RNA (1995), *1*:225–233. Cambridge University Press. Printed in the USA.

REVIEW

Regulation of the hepatitis delta virus ribozymes: To cleave or not to cleave?

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INTRODUCTION AND BACKGROUND

Hepatitis delta virus (HDV) is a human pathogen with unique characteristics that are of potential interest to students of RNA structure, RNA–protein interactions, transcription, editing, RNA processing, and RNA catalysis. We will briefly summarize the HDV replicative cycle in an effort to highlight some of these areas, and then focus on the ribozymes and the manner in which they are thought to be regulated within the host cell.

HDV is a subviral agent whose propagation depends upon the presence of a helper virus (Rizzetto et al., 1980). Hepatitis B virus (HBV) is required for the provision of the envelope proteins that package the HDV genome into an infectious virion. However, replication of the HDV genome can occur in the absence of HBV (Kuo et al., 1989). HDV has a circular, single-stranded RNA genome that, at 1,679 nt in length, is the smallest genome found among agents that infect animals (Chen et al., 1986; Kos et al., 1986; Wang et al., 1986). The genome has the potential to fold into an unbranched rodlike structure with approximately 72% of its nucleotides involved in standard Watson and Crick base pairs (Zuker & Stiegler, 1981; Wang et al., 1986; Kuo et al., 1988a). This rodlike structure can be thought of as a single, extremely long RNA hairpin (stem and loop) in which the two termini at the bottom of the stem are covalently joined by a second loop. The HDV genome, which contains 601 paired bases, is the longest predicted RNA structure of its type (Zuker & Stiegler, 1981). Although there is extensive G-C rich pairing throughout the stem, no region of perfect duplex exceeds 12 bp before it is interrupted by either a mismatch or a bulge. A function for such discontinuities has not been established, although they could have evolved in response to various host mechanisms that inactivate perfect duplex RNA (Lazinski & Taylor, 1994b). Within the virion, the genome forms a ribonucleoprotein (RNP) structure together with two forms of the only protein that HDV is known to encode, the delta antigen (Ryu et al., 1993). This protein contains two arginine-rich motifs that are needed for its specific interaction with HDV RNA (Lee et al., 1993). Similar RNA-binding motifs are present in bacteriophage antitermination proteins and in the HIV tat and rev transactivators (Lazinski et al., 1989).

Upon entry into the cell, the genomic RNP is transported to the nucleus, where it is replicated via RNA-directed transcription. Infectious genomes encode the small delta antigen variant δAg-S. Synthesis of this protein is required for genome replication to occur (Kuo et al., 1989). However, due to its small size (195 amino acids) and lack of homology with any known polymerase, δAg-S is not thought to transcribe the genomic RNA; rather, a host polymerase is believed to provide this function. In vitro, RNA polymerase II will function in the RNA-directed transcription of the HDV genome and is thought to act similarly in vivo (Macnaughton et al., 1991; Fu & Taylor, 1993). That is, HDV is thought to redirect for its own use a host polymerase that normally transcribes DNA templates. Transcription of the genome results in the synthesis of an mRNA (Fig. 1) that encodes the δAg-S open reading frame (Hsieh et al., 1990). This 700-nt RNA contains a unique 5′ end that may arise as a consequence of the initiation of transcription, and a 3′ end that is polyadenylated by the host processing apparatus (Hsieh et al., 1990). The same AAUAAA motif that typically specifies the polyadenylation of polymerase II mRNA following DNA-directed transcription is also required for the production of the mature HDV message from its RNA template (Hsieh et al., 1990).

During replication, HDV RNA is specifically edited at a site corresponding to the stop codon of the δAg-S mRNA (Luo et al., 1990; Casey et al., 1992; Zheng et al., 1992). As a consequence, this UAG codon is changed to UGG, resulting in the synthesis of a variant of the delta antigen (δAg-L) that contains an additional 19

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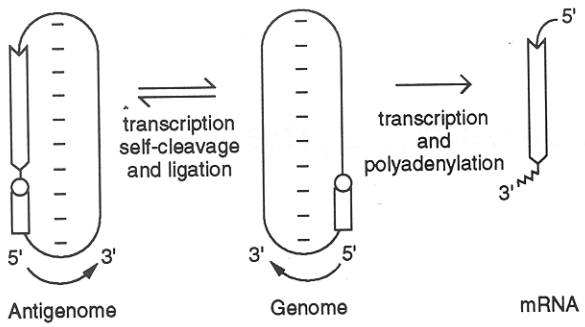

FIGURE 1. The HDV RNAs. Ribozymes are depicted as rectangles with an overlapping circle to indicate the adjacent site of self-cleavage. The delta antigen open reading frame is represented as an open arrow, and dashes indicate base-pairing within the predicted rodlike structure.

amino acids at its carboxy-terminus. δAg-L acts as a dominant repressor of replication and is also required for the packaging of the HDV RNP into HBV envelope particles (Chao et al., 1990; Chang et al., 1991; Ryu et al., 1992). Thus, the editing of HDV RNA represents an essential step in the completion of the viral propagative cycle.

In addition to mRNA, transcription from the HDV genome generates a relative excess of unit-length and multimeric RNAs of antigenomic polarity (Chen et al., 1986). Existence of the latter provides evidence in support of a rolling-circle model for HDV replication (see Fig. 2) in which transcription is thought to proceed multiple times around the circular template (Taylor, 1990). Within the resulting antigenomic multimer, a single *cis*-acting ribozyme that self-cleaves at a unique site is present at monomeric intervals (Sharmeen et al., 1988). Thus, two self-cleavage events will release an antigenomic linear monomer (Fig. 2, step 3). Ligation of the two termini of the monomer produces a circular replication intermediate referred to as the antigenome (Fig. 2, step 4). Ligation of such termini has been observed in vitro in the absence of protein (Sharmeen et al., 1989). However, this reaction requires a splint (or guide) RNA, derived from HDV sequences on the opposing side of the rodlike structure. In addition, the in vitro reaction appears to generate both 3′,5′ and 2′,5′-phosphodiester bonds, in equimolar proportions. In contrast, it was recently observed that within transfected cells, the ligation of HDV RNA displays no requirement for a splint, and seems to produce only 3′,5′ linkages (Lazinski & Taylor, 1995). These and other observations are consistent with the possibility that HDV RNA might be a substrate for a host ligase; however, this has not yet been tested directly.

Once generated, the circular antigenome is thought to serve as template for genome synthesis via an analogous rolling-circle mechanism (Fig. 2, steps 5–8). Like the antigenome, the unit-length genome contains a single *cis*-acting ribozyme that is thought to resolve multimeric replication intermediates (Kuo et al., 1988b). Consistent with such a role, a direct correlation has been established between the ability of a mutant HDV sequence to self-cleave in vitro and to replicate in vivo (Macnaughton et al., 1993). Because both the circular genome and the circular antigenome are thought to serve as templates for transcription, the term "double rolling-circle mechanism" best describes HDV replication (Macnaughton et al., 1993).

The HDV genome's small size and circular rodlike structure, its ability to recruit a host enzyme for its RNA-directed replication, and its use of ribozymes to process rolling-circle replication intermediates distinguish this virus from all other animal pathogens. However, these same properties are shared with two families of infectious agents of plants, the viroids and the viroid-like satellite RNAs (virusoids). These agents have small, circular, rodlike RNA genomes (246–375 nt in length) that are likewise thought to replicate via a rolling-circle mechanism (Diener, 1993). A number of virusoids and at least two viroids encode ribozymes (of either the hammerhead or hairpin type) that can cleave multimeric replication intermediates (Symons, 1994). Furthermore, like HDV, the viroids are thought to recruit the host polymerase II enzyme for their RNA-directed transcription (Schindler & Mühlbach, 1992). Both the viroid and HDV genomes are also similar in that they contain multiple, long homopyrimidine and homopurine tracts and ultraviolet light-inducible sites of cross-linking and share limited sequence homology (Branch et al., 1989, 1993). The major discriminating feature between HDV and these plant agents is that the former encodes a protein (expressed by an mRNA) and, as a consequence, has a much larger genome.

RIBOZYME STRUCTURE AND FUNCTION

The positions of the genomic and antigenomic ribozymes within their respective RNAs, and in relation to their sites of cleavage, are indicated in Figure 1. In each case, the ribozyme functions as a contiguous sequence that is located near the bottom and on one side of the rodlike structure. Due to the semicomplementary nature of this structure and the perfect complementarity shared between the genome and the antigenome, the two ribozymes display 80% sequence identity when gaps are allowed in the alignment (Kuo et al., 1988b; Been, 1994). The genomic and antigenomic ribozymes are also related in a number of other ways. As with the hammerhead and hairpin ribozymes, each HDV ribozyme uses a divalent metal ion in a transesterification reaction that generates 2′,3′-cyclic phosphate and 5′-hydroxyl termini (Kuo et al., 1988b; Sharmeen et al., 1988). However, the HDV ribozymes differ from those of the plant agents in that the former demonstrate significant activity in the presence of denaturants and can cleave optimally with as little as

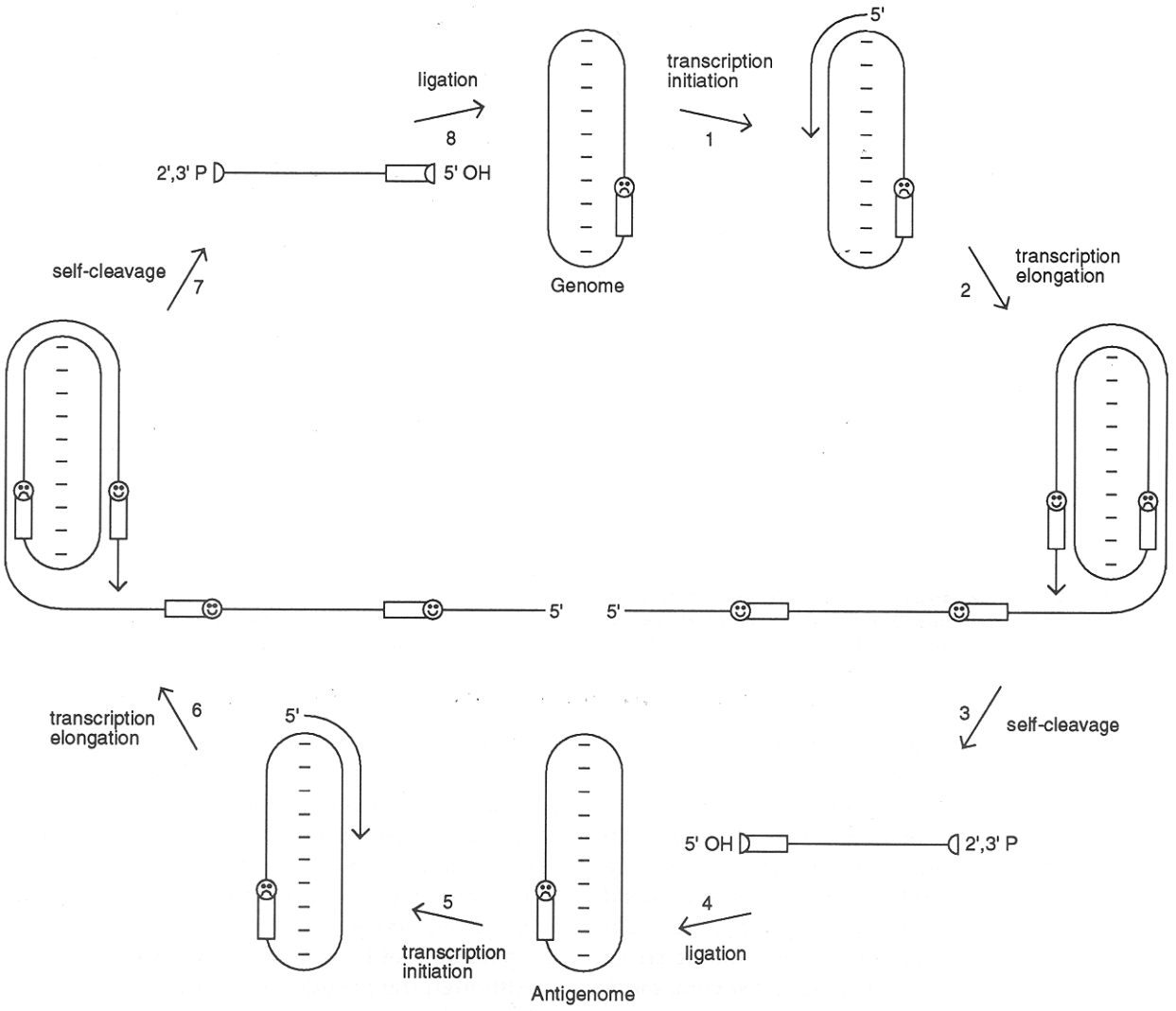

FIGURE 2. A double rolling-circle model for HDV replication. For simplicity, the regulated synthesis of mRNA versus full-length antigenomic RNA via suppression of the polyadenylation signal is not considered in this model (see Hsieh & Taylor, 1991). Ribozymes are depicted as rectangles with an overlapping circle to indicate the adjacent site of self-cleavage. A happy face within the circle indicates an active ribozyme, and a sad face depicts an inactivated ribozyme.

a single nucleotide 5′ to the site of cleavage (Perrotta & Been, 1990; Rosenstein & Been, 1991; Smith & Dinter-Gottlieb, 1991). Because the HDV ribozymes make no essential interactions with their associated 5′ flanking sequences, they can be joined in *cis* to heterologous upstream sequences and still cleave efficiently. This property has been exploited in studies of the replication of both vesicular stomatitis virus (VSV) and flock house virus (FHV) (Pattnaik et al., 1992; Ball, 1994). An HDV ribozyme cassette was strategically engineered downstream of either VSV or FHV sequences so that self-cleavage generated the desired 3′ end.

For *cis*-cleaving ribozymes such as the hammerhead and the hairpin, it has been possible to divide the RNA into two fragments: a substrate and a *trans*-acting enzyme domain (Haseloff & Gerlach, 1988; Berzal-Herranz et al., 1993). The HDV ribozymes have been similarly segmented, and several substrate/enzyme combinations have been reported (Branch & Robertson, 1991; Perrotta & Been, 1992; Wu et al., 1992). The creation of such *trans*-acting ribozymes is useful because it both facilitates genetic studies aimed at elucidating ribozyme structure and enables the determination of Michaelis-Menten constants such as k_{cat} and K_m. Thus far, HDV *trans*-acting ribozymes have been less active than comparable hammerhead and hairpin derivatives as judged by their lower k_{cat}/K_m (Perrotta & Been, 1992). Nevertheless, there are efforts under way to engineer *trans*-acting HDV ribozymes so as to target and cleave specific host or viral messages for their potential use as therapeutic agents (Shih et al., 1993).

Given that the genomic and antigenomic ribozymes display so many similarities, it is likely that they adopt a common structure during catalysis. Several structural

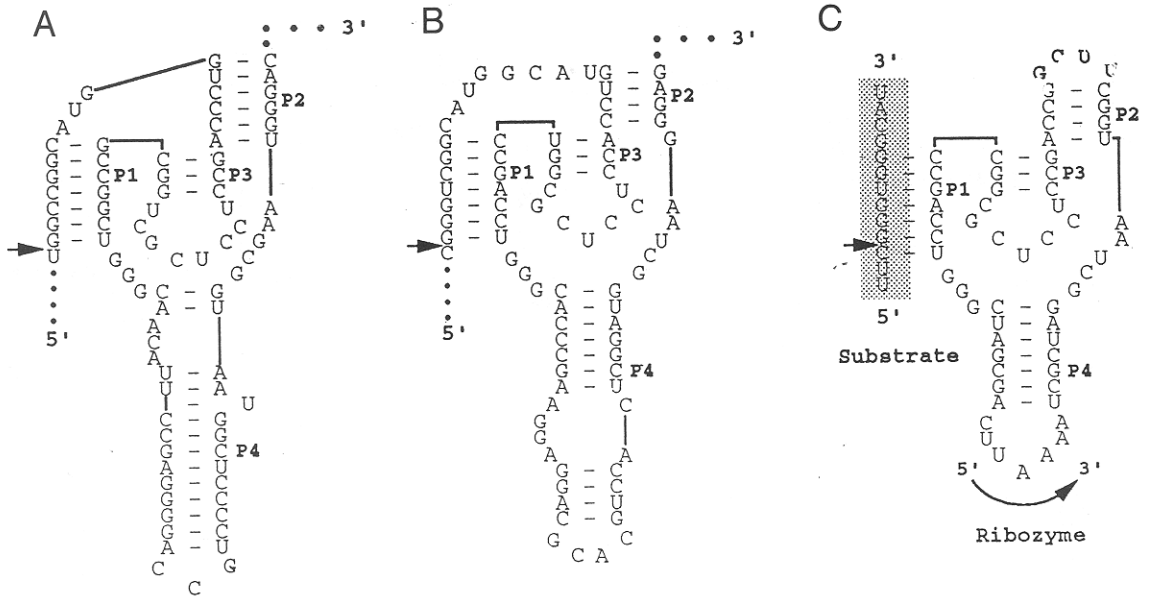

FIGURE 3. Two-dimensional "pseudoknot" structural models for the HDV ribozymes as adapted from Been (1994). **A:** The genomic ribozyme. **B:** The antigenomic ribozyme. **C:** A *trans*-acting circular antigenomic derivative. Arrows point to the cleavage sites, and P1–P4 indicate the four proposed regions of base-pairing.

models have been proposed by different laboratories, and most of them attempt to depict the two ribozymes in analogous conformations (Branch & Robertson, 1991; Perrotta & Been, 1991; Smith et al., 1992; Wu et al., 1992). Nevertheless, none of these models are similar to those proposed for either the hammerhead or hairpin enzymes, and there is general agreement that the ribozymes of HDV constitute a unique structural class of catalytic RNA. Among the several models for the HDV ribozymes, the pseudoknot structure proposed by Perrotta and Been (1991) is most consistent with available data. The genomic and antigenomic versions of this model are depicted in Figure 3A and B, respectively. Of the four pairings shown in each figure, the P1 and P4 stems are elements common to all structural models proposed for the HDV ribozymes. However, the pseudoknot model differs from others in its prediction of the P2 and P3 stems, the former constituting the pseudoknot interaction.

Both genetic and biochemical data obtained from a number of different laboratories support the pseudoknot model (Perrotta & Been, 1993; Thill et al., 1993; Wu et al., 1993; Kumar et al., 1994). The most convincing evidence for the existence of the pseudoknot interaction itself comes from genetic manipulations of the P2 stem. Mutations that reduce the predicted base-pairing in this stem decrease ribozyme activity, whereas increased activity is observed with second site compensatory mutants that restore pairing (Perrotta & Been, 1993). In addition, a circular *trans*-acting antigenomic derivative has been created and is shown in Figure 3C (Puttaraju et al., 1993). In this case, the predicted P2 stem is "forced" to form by the covalent joining of its two ends with a specific tetraloop sequence (UUCG) that is known to impart extraordinary stability to its associated hairpins (Cheong et al., 1990). Such an arrangement should preclude the involvement of the P2 residues in alternate pairings, as is proposed in each of the other models. Thus, the activity of this circular *trans*-acting ribozyme demonstrates that formation of the pseudoknot P2 stem is compatible with catalysis.

Although the pseudoknot model provides a useful framework, it should not be thought of as a complete and accurate portrayal of ribozyme structure. It depicts only standard Watson and Crick base pairs in a two-dimensional representation without accounting for the important tertiary interactions involved in the assembly of the enzyme's active core. Such interactions are thought to be promoted by many of the residues within the P3 loop, as well as the P1/P4 and P4/P2 joining regions (Been, 1994). However, the exact manner in which these residues associate and coordinate the divalent metal ion is not known. Recently, by combining computer-assisted modeling with biochemical, mutational, and HDV isolate sequence data, a three-dimensional structure has been proposed (Tanner et al., 1994). This proposed structure, based upon the pseudoknot two-dimensional model, makes several testable predictions concerning the spatial arrangement of some critical bases within the active core. However, a more complete understanding of the structure and function of the HDV ribozyme may ultimately await the determination of its X-ray diffraction crystal structure. The structure of the hammerhead ribozyme, as complexed with a DNA "substrate," has recently been solved and has provided a number of new insights into the mecha-

nism of catalysis (Pley et al., 1994). Given the relatively large size of the HDV ribozymes, however, comparable undertakings with these enzymes would currently be formidable.

THE "ROLLING-CIRCLE PARADOX"

It is important to remember that, in nature, the HDV ribozymes function neither as 85-nt oligomers nor in protein-free environments. During rolling-circle replication in the host nucleus, the ribozymes must cleave their substrates in the context of flanking HDV sequences. As with the viroids and virusoids, HDV is confronted by an apparent paradox implicit in the rolling-circle mechanism. Its multimeric RNA intermediate contains a particular sequence that is repeated in monomeric intervals and that serves as the substrate for cleavage (Fig. 2, step 3). After cleavage, the resulting linear monomer is ligated so that the cleavage sequence is actually reconstituted (Fig. 2, step 4). If this reconstituted sequence were to be re-cleaved, then the circular product would be linearized and further rolling-circle replication would be impossible. Thus, the processing of this sequence must be activated in the multimeric precursor but then attenuated in the circular product. How can this be achieved? We will first discuss three possible solutions to the "rolling-circle paradox" thought to be employed by certain plant agents, and then contrast these with a recently proposed mechanism for HDV processing.

For the majority of viroid agents, processing during rolling-circle replication is not believed to be autolytic because, in these cases, self-cleaving ribozymes have not been identified (Diener, 1993). Rather, a host ribonuclease is thought to cleave specific RNA structures repeated at monomeric intervals within the multimeric intermediate. In reconstitution experiments, RNase T1 can be substituted for the not yet identified host enzyme and can process multimeric intermediates of the potato spindle tuber viroid (PSTV) to yield infectious circular monomers (Tsagris et al., 1991; Tabler et al., 1992). The minimal RNA substrate requirements and sites of cleavage for this reaction have been determined. The structure recognized by the ribonuclease can form only when the sequences flanking each side of the cleavage site are repeated twice in the substrate RNA (Steger et al., 1992). Therefore, PSTV is proposed to have solved the "rolling-circle paradox" by creating a processing substrate that is present in its multimer but absent from its monomeric circle.

The avocado sunblotch viroid (ASBV) is distinct from all other viroids save one, in that it is known to encode a ribozyme that processes its replication intermediates. In the ASBV monomer, this hammerhead motif does not adopt an active structure due to the small stem size and sterically constrained loop of its hairpin III sequence. However, in the multimer, two copies of the motif are thought to fold into what is called a double-hammerhead structure (Forster et al., 1988). In this case, the stem length of hairpin III is extended and steric hindrance is reduced so that self-cleavage can occur. Therefore, the solution to the "rolling-circle paradox" used by ASBV differs from that of PSTV in that a ribozyme, rather than a substrate, is active in the multimer but absent from the monomeric circle.

As a third example from the plant agents, we speculate about a solution to the "rolling-circle paradox" that might be employed by RNA2 of the *Solanum nodiflorum* mottle virus (SNMV). A hammerhead ribozyme, encoded by this virusoid, cleaves multimeric replication intermediates; yet here, the single-hammerhead structure is thought to be active. Following self-cleavage of the multimer, the two resulting termini in the monomer are ligated to form the circular product. A biochemical analysis of this ligation junction revealed the presence of an additional phosphate joined to a 2′ hydroxyl group in a 2′ phosphomonoester, 3′-5′ phosphodiester linkage (Kiberstis et al., 1985). If ligation had occurred via a reversal of the self-cleavage reaction, then this additional 2′ phosphomonoester bond would not be expected. However, such a linkage would be created if ligation were mediated by a host ligase such as that used during tRNA splicing (Greer et al., 1983). Furthermore, given our understanding of the mechanism of hammerhead-mediated cleavage, it seems likely that the presence of the 2′ phosphate moiety should protect the 3′-5′ phosphodiester bond from cleavage by this ribozyme. Thus, for SNMV, the cleavage substrate would be "on" in the multimer, but "turned off" in the circular monomer by the action of the ligase.

When considering possible solutions that might be used by HDV in response to the "rolling-circle paradox," it is apparent that none of the above three plant agent precedents apply. Unlike the first two examples, for HDV both the cleavage site and ribozyme are active when present in a single copy. This has been verified not only in vitro (Sharmeen et al., 1988) but also within transfected cells (Pattnaik et al., 1992). Furthermore, in contrast to the third example, recent evidence demonstrates that the ligation junction within the circular HDV genome is a substrate, and under certain experimental conditions can be cleaved by its adjacent ribozyme (Lazinski & Taylor, 1995). Thus, the monomeric genome contains a potentially active ribozyme and site of cleavage, but these are normally maintained in a dormant state so that the integrity of the circular conformation is preserved. How then is the HDV ribozyme first activated in the multimeric precursor and then attenuated in the monomeric circle? Two recent studies have addressed this issue and, in the latter, a solution to the "rolling-circle paradox" is proposed (Lazinski & Taylor, 1994a, 1995). We will first present the proposed mechanism and then review its support-

ing evidence. Although the model is based solely on an analysis of the processing of genomic RNA, given that the structures and locations of the two HDV ribozymes are analogous, it should be equally applicable to the processing of antigenomic RNA.

HDV RIBOZYME REGULATION

As shown in Figure 1, each of the 84 nucleotides HDV ribozymes is located strictly on a single side of the rodlike structure. Any base-pairing between the ribozyme sequences and their partners on the opposite side of the rodlike structure is expected to prevent the formation of the active pseudoknot structure (Fig. 3A,B). Therefore, the sequences on the opposite side of the rodlike structure are proposed to attenuate ribozyme activity in the circular product. Because these same attenuator sequences are also present within the multimeric precursor, they could potentially interfere with the processing of that molecule. However, it is also proposed that host factors interact transiently with the multimeric precursor so as to prevent the ribozyme/attenuator interaction and thereby promote processing (see Fig. 4). Thus, during HDV replication, the ribozyme is thought to be "turned on" in the multimer by a host activity and then "turned off" in the monomeric circle via its participation with the attenuator sequences in the rodlike structure.

The proposed mechanism for the processing of HDV RNA is based on a number of recent findings. The processing of multimeric HDV RNAs was analyzed following DNA-directed transcription from transfected plasmids. It was found that both self-cleavage and ligation can occur efficiently even in the absence of HDV replication (Lazinski & Taylor, 1994a). Several deletion mutants were constructed and their competence for processing was determined either in the presence or in the absence of the delta antigens. A 348-nt derivative of HDV, similar in size to a viroid, was shown to be properly processed into a circular monomer. Such processing occurred in the absence of the delta antigens and was not significantly affected by their presence (Lazinski & Taylor, 1994a). Thus, neither the top four-fifths of the rodlike structure nor the delta antigens play a necessary role in the processing of HDV RNA.

The processing of this same viroid-sized mutant was then examined in vitro following synthesis by T7 RNA polymerase (T7 RNAP). Under these conditions, self-cleavage was inhibited and none of the monomeric 348 nucleotides species was detected (Lazinski & Taylor, 1995). Similar results were obtained following T7 RNAP initiated transcription in vivo, in either *Escherichia coli* or *Saccharomyces cerevisiae*. However, when the same T7 polymerase was used to transcribe the same template in a human hepatoma cell line (Huh7),

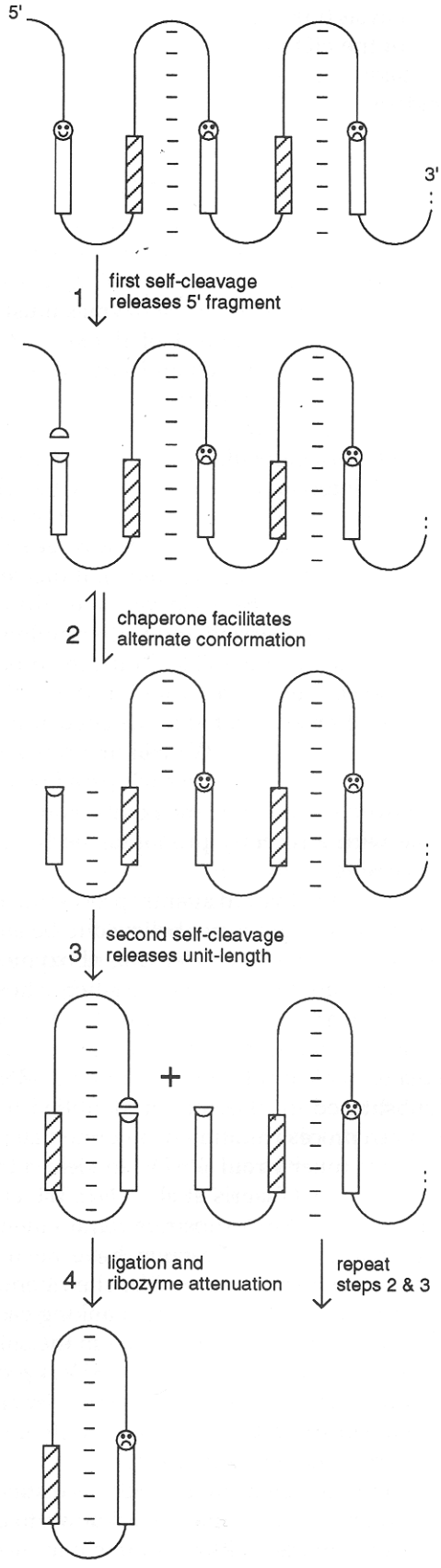

FIGURE 4. Possible pathway for the processing of a genomic HDV multimeric precursor. A rightward-hatched rectangle denotes attenuator sequences, and dashes indicate base-pairing within the predicted rodlike structure. Otherwise, conventions are as in Figure 2.

self-cleavage of the resulting precursor was not inhibited and monomeric 348 nucleotide linear and circular products resulted (Lazinski & Taylor, 1995). Thus, HDV precursors contain sequences capable of inhibiting the self-cleavage reaction, and yet, such inhibition can be prevented when expression occurs within the context of the natural host cell.

Deletion analysis was carried out in order to identify those HDV sequences responsible for ribozyme inhibition. Not surprisingly, the sequences opposite to, and that can potentially base-pair with, the ribozyme in the rodlike structure were found to be responsible for such inhibition. When these attenuator sequences were excised from the primary transcript, efficient self-cleavage was observed in vitro, in bacteria, and in yeast, so that the monomeric product resulted (Lazinski & Taylor, 1995). Furthermore, it was found that not all of the attenuator sequences that are predicted to pair with the ribozyme in the rodlike structure are required to inhibit self-cleavage. Two nonoverlapping subfragments inhibited self-cleavage as efficiently as the whole region did. In addition, inhibition was observed following the replacement of all of the natural attenuator sequences with a small, dissimilar 21-nt synthetic sequence designed to base-pair with the ribozyme's active core (Lazinski & Taylor, 1995). Thus, no particular structure or sequence is required for ribozyme inhibition; all that is needed is a capacity to disrupt the ribozyme's active conformation through base-pairing.

The cleavage and subsequent ligation of this set of mutant HDV RNA precursors was also examined following expression in Huh7 cells. Consistent with what was observed with the 348-nt mutant, each precursor was processed into the monomer form whether or not it contained attenuator sequences. However, only those excised monomers that contained attenuator sequences existed as circular RNAs. In contrast, monomers that lacked attenuator sequences were present as linear molecules (Lazinski & Taylor, 1995). It was therefore proposed that, even though attenuator sequences do not inhibit the self-cleavage of precursor molecules within the mammalian cell, they do inhibit the subsequent re-cleavage of the circular product. In other words, the circular monomer contains both an active ribozyme and a site of self-cleavage; however, these are inactivated by base-pairing in the rodlike structure. This model was further tested. As represented in Figure 5, circular monomers generated within transfected cells and isolated as total RNA were denatured and renatured in the presence of an RNA that is 100% complementary to the attenuator sequences. The attenuator sequences can thus form a more stable interaction with this RNA than with the ribozyme sequences (72% complementarity). As predicted, the ribozyme was freed from inhibition imposed by the rodlike structure, and efficient self-cleavage of the circular molecule was observed (Lazinski & Taylor, 1995).

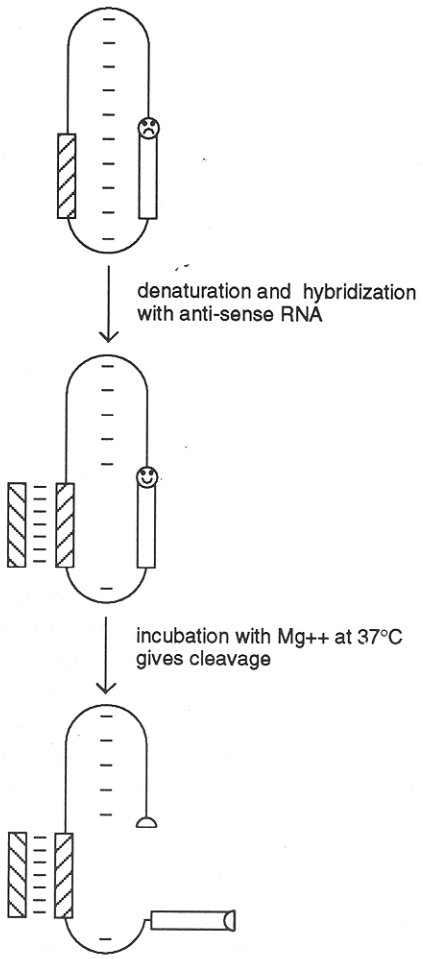

FIGURE 5. Disruption of the rodlike structure can activate self-cleavage of a circular monomer. A leftward-hatched rectangle denotes an antisense RNA that hybridizes to the attenuator sequences. Otherwise, conventions are as in Figure 4.

Additional evidence concerning a role for host factors in the processing of precursor RNA is derived from a study of HDV RNA-directed transcription in nuclear homogenates. Because the resulting products of such transcription were of monomeric length, it was inferred that the self-cleavage of nascent transcripts was efficient (Fu & Taylor, 1993). Thus, any host factor required for the processing of HDV RNA should be active in these homogenates. Preliminary evidence confirms this and indicates that this protein(s) can even activate the self-cleavage of exogenously added precursor RNA (D.W. Lazinski & J.M. Taylor, unpubl. results). The manner in which this mammalian cell-specific factor(s) activates the HDV ribozymes has not yet been determined. However, a clue may be provided by results obtained from a protein-free in vitro reaction. In this case, activation of the ribozymes in the multimeric precursor could be accomplished when the attenuator/ribozyme interaction was transiently disrupted by heat denaturation (Lazinski & Taylor, 1995). One interpretation of this finding is that the heat denaturation/

renaturation step enables the RNA precursor to refold into an activated form, as is diagrammed in step 2 of Figure 4. It is therefore tempting to speculate that a host factor(s) possessing RNA chaperone activity might similarly promote such interconversion between alternative conformations at physiological temperatures.

RNA chaperones constitute a class of nonspecific nucleic acid binding proteins that are capable of promoting both the denaturation and renaturation of duplex structures (Pontius & Berg, 1990; Portman & Dreyfuss, 1994). Such proteins have been used to enhance the turnover rate at which a ribozyme cleaves its substrate in vitro (Tsuchihashi et al., 1993; Herschlag et al., 1994) and can affect the structure of nascent transcripts in vivo (Dreyfuss et al., 1993). Figure 4 illustrates only one of a number of possible pathways by which an RNA chaperone could alter the folding of the HDV multimer and thereby activate self-cleavage. In an alternate model, a host protein that specifically recognizes and binds to the ribozyme's active conformation might prevent the formation of the rodlike structure and thereby stimulate self-cleavage of the RNA precursor. Both the HDV genomic and antigenomic ribozymes each contain a region that is homologous to a portion of the RNA component of the signal recognition particle (7SL RNA) (Negro et al., 1989, 1991). Could a host factor(s) that normally binds to 7SL RNA promote HDV processing? Clearly, more studies will be required to both characterize the activity of the host factor(s) and, ultimately, determine its identity and mechanism of action.

PROSPECTS

The regulation of RNA processing represents an important step in the control of eukaryotic gene expression. There are numerous examples of RNA precursors that are alternatively spliced during differentiation and development. In at least one case, the relative abundance of a particular RNA chaperone (hnRNP A1) has been shown to dictate the splicing outcome (Mayeda & Krainer, 1992; Caceres et al., 1994). However, the study of such processes has been hampered, in part, by the vast complexity of the associated RNA processing machinery. In contrast, the relative simplicity of HDV processing should facilitate mechanistic studies. In this case, cleavage is self-catalyzed and the activation of self-cleavage may involve only one or a few host factors. In addition to their interaction with HDV RNA, such factors are likely to participate in the maturation of host RNAs. Thus, future studies of the processing of HDV RNA may shed light on analogous mechanisms used by the host cell.

ACKNOWLEDGMENTS

J.M.T. was supported by grants AI-26522, AI-31927, and CA-06927 from the National Institutes for Health and by an appropriation from the Commonwealth of Pennsylvania. We thank Drs. C. Seeger, R. Krug, W. Mason, and R. Katz for their constructive comments on the manuscript and Ms. K. Trush for the graphics.

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