding to RNA targets, which has been extensively investigated for the hammerhead ribozyme. This catalytic RNA is a paradigm system for the study of RNA – drug interactions because, in addition to the binding affinity, the specificity of RNA recognition can be analyzed by monitoring inhibition of the ribozyme. Following on the experimental observation that neomycin B (**1** in Figure 3) inhibits the self-cleavage of the hammerhead ribozyme by competing with the binding of catalytic metal ions to the RNA,[29] it has been shown that solution conformers of aminoglycosides provide conformationally constrained scaffolds carrying cationic ammonium groups in the proper spatial orientation so as to simultaneously displace several Mg^{II} ions from their binding sites in electronegative pockets of the hammerhead fold (Figure 4C).[33] MD simulations of hammerhead RNA – aminoglycoside complexes suggest that ammonium groups of the aminoglycosides can mimic the interaction of Mg^{II} ions with the RNA down to an atomic level (Figure 5A, B).

Experimental studies on hammerhead RNA – drug complexes containing designed aminoglycoside derivatives in which the number, the basicity, or the flexibility of the attachment of ammonium groups has been systematically altered[30, 128, 192] support the model of structural electrostatic complementarity.[34] When either the number or the charge density of ammonium groups in aminoglycosides is increased, the inhibitory activity of the compounds is enhanced.[30, 34, 192] The binding specificity of aminoglycosides is diminished when the conformational constraint on the aminoglycoside scaffold is partially relieved by replacing one of the ring substructures by a more flexible aliphatic chain carrying one or more amine groups (Compounds **3** and **4** in Figure 3).[128]

The concept of structural electrostatic complementarity has been successfully used to explain the structural basis of aminoglycoside inhibition of self-splicing group I introns.[197] The Mg^{II} ions needed for catalysis could be displaced from the active site of a group I intron by docking solution conformations of neomycin B to the RNA.

While the direct participation of metal ions in RNA function may be limited to ribozymes, the role of cations in stabilizing the three-dimensional 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. A hierarchical procedure has been developed to, first, calculate electronegative

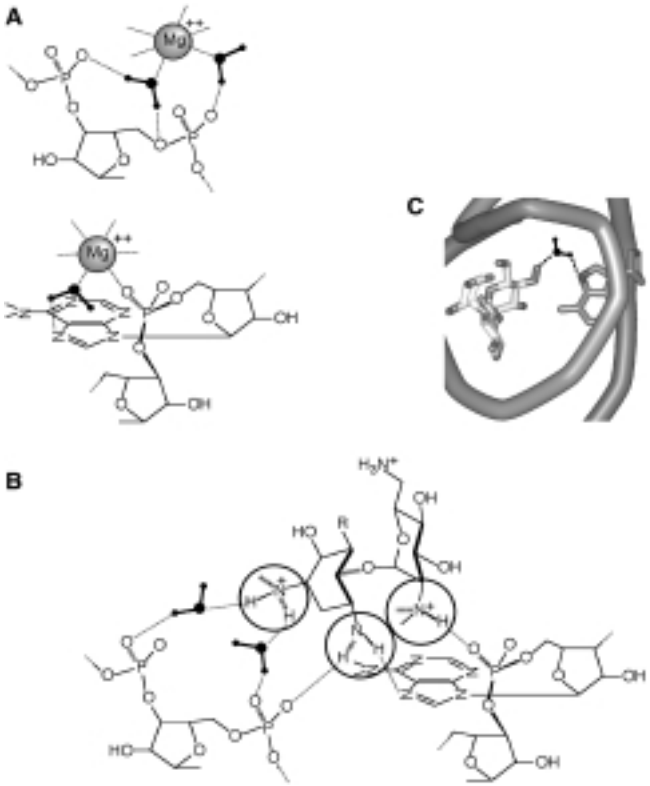

Figure 5. The Mg^{II} ions bound to the hammerhead ribozyme in the crystal structure.[218] (A) can be displaced by ammonium groups of aminoglycosides engaging in interactions with the RNA which resemble the contacts of the metal ions down to an atomic level (B).[33] Water molecules (represented by black ball-and-stick models) mediate the binding by conferring a certain plasticity in the contacts. C: A proposed water-mediated contact between a hydroxyl group of the drug (stick representation) and the N7 atom of an adenine of the RNA (tube representation) in the solution structure of a tobramycin – aptamer RNA complex.[194] Figures A and B were adapted from Ref. [33], Figure C was prepared from coordinates kindly provided by D. J. Patel.

pockets in RNA folds[196] and, second, predict, on the basis of structural electrostatic complementarity, docking sites for cationic compounds.[50, 198] The prediction method was used to construct complexes of aminoglycosides bound to HIV-1 TAR which explain, on a molecular basis, the experimentally established allosteric mechanism[199] by which neomycin induces dissociation of Tat – TAR complexes.[50]

4.3. H-Bonds and Water-Mediated Contacts

For the H-bond forming capacity of single atoms in RNA – small molecule complexes, the observations from protein – RNA complexes (see Section 3.2) apply accordingly. It has already been pointed out that polar hydrogen bonds, predominantly between phosphate groups of the RNA backbone as acceptors and hydrogen atoms of the small molecule, can contribute significantly to RNA – drug binding. A large portion of the available data on H-bonding in RNA – drug complexes stems from studies on RNA – aminoglycoside complexes,[200] among them NMR studies on aminoglycoside complexes of aptamer RNAs[194, 195, 201] and oligonucleotides derived from the eubacterial A site rRNA.[122, 124, 125]

In experimentally determined three-dimensional structures of RNA – aminoglycoside complexes, both the ammonium and hydroxyl groups of the drugs are H-bond donors which interact predominantly with phosphate oxygen atoms of the RNA backbone (Figure 4A), and with the N7 and O4 atoms of purines and uridine, respectively.[122, 124, 125, 194, 195, 201] Similar observations have been made in model complexes obtained by docking of aminoglycosides to RNA targets.[33, 202]

The stereochemical variety of substitution sites for the ammonium and hydroxyl groups in aminoglycosides, along with their constrained conformational flexibility, provides a versatile tool for orienting 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 distinct compounds of these family of drugs with high affinity. Thus, it has been suggested that the principal sugar building blocks of aminglycosides could be used as synthons for the design of RNA-targeted drugs.[33] Both conventional synthetic chemistry[128, 129, 131] and combinatorial synthesis[79, 130] have been used to simplify aminoglycoside building blocks and to obtain synthetically readily accessible carbohydrate mimetics (Compounds **3** and **4** in Figure 3).[132, 203]

H-bond formation involving the 2-amino group of guanine is likely to be responsible for the shallow-groove recognition of G – U wobble pairs by a small peptide.[179] Likewise, a specific H-bond between the 2-amino group of guanine and a carbonyl group of the isoalloxazine moiety has been proposed in a model for the shallow-groove recognition of G – U pairs by photocleaving flavin derivatives (**8** in Figure 3).[204, 205]

Water may participate in H-bond formation in RNA – small molecule complexes, as has been suggested for a tobramycin – RNA aptamer complex in which a water-mediated H-bond between a hydroxyl group of the drug and an adenine N7 atom could occur (Figure 5C).[194] Such water-mediated H-bond contacts between the RNA and the small molecule, again preferably to phosphate oxygen atoms and N7 atoms of purines, have frequently been observed in MD simulations of hammerhead RNA – aminoglycoside complexes (Figure 5B).[33]

4.4. Stacking and Intercalation

The bases in RNA folds provide surfaces for stacking interactions driven by hydrophobic effects, that is, the exclusion of polar solvent from the proximity of the flat unpolar surfaces, van der Waals interactions and, eventually, interactions between aromatic π-electron systems.[206] The full range of these interactions contributes in classical intercalation of planar aromatic moieties between nucleic acid base pairs which is the binding mode of a variety of DNA-targeted antibiotics and antitumor agents.[207] Different classes of DNA intercalators have been systematically studied for their affinity for RNA[208] and most of the DNA binders were found to intercalate as well in A-form RNA duplexes.[190] For both classical intercalators such as ethidium,[209] and com-

pounds intercalating in a partial "bookmarking" fashion into single-stranded base stacks, such as copper phenanthroline,[210] the interactions with RNA have been analyzed in modeled complexes of HIV TAR and tRNA, respectively.

Due to the physical nature of the interactions involved, classical intercalation is relatively nonspecific. In an attempt at rational design of inhibitors targeting the HIV Tat–TAR complex, an aromatic acridine moiety for stacking and a flexible polycationic anchor for electrostatic contacts with the RNA backbone have been connected through an aliphatic linker to give rise to the inhibitor of protein–ribonucleotide sequences (In-PRiNts;[45] **7** in Figure 3). While this approach yielded a number of promising Tat–TAR inhibitors, large activity differences, not easily linked to compound structure, were found.[45] This suggests that additional restrictions, such as constrained conformational flexibility, might increase inhibitor specificity. For the In-PRiNts type of RNA binders, a stacking interaction different from classical intercalation has been proposed which involves additional H-bonding between the substituted acridine moiety and the RNA base pair edges.[45] Nonclassical threading intercalation has also been discussed as the binding mode of synthetic diphenylfuran cations (**11** in Figure 3) which selectively inhibit HIV Rev binding to RRE RNA.[77]

Recently, specific inhibition of the HIV Tat–TAR interaction has been reported for derivatives of quinoxaline-2,3-dione and 2,4-diaminoquinozaline (**9** and **10** in Figure 3) which specifically recognize an internal bulge and a loop in TAR RNA, respectively.[46] Although detailed structural models for the TAR–drug complexes are not available for these two compounds, it is likely that stacking interactions contribute to their RNA-binding affinity as both molecules contain flat aromatic moieties. The presence of several potential H-bond donating and accepting groups in the quinoxalinediones and diaminoquinozalines suggests that these compounds, similar to the In-PRiNts, might form additional H-bonds within the stacking plane. Extended interactions, as observed in the In-PRiNts, diphenylfurans, quinoxalinediones, and diaminoquinozalines, increase the specificity of intercalation, attested also by the frequent use of combinations of stacking and H-bonding as the recognition principle in RNA aptamers.[177]

Sufficiently planar, unpolar molecule fragments can engage in a more general fashion in stacking interactions driven by van der Waals forces and hydrophobic effects. Spectacular examples are seen in aminoglycosides which stack the less polar side of six-membered sugar rings on purine bases (Figure 4B) in the three-dimensional structures of paromomycin and gentamicin bound to an A site oligonucleotide[122, 125] and tobramycin bound to aptamer RNA.[194] An inverse and maybe more fragile situation with a flat aromatic molecule stacking on the ribose moiety of the RNA backbone is found in the model of a complex between a photocleaving flavin derivative (**8** in Figure 3) bound to the shallow groove of an RNA helix with a central G–U wobble pair.[205] Stacking of the isoalloxazine core of the flavin on a ribose orients the small molecule 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.

4.5. Shape Complementarity and Conformational Adaption

The complementarity of molecular shapes can provide a major driving force in the formation of biomolecular complexes providing high binding affinity and specific ligand discrimination. This is most impressively demonstrated by the three-dimensional structures of RNA aptamer complexes.[177, 186, 187] Characteristically, aptamers encapsulate their substrate within a tightly fitting cavity. For example, in a tobramycin–RNA aptamer complex, 75% of the accessible surface area of the aminoglycoside is buried inside the RNA fold.[194] Ligand binding to aptamer RNAs is typically associated with major conformational changes as the nucleic acids adapt to their substrate.[177, 187]

For natural RNA targets, which, in contrast to aptamers, did not evolve in order to tightly bind to a specific small molecule, shape complementarity with effector compounds might be more difficult to achieve. It has been pointed out that in complexes of aminoglycosides bound to natural targets, considerably less accessible surface of the drugs is buried relative to aptamers.[50] In the complexes between eubacterial ribosomal A site oligonucleotides and aminoglycosides, however, a reasonably good complementarity between the binding surfaces of the RNA and the drugs are observed (Figure 4D).[122, 125]

Shape complementarity can account for remarkable site discrimination, as was reported for a photocleaving phenanthroline rhodium complex that selectively recognizes and cleaves G–U wobble base pairs within double-helical regions of folded RNAs, such as tRNAs.[211]

Conformational changes in RNA upon binding of other molecules can be small, as for the A site oligonucleotide–aminoglycoside system,[123] or considerably larger, as for the HIV TAR RNA interacting with argininamide.[52] The development of small molecules that, upon binding to an RNA fold, prevent a conformational change necessary for the biological function of the target, is another promising strategy in the search for therapeutic compounds. The thiazole-containing peptide antibiotic thiostrepton recognizes a complex structure in the conserved GTPase center of eubacterial 23S rRNA and stabilizes a tertiary interaction which might be required to be flexible for a functional interaction with the ribosomal L11 protein.[136–138] Rational design of effector molecules interfering with RNA conformational flexibility requires a detailed picture of the three-dimensional structure and function of the RNA target. Recently, extensive molecular modeling and simulation studies have led to the suggestion that the allosteric mechanism by which neomycin B induces dissociation of HIV Tat–TAR complexes[199] is based on a conformational arrest in which the aminoglycoside locks TAR in a conformer with low affinity for Tat.[50]

Finally, shape complementarity in RNA–ligand interactions is mediated and enhanced by structural water molecules at the complex interface, as they are commonly found in protein–drug complexes.[212] By reorienting, water molecules can provide a certain plasticity in H-bond formation (see Section 4.3) 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,[213] special NMR spectroscopic techniques,[214] and MD simulations.[215]

5. Summary and Outlook

We are beginning to uncover the many pathological processes involving RNA molecules as key players. It is therefore becoming evident that RNA is a potential prime target for therapeutic intervention. Small molecules may interfere with RNA functions by a number of mechanisms. By binding to an RNA fold, small molecules can alter the conformation or the flexibility of the RNA, and, thereby, inhibit the interaction with proteins or even enhance protein–RNA association. Thus, RNA-binding effectors can trigger responses that depend on either the formation or the disruption of protein–RNA complexes. Outstanding examples are the aminoglycosides and thiostrepton discussed above, which interfere with the function of protein–RNA complexes by preventing their formation (aminoglycosides) or by increasing the rigidity of the RNA component (thiostrepton). Another class of RNA-targeted molecules may act as interface inhibitors and directly prevent the binding of proteins.

The sequencing of the whole human genome will cause an unprecedented leap in the number of potential drug targets, namely RNA transcripts and their various processed active forms. The exorbitant number of available RNA targets requires efficient novel approaches of high-throughput screening (HTS) for small molecules interfering with RNA functions. The development of compounds identified by HTS into highly specific effectors targeted at distinct RNA folds is hardly imaginable without a detailed structural understanding of RNA recognition. This structural knowledge comes from analyses employing X-ray crystallography and NMR spectroscopy on RNA alone, as well as in complexes with proteins and small molecules. With our growing understanding of the RNA three-dimensional structure and function, firm grounds will be provided for the development of specific RNA-directed effector molecules.

Structure-based approaches will be at the heart of RNA-targeted drug design and the evolution of lead compounds identified by HTS into therapeutics. This is particularly true if our efforts in targeting RNA go beyond the group of RNAs which are unique to eubacteria and certain organelles, and if we set out to develop drugs which exploit the minimal structural differences between RNA targets common to humans and pathogens.

Our view on drug design may still be biased by the protein world and some of the difficulties encountered in the search for RNA-binding compounds are due to limitations in applying methods developed for studying protein targets. Just as new experimental tools have been developed for studying RNA in the laboratory, theoretical investigations of RNA and its interactions call for novel approaches. Initial steps have been made in the synthesis of RNA-targeted compounds and tools are emerging for their rational design.

The physical laws describing intermolecular interactions are the same for proteins and RNAs. The example of electrostatic forces shows, however, that the focus on energetic contributions is shifted and requires a higher level of sophistication in theoretical treatment. The emerging improvements in algorithms and techniques for studying RNA complexes by crystallography and NMR spectroscopy, along with novel molecular design approaches, will be profitable for structure-based drug design, irrespective of whether the target is an RNA or a protein.

While I have tried to cover the literature until September 1999, the selection of references was subjective, and I apologize for any contribution omitted from the review. I thank Dinshaw J. Patel for support and helpful discussions, and Eric Westhof, Eugene Skripkin, and Seema Qamar for useful comments. I am especially grateful to my wife, Tatjana Singer, for critical reading of the manuscript and numerous valuable suggestions. Research in the laboratory of D.J.P is supported by the NIH (GM-54777).

Received: May 17, 1999 [A 344]

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