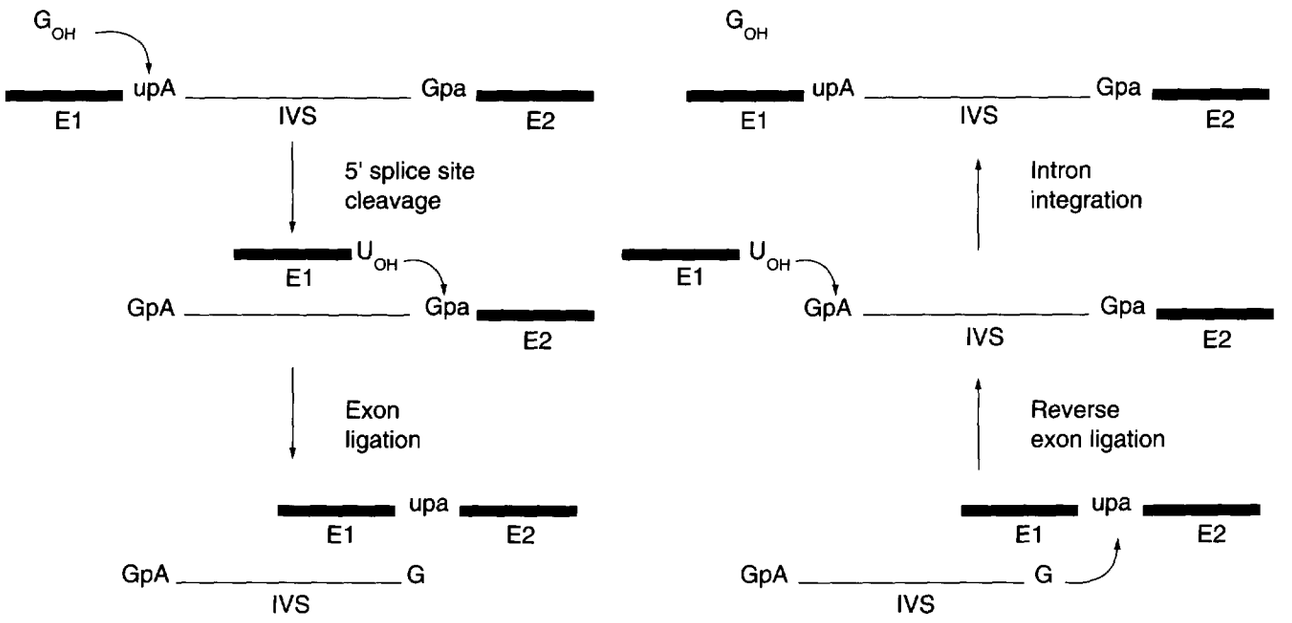

Figure 1. *Splicing of group I introns: forward and reverse reactions. The letter 'E' refers to exons, 'IVS' refers to the intervening sequence. A three-letter sequence, such as Gpa or GpA, indicates two nucleotides connected by a phosphodiester bond. In the forward reaction, attack of the required GTP cofactor at the 5′-splice site releases the 5′-exon. The 5′-exon then attacks the 3′-splice site resulting in exon ligation and excision of the IVS. The reverse reaction follows the same pathway in the opposite direction. After binding of the ligated exon RNA in the active site of the linear IVS, attack of the 3′-terminal hydroxyl of the IVS on the phosphate at the splice junction results in the addition of the 3′-exon to the 3′-end of the IVS. In the second step, the 3′-hydroxyl of the 5′-exon attacks the phosphate of the first phosphodiester bond of the IVS, releasing G and joining the 5′-exon to the 5′-end of the IVS. Exon sequences are in lower case, IVS sequences in upper case. Reproduced from Ref. 79 with permission.*

5′-ends of the ribozyme. For the HP ribozyme, four nucleotides on the 5′-side and a variable number on the 3′-side of the cleavage site can be modified. In the HDV ribozyme only seven nucleotides at the 5′-side of the cleavage site need be changed to alter the specificity. These ribozymes are relatively small and they can be made either by chemical synthesis or by transcription from viral or non-viral vectors.

Increasing the length of the complementary binding sequences in the ribozyme should improve the specificity of binding. A recognition sequence of approximately 15 nucleotides – seven each in the 3′- and 5′-arms and one at the catalytic core – should be optimal for recognition, specificity and turnover. This nucleotide length may be unique in the human genome; however, this does not take into account the bias in the genetic code, particularly in regions that are transcribed as mRNA. Nevertheless, this target size of 15 nucleotides will ensure reasonable uniqueness and therapeutic specificity. It may not be desirable to extend the recognition sequence beyond 15 nucleotides because, beyond that point, the binding affinity increases to the point where the off-rate of the cleaved product from the enzyme is too slow to permit efficient catalysis[22].

Scope of ribozyme technology

Ribozymes provide a broad technology that is potentially applicable to human disease diagnosis and therapy, agriculture and animal health because a ribozyme will, in principle, selectively bind and cleave any target RNA (Ref. 11). Highly specific control of gene expression by ribozyme cleavage and consequent nuclease destruction of mRNA fragments can be envisaged. The sequence-specific enzymatic activity, and the relative ease with which a lead ribozyme can be designed, are substantial advantages that may result in few side-effects, high potency and a substantially reduced drug discovery time.

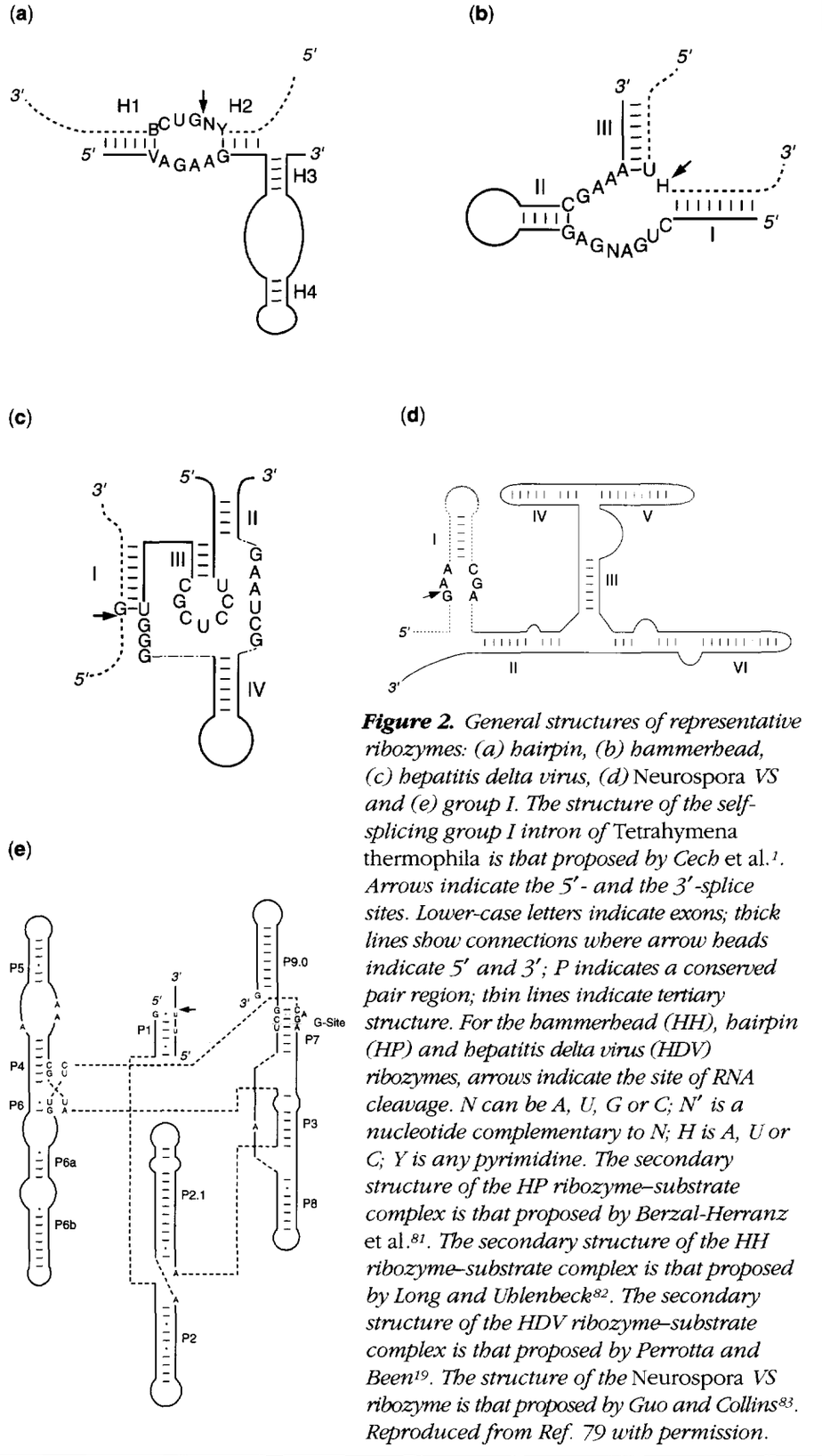

Figure 2. *General structures of representative ribozymes: (a) hairpin, (b) hammerhead, (c) hepatitis delta virus, (d)* Neurospora *VS and (e) group I. The structure of the self-splicing group I intron of* Tetrahymena thermophila *is that proposed by Cech* et al.[1]. *Arrows indicate the 5′- and the 3′-splice sites. Lower-case letters indicate exons; thick lines show connections where arrow heads indicate 5′ and 3′; P indicates a conserved pair region; thin lines indicate tertiary structure. For the hammerhead (HH), hairpin (HP) and hepatitis delta virus (HDV) ribozymes, arrows indicate the site of RNA cleavage. N can be A, U, G or C; N′ is a nucleotide complementary to N; H is A, U or C; Y is any pyrimidine. The secondary structure of the HP ribozyme–substrate complex is that proposed by Berzal-Herranz* et al.[81]. *The secondary structure of the HH ribozyme–substrate complex is that proposed by Long and Uhlenbeck[82]. The secondary structure of the HDV ribozyme–substrate complex is that proposed by Perrotta and Been[19]. The structure of the* Neurospora *VS ribozyme is that proposed by Guo and Collins[83]. Reproduced from Ref. 79 with permission.*

Chemistry of ribozymes

Mechanism of action

Ribozymes cleave their target either by transesterification or by hydrolysis (Figure 1). In the cleavage reaction the formation of a 2′,3′-cyclic phosphate and 5′-hydroxyl are typical products. If the ribozyme is present in excess, as is often the case in practice, but at a concentration that is not saturating with respect to the substrate, the cleavage rate is determined by the second-order rate constant K_{cat}/K_m. Values of approximately 10^8 $mol^{-1}min^{-1}$ are frequently observed[17]. If the substrate is in excess, and at saturating concentrations, ribozymes are rate-limited by release of the product[23]. Improvements in ribozyme turnover will depend upon research to modify ribozymes in a way to increase product release from the complex.

Stability in biological milieu

The instability of unmodified RNA is a significant challenge to the use of ribozymes as human therapeutic agents.

Structural modifications have been applied to synthetic oligonucleotides to enhance resistance to nuclease[24–28]. Substitution of the 2′-*O*-Me-modified nucleotide at all positions in a HH ribozyme, except G5, G8, A9, A15.1 and G15.2, gave rise to a catalytically active molecule but with a significantly decreased K_{cat} value[28]. These molecules showed a 1,000-fold increase in stability when compared to an all-RNA ribozyme. In another investigation, a per-substituted 2′-*O*-allyl-containing ribozyme with ribose residues at positions U4,

G5, A6, G8, G12 and A15.1 retained 20% of the catalytic activity of an all-RNA ribozyme. As in the previous case, the stability of this ribozyme increased[29].

Two phosphorothioate linkages at C3 and U4, with replacement of U7 by adenosine or guanosine in a phosphorothioate-DNA/RNA chimera, further stabilize the molecule, but the catalytic activities of these chimeras are significantly reduced[30]. Substitution of all the pyrimidine nucleotides in a HH ribozyme by their 2′-amino or 2′-fluoro analogs resulted in increased stability[31].

Beigelman and coworkers[32,33] constructed a considerably improved stable HH motif by selective modification of the 2′-modified sugars. Most of these were substituted as 2′-*O*-Me residues as described above. The remaining 2′-modifications were introduced at position U4 or U7. Two of these ribozymes, U4- and U7-2′-amino and U4-2′-*C*-allyl, have wild-type catalytic activity and a 5–8-h half-life in human serum. The addition of a 3′–3′-linked dT or an abasic nucleoside to the 3′-terminus of these ribozymes increases their half-lifes in serum to 3 days (Figure 3; Table 1).

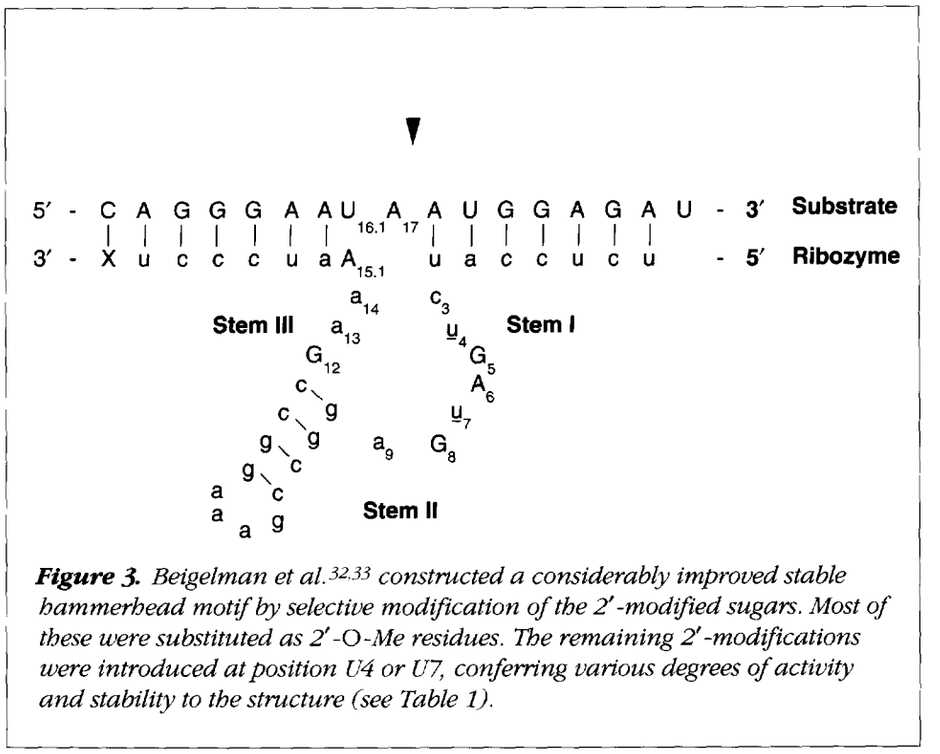

Figure 3. *Beigelman et al.[32,33] constructed a considerably improved stable hammerhead motif by selective modification of the 2′-modified sugars. Most of these were substituted as 2′-O-Me residues. The remaining 2′-modifications were introduced at position U4 or U7, conferring various degrees of activity and stability to the structure (see Table 1).*

Delivery of synthetic ribozymes

There are two methods for the delivery of oligonucleotides. In the first, a chemically synthesized or 'free' ribozyme is coupled, physically or chemically, to a lipid or other molecule. The molecule complexed to the ribozyme makes use of either a nonspecific entry mechanism (as with a lipid carrier) or a somewhat more specific entry method such as receptor-mediated endocytosis. In the second method, an oligonucleotide is incorporated into a vector, which codes for the oligonucleotide. Transfection or infection of the cell depends upon artificial enhancement of cell permeability or relies on the inherent characteristics of the virus.

Many lipid vehicles have been studied. They can encapsulate relatively large quantities of drug molecules, either within the aqueous interior or dissolved into the hydrocarbon regions of the bilayers[34,35]. When attached to the appropriate antibody or other ligand, liposomes can sometimes facilitate cell-specific attachment and entry[36–39].

However, liposomes are removed rapidly by macrophages of the reticuloendothelial system. This can be avoided partially by modifying the character of the bilayer, or by using a polyethylene glycol coating to prevent nonspecific adsorption of serum proteins and thus nonspecific recognition of liposomes by macrophages[40–42]. It is not yet possible to target liposomes to a specific tissue.

An alternative method is the use of cationic lipids. These contain a nonpolar long-chain fatty acid – usually C_{16} or C_{18} – complexed to a polyamine and are believed to complex with the oligonucleotide in such a way that it is not internalized into a liposome-type structure.

The use of lipids and other factors to enhance exogenous delivery and effectiveness of oligonucleotides is encouraging, but their utility still remains empirical and must be decided experimentally for each ribozyme in each cell or tissue of interest.

Biology of ribozymes

Proof of principle

To demonstrate the potential of ribozymes, cleavage mRNA targets must be correlated with biochemical and physiological changes. In a study of HH ribozymes in monkey cells[43], ribozymes designed against chloramphenicol acetyltransferase (CAT) were cloned into a mammalian expression

Table 1. Increased net activity and improved stability conferred by each of the 2′-modifications at U4 or U7 compared to the unmodified (all-RNA) ribozyme (see Figure 3)[a]

Rz[b]	2′ modification (U4/U7)	Activity t_A (min)[c]	Stability t_S (min)[d]	Relative stability/activity β (Rz *n*) = $\frac{t_S/t_A \text{ (Rz } n)}{t_S/t_A \text{ (Rz 1)}}$
6	OH/*O*-Me	1	0.1	1
7	*O*-Me/*O*-Me	4	260	650
8	$=CH_2$/*O*-Me	6.5	250	380
9	*O*-Me/$=CH_2$	8	320	400
10	$=CH_2/=CH_2$	8.5	250	300
11	$=CF_2$/*O*-Me	4.5	400	900
12	*O*-Me/$=CF_2$	5.5	250	220
13	$=CF_2/=CF_2$	>15	380	250
14	F/*O*-Me	3	300	1000
15	*O*-Me/F	8	300	373
16	F/F	3.5	300	850
17	H/*O*-Me	5.5	250	450
18	*O*-Me/H	>10	250	<250
19	H/H	4	280	700
20	araF/*O*-Me	5.5	500	900
21	*O*-Me/araF	4	350	875
22	araF/araF	>15	500	<330
23	NH_2/*O*-Me	10	500	500
24	*O*-Me/NH_2	5.5	300	900
25	NH_2/NH_2	2	300	1500
26	*C*-Allyl/*O*-Me	3	>500	>1700
27	*O*-Me/*C*-Allyl	3	300	1000
28	*C*-Allyl/*C*-Allyl	3	300	1000
29	*C*-Allyl/*O*-Me + iT	3	16000	53000
30	NH_2/NH_2 + iT	2	16000	80000

[a]Reproduced from Ref. 33 with permission

[b]Rz, ribozyme; Rz *n*, modified ribozyme; Rz 1, unmodified ribozyme

[c]t_A, time required to cleave one-half of the substrate in solution under standard conditions (Tris 50 mM, pH 7.5; $MgCl_2$ 10 mM; substrate 1 nM; ribozyme at three concentrations 8 nM)

[d]t_S, time required for one-half of the ribozyme to be destroyed in human serum at 37°C

vector. The ribozymes specifically suppressed CAT expression by up to 60% when compared with an inactive ribozyme and a corresponding antisense control.

DNA encoding a ribozyme–tRNA construct has been microinjected into the nucleus of frog oocytes[44]. It remained largely in the nucleus, but small amounts were transported to the cytoplasm. Reduction of the coinjected U7snRNA target, present in the cytoplasm, was observed after 10 and 20 h.

The studies of L'Huiller and coworkers[45] demonstrated the specificity of a ribozyme directed against α-lactalbumin. A ribozyme directed against 28S RNA cleaved the 28S RNA but not related RNA. This showed that the ribozyme could detect its substrate among other RNAs present in the cell[46].

Other studies at the cellular level have been reported, and it is clear that 'proof of principle' for ribozymes has been demonstrated in cell culture[47,48].

As an example of ribozyme function *in vivo*, Efrat and coworkers[49] sought to generate a mouse model for maturity-onset diabetes in the young by reducing glucokinase activity in β-cells of the pancreas without affecting its function in the liver. To do this, a glucokinase ribozyme was expressed in β-cells in transgenic mice. These mice manifested only one-third of the normal islet glucokinase activity, and insulin secretion and response to glucose were impaired. Interestingly, the mice remained euglycemic, which suggests that glucokinase deficiency in the liver is important in the induction of diabetes in maturity-onset diabetes in the young. These mice provide an animal model for studying the interaction of genetic and environmental diabetogenic factors in a system with reduced islet glucokinase activity. This is an example of the use of ribozyme specificity to study physiology in intact animals.

One dramatic example of ribozyme function *in vivo* has been shown in a study in *Drosophila*[50]. Transgenic eggs carrying a ribozyme against the *fushi tarazu* (*ftz*) gene under the control of a heat-inducible promoter were generated. These investigations distinguished the two developmental phases of the *ftz* gene using timed heat-induction of the ribozyme. Induction of the ribozyme against the first of these development phases – production of a seven-stripe cuticle pattern – created cuticle defects in larvae. Activation of the ribozyme in other eggs later during neurogenesis inhibited CNS development without disturbing the antecedent cuticle pattern. These experiments demonstrated the specific induction of ribozymes at different points in *Drosophila* development, that activation of the ribozyme causes the same phenotypic mutations in segmentation and neurogenesis that occur in known *ftz* mutations, and that the presence of the ribozyme caused no other biological or biochemical damage to the organism.

In another study, a plasmid carrying a ribozyme against β_2-microglobulin was injected into the male pronucleus of fertilized oocytes[51]. Ribozymes were expressed in the lung, kidney and spleen. Expression was accompanied by a reduction in β_2-microglobulin mRNA that was greater than 90% in the lungs of individual mice; smaller reductions were observed in kidney and spleen. Such a study not only provides convincing proof of principle *in vivo*, but also confirms that continued expression of a ribozyme directed against a nonessential function will not result in any obvious toxicity in an animal.

Vector delivery

All studies of viral delivery of ribozymes have so far used retroviruses. Sullenger and Cech[52] found that inclusion of a retroviral packaging signal as part of the ribozyme gave a 90% reduction in titer of a targeted retrovirus, because the ribozyme was colocalized with its viral genomic RNA target. The ribozyme had no effect on the same target localized in the cytoplasm.

Adeno-associated virus (AAV) is a vector that can transfect diverse cell types. Samulski established a vector consisting only of the terminal repeats of AAV[53]. This could express *tar* antisense and block HIV replication. AAV has also been used to transform $CD34^+$ cells with a transduction efficiency of approximately 80% in the presence of interleukin 3 and granulocyte/macrophage-colony-stimulating factor[53].

Adenoviruses can express genes as well as enhance delivery of various large molecules, such as dextrans, proteins and plasmid DNA linked to ligands. The latter occurs whether they are replication-competent or replication-deficient[54,55]. Gao and coworkers[56] delivered genes to airway epithelium via an adenovirus–polylysine–DNA complex.

Targeting ribozyme-containing vectors to particular tissues or cells can be accomplished via an aerosol to the lung epithelium, to a target tissue *ex vivo* (e.g. bone marrow), which is then reinfused; by widespread delivery via a vector such as AAV, which would infect many cells and tissues; or by intracellular targeting.

Therapeutic applications

Therapeutic applications of ribozymes are potentially broad and include use in viral infections, in cancer where an oncogene product is known, and in disease states that are associated with overexpression of a particular gene. This review concentrates on examples from viral infections and cancer.

Viral diseases

HIV. One of the earliest descriptions of the activity of ribozymes against viral diseases was that of Sarver and coworkers[57], who demonstrated cleavage of HIV sequences in a cell-free system by HH ribozymes and a reduction in p24 antigen levels of approximately 98%. Longer-term toxicity studies showed that ribozyme-containing cells behaved as their control nonribozyme-expressing counterparts when followed for 9 months[58].

A subsequent study[59] demonstrated that stable MT_4 transformants that express the ribozyme under the control of the herpes simplex virus thymidine kinase promoter were only modestly resistant to HIV infection; virus production was simply delayed. The highest level of resistance was observed in MT_4 cells transformed with a vector containing the *tar* regulatory element to allow ribozyme expression in a *tat*-inducible manner. No HIV production was observed 22 days after infection of these cells. This study illustrates the importance of high expression levels of the ribozyme in HIV-infected cells. An inducible promoter that upregulates the ribozyme in the presence of this infection may enhance efficacy even further.

A similar strategy[60] has used a HP ribozyme to cleave the 5′-leader sequence of HIV. Expression of the HP ribozyme under the control of a β-actin promoter inhibited HIV expression. In a follow-up investigation[61], the HP ribozyme inhibited HIV expression by up to 95% in a transient transfection assay. The authors also showed that the HIV RNA cleavage products were degraded with high specificity.

The above studies support the conclusion that, by cleaving at the 5′-leader sequence, the ribozyme removes the RNA cap so that the mRNA is poorly translated and is probably more quickly degraded. The 5′-leader sequence is highly conserved among most HIV isolates and is a theoretically more important therapeutic target. Of the available HIV strains, only 'MN' contains one nucleotide substitution in the 5′-leader sequence cleaved by a HP ribozyme. There are differences among some HIV strains in other portions of the 5′-leader[62].

Another way to enhance ribozyme effectiveness in solution is through the use of multiple ribozymes[63]. Ribozymes, flanked by *cis*-acting ribozymes, upon transcription are trimmed and liberated independently. Their activities are also proportional to the number of units. The tandem *cis*-cleaving ribozymes (n = 1–10) could generate independent ribozymes specific for different target sites *in vitro* without sacrificing the activity of any individual ribozyme. The

activities of connected ribozymes reached a plateau at values of about $n = 3$.

In cells, a multiple ribozyme transcript may be more active against HIV than the corresponding individual ribozymes[64]. A transcription unit containing nine ribozymes was more active than transcripts with fewer catalytic units. The individual ribozymes were arranged in tandem. The multitarget ribozymes retained the specificity of monoribozymes but were more efficient per ribozyme RNA copy and remained active when part of a large transcript.

The above provides encouragement regarding the potential use of ribozymes in the management of HIV infection. Therapeutic efficacy will require high efficiency of transfection or transduction of the gene coding for the ribozyme. The optimum viral vector and expression unit are not yet certain.

Other viruses. Ribozyme inhibition of other viruses also has been studied. Xing and Whitton[65] prepared ribozymes which cleave the RNA genome of lymphocytic choriomeningitis virus (LCMV) – a prototype arenavirus. The efficiency of the cleavage was site-dependent and the secondary structure at the target site could abolish ribozyme cleavage. An anti-LCMV ribozyme expressed in tissue-culture cells diminished viral RNA levels and reduced infectious virus yield approximately 100-fold[66]. This effect was shown to be specific because yields of related arenavirus were not similarly curtailed.

Inhibition of oncogene function

The crucial differences between normal cells and cancer cells appear to stem from discrete changes in specific genes controlling proliferation. They fall into two categories: tumor-suppressor genes and oncogenes.

Oncogenes are evolutionarily conserved and induce cellular transformation either when naturally incorporated into a retrovirus or when their DNA is transfected into tissue-culture cells. Most of the known oncogenes were originally isolated as viruses containing genes of nonprimate origin. These genes are mutations of protooncogenes, which are normally found in cells and are activated during embryogenesis, cell growth or specific tissue regeneration. Because oncogenes are overexpressed and produce RNA that is distinguishable from the protooncogene, they are potentially excellent targets for ribozyme therapeutic activity.

H-ras *Gene.* Monia and coworkers[67] demonstrated a fivefold discrimination between a point mutation in the twelfth codon of H-*ras* and the wild-type H-*ras* target sequence. The authors also showed that discrimination correlated with, and was limited by, the difference in thermodynamic stability of the hybrids formed between mutants or wild-type sequences.

This twelfth codon mutation (GGC to GUC) in H-*ras* creates a consensus HH ribozyme target site[68,69]. An H-*ras*-dependent cell line, stably transformed with a β-actin expression vector encoding a HH ribozyme, showed reduced H-*ras* expression and reduced rates of cell proliferation. There was a decrease in the H-*ras* RNA and in the p21 protein derived from H-*ras* expression. The ribozyme described, designed to cleave the mRNA of the H-*ras* gene, was expressed in human bladder carcinoma EJ cells. These were injected by an orthotopic (transurethral) implantation model to recapitulate the invasive potential of bladder carcinoma. The EJ transfected cells preserved the malignant phenotype in these mice and caused highly invasive tumors and death. In contrast, in the EJ clones transfected with the ribozyme-expressing vector, there was a dramatic reduction in the malignant phenotype. The tumors displayed limited invasive capacity and there was a significant increase in survival, approximately double that of the control cells (a median of 75 days versus a median of 47 days). Histology of the bladders demonstrated that the control tumors consisted of cells with highly invasive properties, with nests of neoplastic cells dispersed throughout the tubules of the normal kidney. In contrast, the ribozyme clones produced tumor nodules that compressed surrounding tubules of the normal kidney but with no evidence of invasion in the vascular spaces. Ribozymes were detected by PCR assay in tumor-bearing mice that were alive at 86–90 days. Thus, this experiment demonstrated the ability of a ribozyme directed against H-*ras* mRNA to reverse the phenotypic expression of H-*ras* *in vitro* and *in vivo*.

Ribozymes can inhibit the transformation of NIH 3T3 cells about 50% (Refs 70,71). When the activated c-Ha-*ras* gene was cotransfected with the ribozyme-encoding gene, colonies were isolated that were morphologically different from those containing only the c-Ha-*ras* gene.

c-fos *Gene.* The c-*fos* gene has been implicated in signal transduction, DNA synthesis and resistance to antineoplastic agents[71]. It is one of the earliest genes to be activated by mitogenic stimuli. The linkage between expression of c-*fos*

and DNA synthesis genes is supported by data showing that cisplatin administration leads to a sequential induction of c-*fos* followed by a dTMP synthase and DNA polymerase[72]. A ribozyme constructed against a site in c-*fos* mRNA resulted in decreased c-*fos* gene expression, increased sensitivity to chemotherapeutic agents (including cisplatin), and a significant decrease in dTMP synthase, DNA polymerase β, topoisomerase I and hMTII-A (human metallothionein) gene expression[71–74].

bcr/abl *Gene.* Snyder and coworkers[75] demonstrated ribozyme-mediated inhibition of *bcr/abl* gene expression in a cell line that was positive for the Philadelphia chromosome. The mRNA transcript resulting from the fusion gene is translated into a p210 protein with augmented tyrosine kinase activity. This $p210^{bcr/abl}$ protein and its corresponding RNA are found in virtually all patients with chronic myelocytic leukemia (CML), and in about 50% of patients with Philadelphia-chromosome-positive acute lymphoblastic leukemia. In this study, a ribozyme was directed against the junction sequence in *bcr/abl.* The ribozyme decreased levels of detectable *bcr/abl* mRNA in these cells, inhibited expression of the *bcr/abl* gene product $p210^{bcr/abl}$ completely, and inhibited cell growth by 84%. This is a significantly greater effect than could be achieved with an antisense oligonucleotide. There was no significant inhibition by liposome vector alone, sense oligonucleotide or an unrelated ribozyme.

Wright and coworkers[76] also demonstrated ribozyme-mediated cleavage of *bcr/abl,* and a similar study by Shore and coworkers[77] showed ribozyme-mediated cleavage of the *bcr/abl* oncogene transcript. These investigators designed a ribozyme to cleave a GUU triplet adjacent to the junction of the c-*bcr* and c-*abl* fused genes. The ribozyme efficiently cleaved the RNA transcript *in vitro,* and introduction of the recombinant retroviruses into the CML blast crisis cell line K562 resulted in the elimination of the p210 protein kinase activity in several single-cell clones infected with the ribozyme expression cassette.

rex/tax *Gene.* Cantor and coworkers[78] developed a ribozyme that cleaved *rex/tax* mRNA and inhibited bovine leukemia virus expression. The transactivating protein, tax, stimulates the long terminal repeat to promote viral transcription and may be involved in tumorigenesis. Rex is involved in the transition from early expression of regulatory proteins to later expression of viral structural proteins. A ribozyme designed against both target sequences cleaved more than 80% of the target RNA *in vitro.* In cells expressing the ribozyme, viral expression was inhibited significantly – as measured by bovine leukemia virus core protein p24 (61% inhibition) and reverse transcriptase activity (92% inhibition).

Therapeutic potential

Ribozymes offer many therapeutic possibilities in clinical medicine. The mRNA for any protein that is causative in a disease is a potential ribozyme target. Similarly, microorganisms, especially viruses, are targets for ribozyme therapy. The specificity of the ribozyme is important; the ability to eliminate a specific mRNA in a cell without damage to other normal cellular RNA molecules is a significant advance in therapeutics and one which may not apply to other therapeutic modalities.

Chemically synthesized ribozymes can be delivered to a variety of target organs or tissues topically. The direct application of a ribozyme to the arterial wall may be used to modify the restenotic process after angioplasty. A synthetic ribozyme could be injected into a joint space to suppress an inflammatory process by eliminating a particular cytokine or enzyme in that process. A viral infection in the lung, especially one confined to the bronchial epithelium, could be treated by aerosolization of a chemically synthesized ribozyme.

Ribozymes delivered by a vector will be of more use in systemic diseases (infectious or noninfectious) and diseases of a chronic nature (local or systemic) where long-term expression of the ribozyme is important. The most prominent example is HIV. Elimination of the virus from bone marrow could allow reconstitution of the immune system with cells resistant to HIV. A leukemic process resulting from the presence of a new gene product, such as *bcr-abl,* is a natural target for a ribozyme since the fusion gene is not found in normal cells. Oncogenes associated with other types of neoplasia are also targets.

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Metabotropic glutamate receptors: potential drug targets

Thomas Knöpfel and Fabrizio Gasparini

The neurotransmitter glutamate activates not only ionotropic receptors, which mediate fast excitatory synaptic transmission, but also metabotropic receptors. The latter form a large, heterogeneous family of G protein-coupled receptors with specific functions in normal as well as in pathological situations. The diverse cellular responses mediated by metabotropic glutamate receptors and their distinct expression patterns in different brain systems render these receptors potential drug targets with a variety of possible mechanisms of action.

Glutamate activates not only ionotropic receptors but also metabotropic receptors[1,2]. Following expression cloning of a cDNA encoding the first rat metabotropic glutamate receptor (mGluR)[3,4], a family of related mGluR cDNAs was isolated by cross-hybridization and PCR amplification. Eight different subtypes have been described, termed $mGluR_1$ through $mGluR_8$ (Refs 5–12). In contrast to many other G protein-coupled receptors, the existence of functionally and pharmacologically distinct mGluR subtypes has been established by molecular cloning and molecular pharmacological studies rather than by deduction from classical pharmacological observations. Thus, the signal transduction mechanism for each newly cloned subtype was elucidated first in recombinant non-neuronal cells by testing agonist-induced effects on intracellular messengers. Such studies revealed that mGluRs either activate phospholipase C or inhibit adenylate cyclase. Following characterization of the transduction pathway in recombinant cells, the rank order of potency of several standard agonists was established for each mGluR subtype.

From these characterization studies three groups of mGluR subtypes were defined (Box 1)[13]. Group I includes $mGluR_1$ and $mGluR_5$, which both couple to phospholipase C and show a very similar agonist selectivity. Group I mGluRs are potently activated by quisqualate[3,5,8,14] and are activated selectively by (*S*)-3,5-dihydroxyphenylglycine (3,5-DHPG)[15]. Group II, which includes $mGluR_2$ and $mGluR_3$, and group III, consisting of $mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$, inhibit adenylate cyclase and, consequently, suppress elevations in cAMP levels[6,7,9–12]. However, the agonist selectivity differs markedly between groups II and III. Group II is effectively activated by (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxypropyl)-glycine (DCG-IV)[16], (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)-glycine (L-CCG-I)[17] and (2*S*,1′*S*,2′*R*,3′*R*)-2-(2-carboxy-3-methoxymethylcyclopropyl)glycine (*trans*-MCG-I)[18], whereas (*S*)-2-amino-4-phosphonobutyric acid (L-AP4) and (*S*)-serine *O*-phosphate (L-SOP) are potent agonists for group III[11,12,19,20]. This functional grouping correlates well with similarities in the deduced amino acid sequences of the receptor proteins.

Prototypic agonists and antagonists

All presently known agonists at mGluR subtypes share the *S*-configurated α-amino group and a remote acidic function. The rank order of potency of agonists at the different subtypes suggests not only an interaction of each subgroup with different glutamate conformations but also a different interaction between the second acidic function and the receptor subtypes. Using selectivity rather than potency as a selection criterion, the following prototypic agonists can be assigned to each group: 3,5-DHPG for group I, *trans*-MCG-I for group II and L-AP4 for group III mGluRs. The structures of these compounds

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