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Genetics

Ultraviolet light-induced crosslinking reveals a unique region of local tertiary structure in potato spindle tuber viroid and HeLa 5S RNA

(viroid RNA structure/5S rRNA structure/RNA tertiary structural element/ultraviolet crosslinking)

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ABSTRACT **The positions of intramolecular crosslinks induced by irradiation with ultraviolet light were mapped into potato spindle tuber viroid RNA and HeLa 5S rRNA. Crosslinking in each of these molecules occurred at a single major site, which was located by RNA fingerprinting and secondary analysis (and additional primer extension studies in the case of the viroid). Various lines of evidence suggest that these crosslinks identify a previously undescribed element of local tertiary structure common to these two widely divergent RNA molecules: (*i*) both crosslinks occur in an identical eight-base context, with the sequence 5′ GGGAA 3′ on one side and the sequence 5′ UAC 3′ on the other; (*ii*) both crosslinks connect bases that are not thought to be involved in conventional hydrogen bonding, within regions usually depicted as single-stranded loops flanked by short helical segments; and (*iii*) both crosslinks connect a purine and a pyrimidine residue, and both may generate the same G-U dimer. Furthermore, it is likely that the crosslinking site is of functional significance because it is located within the most highly conserved region of the viroid sequence and involves bases that are essentially invariant among eukaryotic 5S rRNA molecules.**

Structural maps have been developed for a large number of RNA molecules. These maps usually depict helical regions interspersed with loops and bulges of various lengths. Except for the map of tRNA, which is drawn from crystallographic data, RNA structural maps are largely based on studies of nuclease sensitivity, evolutionary data, and computer analysis. In most instances, these techniques are used to assign a nucleotide residue to one of only two categories: based-paired or unbonded.

However, various lines of evidence suggest that many RNAs have additional structural elements. For example, (*i*) the crystal structure of tRNA contains several non-Watson–Crick bonds (1, 2); (*ii*) the nuclear magnetic resonance spectrum of *Escherichia coli* 5S rRNA contains peaks in addition to those resulting from conventional hydrogen bonding (3); and (*iii*) the autocatalytic cleavage of the rRNA precursor of *Tetrahymena* (4) and the ability of M1 RNA from *E. coli* to cleave tRNA precursors (5) suggest that these RNA molecules may contain regions analogous to the active sites of protein enzymes. In the studies to be described here, we present evidence that two other biologically active RNA molecules, HeLa 5S rRNA and potato spindle tuber viroid (PSTV) RNA are exceptionally susceptible to crosslinking upon exposure to UV light and that they both contain a previously undescribed element of local tertiary structure.

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MATERIALS AND METHODS

Gel Procedures. Two-dimensional gel electrophoretic analysis (6, 7) was carried out as described (8) by using a 5% nondenaturing gel containing a Tris acetate buffer (0.040 M Tris, pH 7.2/0.020 M sodium acetate/0.001 M EDTA; ref. 6) for the first dimension and a 7 M urea gel (9) containing a Tris borate buffer (0.045 M Tris, pH 8.2/0.044 M boric acid/0.0014 M EDTA) for the second dimension.

RNA-RNA Crosslinking by Irradiation with UV Light. Nucleic acids (either in a nondenaturing gel or merely resuspended in a small amount of the Tris acetate buffer) were placed on a protective sheet of Saran Wrap and irradiated with a UV light box (Ultra-Violet Products, San Gabriel, CA) equipped with four shortwave tube bulbs for 1–8 min. For irradiation of samples resuspended in a small drop of buffer (or in a quartz cuvette), a prechilled UV box was used to prevent excessive heating. In addition, 1-min intervals of UV exposure were followed by 30-sec cooling periods. For irradiation of gel strips, plastic bags filled with ice and water were placed on top of the strips for cooling.

Procedures for *in Vitro* Labeling. *In vitro* iodination of RNA was carried out as described (10, 11). For 5′-end-labeling of RNase T1-resistant oligonucleotides, small quantities of RNA (ranging from 2 to 200 ng) were digested with 2 μl of RNase T1 (30 μg/ml), treated with calf alkaline phosphatase, heated, and incubated with polynucleotide kinase and [γ-^{32}P]ATP (12). Labeling of a DNA oligomer was carried out under similar conditions.

One- and Two-Dimensional Oligonucleotide (Fingerprinting) Analysis. Internally labeled RNA samples were incubated with 2 μl of either RNase T1 (Sankyo, Calbiochem) or pancreatic RNase (Worthington) at 1 mg/ml in the presence of 10 μg of *E. coli* tRNA under conditions as described (11, 13, 14). After digestion, samples were either fractionated by electrophoresis in 20% polyacrylamide gels containing 7 M urea for one-dimensional analysis or were spotted onto cellulose acetate strips as the first step toward the preparation of standard two-dimensional fingerprints (13).

Classical Secondary Analysis. Oligonucleotides were recovered either by soaking excised gel bands overnight at 37°C in 100 μl of water or by eluting fingerprint spots from DEAE thin-layer plates (13). Secondary enzymatic digestion of oligonucleotides and fractionation of the products were carried out as described (11, 13, 15). Radiolabeled markers (derived from oligonucleotides of known sequence) were included in each experiment.

Mapping the PSTV Crosslinking Site by Partial Alkaline Hydrolysis. RNase T1-resistant oligonucleotides were subjected to partial alkaline hydrolysis under conditions tested to yield (on average) one cut or less per oligonucleotide. The

Abbreviation: PSTV, potato spindle tuber viroid.

hydrolysis was carried out in 10 μl of 0.050 M sodium bicarbonate/carbonate at pH 9.2, in the presence of 5 μg of carrier tRNA for 27 min at 90°C, and then samples were fractionated in 20% polyacrylamide gels containing 7 M urea and the Tris borate buffer described above. Treatment of conventional, linear oligonucleotides under these mild conditions generated a continuous ladder of gel bands (typically used for sequence analysis); size markers were used for positive identification of specific positions in the sequence of a given oligonucleotide. It was expected that a single cut in a crosslinked oligonucleotide would not give all possible sizes of digestion products and that the missing bands would allow us to infer the location of the crosslink.

RESULTS

Exposure of Phenol-Extracted RNAs to UV Light. A variety of conditions were used for UV-irradiation of nucleic acids extracted from tomato plants and ^{32}P-labeled HeLa cells. The experiment shown in Fig. 1 *A* and *B* illustrates a simple two-dimensional gel assay that can be used to seek UV-induced electrophoretic variants of plant viroids and other RNAs. Nucleic acids of PSTV-infected plants were exposed to UV light from a standard UV source (of the type commonly used for stimulating fluorescence from ethidium bromide-stained nucleic acids) and then were fractionated in a 5% nondenaturing polyacrylamide gel alongside an untreated control sample. The individual lanes were cut out and the samples were run directly into a 5% polyacrylamide gel containing 7 M urea, stained with ethidium bromide, and photographed. After UV treatment, a new form of PSTV (identified by the arrow in Fig. 1*B*) was seen migrating between the circular and linear viroid RNAs.

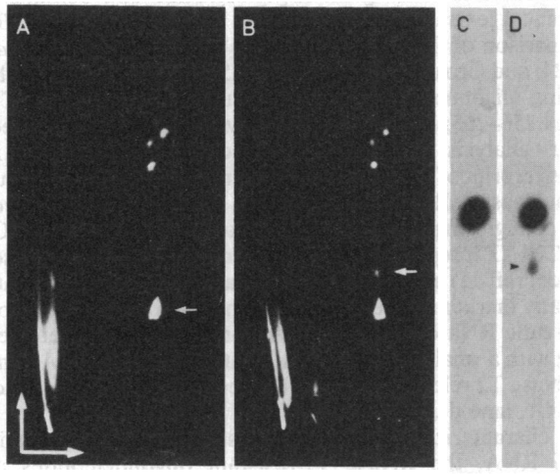

FIG. 1. Detection of crosslinked RNAs by two-dimensional gel electrophoresis. (*A* and *B*) To generate crosslinked PSTV, a sample containing 25 μg of nucleic acids from the LiCl supernatant fraction purified from PSTV-infected tomato plants as described (6, 16, 17) was dissolved in Tris acetate buffer (6) and then exposed to UV light for 7 min. In the two-dimensional gel pattern of nucleic acids from a control sample (*A*), an arrow identifies the position of circular PSTV. The arrow in *B* marks the position of crosslinked PSTV, which is present in the irradiated sample. (*C* and *D*) Aliquots of highly purified 5S rRNA, prepared from ^{32}P-labeled HeLa cells as described (8, 18) were mixed with 10 μg of tRNA and then fractionated in a nondenaturing gel. The sample shown in *C* received no treatment, whereas the sample shown in *D* was exposed to UV light for 4 min (60-sec intervals were punctuated by 30-sec cooling periods). Both samples were then fractionated in 12% polyacrylamide gels containing 7 M urea. The arrowhead in *D* indicates the position of crosslinked 5S rRNA. The pair of horizontal and vertical arrows in *A* designates the directions of first and second dimension separations, respectively.

In other experiments, UV-irradiation was carried out on RNA contained in a nondenaturing polyacrylamide gel. In this configuration, it was possible to keep heating and evaporation to a minimum. However, identical results were obtained (*i*) when RNA in a Tris acetate buffer solution (6) was pipetted directly onto a film of Saran Wrap for UV treatment (as in Fig. 1*B*), (*ii*) when irradiated through the walls of a quartz cuvette, or (*iii*) while in a gel (see figure 1 of ref. 8). About 25% of the circular PSTV in a sample can be converted to the altered form by irradiation, suggesting that essentially all of the circular molecules have the potential for this modification. However, prolonged UV exposure resulted in RNA breakdown. Thus, for RNA samples contained in 1.5-mm-thick gels, irradiation times of 1–5 min were used in most experiments.

Purification of 5S rRNA after Irradiation. Preliminary studies using nucleic acids of tomato plants indicated that irradiation produces a derivative of tomato 5S rRNA (8), suggesting that 5S rRNA from other eukaryotes might be susceptible to a similar reaction. ^{32}P-labeled 5S rRNA from HeLa cells was used to test this possibility because it is relatively easy to purify and has been studied extensively in the past (19, 20). UV-irradiation of ^{32}P-labeled HeLa 5S rRNA produced a single major new species, which migrated as a distinct spot upon electrophoresis in a 12% polyacrylamide gel containing 7 M urea and appeared to be about 5 times more intense than other minor forms (compare Fig. 1 *C* and *D*). The major 5S derivative and 5S rRNA that received no UV treatment at any point during its purification were prepared as described (8) and analyzed by fingerprinting.

Mapping the Crosslink in 5S rRNA. Two-dimensional fingerprinting analysis was carried out on both pancreatic RNase and RNase T1-resistant oligonucleotides of 5S rRNA and its crosslinked derivative. The pancreatic RNase fingerprint of the crosslinked form of 5S rRNA is missing the oligonucleotide GGGAAU* (bases 97–102; ref. 19) and contains one new spot (compare Fig. 2 *A* and *B*). This suggests (*i*) that crosslinking has occurred at a single site in the 5S molecule, (*ii*) that the crosslink involves one of the bases from the oligonucleotide GGGAAU, and (*iii*) that the second base present in the crosslink is part of an oligonucleotide that does not form a unique spot in the fingerprint (so that its absence goes undetected). Secondary analysis of the spots marked with numbers in Fig. 2 provided further important information concerning the crosslinking site. For example, electrophoresis of spots 1a (Fig. 2*A*) and 1b (Fig. 2*B*) on DEAE paper (in pH 1.9 buffer; ref. 13) revealed that 1a contains the oligonucleotides GAU and AGU while 1b contains GAU but lacks AGU (an oligonucleotide that occurs only once in the sequence of HeLa 5S rRNA). Furthermore, RNase T1 digestion of spot 3, the crosslink-specific spot (Fig. 2*B*), generated products comigrating with AAU, AG, and G, plus additional material with an anomalous mobility. These data indicate that the crosslink joins uridine-76 from the oligonucleotide AGU to one of the guanosine residues from the oligonucleotide GGGAAU.

This conclusion is strengthened by analysis of RNase T1-resistant oligonucleotides of 5S rRNA and those of its crosslinked derivative shown in Fig. 2 *C* and *D*. The RNase T1 fingerprint of the crosslinked 5S rRNA is missing spot 4, UACUUG (bases 76–81) and contains two new oligonucleotides (see Fig. 2*D*, spots 6 and 7) not present in RNase T1 digests of 5S rRNA itself. In addition, the oligonucleotide

*Phosphate groups are depicted only when they occur at the 5′ end of an oligonucleotide. Unless otherwise indicated, oligonucleotides are written in the 5′ to 3′ direction (left-to-right) and are assumed to have 5′ hydroxyl and 3′ phosphate termini.

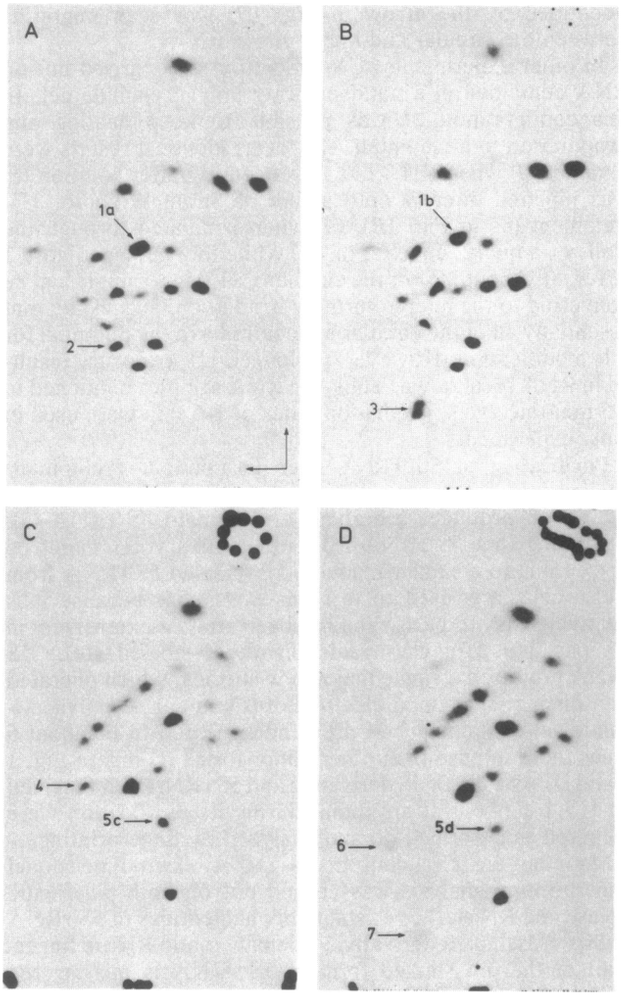

FIG. 2. Two-dimensional fingerprinting analysis of HeLa 5S rRNA and its crosslinked derivative. Pancreatic RNase-resistant oligonucleotides of 5S rRNA (*A*), UV-crosslinked 5S rRNA (*B*), and RNase T1-resistant oligonucleotides of control (*C*) and crosslinked 5S rRNA (*D*) were prepared by digestion of each RNA under conditions as described above and then fractionated to give a two-dimensional array. RNA was eluted from the spots designated by the arrows 1–7 for secondary analysis. The horizontal and vertical arrows at the bottom of *A* indicate the directions of high-voltage electrophoresis and ascending RNA homochromatography (13), respectively.

AAUACCG (bases 100–106) is present in reduced yield [compare the intensity of spots 5c (Fig. 2*C*) and 5d (Fig. 2*D*)]. Inefficient cleavage by RNase T1 at the guanosine-99 residue might be expected if this residue were involved in a crosslink, since RNase T1 is a guanosine-specific ribonuclease (21). Incomplete cleavage after guanosine-99, giving rise to the partial product GAAUACCG at some frequency, could explain why two crosslink-specific spots are present and account for the reduction in AAUACCG as well. Secondary analysis of the two crosslink-specific oligonucleotides, spots 6 and 7 (Fig. 2*D*), supported the conclusion that the crosslink connects uridine-76 to guanosine-99 (data not shown).

Mapping the Crosslinking Site Present in PSTV. To provide material for detailed analysis, the crosslinked form of PSTV was recovered from urea gels (17, 22) and iodinated *in vitro* (10, 11). This approach provided samples of crosslinked PSTV (Fig. 3*C*, lane b) that were free of circular (Fig. 3*C*, lane a) or linear (Fig. 3*C*, lane c) forms. An extensive description of the electrophoretic properties of the crosslinked viroid RNA appears elsewhere (8).

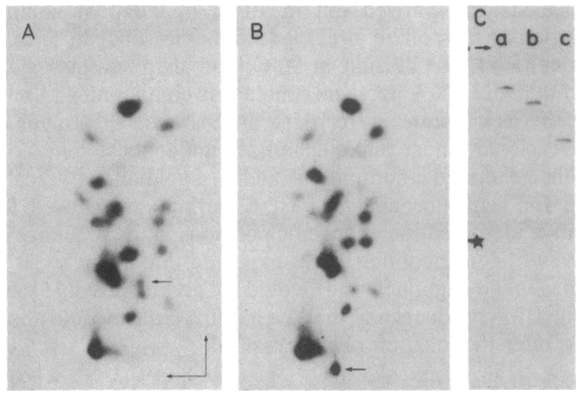

FIG. 3. RNase T1 fingerprinting analysis of circular and crosslinked PSTV. ^{125}I-labeled circular (*A*) and crosslinked (*B*) PSTV were digested with RNase T1 and then fractionated into two-dimensional patterns by standard techniques (13). The arrow in *A* marks the oligonucleotide CUACUACCCG, which is missing from the fingerprint of the crosslinked PSTV, while the arrow in *B* marks an oligonucleotide unique to the crosslinked form. The pair of horizontal and vertical arrows in *A* designates the directions of first (high-voltage electrophoresis) and second (ascending RNA homochromatography) dimensional separations. (*C*) A 5% polyacrylamide gel containing 7 M urea was used to fractionate ^{125}I-labeled circular (lane a), crosslinked (lane b), and linear (lane c) PSTV. An arrow indicates the origin of electrophoresis of the gel; a star denotes the position of xylene cyanol.

Inspection of RNase T1 fingerprints of ^{125}I-labeled PSTV circular and crosslinked RNA showed only two qualitative differences that were consistently observed. The crosslinked viroid RNA is missing an oligonucleotide and contains a prominent extra spot (marked by an arrow in Fig. 3*B*). Comparison of these fingerprints to those of Dickson *et al.* (22–24) and Gross and co-workers (25, 26) suggested that the missing oligonucleotide has the sequence CUACUACCCG (bases 256–265; ref. 26). For positive identification by secondary analysis, RNA was recovered from the extra spot in the fingerprint of crosslinked PSTV and digested with various ribonucleases. RNase U2 digestion produced oligonucleotides comigrating with ^{125}I-labeled markers for CUA, CCCG, and CCUG (prepared from oligonucleotides of known sequence; ref. 11) in addition to material that did not comigrate with any marker species (Fig. 4*A*, lane b). After digestion by pancreatic RNase, ^{125}I-labeled AC and C could be detected along with a small amount of resistant material (Fig. 4*A*, lane c). RNase T2 released free ^{125}I-labeled C as the only product (Fig. 4*A*, lane d).

To characterize further the crosslinking site present in viroid RNA, the RNase T1-resistant oligonucleotides of circular and crosslinked PSTV were labeled at their 5′ ends by polynucleotide kinase and [γ-^{32}P]ATP and then were fractionated either by electrophoresis in a 20% polyacrylamide gel to give a one-dimensional array (Fig. 4*B*) or by high-voltage electrophoresis followed by homochromatography to give a two-dimensional fingerprint (data not shown). The pattern of oligonucleotides from crosslinked PSTV contained a new band (indicated by an arrowhead in Fig. 4*B*), which was eluted and analyzed by a variety of tests. Digestion of the crosslink-specific oligonucleotide with P1 nuclease [which, unlike all other nucleases mentioned in this paper, creates 5′ phosphate and 3′ hydroxyl termini (27)] released pCoH as the only product (see Fig. 4*C*); while digestion with RNase U2 released a single oligonucleotide that comigrated with pCUA (data not shown).

Since CUA occurs at the 5′ end of only one RNase T1-resistant oligonucleotide in PSTV (26, 28), these data indicate that only one of the two possible 5′ ends present in

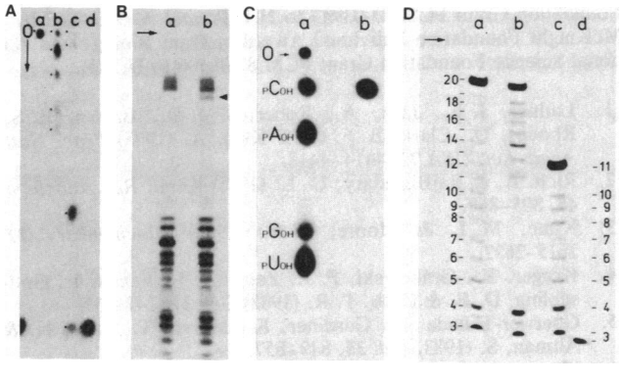

FIG. 4. Secondary analysis of crosslinked PSTV. (*A*) The ^{125}I-labeled, crosslink-specific oligonucleotide (identified by the arrow in Fig. 3*B*) was purified, treated by a variety of procedures as described (11), and then fractionated by high-voltage electrophoresis on Whatman DE 81 DEAE paper (pH 1.9). Aliquots received the following treatments: resuspension in water (lane a); incubation with RNase U2 (Sankyo) (lane b); pancreatic RNase (lane c); and RNase T2 (Sankyo), prepared as described in ref. 13 (lane d). Spots migrating as ^{125}I-labeled CUA, CCCG, and CCUG (fastest to slowest) are marked by dots adjacent to lane b, along with two additional spots near the origin. Products migrating as ^{125}I-labeled cytidine and AC are similarly noted for lane c, which also has two faint, slow spots. (*B*) Circular (lane a) and crosslinked (lane b) PSTV were digested with RNase T1, labeled with [γ-^{32}P]ATP and polynucleotide kinase, and then fractionated in an 0.3-mm thick gel of 20% polyacrylamide/7 M urea. An arrowhead denotes the position of an oligonucleotide specific to the crosslinked RNA, which was excised and characterized further as described in *C* and *D*. An arrow marks the position of xylene cyanol blue. (*C*) The 5′-end-labeled, crosslink-specific oligonucleotide was incubated in P1 nuclease (15) and then fractionated by high-voltage electrophoresis (13) on Whatman 3 MM paper (pH 3.5) (lane b). To provide markers for the nucleoside 5′ monophosphates, ^{32}P-labeled heterogeneous nuclear RNA from HeLA cells (lane a) and end-labeled PSTV oligonucleotides of known sequence (not shown) were treated in parallel. "O" indicates the origin of electrophoresis. (*D*) The 5′-end-labeled crosslink-specific oligonucleotide was cleaved by alkaline hydrolysis and then fractionated by electrophoresis (lane b). Partial alkaline digests of the PSTV-derived oligonucleotides CUUUUUCUCUAUCUUACUUG (lane a) and AACUAAACUCG (lane c), each labeled with ^{32}P at its 5′ terminus, were fractionated in parallel, along with ^{32}P-labeled AAC (lane d). An arrow marks the position of xylene cyanol blue. Electrophoresis was from top to bottom (as indicated in *A*).

the crosslink-specific oligonucleotide was labeled by [γ-^{32}P]ATP and polynucleotide kinase and that the crosslink involves a base in the second CUA of the oligonucleotide CUACUACCCG. These conclusions take into account (*i*) the absence of the spot CUACUACCCG from the fingerprints of crosslinked PSTV; (*ii*) the presence of ^{125}I-labeled CUA and CCCG in the secondary products of the crosslink-specific oligonucleotide, and (*iii*) the release of pCUA as the only RNase U2 product of the 5′-end-labeled crosslink-specific oligonucleotide. Furthermore, electrophoresis of the 5′-end-labeled fragments created by limited alkaline hydrolysis of the crosslink-specific oligonucleotide indicated that a highly resistant bond is present at the fifth base from the 5′ terminus. This resistance is reflected by a gap in the ladder of digestion products, which begins at the point in the sequence corresponding to uridine-260 (see Fig. 4*D*, lane b). Since the continuous ladder of gel bands resumes at the position expected for an oligonucleotide about 11 bases long, it appears that the oligonucleotide pCUACUACCCG$_{OH}$ is connected to a second oligonucleotide at least 6 bases long (assuming that the detectable digestion products result from random cleavage at only one phosphodiester bond per oligonucleotide).

A variety of procedures was used to determine the oligonucleotide linked to uridine-260. Analysis of two-dimensional fingerprint patterns of kinase-labeled oligonucleotides first suggested that the spot corresponding to pAAACCUG$_{OH}$ (bases 99–105) was present in reduced yield in RNase T1 digests of the crosslinked RNA (data not shown), suggesting a source of the ^{125}I-labeled CCUG detected by secondary analysis of the crosslink-specific oligonucleotide recovered from iodinated RNA (see Fig. 4*A*). In addition, primer extension experiments showed that reverse transcription is blocked at guanosine-98 when the crosslinked RNA is used as the template RNA (Fig. 5). By analogy to the results of 5S rRNA studies in which RNase T1 was found to cleave guanosine-99, the crosslinking site in 5S rRNA, with reduced efficiency, crosslinking of PSTV at guanosine-98 could produce the oligonucleotide $\begin{matrix}5'\ \text{GAAACCUG}\ 3' \\ \quad\quad\quad\quad | \\ 3'\ \text{GCCCAUCAUC}\ 5'\end{matrix}$ and explain the low yields of AAACCUG. Thus, these results indicate that irradiation of PSTV with UV light leads to the crosslinking of guanosine-98 to uridine-260.

The PSTV strain used in this study causes severe disease symptoms when inoculated onto tomato plants (28, 30). However, other strains of PSTV and RNA of citrus exocortis viroid also gave rise to crosslinked RNAs (data not shown). These studies were carried out on purified RNAs, but this approach also could be applied to conformational studies of RNA–protein complexes and of RNA molecules *in vivo*.

DISCUSSION

RNA fingerprinting, secondary analysis, and primer extension were used to identify sites in PSTV and HeLa 5S rRNA that are highly susceptible to crosslinking upon exposure to UV light. Portions of HeLa 5S rRNA and viroid RNA

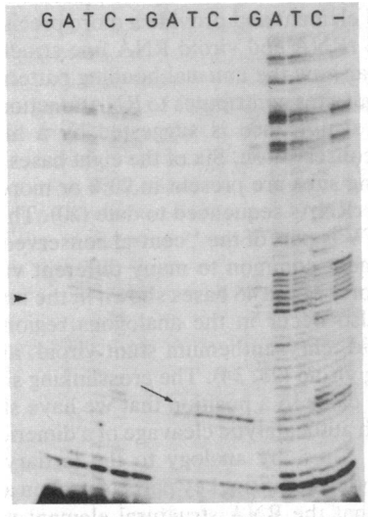

FIG. 5. Mapping the PSTV crosslinking site by primer extension. A small amount (15–25 ng) of PSTV circular (*Left*) crosslinked (*Center*) and linear (*Right*) RNA was hybridized to 60 ng of a [^{32}P]DNA oligomer (complementary to PSTV bases 110–129) in 2 μl of 0.1 M KCl, and then one-sixth of each RNA·DNA hybridization mixture was extended by avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL) under conditions similar to those described by Zaug *et al.* (29). Products of primer extension reactions carried out in the presence of dideoxy-GTP, -ATP, -TTP, or -CTP are noted ("G, A, T, or C"). Reactions without any dideoxynucleoside triphosphate are marked blank ("–"). An arrow indicates the position of the longest transcripts of crosslinked PSTV. An arrowhead shows the position of xylene cyanol blue on this gel, which is composed of 12% polyacrylamide, the Tris borate buffer described above, and 8 M urea.

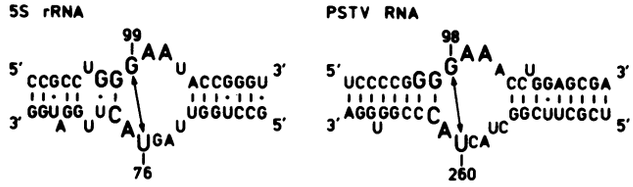

FIG. 6. Schematic depiction of the UV-induced crosslinking sites in HeLa 5S rRNA and PSTV. In the portions of HeLa 5S and PSTV presented here (redrawn from refs. 20 and 26, respectively), Watson–Crick base pairs are indicated by short vertical lines; G·U base pairs are represented by dots; boldface letters denote bases present at the crosslinking site in both molecules. Double-headed arrows mark the positions of covalent attachment.

containing the crosslinking sites and flanking sequences are shown in Fig. 6. Comparison of these partial maps reveals a number of interesting similarities that suggest that the crosslinking sites are closely related members of a novel class of tertiary structural elements. Crosslinks in both RNAs occur in the identical eight-base context (emphasized in Fig. 6 by boldface lettering) and appear to connect a guanosine and a uridine residue in each case. Studies of UV-treated 16S rRNA of *E. coli* previously indicated that purines may participate in certain crosslinking reactions (31, 32).

Structural studies of 5S rRNA (20) and PSTV (33) demonstrated that the bases sensitive to UV-irradiation are not themselves involved in Watson–Crick base pairing but are flanked by regions composed of seven to nine base pairs. Despite the absence of conventional bonding patterns at the crosslinking sites, our data indicate that the guanosine and uridine residues that become covalently attached upon UV-irradiation must lie in close proximity to each other. After crosslinking, the bonds are stable to boiling and treatment with glyoxal (8). Detailed chemical analysis of the points of covalent attachment should clarify the placement of bases in this structural element and provide a more precise picture of eukaryotic 5S rRNA and viroid RNA fine structure.

It is not clear how the unusual bonding pattern present at the crosslinking sites contributes to RNA function; however, its biological significance is suggested by a high level of evolutionary conservation. Six of the eight bases common to the crosslinking sites are present in 90% or more of the 125 eukaryotic 5S rRNAs sequenced to date (20). The crosslinking site in PSTV is part of the "central conserved region," a sequence element common to many different viroid RNAs (28, 34, 35). Forty of the 46 bases shown in the map of PSTV (see Fig. 6) also occur in the analogous regions of citrus exocortis viroid, chrysanthemum stunt viroid, and coconut cadang cadang viroid (28, 34). The crosslinking site in PSTV is also located close to a position that we have shown to be associated with autocatalytic cleavage of a dimeric transcript of PSTV (12). Thus, by analogy to the tertiary structural elements that often comprise key parts of protein active sites, it is possible that the RNA structural element revealed by these studies could help form the active site for RNA-catalyzed cleavage or other RNA-catalyzed reactions. Future studies seeking similar elements of local tertiary structure in other RNA molecules that promote cleavage, such as M1 RNA of *E. coli* (5) and the rRNA precursor of *Tetrahymena* (4), may shed further light on this possibility.

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