Oligomerization of Intervening Sequence RNA Molecules in the Absence of Proteins

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Abstract. *The intervening sequence RNA excised from the ribosomal RNA precursor of* Tetrahymena *forms linear and circular oligomers when exposed to a heating-cooling treatment in vitro. The reactions require no protein or external energy source. Oligomerization is different from other self-catalyzed reactions of the intervening sequence RNA in that it involves intermolecular rather than intramolecular recombination, producing RNA molecules that are substantially larger than the original. The observation that RNA molecules can catalyze their own oligomerization has possible implications for the evolution of chromosomes and for the replicative cycle of plant viroids and virus-associated RNA's.*

In eukaryotes, formation of mature, functional RNA molecules often requires the removal of intervening sequences by a process called RNA splicing (*1*). In one class of RNA splicing reactions, the IVS RNA (*2*) mediates both its own excision from the precursor RNA and the ligation of the flanking sequences (exons). Examples of such self-catalyzed RNA splicing are found in the nuclear ribosomal RNA (rRNA) IVS of the ciliated protozoan, *Tetrahymena thermophila*, and in the mitochondrial group I messenger RNA and rRNA intervening sequences in yeast and other fungi (*3, 4*). These reactions are of interest because they are responsible for essential steps in gene expression. In a broader context, they represent examples of catalytic activity intrinsic to RNA molecules, a departure from the general scheme of cellular reactions being catalyzed by protein enzymes.

The *Tetrahymena* rRNA IVS excises itself from the pre-rRNA in the form of a linear RNA molecule (L IVS RNA), 414 nt (nucleotides) in length. The IVS then mediates its conversion into a covalently closed circular form, C IVS RNA (*5*). Cyclization occurs by attack of the 3′ hydroxyl group of the terminal guanosine of the linear IVS RNA on the phosphate at position 16 in the chain, with concomitant release of an oligonucleotide containing the first 15 nt of the molecule (*6*). Cyclization is thus a cleavage-ligation reaction, occurring by the same transesterification mechanism as the original splicing reaction (*6*). In fact, cyclization might take place in the same active site as the first step of RNA splicing, the attack of a free guanosine nucleotide at the 5′ splice site (*7–9*). The C IVS RNA undergoes site-specific hydrolysis at the phosphodiester bond formed during cyclization, reforming a linear molecule (L − 15 IVS RNA); this reaction is also mediated by the folded structure of the RNA molecule (*10*).

In both the splicing and the cyclization reactions, the sequence of the RNA chains is rearranged. Thus, the reactions can be characterized as intramolecular recombination reactions. At each step in the series of reactions, the RNA molecules get smaller. In an effort to promote reversal of the RNA splicing reaction, L IVS RNA + ligated exons → pre-rRNA, we heated the RNA samples above their denaturation temperature and then allowed renaturation. No evidence was obtained for reverse splicing under these conditions. Instead, oligomers of the IVS RNA were unexpectedly generated. This oligomerization reaction shows that the ability to undergo intermolecular recombination is intrinsic to certain nucleic acid molecules. The reactions are intriguing because they produce RNA molecules larger than the starting material.

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Formation of IVS RNA oligomers. When linear IVS RNA is incubated at 42°C in the presence of Mg^{2+}, it is converted to circular IVS RNA (*6, 10*). Cyclization also takes place with high efficiency at 30°C in solutions containing 45 percent formamide and Mg^{2+} (Fig. 1A). Under various ionic conditions at 30° to 42°C, no products with molecular weights higher than that of the starting material are formed. In contrast, when a solution of L IVS RNA was heated to 95°C and then cooled to 42°C in the presence of Mg^{2+}, several discrete higher molecular weight RNA's were produced (Fig. 1A). Typically the species migrating more slowly than C IVS RNA represented 37 percent of the total RNA in the reaction. Denaturation of the RNA in the presence of glyoxal (*11*) did not decrease the relative proportion of these large RNA's, providing evidence that they were covalently joined rather than being some sort of noncovalent aggregates (*12*). Two of the products had measured molecular weights of 820 and 1200 nt; as described below, they are linear dimers and trimers of the IVS RNA. Two products of even lower electrophoretic mobility are circular dimers and trimers of the IVS RNA. Less prominent products of even higher molecular weight were observed but have not been characterized; they are presumably higher oligomers of the IVS RNA.

The circular nature of RNA species C_2 was indicated by two independent criteria. The first was its behavior during electrophoresis on an 8*M* urea–polyacrylamide gel. The apparent molecular weight of C_2 increased rapidly with the percentage of polyacrylamide in the gel, a trend which paralleled that of the C IVS (*12*). Such behavior is typical for circular nucleic acids although it is also found with quasi-circular forms like the lariats produced during nuclear messenger RNA splicing (*5, 13*). Second, C_2 could be converted to a discrete linear form by nicking. Limited ribonuclease (RNase) T1 digestion of C_2 converted a substantial fraction of the molecules to a form that migrated slightly faster than L_2 (Fig. 1B), just as limited RNase treatment of C IVS RNA gave partial conversion to a form that migrated slightly faster than L IVS RNA (because C IVS contains 15 nt less than L IVS).

The oligomerization treatment was repeated with L IVS RNA that was labeled with ^{32}P at the 5′ end and uniformly labeled with ^{3}H. In addition to the L IVS RNA, the putative linear oligomers retained ^{32}P radioactivity; the putative circular molecules were unlabeled (Fig. 1C, lanes 5 and 6). The ratios of ^{32}P to ^{3}H

were determined for species L, L_2, and L_3. When the ratio for L was normalized to 1.00, the ratios for L_2 and L_3 were 0.60 and 0.42, respectively. These ratios were in reasonable agreement with the ratios of 0.51 and 0.34 predicted if a single 5′-terminal label were retained in each linear molecule. This result immediately suggested a mechanism for oligomerization analogous to that for IVS RNA cyclization. If oligomerization occurred by addition of the 3′ end of one molecule to the cyclization site of another, with release of a 5′-terminal 15-nt fragment from the molecule being attacked, then each linear oligomer would retain a single copy of the original labeled 5′ end. Subsequent cyclization would release the remaining 15-nt fragment, leaving circular oligomers with no ^{32}P label.

The structure of the L_2 and C_2 species were confirmed by dideoxynucleotide sequence analysis (*14*). The L_2 RNA had exactly the sequence of a mixture of L and C IVS (Fig. 2). This is the expected result if, on each molecule, one primer hybridized near the 5′ end and was extended through the 5′-terminal 15 nt before terminating at the end of the molecule, while the other primer hybridized to its complementary sequence in the 3′ half of the dimer and was extended through the ligation junction into the 5′ half of the dimer. The C_2 RNA had the same sequence as C IVS RNA (*12*). This is the expected result for a dimer in which the G residue at the end of each monomer unit (G^{414}) is joined to the A residue at position 16 of the other monomer unit.

Properties of the oligomerization and cyclization reactions. The temperature dependence of oligomerization was determined by heating RNA at 95°C for 2 minutes, then adding Mg^{2+} and shifting the solution to various lower temperatures. The optimum temperature was about 55°C, with no oligomerization evident at 75°C or above (Fig. 3A). The temperature dependence of oligomerization is similar to that of cyclization of L IVS RNA, which appears maximal at 65°C in Fig. 3A.

Formation of the linear oligomers was complete within 15 seconds after the samples were shifted to 42°C, while cyclization of L IVS RNA and the linear oligomers was complete within a minute (Fig. 3B). In all previous experiments, reaction times of 10 to 30 minutes were used; thus, the observed extents of oligomerization were not limited by the reaction time.

The oligomerization reaction was independent of IVS RNA concentration above ~1 n*M* RNA, but the amount of reaction decreased at lower RNA concentrations (Fig. 3, B and C). The data are interpreted to indicate that oligomerization is intrinsically concentration-dependent. It competes effectively with the cyclization reaction only at the higher concentrations. Whatever RNA cannot oligomerize is folded in such a way that it can undergo cyclization. In a separate experiment starting with purified L IVS RNA, we confirmed that the cyclization reaction was not intrinsically concentration dependent (*12*).

In analogy with cyclization of L IVS RNA, it seemed most reasonable that the circular oligomers were formed by cyclization of the linear oligomers. The existence of such a reaction pathway was demonstrated by incubation of isolated L_2 and L_3 RNA's under cyclization conditions (Fig. 4). L_2 RNA was largely converted to C_2, C, L − 15, and an unidentified species with mobility intermediate between that of L_2 and C. The L − 15 IVS RNA could arise from site-specific hydrolysis of C IVS RNA (*10*) or from hydrolysis at the equivalent bond in L_2 RNA. The L_3 RNA was partially converted to C_3, C_2, C, and an unidentified species with mobility intermediate between that of C_3 and C_2. Thus, each linear oligomer gave rise to a family of circular RNA's containing the same or a smaller number of monomer units.

Activity of other RNA's in the reaction. The attacking group in the normal oligomerization reaction is the 3′-terminal G-OH of the IVS RNA. To investigate whether one of the two molecules in a dimerization reaction could be replaced by a non-IVS RNA species, we first tested whether the attacking group could be replaced by the mononucleotide guanosine 5′-triphosphate (GTP). If a reaction analogous to dimerization took place, GTP would be added 15 nt from the 5′ end of the L IVS RNA, producing a G-(L − 15) RNA (an L − 15 RNA with a 5′-terminal pppG). The same reaction on L_2 and L_3 would produce G-(L_2 − 15) and G-(L_3 − 15), respectively.

A mixture of [^{32}P]GTP and unlabeled L IVS RNA was treated under oligomer-

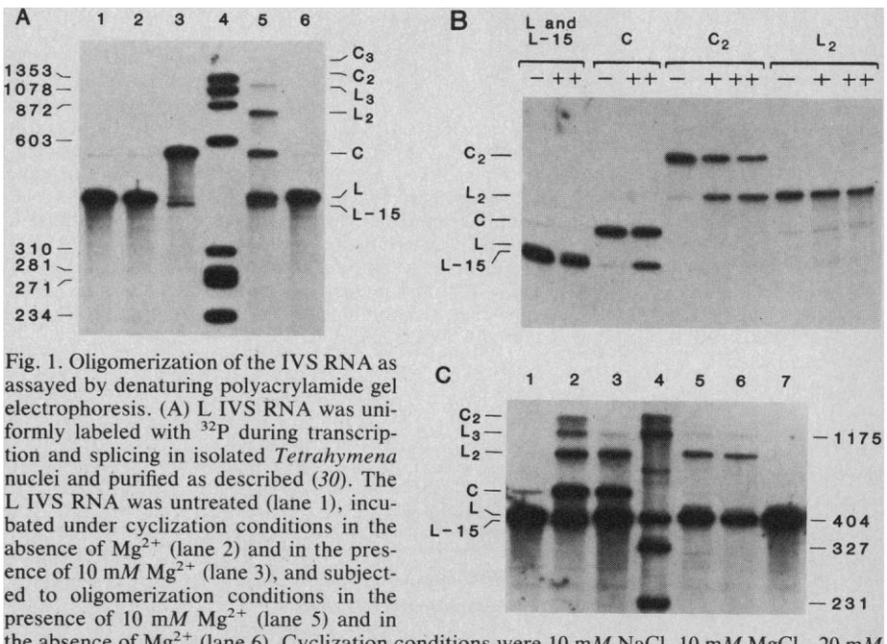


Fig. 1. Oligomerization of the IVS RNA as assayed by denaturing polyacrylamide gel electrophoresis. (A) L IVS RNA was uniformly labeled with ^{32}P during transcription and splicing in isolated *Tetrahymena* nuclei and purified as described (*30*). The L IVS RNA was untreated (lane 1), incubated under cyclization conditions in the absence of Mg^{2+} (lane 2) and in the presence of 10 m*M* Mg^{2+} (lane 3), and subjected to oligomerization conditions in the presence of 10 m*M* Mg^{2+} (lane 5) and in the absence of Mg^{2+} (lane 6). Cyclization conditions were 10 m*M* NaCl, 10 m*M* $MgCl_2$, 20 m*M* tris-HCl (*p*H 7.5), 45 percent formamide, 30°C for 30 minutes. Similar results were obtained at 42°C in the absence of formamide. The standard oligomerization treatment involved heating the RNA in 10 m*M* NaCl, 20 m*M* tris-HCl, *p*H 7.5, at 95°C for 2 minutes, then adding Mg^{2+} to a final concentration of 10 m*M*, and immediately transferring the sample tube to a 42°C water bath. Incubation at 42°C continued for 30 minutes. All reactions were stopped by the addition of EDTA (20 m*M* final concentration) and precipitation with ethanol. Molecular weight markers were Hae III restriction endonuclease fragments of ϕX174 DNA (lane 4). Nucleic acids were analyzed by electrophoresis in a 4 percent polyacrylamide, 8*M* urea gel (*31*), an autoradiogram of which is shown. (B) Identification of circular RNA species by their conversion to a discrete linear form upon limited ribonuclease T1 treatment (*5*). Purified L IVS, C IVS, C_2 and L_2 RNA's were (−) untreated; (+) treated with ribonuclease T1 (8.3 unit/ml) or (++) with 12 unit/ml, all in 7*M* urea for 30 minutes at 50°C. The C and C_2 RNA's were substantially converted to products with mobilities close to those of the corresponding linear species. (C) L IVS RNA, uniformly labeled with ^{32}P as in (A), was subjected to standard oligomerization conditions in the absence of Mg^{2+} (lane 1) or with Mg^{2+} (lanes 2 and 3). The L IVS RNA was labeled at the 5′ end with [α-^{32}P]GTP during RNA splicing in vitro (*31*) and then treated in parallel in the presence of Mg^{2+} (lanes 5 and 6) or in its absence (lane 7). Molecular weight markers (lane 4) were Taq I restriction endonuclease fragments of ϕX174 DNA.

ization conditions. Radioactivity was incorporated into molecules of approximately the sizes expected for L − 15, L_2 − 15, and L_3 − 15 (Fig. 5A). (The resolution of such a gel is insufficient to distinguish between L_2 and L_2 − 15 or between L_3 and L_3 − 15.) The reaction required Mg^{2+} and was specific for the guanosine nucleotide (Fig. 5B). No reaction occurred when *Escherichia coli* 16*S* rRNA was substituted for the L IVS RNA. The amount of reaction was increased with L IVS RNA that had a portion of its 3′ ends blocked with cordycepin (3′-deoxyadenosine) to inhibit it from reacting intramolecularly (cyclization) or from serving as the attacking group in oligomerization. Direct sequence analysis of the molecules of size ~400 nt confirmed that the GTP had been covalently added to the RNA (*12*). The sequencing gel clearly showed a superimposition of two sequences. One sequence was that expected for addition of GTP to the cyclization site (A^{16}), a reaction that is the mononucleotide equivalent of dimerization. The other sequence surprisingly was that of L IVS RNA labeled with ^{32}P at the 5′ end, perhaps the product of IVS-catalyzed exchange of the 5′-terminal G.

Thus, one of the two IVS RNA molecules in a dimerization reaction can be replaced by GTP. The oligoribonucleotides C_4G_{OH} and $AGUACUCG_{OH}$ also add to the cyclization site of the IVS RNA with an efficiency at least as high as that of GTP (*15*). Therefore a variety of RNA's terminating in G-OH can react with the IVS RNA to form ligated products.

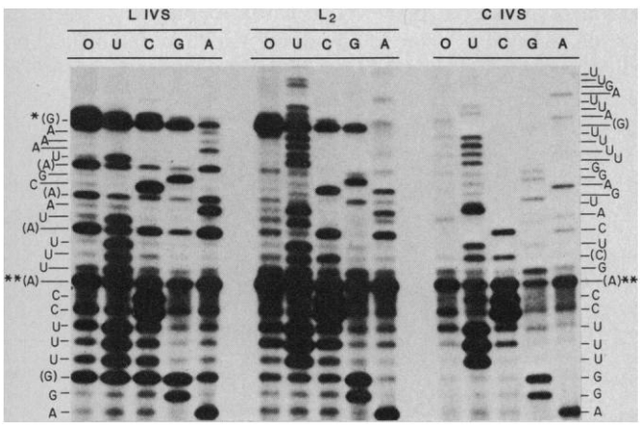


Fig. 2. Nucleotide sequence analysis of L_2 RNA. A DNA primer complementary to nucleotides 32 to 45 of the IVS was hybridized to the gel-purified RNA and extended by AMV reverse transcriptase. Each reaction contained all four deoxynucleoside triphosphates and a single ddNTP (dideoxynucleoside triphosphate) (*14*). Lanes U, C, G, and A indicate the nucleotide complementary to the ddNTP added; lane O, no ddNTP added. The sequence of L IVS is shown to the left and the sequence of C IVS to the right; nucleotides in parentheses could not be clearly identified in this experiment. (*) Strong stop for reverse transcription at the 5′ end of L IVS RNA. (**) Strong stop at the 5′ end of the L − 15 IVS RNA that contaminated all samples; this is the cyclization junction in C IVS RNA. Below (**), the sequence of all three RNA's is the same. Between (**) and (*), the L_2 RNA sequence is a mixture of those of the L and C IVS RNA's. Above (*), the L_2 RNA sequence is that of C IVS RNA.

Mechanism of IVS RNA oligomerization. The dimerization of L IVS RNA occurs by attack of the 3′-terminal G-OH of one molecule exactly at the cyclization site of the other molecule. The Mg^{2+} dependence and temperature dependence of the reaction also suggest a close relation to cyclization (Fig. 6A). These features lead to a two-step model for dimerization (Fig. 6B).

The first step is the formation of a noncovalent complex during cooling of the molecules from 95°C to the reaction temperature. This step accounts for the concentration dependence of the amount of dimer formed. The process is amazingly efficient. Typically 80 percent of the molecules that have been heated and cooled can react, half of them by cyclization and half by oligomerization. Thus the complexes must not be random aggregates of RNA, but rather very specific complexes in which part of one molecule displaces its homologous part in the structure of the other molecule. The high rate of the process at relatively low concentrations of nucleic acid is also unexpected. In this regard, it is interesting that dimerization is dependent on the presence of Mg^{2+} during the cooling step as well as during the reaction.

The second step of dimerization is covalent bond formation. The reaction occurs by the same transesterification mechanism as IVS RNA cyclization (*6*), and it occurs at the same site (the U^{15}–A^{16} bond of the IVS).

The formation of some of the circular oligomers, $L_2 \rightarrow C_2$ and $L_3 \rightarrow C_3$, is easily explained by the same model (Fig. 6C). Each linear oligomer has one 3′ end and one normal cyclization site, and intramolecular reaction between these sites occurs despite the presence of extra nucleotides in the middle of the molecule. The mechanism by which smaller circles are formed, such as $L_3 \rightarrow C_2$ and C, is less obvious. The most straightforward possibility is that the 3′-terminal G-OH of a linear oligomer can attack the phosphate that follows the terminal G (G^{414}) of another monomer unit in the same oligomer, as shown in Fig. 6C. This would be an unusual cyclization site, because it is not preceded by the tripyrimidine sequence that is required for the

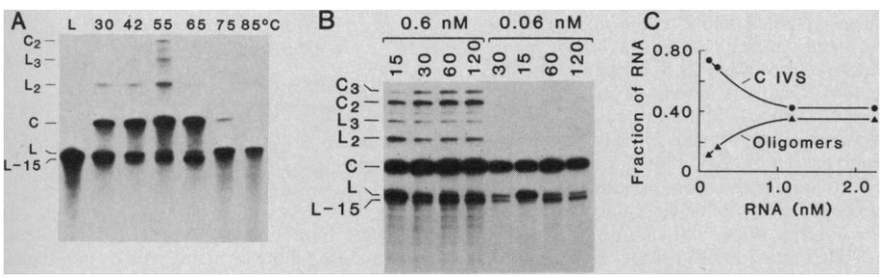
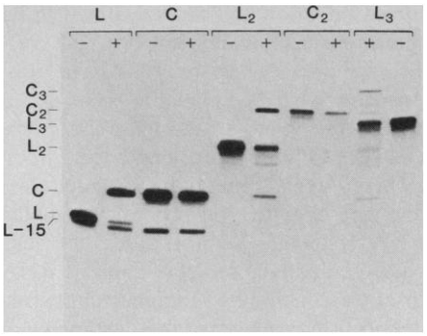


Fig. 3. (A) Temperature dependence of oligomerization. L IVS RNA samples were heated at 95°C for 2 minutes. $MgCl_2$ was then added to a final concentration of 10 m*M*, and the RNA was incubated for an additional 10 minutes at the indicated temperature; (lane L) untreated L IVS RNA. (B) Time and concentration dependence of oligomerization. L IVS RNA was treated under oligomerization conditions at two concentrations. The concentration of the ^{32}P-labeled IVS RNA is given. Because IVS RNA was prepared from nuclei, the preparation contains a small amount of unlabeled IVS RNA transcribed in vivo (*17*). Thus, the relative RNA concentrations of the samples are precise, but the absolute concentrations may be underestimated. Numbers give time in seconds after RNA was shifted from 95° to 42°C. Approximately equal amounts of radioactivity were placed on each lane of the gel. (C) Concentration dependence of oligomerization. L IVS RNA at four different concentrations was heated to 95°C, Mg^{2+} was added, and the samples were shifted to 42°C for 15 seconds before the reactions were stopped with EDTA. Fraction of RNA in (●) C IVS and in (▲) all oligomeric species was determined by liquid scintillation counting of a sliced gel. The remaining fraction of RNA not indicated on the graph consisted of L IVS and L − 15 IVS (reopened C IVS). A repeat of the experiment except for a 2-minute incubation at 42°C gave equivalent results.

normal cyclization of L IVS RNA (*7, 8*). There is, however, a precedent for cyclization occurring after a guanosine residue in certain mutated IVS RNA's (*8*). Furthermore, the phosphate esters following G^{414} in both C IVS RNA and prerRNA are particularly susceptible to hydrolysis, which indicates that this phosphate may be primed for nucleophilic attack in general (*7, 9, 10*). Thus, cyclization to this site in an oligomer is chemically reasonable. There is an alternative possibility for the formation of the smaller circles. The oligomer could first undergo hydrolysis at the labile phosphodiester bond that follows one of its G^{414} residues. The newly created 3′-terminal G-OH would then attack at the normal cyclization site 15 nt from the 5′ end of the oligomer, producing a circle smaller than the original oligomer. The remaining linear molecule containing the 3′ region of the original oligomer would still have a 3′-terminal G-OH. It would not have a major cyclization site (U^{15}–A^{16}), but could still cyclize to a position 4 nt from its 5′ end (U^{19}–U^{20}) as described by Zaug *et al.* (*10*).

Oligomerization and its possible role in evolution. Our data show that the ability to undergo intermolecular ligation to form products of higher molecular weight is intrinsic to certain RNA molecules. The reactions occur by transesterification, and therefore require no external energy source such as the ATP (adenosine triphosphate) used by the protein enzyme RNA ligase (*16*). Oligomerization requires the folded structure of the RNA molecule, as demonstrated by the temperature dependence of the reaction. It also requires denaturation of the RNA followed by renaturation, conditions achieved by a heating-cooling treatment in our experiments. Denaturation-renaturation could potentially be accomplished in other ways, such as extreme changes in ionic conditions or interaction of the RNA with proteins. Oligomeric IVS RNA's are not detected in nuclear RNA from *Tetrahymena* grown under a variety of conditions (*17*), so oligomerization is unlikely to be important in normal metabolism of the IVS RNA.

It is generally thought probable that RNA, not DNA, was the primordial genetic material (*18*). Self-catalyzed RNA oligomerization provides a possible pathway for rapid, block-wise joining of smaller RNA molecules to form an RNA chromosome. In present-day cells, having genes linked together in chromosomes is important for ensuring the accurate division of the genetic material in



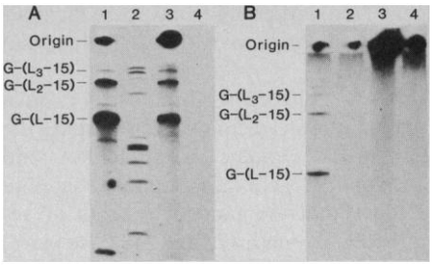


Fig. 4 (left). Cyclization of linear IVS RNA oligomers. Purified RNA species labeled uniformly with ^{32}P were (−) not treated or (+) incubated under cyclization conditions (legend to Fig. 1A). RNA was analyzed by electrophoresis in an 8*M* urea, 4 percent polyacrylamide gel. Fig. 5 (right). Reaction of GTP with L IVS RNA under oligomerization conditions. (A) A solution containing 3 μ*M* [α-^{32}P]GTP and 3 n*M* ^{3}H-labeled L IVS RNA was treated according to the standard oligomerization protocol with incubation for 10 minutes at 50°C. Products were analyzed by electrophoresis on a denaturing 4 percent polyacrylamide gel; an autoradiogram showing ^{32}P radioactivity is shown. (Lane 1) The IVS RNA in the reaction had been treated with cordycepin and *E. coli* poly(A) polymerase to block its 3′ end (20 percent of ends blocked). (Lane 2) Hae III restriction fragments of φX174 DNA. (Lane 3) Reaction with normal unblocked IVS RNA. (Lane 4) The same reaction as in lane 3 but Mg^{2+} was omitted. (B) Specificity of the reaction for guanosine. Reactions as in (A) with 3′ end-blocked IVS RNA; standard oligomerization protocol except where indicated. (Lane 1) [α-^{32}P]GTP; (lane 2) [α-^{32}P]GTP, Mg^{2+} omitted; (lane 3) [α-^{32}P]ATP; (lane 4) [α-^{32}P]UTP.

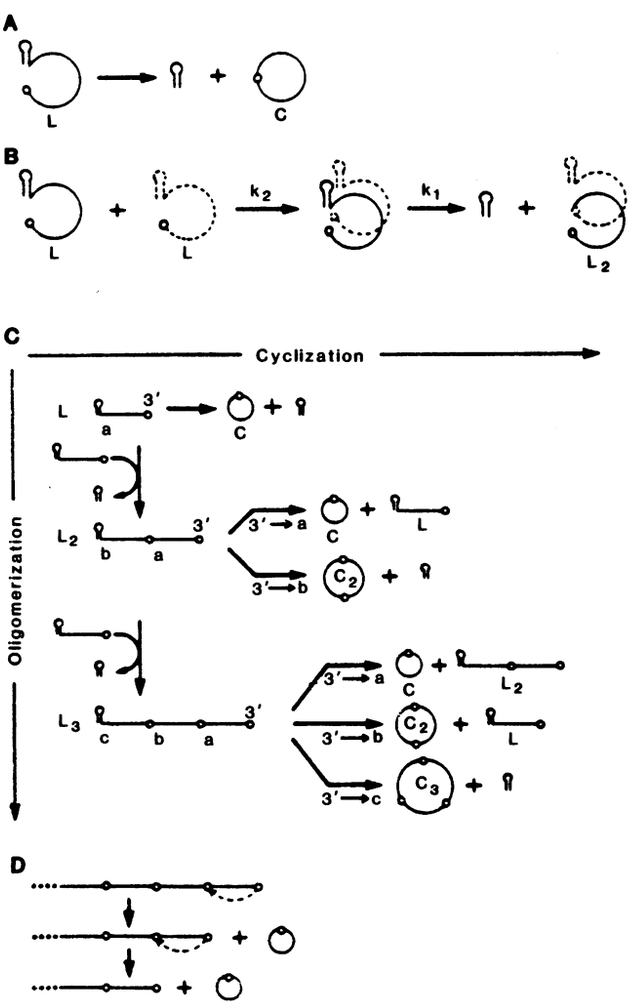


Fig. 6. Comparison of the IVS RNA cyclization and oligomerization reactions. (A) IVS RNA cyclization (*6*). The L IVS RNA is represented schematically with its 3′ hydroxyl (○) in proximity to the reaction site at residue 16 from the 5′ end of the molecule. The 5′-terminal 15 nt are shown in the form of a hairpin structure for convenience; these nucleotides are probably not folded in a hairpin prior to cyclization (*7, 8*). (B) Model for IVS dimerization. During cooling from 95°C to 42°C, two L IVS RNA molecules form a noncovalent intermolecular complex in which the 3′ end of one molecule occupies the cyclization site of the other. This is the concentration-dependent step, presumably following second-order kinetics. Attack by the 3′-OH at the cyclization site results in formation of a linear dimer with release of one 15-nt fragment. (C) Model for IVS oligomerization and subsequent cyclization of the oligomers. No attempt is made to represent the folded structure of the molecules or the noncovalent complexes that are proposed to precede the reaction [as in (B)]. (D) Model for the final step in the replication of viroid RNA. A plus-strand linear oligomer is sequentially converted to infectious plus-strand circular monomers.

mitosis and meiosis. The linkage of RNA genes into larger units may have facilitated the accurate partition of primordial genomes.

RNA oligomerization may have played a more recent role in shaping the eukaryotic genome. The monkey and *Drosophila* genomes contain satellite DNA's that are tandem repeats of sequences of length 172 and 359 base pairs, respectively (*19*). A possible scenario for the generation of this particular type of highly repeated sequence might involve transcription of an isolated copy of the sequence, oligomerization of the resulting RNA, reverse transcription to give a DNA copy, and insertion into the genome. At present there is no reason to favor this hypothesis over the more conventional theories, such as unequal crossing over during recombination or DNA amplification (*20*).

Oligomerization and cyclization of plant viroids and virus-associated RNA's. Plant viroids, the smallest infectious elements identified, are single-stranded, covalently closed circles of RNA (*21*) approximately the size of the *Tetrahymena* rRNA IVS. Viroid RNA's contain sequences homologous to the conserved sequence elements that define group I introns, the group to which the *Tetrahymena* IVS belongs (*22*). Peanut stunt virus–associated RNA 5 (PARNA 5), a linear RNA of 393 nt, contains the same conserved sequences (*23*). The six conserved sequence elements, each 5 to 8 nt in length, are thought to interact in a pairwise manner to fold the introns into a common core secondary structure (*24*). The occurrence of the same sequence elements in the same 5′ to 3′ order along the viroid RNA's suggests that the biologically active conformation of the viroid might involve a core structure similar to that of the introns, folding of the RNA perhaps being facilitated by proteins in vivo (*22*).

The possible relation between viroids and group I introns becomes even more interesting when one considers the similarity between certain steps in the viroid replication cycle and the IVS RNA oligomerization and cyclization reactions reported above. Multimeric plus strands and minus strands (*25*) both arise during viroid infection and are presumed to be intermediates in replication (*26, 27*). Branch and Robertson (*27*) have proposed a viroid replication scheme in which the plus strand oligomers are cleaved to monomer length and then cyclized to give infectious viroid circles, and they note that this process resembles RNA splicing. We now add that self-catalyzed conversion of oligomeric RNA to circles, including monomer circles, is an intrinsic property of the *Tetrahymena* IVS RNA oligomers, and therefore perhaps of viroid oligomers. More specifically, oligomeric linear plus-strand RNA could be converted to monomeric viroid circles in a progressive 3′ to 5′ order as shown in Fig. 6D. Such a mechanism differs from those proposed previously (*27, 28*) in that the oligomers would not be cleaved to monomers before being cyclized. Another prediction of the model is that the oligomeric precursor would have a 3′-terminal hydroxyl group, presumably a G-OH. The formation of oligomers of viroids (*26, 27*) and virus-associated RNA's (*23, 29*) could also involve elements of self-catalyzed RNA ligation similar to that found for the *Tetrahymena* IVS RNA.

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