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Challenges and Perspectives in Nucleic Acid Enzyme Engineering

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Abstract Engineering of nucleic acids has been a goal in research for many years. Since the discovery of catalytic nucleic acids (ribozymes and DNAzymes), this field has attracted even more attention. One reason for the increased interest is that a large number of ribozymes have been engineered that catalyze a broad range of reactions of relevance to the origin of life. Another reason is that the structures of ribozymes or DNAzymes have been modulated such that activity is dependent on allosteric regulation by an external cofactor. Such constructs have great potential for application as biosensors in medicinal or environmental diagnostics, and as molecular tools for control of cellular processes. In addition to the development of nucleic acid enzymes by in vitro selection, rational design is a powerful strategy for the engineering of ribozymes or DNAzymes with tailored features. The structures and mechanisms of a large number of nucleic acid catalysts are now well understood. Therefore, specific design of their functional properties by structural modulation is a good option for the development of custom-made molecular tools. For rational design, several parameters have to be considered, and a number of tools are available to help/guide sequence design. Here, we discuss sequence, structural and functional design using the example of hairpin ribozyme variants to highlight the challenges and opportunities of rational nucleic enzyme engineering.

Keywords Cleavage, Engineering, Ligation, Recombination, Ribozyme

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1 Introduction

Over the past decade, the engineering of nucleic acid enzymes has become a powerful area of research with potential applications in the fields of chemical and molecular biology and medicinal and environmental diagnostics [1]. Ribozymes and DNAzymes are versatile molecular tools and their relevance for the aforementioned research fields has constantly grown over the past few years. Ribozyme applications in molecular biology range from simple cleavage or ligation of a defined RNA target [2], to the introduction of sequence alterations and/or modifications of the desired target RNA, to regulation of gene expression when combined with a suitable sensor module (e.g., an aptamer) [3, 4].

There are two major strategies for nucleic acid enzyme engineering: (1) in vitro evolution, which is based on selection of a nucleic acid molecule with desired properties from a library of random sequences, and (2) rational design, which starts from a known ribozyme or DNAzyme and is based on structural manipulation to affect the function in a predefined way. In vitro evolution has allowed development of many nucleic acid enzymes with novel activities and, thus, greatly enlarged the repertoire of nucleic acid catalysis [5, 6], whereas rational design has been more focused on using the intrinsic catalytic features of ribozymes and DNAzymes for novel developments. For the latter, deep knowledge of the structure and mechanism is of utmost importance. Over the years, an enormous amount of data has been collected on the structure and mechanism of nucleic acid enzymes [2, 7]. We now understand many RNA- and DNA-based catalysts well enough to turn them into useful tools. The past decade has seen impressive developments based on the usage of known catalytic nucleic acid structures [8]. For example, self-splicing group I introns have been designed to support RNA circularization [9, 10]; several ribozymes and DNAzymes have been engineered for regulation by allosteric cofactors or temperature [3]; and hairpin ribozyme descendants have been designed to support RNA repair, recombination, oligomerization, and circularization [11]. Looking into the literature, it is fascinating to see how well the engineered nucleic acid catalysts perform the intended action. However, often it takes a long time and much effort to reach that point.

Many aspects need to be considered when designing even an already extensively characterized ribozyme for a novel application. Many hurdles and challenges, including sequence design, site-specificity, structural design, and target accessibility, need to be overcome. Therefore, the design of a new ribozyme-based application

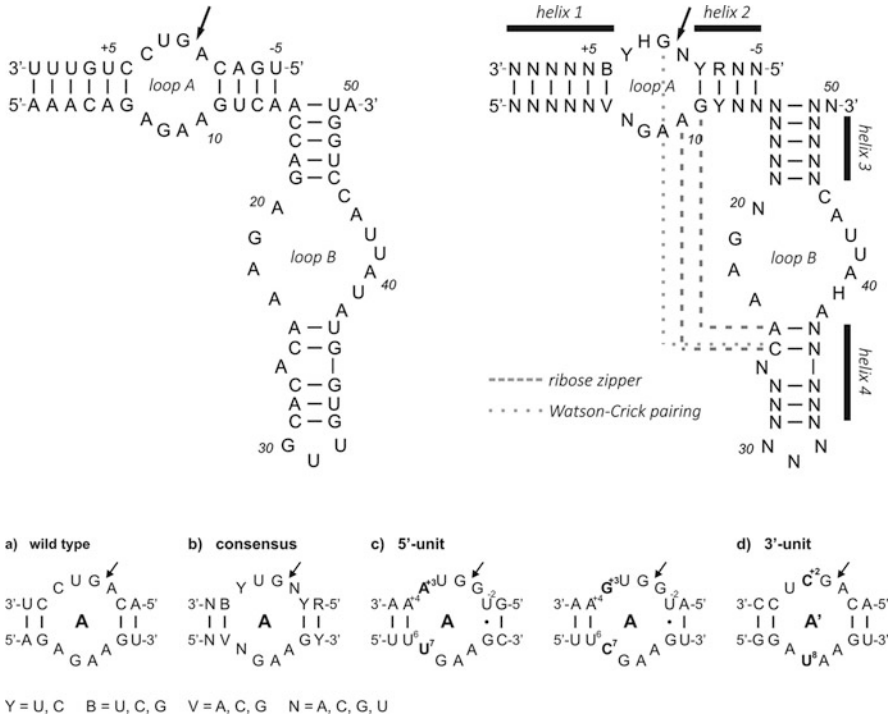

Fig. 1 Secondary structure of the hairpin ribozyme (*left*) and conserved nucleobases (*right*) with tertiary contacts. (**a**–**c**) Active sequence variants of the loop A motif

requires good guidelines to achieve a functional system. Design can be divided into three major parts. The first part covers sequence design, the second part deals with structural aspects that need to be taken into account for a certain application, and, third, functional design into novel activities plays a role. In this chapter, we concentrate on ribozymes (not DNAzymes) in our discussion of the challenges of rational design. In particular, we focus on the hairpin ribozyme (Fig. 1), because it is a well-studied naturally occurring RNA that we have used in our laboratory for a number of engineering projects.

2 Sequence Design

Most naturally occurring ribozymes catalyze similar reactions, which are cleavage and/or ligation of phosphodiester bonds by transesterification or hydrolysis. These activities are required for applications in molecular biology and medicine, such as specific cleavage of a defined target RNA or joining two RNA fragments. However, the ribozyme sequence needs to be adapted to bind the chosen substrate, and one has to decide which ribozyme is the most suitable for the intended ribozyme-based

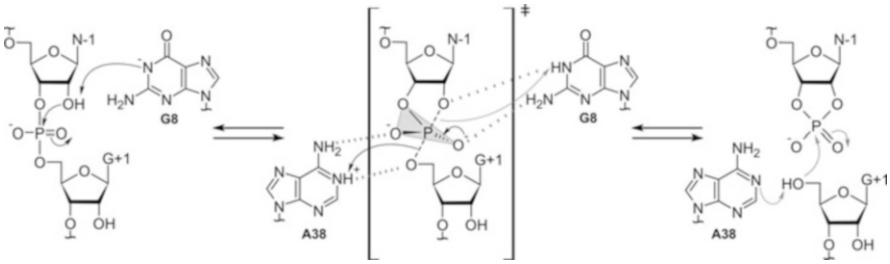

Fig. 2 Mechanism of the reversible cleavage of a phosphodiester bond catalyzed by the hairpin ribozyme

application. The hammerhead, hairpin, and hepatitis delta virus ribozymes are excellent for RNA cleavage and are useful tools for processes such as knocking down gene expression by cleaving a target mRNA [12, 13]. Group I ribozymes and hairpin ribozymes can be used for RNA sequence alteration [14–16] or, among other ribozyme motifs, for the introduction of modifications into the desired RNA strand [17, 18]. The hairpin ribozyme is employable in various ways because of its flexible adaptability to a desired target, activity, and application. In addition, this ribozyme has been extensively studied over the past decades. The three-dimensional structure has been solved and the reaction mechanism is well understood [19] (Fig. 2).

A large part of the hairpin ribozyme sequence consists of variable nucleotides, which makes it relatively easy to tailor the ribozyme for specific RNA targets. Furthermore, with a length of 50 nucleotides (nt), the minimal structure of the hairpin ribozyme (a *trans*-acting ribozyme) represents a relatively small catalytic RNA, which is easy to handle (low tendency to misfold) and synthesize. The minimal hairpin ribozyme consists of four base-paired helices (H1–H4) and two loops (A and B) (Fig. 1). The cleavage/ligation site is located in loop A. The active conformation is formed by docking of loops A and B. Interestingly, the helical junction has a tremendous effect on the stability of the docked conformer [20]. Four-way junctions provide a stable scaffold that, in the case of the hairpin ribozyme, enables stabilization of the tertiary structure and thus promotes ligation [21] (Fig. 3). A hairpin ribozyme with a four-way junction binds its cleavage product with higher affinity than the minimal hairpin motif does, because tertiary interactions within the folded structure contribute to product binding. Two-way and three-way junctions are less stable, but more sensitive to regulation by ligands [20]. The crystal structure of the hairpin ribozyme was solved by Rupert and Ferré d'Amaré and gives insight into the catalytic mechanism, which is thought to proceed by general acid–base catalysis [22, 23].

The most crucial aspect for sequence design is the consensus sequence of the ribozyme, which defines the ribozyme's adaptability to a particular target RNA. For the hairpin ribozyme, the conserved nucleobases essential for formation of the catalytically active structure and for active site chemistry are only located within loop A and loop B [24–28]. The helical regions are fully variable and can be easily

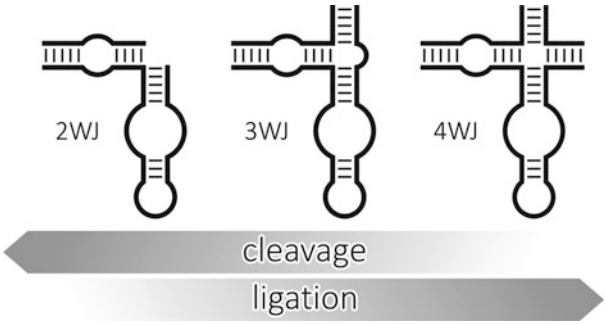

Fig. 3 Influence of the hairpin ribozyme structure containing a two-way (*2WJ*), three-way (*3WJ*), or four-way (*4WJ*) junction on cleavage/ligation activity

adapted to the target RNA. For a *trans*-acting ribozyme, loop A is formed upon binding of the substrate to the ribozyme. Therefore, it is important to screen the target RNA for the required consensus sequence 5′-Y_{-2} N_{-1}↓$G_{+1}U_{+2}Y_{+3}B_{+4}$-3′ (with N = A, C, G, or U; Y = C or U; B = C, G, or U) to ensure excellent ribozyme activity (Fig. 1b). However, deviations from the consensus sequence do not necessarily result in loss of ribozyme activity. Although the presence of G+1 is indispensable [24, 26, 29, 30], other deviations are more tolerated. As shown previously, A+4, although not allowed according to the consensus sequence mentioned above, does not lead to a significant decrease in ribozyme activity; furthermore, loss of activity caused by deviations from U+2 and Y+3 in the substrate strand can be restored by compensatory mutations in the ribozyme strand [31–33] (Fig. 1c).

Compensatory mutations can be found by careful checking of the hairpin ribozyme crystal structure and by trial and error activity tests. This strategy requires some effort; however, it has allowed the design of ribozymes and processing of substrates beyond the consensus sequence. Interestingly, there are also mutations that strongly influence the cleavage–ligation equilibrium of the hairpin ribozyme. In the wild type, ribozyme ligation is favored over cleavage, but mutations of A9 and A10 eliminate ligation activity and leave cleavage activity fully intact [34]. Moreover, mutation of A10→G enhances cleavage fivefold but prevents ligation, and substrates with A10→C are ligated but virtually uncleaved [32]. Thus, a single point mutation can have both quantitative and qualitative effects on activity and can be of great importance in rational design.

Another important aspect of sequence design is the length of the duplex formed between ribozyme and substrate upon binding, as it can strongly influence the preference for cleavage or ligation catalysis depending on the stability of the ribozyme–substrate/product complex. When the duplex is relatively short, resulting in a less stable ribozyme–substrate complex (but stable enough to form a catalytically competent structure), then dissociation of cleavage products (with a length of about 5–8 nt) is fast, and cleavage is favored over ligation. For this reason, the equilibrium is shifted toward cleavage in minimal hairpin ribozymes consisting of

just two hinged loop A and loop B domains. On the other hand, a longer duplex leads to tighter bound substrates because of the large thermodynamic contribution of the Watson–Crick base pairs. When fragments (with a length of $\geq$10 nt) are tightly bound to the ribozyme, dissociation is not favored and the hairpin ribozyme preferentially undergoes ligation. Increased stability of the ribozyme–substrate complex can be achieved by lengthening the 3′-end of the ribozyme via a three-way junction (Fig. 3).

Three-way junction hairpin ribozymes can be used for RNA ligation. Thereby, two RNA substrates can be joined to form a long-mer RNA, which would be inaccessible by chemical synthesis. The ability to produce long-mer RNAs becomes even more important when modifications are site-specifically introduced. The RNA fragment that contains the desired modification (e.g., fluorescent dye or biotin) can be chemically synthesized and subsequently ligated to a second RNA fragment in a reaction supported by the three-way junction hairpin ribozyme. Three-way junction hairpin ribozymes can also be used for RNA recombination. When combining two three-way junction hairpin ribozymes into one molecule, a new type of catalytic RNA is generated, which we have named "twin ribozyme" (Fig. 4).

Twin ribozymes are capable of cleavage and ligation of a suitable RNA substrate at two defined positions, allowing the exchange of a short patch of RNA for an externally added oligonucleotide [35]. The twin ribozyme-mediated exchange reaction enables sequence alteration or introduction of modifications into the target RNA. However, because binding of the externally given oligonucleotide competes with re-association of the internal cleavage fragment, the equilibrium needs to be shifted toward binding of the external oligonucleotide to ensure optimal sequence exchange. This shift can be achieved by promoting dissociation of the internal cleavage fragment. Therefore, binding of the substrate RNA is designed such that a destabilizing structure (e.g., mismatch or bulge) is formed within the sequence patch to be cut out. Consequently, dissociation of the cleavage fragment is promoted. The externally added oligonucleotide forms a contiguous duplex with the ribozyme and is preferentially bound because of its more stable and, therefore, favored structure (Fig. 4). Finally, after twin ribozyme-mediated ligation, the desired product is formed. Depending on the design of the ribozyme–substrate complex, twin ribozymes can mediate the exchange of fragments of the same length [18] or exchange of short fragments by longer versions [35] and vice versa [36]. A crucial aspect for optimal twin ribozyme-mediated sequence exchange is the length of the fragment to be cut out. It is very important that the gap between the two cleavage/ligation sites is not too large, otherwise dissociation of the cut-out fragment and, consequently, exchange with the repair oligonucleotide is dramatically hampered. As a guideline, the optimal length of the fragment to be cut out should be 12–18 nt to ensure sufficient dissociation [18, 31, 32, 35, 36].

Thus, a number of points have to be considered when designing the sequence of a ribozyme for a certain target and application. First, a suitable naturally occurring or previously in vitro selected nucleic acid enzyme has to be defined as the precursor or starting point for design. Next, the sequence of the ribozyme/

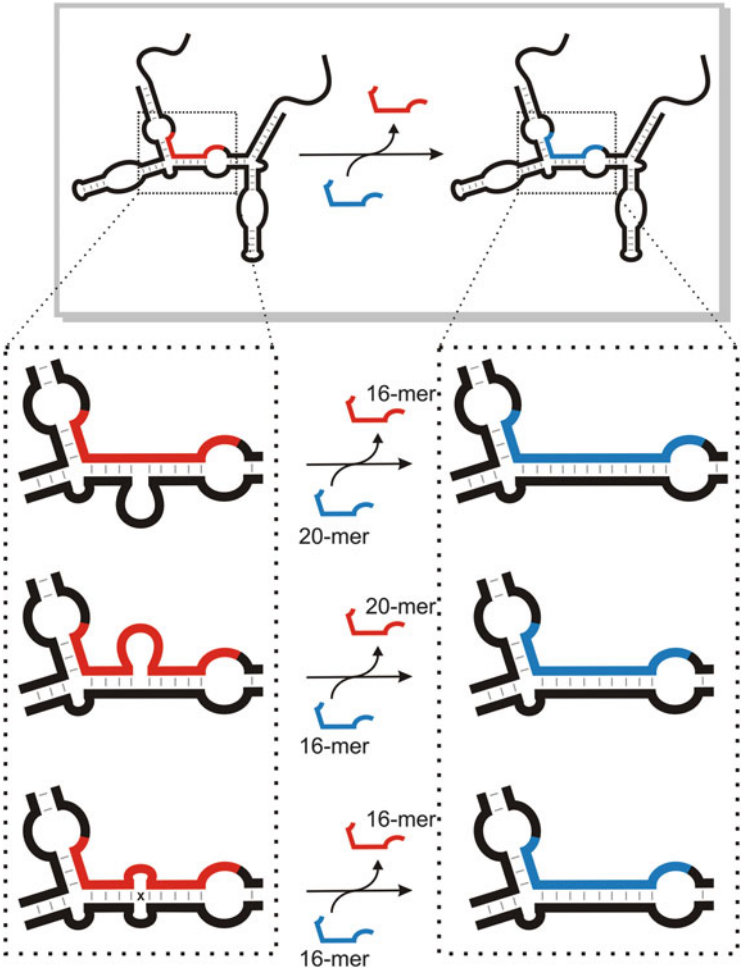

Fig. 4 Twin ribozyme-mediated fragment exchange reactions. The *red* fragment is cut out (cleavage is favored) and replaced with the *blue* fragment (ligation is favored). To shift the equilibrium toward product formation, substrate binding is designed to lead to the formation of a destabilizing structure within the sequence patch to be cut out (e.g., mismatches or bulges). In this case, dissociation of the formed cleavage fragment is promoted. In contrast, the externally added oligonucleotide forms a contiguous duplex with the ribozyme and undergoes preferential ligation because of its favorable stable structure. Upon twin ribozyme-mediated ligation, the desired product is formed

DNAzyme that is involved in substrate binding needs to be adapted to recognize and process the chosen target. This requires ensuring that sequence changes do not inhibit the activity of the nucleic acid enzyme. Last, because sequence changes can affect the reaction equilibrium (e.g., between cleavage and ligation), they need to be considered (or maybe even used on purpose) to favor one or the other activity.

These key aspects apply to all engineering work, independent of the specific ribozyme/DNAzyme and application.

3 Structural Design

To develop a rationally designed system, it is essential to verify that the engineered nucleic acid sequence folds into the intended secondary structure in the presence of its substrate. Although the helical parts of most ribozymes and DNAzymes are freely selectable, it is possible that the designed sequence folds into an energetically preferred secondary structure that is different from the structure of the active state, thus forming an inactive RNA or DNA. In addition, sequence changes necessary to meet the expected application can result in unwanted interactions. Such challenges include targets that do not bind to the substrate binding site but instead bind to the ribozyme sequence at another site, strands that favor monomolecular over bimolecular folding, and a thermodynamically favored dimer instead of an intramolecularly folded nucleic acid strand. For every substituted, inserted, or deleted nucleotide, the secondary structure of the overall system has to be rechecked to ensure that it still folds into the intended active conformation. If not, the mutation has to be reversed or (more challenging) compensatory mutations found and inserted.

A first indication of proper folding can be achieved with computer-aided folding algorithms, which focus on RNA folding but can also be applied to predict DNA folding. For prediction of RNA secondary structure, several software applications have been developed and are freely accessible; examples include RNAstructure [37], Vienna RNA Package [38, 39], and Mfold [40] (Table 1). The most popular method for predicting RNA secondary structure is based on calculating the minimal free energy of structural motifs that are formed by base-pairing within the RNA. The Gibbs free energy change can be determined by summing the individual base-paring energies. The secondary structure with the lowest Gibbs free energy change is generally the preferred structure. However, these programs only calculate Watson–Crick and wobble base pairs (G–U), and do not consider noncanonical base pairs such as Hoogsten base pairs. Pseudoknots are also usually ignored in order to gain higher calculation efficiency. Furthermore, because secondary structure prediction is a modeling approach, the calculated structure with the lowest free energy does not necessarily correspond to the actual secondary structure formed under the chosen reaction conditions. This crucial aspect should always be taken into account when using computational methods for predicting RNA secondary structure [41]. The method works very well for short and simple structures such as the hairpin ribozyme. However, the method is not accurate for large RNAs, as exemplified by the fact that only 50% of the base pairs of *Escherichia coli* 16S rRNA were predicted correctly [42].

Table 1 Useful programs for sequence design of ribozymes

Program	Description	URL
RNAstructure	RNA secondary structure prediction and prediction of the consensus secondary structure of two or more sequences (Dynalign or Multialign)	http://rna.urmc.rochester.edu/rnastructure.html
Vienna RNA Package	RNA secondary structure prediction (RNAfold) and RNA sequence design using constraint secondary structures (RNAinverse)	http://www.tbi.univie.ac.at/RNA/
RNAshapes	Secondary structure prediction of multiple sequences, followed by determination of a conserved structure	http://bibiserv.techfak.uni-bielefeld.de/rnashapes
RNA Designer	RNA sequence design using constraint secondary structures	http://www.rnasoft.ca/cgi-bin/RNAsoft/RNAdesigner/rnadesign.pl
RNAdesign	RNA design with multiple target secondary structures	http://www.bioinf.uni-leipzig.de/~choener/rnadesign/
VARNA	Visualization and drawing of RNA secondary structures	http://varna.lri.fr/

Refinement of the secondary structure prediction can be achieved by incorporating experimental information into the prediction algorithm [43]. Thus, prediction of the 16S RNA secondary structure was improved to 72% accuracy by including experimental data obtained from chemical probing experiments, and up to 95% accuracy by selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) [42]. Therefore, one should keep in mind that computer-aided prediction of secondary structure works best for smaller RNAs. For larger RNAs, the accuracy of prediction is significantly improved by additional experimental data, most favorably from SHAPE analysis. The software platform's RNA structure offers the possibility of directly feeding in experimental SHAPE data, which then are considered in the structure calculation. The emerging abundance of experimental data not only helps to refine prediction of secondary structure when using current folding algorithms, but also to improve the prediction algorithms or to develop new, more accurate algorithms.

As an alternative to verifying the secondary structure of a designed RNA, one can apply a method that allows inverse RNA sequence design. In contrast to the abovementioned approach, sequence design proceeds in the opposite way. First, the desired secondary structure is defined and then an inverse RNA sequence design program, such as RNAinverse (included in the Vienna RNA package [44]) or RNA Designer [45], determines the RNA sequence with the lowest free energy that gives the predefined secondary structure. Because the entire RNA sequence may not be variable, it is possible to specify nucleotides at defined positions within the secondary structure. More recently, several inverse folding programs (e.g., MODENA) have been developed that even allow the design of RNA sequences that fold into

multiple target secondary structures [46, 47]. This tool could be very useful for the design of riboswitches, aptazymes, or multiple substrate-processing ribozymes.

It should be mentioned that all efforts in ribozyme design are useless if the ribozyme binding site within the target RNA is not accessible. This applies not only to ribozyme design, but also to antisense oligonucleotides (ASOs), short interfering RNAs (siRNAs), and guide-RNAs that direct a specific enzyme to the desired processing location. As previously shown, a twin ribozyme was developed by rational design and was able to repair a three-base deletion within a short model substrate based on *CTNNB1* mRNA with a yield of 30% [32]. However, after adaption of the twin ribozyme to the entire *CTNNB1*-ΔS45 mRNA repair, the reaction failed. SHAPE analysis to refine secondary structure prediction revealed that the mRNA folded into an unfavorable structure, such that the twin ribozyme-binding site was blocked. To overcome that challenge one can follow several approaches. A simple and sometimes very helpful technique is the usage of competitor oligonucleotides that assist in defolding the cleavage/ligation of the target RNA [18]. A more systematic approach for detection of ribozyme binding sites deals with preparation of an oligodeoxynucleotide (ODN) library used for an RNaseH assay [48]. Effective cleavage of the RNA–DNA hybrid by RNaseH marks the most accessible sites for ribozyme base-pairing. Another sophisticated technique makes use of RNA–protein hybrid ribozymes that are able to process any RNA target independently of the secondary or tertiary structure [49]. To do so, the constitutive transport element (CTE), an RNA motif that allows interaction with intracellular RNA helicases, has to be conjugated to the ribozyme terminus. The bound RNA helicase assists the ribozyme to bind its target site by unwinding the local secondary structure.

4 Functional Design

As mentioned above, in many of our applications we took advantage of the cleavage/ligation equilibrium of the hairpin ribozyme, which can be easily shifted via temperature adjustment and/or substrate stabilization (or destabilization), thus allowing control of the two reactions. Using the example of a hairpin ribozyme-mediated recombination system (Fig. 5), we discuss the challenges of applying this control for functional design.

As a key feature of the engineered recombination system, two RNA strands without function are cleaved, mutually exchanged, and recombined by a single hairpin ribozyme to yield a functional RNA [14]. The two RNA educts each consist of a nonsense half and a profunctional half linked via the hairpin ribozyme specific cleavage sequence AGUC. The nonsense part was designed such that it binds weakly to the substrate binding site of the ribozyme, whereas the profunctional part binds strongly. Upon cleavage, dissociation of the nonsense part is facilitated, resulting in preferential cleavage without significant back-ligation. Because of the low binding affinity of the nonsense fragment (but strong enough that cleavage can

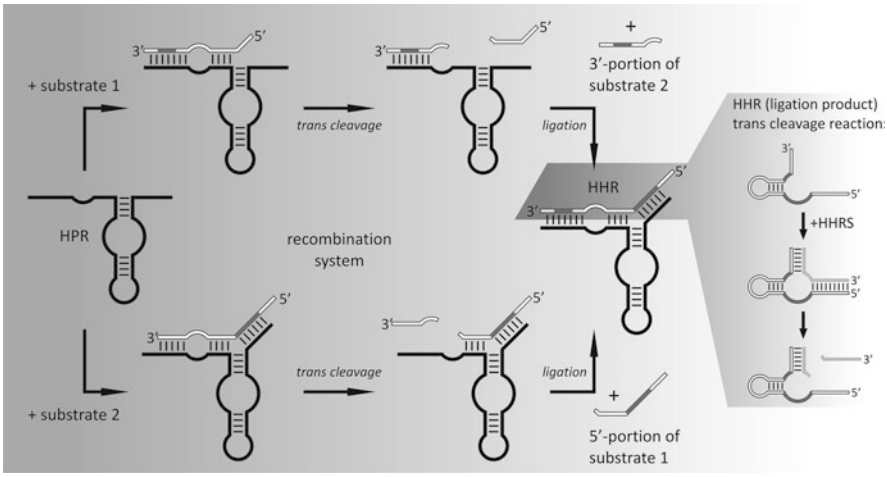

Fig. 5 RNA recombination mediated by the hairpin ribozyme (*HPR*). The recombination product, a functional hammerhead ribozyme (*HHR*), is able to catalyze cleavage of an externally added substrate (*HHRS*), which is depicted in *light grey*. The conserved nucleotide regions of HHR are shown in *dark grey*

occur), exchange between the nonsense and profunctional fragments at the binding site is promoted. However, exchange requires that the profunctional fragments do not bind too tightly so that dissociation and association can occur next to each other. Once both profunctional fragments are bound to one ribozyme, ligation is the favored reaction because of the strong binding of both fragments and the very slow dissociation, such that an active recombination product emerges.

The engineering of this recombination system was challenging because a single ribozyme had to bind two different RNA substrates equally well and catalyze cleavage, releasing the nonsense part and recombining the two profunctional parts. This required finding a good balance between dissociation of one of the profunctional parts from one ribozyme molecule and re-association with another ribozyme molecule for ligation to the profunctional part located there. Thus, a key aspect of design was tuning the lengths of individual helices of ribozyme–substrate complexes. According to the hairpin ribozyme consensus structure, helix 1 needs to consist of four base pairs or longer, and helix 2 is required to have exactly four base pairs. This restriction made it difficult to create a discriminating binding site for one of the substrates. In addition, the four-base pair helix 2 is presumably too weak for the profunctional part to undergo proper ligation. Therefore, we integrated a fifth helix, thus creating a three-way junction hairpin ribozyme (compare Fig. 3); only the substrate with the profunctional part at the 5′-end is bound to this three-way junction. As a side effect, the additional base pairs formed in helix 5 not only make dissociation of the required fragment less favorable, but also enhance overall ribozyme efficiency by facilitating formation of the active conformation [50]. Because the lengths of helices 1 and 5 are not limited, extension of the duplex

formed between the ribozyme and the two profunctional fragments can increase ligation tremendously. However, this would also add to the problem of product inhibition. Strongly hampered product dissociation makes fragment exchange less probable and inhibits multiple turnover reactions. Furthermore, if the recombination product cannot dissociate from the ribozyme, its functionality cannot be exploited and a successful reaction cascade to recombination cannot be verified. Taking into account all these considerations, one can conclude that the lengths of helices between substrates and ribozyme, and thus the binding affinity of substrates, intermediates, and final products, determine the efficiency of fragment exchange and the preference for cleavage or ligation. Therefore, helix length has to be carefully adjusted.

We approached this challenging part of the design in a retro-synthetic way. Starting with design of the functional recombination product (a *trans*-acting hammerhead ribozyme, also quite variable in sequence), we inserted the required AGUC sequence into a nonconserved region for cleavage by the hairpin ribozyme. The ribozyme binding domain was designed for optimal binding of the recombination product with minimal structural distortion of the overall system. We continued by designing the educts (the two RNA strands without function at the beginning of the reaction chain) on the basis of the prior defined binding site. Because the nonsense part of one substrate had to bind less strongly than its profunctional counterpart in the other substrate, we took into account considerations such as mismatches, GU wobble pairs, and shortening of helix length. Nevertheless, it was necessary to achieve a binding capacity that was strong enough to ensure formation of a catalytically competent structure and substrate cleavage (4 bp in helices 1 and 2). Furthermore, undesired interactions of the ribozyme or substrates with the RNAs in the system had to be limited to an insignificant amount. If the defined preconditions could not be met by the designed structure, we went back to the recombination product and the substrate binding site, and re-designed their sequence and length, if necessary going through iterative cycles of design and theoretical verification. Because recombination should proceed in a one-pot reaction, the two initial ribozyme–substrate complexes were designed to have free energy values as close as possible to each other, and to cleave both substrates efficiently enough to deliver sufficient amounts of profunctional fragments for recombination. This required further adjustment of the designed sequences, but still taking into account all the aforementioned conditions. At the end of the design process, we successfully engineered a hairpin ribozyme-based recombination system, composed of two substrates and one ribozyme (Fig. 5), that performed recombination with high yield [14].

5 Summary

Engineering of nucleic acid catalysts by rational design is a powerful tool with potential applications. However, it can be a very challenging task, requiring attention to be paid to a number of factors associated with sequence adaptation, folding and active conformation, and reaction equilibria. Usually, engineering starts from a precursor ribozyme or DNAzyme with known catalytic features. As discussed, one of the key steps is adaptation of the ribozyme sequence to recognize a defined target, ensuring that activity is undamaged. Substrate association and dissociation processes can be influenced by variations in the lengths of substrate binding domains, but the ribozyme/DNAzyme must still be able to fold into the required active conformation. Furthermore, structural modulation of the chosen precursor enzyme can be used to influence the reaction equilibrium (e.g., for the hairpin ribozyme, structural stabilization favors ligation, whereas destabilization favors cleavage).

A number of tools are available to aid rational design, in particular software and platforms for theoretical prediction of nucleic acid secondary structures that can help guide sequence design. One of the most important prerequisites for engineering is that the structure and mechanistic properties of the starting nucleic acid are known. The more data are available, the higher is the chance for successful design. Nowadays, this prerequisite is met by a number of nucleic acids. Many of the ribozymes and DNAzymes known today are understood to a level that allows them to be turned into useful tools.

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