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RNA ligation via 2′-phosphomonoester, 3′,5′-phosphodiester linkage: Requirement of 2′,3′-cyclic phosphate termini and involvement of a 5′-hydroxyl polynucleotide kinase

(eukaryotic RNA ligases/RNA processing/wheat germ)

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ABSTRACT **Extracts of wheat germ contain a RNA ligase activity that catalyzes the conversion of linear polyribonucleotides into covalently closed circles. As reported previously, this enzyme joins two ends of a RNA substrate via a 2′-phosphomonoester, 3′,5′-phosphodiester linkage. In the present work we provide evidence that a 2′,3′-cyclic phosphate group at the 3′ terminus is required for RNA ligation and that the 5′-hydroxyl end is phosphorylated before the two RNA ends are joined. We report on the presence of 5′-hydroxyl polynucleotide kinase and polynucleotide 2′,3′-cyclic phosphate 3′-phosphodiesterase activities in wheat germ extracts. A possible involvement of these enzymes in the ligation process and a potential role of the newly described ligation pathway in RNA processing are discussed.**

The discovery that maturation of eukaryotic RNAs frequently involves excision of introns and subsequent joining of exon sequences (1, 2) raised considerable interest in RNA ligating enzymes. Apart from the joining of exon segments, RNA ligases may participate in circulation of intron sequences (3, 4), viroid molecules (5, 6), and viroid-like components of plant RNA viruses (7).

Two types of RNA ligases have so far been described and studied in some detail. An enzyme from T4-infected *Escherichia coli* cells (8) joins the 5′-phosphorylated end to the 3′-hydroxyl end of RNA molecules. A second type of RNA ligase has been characterized more recently and shown to be involved in tRNA splicing in yeast (9, 10) and in *Xenopus* oocytes (11). It has been demonstrated that the enzyme originating from yeast requires 3′-phosphate and 5′-hydroxyl termini (12).

We have recently identified a third type of RNA ligase; this enzyme, detected in wheat germ extracts, catalyzes the joining of RNA ends to form an unusual 2′-phosphomonoester,3′,5′-phosphodiester linkage $—N^{2'}_{3'}\!\!<^{p}_{p}\!\!>5'N—$ (13). In this work we provide evidence that a 2′,3′-cyclic phosphate group at the 3′ terminus and a phosphate group at the 5′ end are required for RNA ligation by the wheat germ extract. We also report on the presence in wheat germ extracts of 5′-hydroxyl polynucleotide kinase and polynucleotide 2′,3′-cyclic phosphate 3′-phosphodiesterase activities that may be involved in the ligation process.

METHODS

Preparation of Substrates for Ligation. RNA from the SPS isolate of tobacco mosaic virus (TMV) was used for preparation of RNase T1-resistant leader (Ω) oligonucleotide (14).

Preparations of Ω molecules bearing 5′-hydroxyl ends and either exclusively [^{32}P]G$^{3'}$p termini or enriched in [^{32}P]G>p termini were obtained by treatment of either ^{32}P-labeled Ω circles (synthesized from 5′-^{32}P-labeled linear Ω by T4 RNA ligase) or [5′-^{32}P]pCp-labeled Ω (13) with different concentrations of RNase T1. Five million cpm of either type of labeled Ω was digested with 5 or 0.1 unit of RNase T1 in 10 μl for 1 hr at 25°C in the presence of 10 μg of carrier tRNA. The resulting Ω preparations were purified by polyacrylamide gel electrophoresis. Material treated with 5 units of RNase T1 contained exclusively [^{32}P]G$^{3'}$p ends whereas that treated with 0.1 unit contained about 50% of [^{32}P]G>p and 50% of [^{32}P]G$^{3'}$p termini. The preparation enriched in [^{32}P]G>p ends was treated with calf intestine phosphatase in order to remove the label from molecules ending with G$^{3'}$p and yield the radiochemically pure [^{32}P]G>p-terminated Ω. [^{32}P]G$^{2'}$p-terminated Ω was obtained by incubation of [^{32}P]G>p-ended molecules with wheat germ extract followed by isolation of the linear material by gel electrophoresis.

Unlabeled Ω containing exclusively G$^{3'}$p or enriched in G>p termini was obtained by digestion of 0.5 mg of TMV RNA with 10 or 0.1 unit of RNase T1, respectively, in 500 μl of 50 mM Tris·HCl (pH 7.8) for 1.5 hr at 37°C. Alternatively, the Ω preparation enriched in G>p-terminated molecules was obtained by incubation of 0.5 mg of TMV RNA with 1 unit of RNase N1 (Sigma) for 1.5 hr at 37°C in 500 μl of 50 mM Tris·HCl (pH 7.0). 5′-[^{32}P]Phosphorylated Ω substrates, bearing either G$^{3'}$OH ends or enriched in G>p 3′-termini, were prepared by incubation of appropriate unlabeled Ω preparation with T4 polynucleotide kinase and [γ-^{32}P]ATP (14). 5′-Terminal labeling of G$^{3'}$p-terminated Ω was performed at pH 8.3 in order to avoid dephosphorylation at the 3′ end (15).

Incubations with Wheat Germ Extract. Ligation of Ω substrates by a wheat germ extract was assayed as described (13), except that incubations were carried out at 37°C and the mixture contained 50 mM sodium phosphate (pH 7.5) in order to inhibit phosphatases present in the extract.

The phosphorylation of Ω by wheat germ 5′-hydroxyl polynucleotide kinase was performed in 5-μl assay mixtures containing 20 mM Hepes (pH 7.4), 4 mM Mg(OAc)$_2$, 100 mM KOAc, 80 μM spermine, 10% dimethyl sulfoxide, 5 μg of wheat germ extract protein, 4 μg of appropriate unlabeled Ω, and 100 μCi of [γ-^{32}P]ATP (≈1000 Ci/mmol; Amersham; 1 Ci = 3.7 × 10^{10} becquerels). After 10 min of incubation at 37°C, samples were treated with 1 μg of proteinase K (Merck) in 0.1%

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Abbreviations: TMV, tobacco mosaic virus; Ω, RNase T1-resistant 5′-terminal leader fragment of TMV RNA; pN, any nucleoside-5′-monophosphate; Np, any nucleoside-3(2)′-monophosphate; G$^{2'}$p and G$^{3'}$p, guanosine 2′-monophosphate and 3′-monophosphate, respectively; G>p, guanosine 2′,3′-cyclic phosphate.

$NaDodSO_4$ for 30 min at 37°C [in order to eliminate high background of phosphorylated proteins (see Fig. 2*B*, lane a)] and analyzed by gel electrophoresis.

Analytical Procedures. For analysis of the 3′-terminal nucleotides of different Ω preparations, complete digestion with RNase A was followed by thin-layer chromatography on cellulose plates in solvent A [saturated $(NH_4)_2SO_4$/1 M NaOAc/isopropanol, 80:18:2 (vol/vol)]. Analysis of 5′-terminal nucleotides and of RNase T2- or nuclease P1-resistant dinucleotides was performed on cellulose plates in solvent B [isobutyric acid/NH_4OH/H_2O, pH 4.3, 577:38:385 (vol/vol)]. All nuclease digestions were carried out as described (13).

Electrophoresis on 20% polyacrylamide/8 M urea gels was as described (14).

RESULTS

Requirement of a 2′,3′-Cyclic Phosphate Terminus for RNA Ligation. Ω is a 73-nucleotide RNase T1 fragment from TMV RNA that begins with uridine and has AUG at the 3′ end (14). In order to undergo circularization in a wheat germ extract, the Ω fragment bearing a 5′-hydroxyl required the presence of a phosphate at the 3′ end (13). In the circular product this phosphate was recovered in the 2′ position of the guanosine involved in the newly formed 3′,5′-phosphodiester linkage $G^{2'}_{3'}{<}^{p}_{p}{>}5'U$. These results suggested that the 3′-terminal phosphate has either been translocated from the 3′ to the 2′ position during or prior to ligation or that Ω molecules bearing the 2′,3′-cyclic phosphate end are the true substrates in this reaction (13).

To distinguish between the two possibilities, we obtained Ω preparations bearing either 3′-phosphate or 2′,3′-cyclic phosphate termini. The Ω molecules containing exclusively [^{32}P]G$^{3'}$p ends were obtained by treatment of either ^{32}P-labeled Ω circles (synthesized with T4 RNA ligase) or [5′-^{32}P]pCp-labeled Ω (13) with excess of RNase T1. Incubation of the same material with low concentration of RNase T1 followed by treatment with calf intestine phosphatase resulted in formation of radiochemically pure [^{32}P]G>p-terminated Ω. The identity of the 3′ termini of resulting Ω preparations was established by thin-layer chromatography after RNase A digestion (Fig. 1*A*, lanes a and b).

The two different Ω preparations were incubated in a wheat germ extract and the products were analyzed by polyacrylamide gel electrophoresis. Fig. 1*B* indicates that the G>p-terminated Ω served as a substrate in the ligase reaction (lane b), whereas the 3′p-terminated fragments did not yield the circular product (lane a).

Polynucleotide 2′,3′-Cyclic Phosphate 3′-Phosphodiesterase in Wheat Germ Extracts. Because the phosphate from the G>p end was recovered in the 2′ position of the 2′-phosphomonoester,3′,5′-phosphodiester linkage (ref. 13; this work), we investigated whether wheat germ extracts contain a 3′-phosphodiesterase activity acting on 2′,3′-cyclic phosphate-terminated oligonucleotides. We analyzed the 3′ termini of Ω molecules that did not migrate as circular Ω molecules after incubation with a wheat germ extract but remained linear (Fig. 1*B*, lanes a and b). The G$^{2'}$p termini were identified in the fraction that, prior to incubation, contained the G>p-terminated Ω (Fig. 1*C*, lane b). Formation of the G$^{2'}$p termini was not observed when pure G$^{3'}$p-terminated Ω was incubated with the wheat germ extract (Fig. 1*C*, lane a). It should be emphasized that the G>p-terminated Ω yielded exclusively 2′-phosphate-terminated (and not 3′-phosphate-terminated) linear molecules in addition to the circularized product (Fig. 1*C*, lane b). This process was completely inhibited by preincubation of the wheat germ extract at 60°C for 10 min or pretreatment of the extract with proteinase K (not shown). It therefore can be concluded that the wheat germ extract contains a polynucleotide 2′,3′-cyclic phosphate 3′-phosphodiesterase activity.

The findings described above prompted us to ask whether not only Ω molecules ending with a 2′,3′-cyclic phosphate but also those ending with a 2′-phosphate can serve as a substrate for the wheat germ ligase. Fig. 1*B*, lane c, shows the results of an experiment in which the [^{32}P]G$^{2'}$p-terminated Ω (containing exclusively [^{32}P]G$^{2'}$p termini as shown in Fig. 1*A*, lane c) was incubated with a wheat germ extract. It is evident that Ω molecules ending with a 2′-phosphate were not circularized and that the 3′-terminal G$^{2'}$p is retained (Fig. 1*C*, lane c).

5′-Hydroxyl Polynucleotide Kinase in Wheat Germ Extracts. The results described so far support the conclusion that the Ω molecule with a 2′,3′-cyclic phosphate end is the substrate for wheat germ ligase. They also indicate that hydrolysis of cyclic phosphate leading to 2′-phosphate formation may be coupled with other steps of the ligase reaction because preformed [2′-^{32}P]Ω could not be circularized. The question retained as to the origin of the phosphate involved in the 3′,5′-phosphodiester bond of the $G^{2'}_{3'}{<}^{p}_{p}{>}5'U$ linkage.

Incubation of unlabeled Ω, bearing an -OH group at the 5′

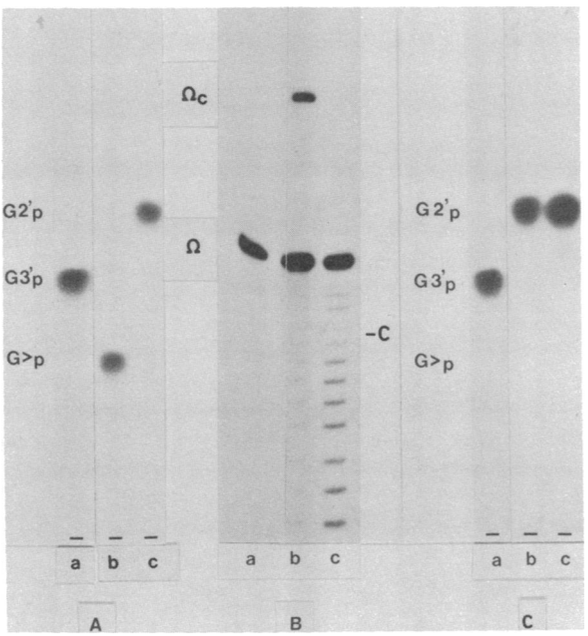

FIG. 1. 2′,3′-Cyclic phosphate terminus is required for circularization of the linear Ω fragment. (*A*) Analysis of the ^{32}P-labeled 3′ terminal nucleotides of different linear Ω preparations used in the ligation experiment shown in *B*. Ω preparations (about 500 cpm) were digested with RNase A and subjected to thin-layer chromatography in solvent A in the presence of G>p, G$^{3'}$p, and G$^{2'}$p markers. (*B*) Circularization of different Ω substrates by wheat germ ligase. Ω molecules (1×10^4 cpm) bearing 5′-hydroxyl termini and different ^{32}P-labeled 3′ ends were incubated in a wheat germ extract, and the products were analyzed by electrophoresis in 20% polyacrylamide/8 M urea gel. Ω substrates contained the following 3′ ends: lane a, G$^{3'}$p; lane b, G>p; lane c, G$^{2'}$p. Positions of linear (Ω) and circular (Ω_c) fragments, and of Coomassie brilliant blue (C) are indicated. (*C*) Analysis of the 3′ termini of radioactive material which, after incubation in wheat germ extract (*B*), electrophoresed in the position of linear Ω. Radioactive Ω fragments were excised from the respective gel lanes in *B*, digested with RNase A, and then subjected to thin-layer chromatography in solvent A.

end and a 3′-phosphate at the other, in a wheat germ extract with [γ-^{32}P]ATP provided evidence that the extract contains polynucleotide kinase activity. Apart from phosphorylation of Ω molecules, labeling of other RNAs present in the wheat germ extract such as tRNAs could also be observed (Fig. 2*A*); addition of Ω fragment decreased the labeling of the endogenous substrates. The phosphorylation reaction was strongly inhibited by ADP and not by AMP, suggesting that ADP may be a product of this reaction. End-group analysis of Ω labeled by the wheat germ kinase revealed that the only labeled product after nuclease P1 digestion was pU (Fig. 3*A*). The identity of the labeled material was further confirmed by sequence analysis (not shown). Hence, the enzyme is a 5′-hydroxyl polynucleotide kinase similar to that described in T4-infected *Escherichia coli* (16) or in other eukaryotic sources (17, 18).

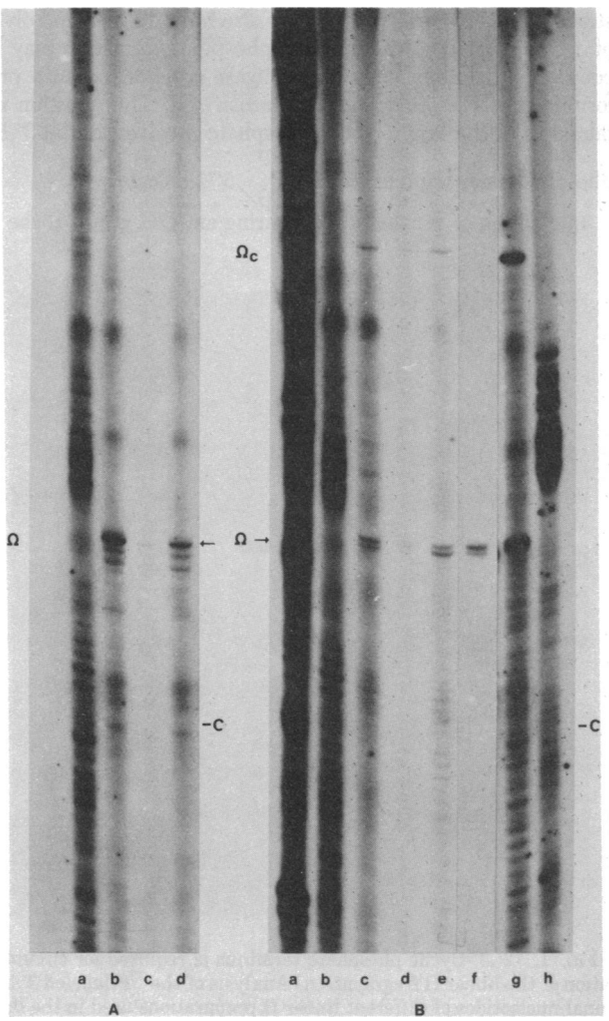

FIG. 2. Wheat germ extracts contain a 5′-hydroxyl polynucleotide kinase activity. (*A*) Unlabeled Ω molecules bearing 5′ hydroxyl and 3′ phosphate termini were incubated in a wheat germ extract in the presence of [γ-^{32}P]ATP. After incubation, samples were treated with proteinase K and analyzed by gel electrophoresis. Lanes: a, without Ω, b, Ω; c, Ω plus 3 mM ADP; d, Ω plus 3 mM AMP. (*B*) Unlabeled Ω preparation enriched in molecules with 5′ hydroxyl and 2′,3′-cyclic phosphate termini (prepared by mild digestion of TMV RNA with RNase T1) was incubated with wheat germ extract as in *A*. Lanes: a and b, without Ω, with proteinase K treatment omitted in a; c, Ω; d, Ω plus 3 mM ADP; e, Ω plus 3 mM AMP; f, Ω preparation as used in c–e, labeled with T4 polynucleotide kinase; incubation in wheat germ extract omitted; g, Ω prepared by RNase N1 digestion of TMV RNA; h, wheat germ tRNA labeled with T4 polynucleotide kinase.

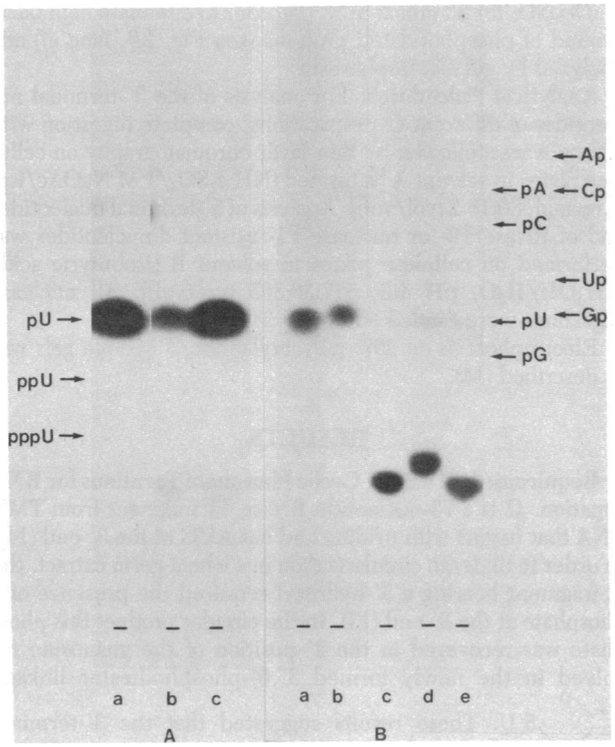

FIG. 3. Products of enzymatic digestion of linear (*A*) and circular (*B*) Ω molecules phosphorylated by wheat germ polynucleotide kinase. (*A*) Linear Ω from Fig. 2*A*, lane b (lane a), Fig. 2*B*, lane c, upper band (lane b), and Fig. 2*B*, lane c, lower band (lane c) was excised from gel and digested with nuclease P1. Samples were analyzed by thin-layer chromatography on cellulose plates in solvent B. Positions of markers are indicated. (*B*) Different circular Ω molecules were subjected to enzymatic treatment and analyzed as in *A*. Lanes: a and b, circular Ω prepared by T4 RNA ligase digested with nuclease P1 and RNase T2, respectively; c and d, circular Ω from Fig. 2*B*, lane c, digested with nuclease P1 and RNase T2, respectively; e, circular Ω from Fig. 1*B*, lane b, digested with nuclease P1. Expected nuclease-resistant dinucleotide products in lanes c, d, and e are pG(2′p,3′p)U, G(2′p,3′p)Up, and pG(2′*p,3′p)U, respectively (* specifies a [^{32}P]phosphate).

5′-Terminal Phosphate Is Incorporated into the 3′,5′-Phosphodiester Bond of the N(2′p,3′p)N Linkage. It was of interest to determine whether the 5′-phosphate is introduced into the phosphodiester bond of the 2′-phosphomonoester,3′,5′-phosphodiester linkage. Therefore, a preparation of unlabeled Ω rich in G>p-terminated molecules, obtained by mild digestion of TMV RNA with RNase T1 or N1, was incubated with [γ-^{32}P]ATP and a wheat germ extract. Fig. 2*B* shows that the Ω oligonucleotide is not only phosphorylated but also circularized under these conditions. [The two bands of labeled linear Ω seen in Fig. 2*B*, lanes c–f, correspond to G>p and 2′ (or 3′)-phosphate-terminated molecules which are separated in a long gel.] Treatment of the labeled circular Ω with RNase T2 or nuclease P1 yielded radioactive products with mobilities expected for G(2′p,3′p)Up and pG(2′p,3′p)U, respectively (Fig. 3*B*, lanes d and e; ref. 13). Treatment with nuclease P1 followed by snake venom phosphodiesterase yielded [^{32}P]pU (not shown). These results indicate that ^{32}P from the 5′ end of Ω was incorporated into the 3′,5′-phosphodiester bond of the G(2′p,3′p)U structure.

To evaluate the mechanism of the ligation reaction in more detail, we tried to separate the two processes observed above—i.e., 5′-phosphorylation from ligation itself. To this end, Ω ending mainly with 2′,3′-cyclic phosphate was ^{32}P-labeled at the 5′ end with T4 polynucleotide kinase. Upon incubation of this material in a wheat germ extract, the circular Ω was formed; the reaction required ATP (Fig. 4*A*, lanes c and d). 3′-Phosphate- or 3′-hydroxyl-terminated [5′-^{32}P]Ω molecules were not circularized (Fig. 4*A*, lanes a and b). The circular material was RNase T1 resistant (Fig. 4*B*, lane a) due to the presence of the G^p_pU linkage (Fig. 4*C*, lanes d and e). The structure of this bond has been confirmed by identification of [^{32}P]pU after nuclease P1 and subsequent snake venom phosphodiesterase digestion (Fig. 4*C*, lane h).

In the following experiment the circular material shown in Fig. 4*A*, lane c, was incubated with a low amount of calf intestine phosphatase. As expected, the ^{32}P label was phosphatase resistant (Fig. 4*B*, lane b). However, after phosphatase treatment, most of the labeled material migrated faster, in the position of the Ω circles produced by T4 RNA ligase. (The observation that Ω circles synthesized by the wheat germ ligase, even though they contain an additional phosphate, migrate more slowly than circles formed by the bacterial enzyme, is surprising. This was confirmed by electrophoretic separation of both forms of circles from their mixture.) This faster-migrating material was now RNase T1 sensitive (Fig. 4*B* lane c), and its digestion with nuclease P1 or RNase T2 yielded pU and Gp, respectively (Fig. 4*C*, lanes f and g), characteristic of Ω circles synthesized by T4 RNA ligase. These results further document that the 5′-phosphate of the linear Ω substrate is incorporated into the 3′,5′-phosphodiester bond of the newly formed N$^{2'}_{3'}$^p_p5′N linkage.

DISCUSSION

The important result of this investigation is that 2′,3′-cyclic phosphate is required for the joining of RNA ends by the RNA ligase activity from wheat germ extracts. The most significant implication of this finding is that the ligation reaction is probably preceded or accompanied by the nucleolytic processing of a longer precursor RNA by a 2′,3′-cyclizing RNase. The action of cyclizing endonucleases appears to be the only possible

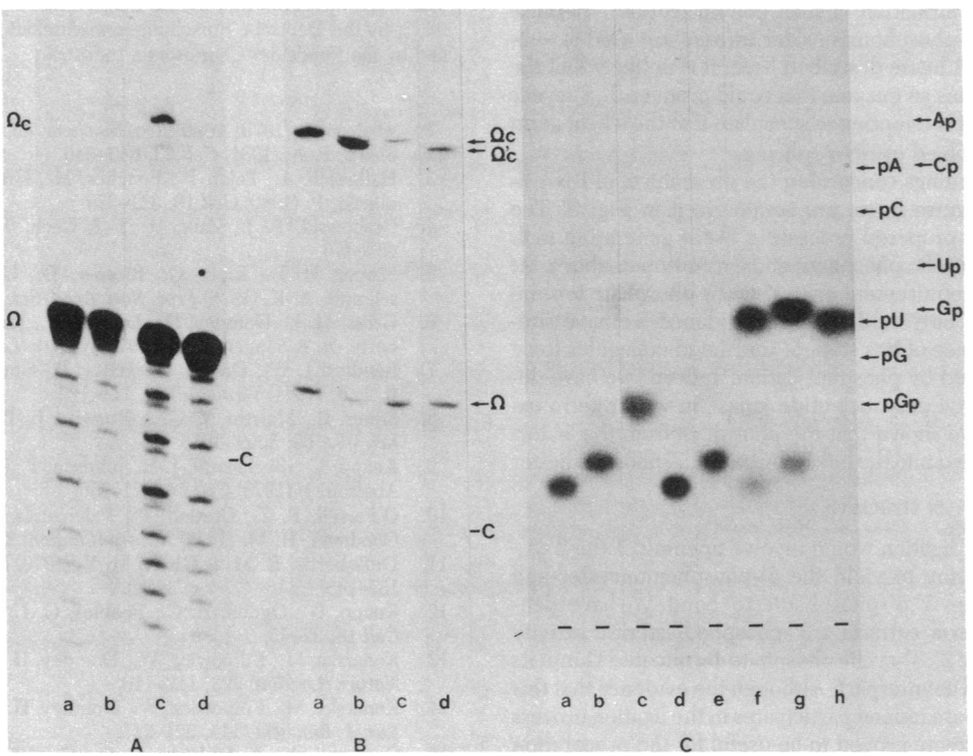

FIG. 4. Analysis of the circular molecules formed from linear Ω substrate bearing [5′-^{32}P]phosphate and 2′,3′-cyclic phosphate termini. (*A*) Requirement of 2′,3′-cyclic phosphate for the circularization reaction. Ω molecules bearing a [5′-^{32}P]phosphate terminus and G$^{3'}$OH (lane a), G$^{3'}$p (lane b), or predominantly G>p (lanes c and d) at the 3′ end were incubated with wheat germ extract and products analyzed by polyacrylamide gel electrophoresis. In lane d, ATP was omitted from the incubation mixture. (*B*) Phosphatase treatment renders the circular product sensitive to RNase T1. The labeled material migrating in position of circular Ω (*A*, lane c) was excised from gel and reelectrophoresed after treatment with RNase T1 (lane a) or low amount (0.5 milliunit/5 μl) of calf intestine phosphatase (10 min at 37°C) (lanes b and c) followed (in lane c) by RNase T1. Lane d shows circular Ω prepared by T4 ligase. Ω, Ω$_c$, and Ω′$_c$ are positions of linear Ω and of circles formed by wheat germ ligase and T4 RNA ligase, respectively. C, position of Coomassie brilliant blue. (*C*) Products of enzymatic digestion of circular Ω. Circular material from Fig. 1*B*, lane b, was digested with: nuclease P1 (lane a), RNase T2 (lane b), or nuclease P1 followed by snake venom phosphodiesterase (lane c). Circular material from Fig. 4*A*, lane c, was treated with nuclease P1 (lane d), RNase T2 (lane e), or nuclease P1 followed by snake venom phosphodiesterase (lane h). Circular material from Fig. 4*A*, lane c, was first subjected to limited phosphatase treatment (see Fig. 4*B*, lanes b and c) and then digested with nuclease P1 (lane f) or RNase T2 (lane g). Samples were analyzed by chromatography on cellulose plates in solvent B. Positions of appropriate markers are indicated. Expected mono- and dinucleotide products are: lane a, pG^{*p}_pU; lane b, G^{*p}_pU; lane c, pG$^{2'*}$p; lane d, pG^p_{*p}U; lane e, G^p_{*p}Up; lane f, *pU and trace of pG^p_{*p}U; lane g, G*p and trace of G^p_{*p}Up; lane h, *pU (* specifies a [^{32}P]phosphate).

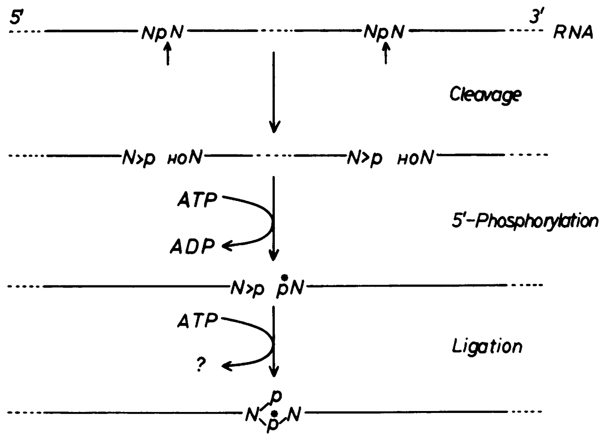

FIG. 5. Postulated ligation pathway leading to the formation of a N<(p)(p)>N linkage. *, fate of phosphate introduced by wheat germ 5'-hydroxyl polynucleotide kinase.

source of polynucleotides bearing a 2',3'-cyclic phosphate end; to our knowledge there are no other pathways described that could lead to the formation of such polynucleotides. Because neither 2'- nor 3'-phosphomonoester termini are used as substrates by the RNA ligase described here, it is unlikely that the wheat germ contains an enzyme that could produce 2',3'-cyclic phosphate ends. It is tempting to speculate that the wheat germ ligase may be involved in RNA splicing.

Our present findings concerning the mechanism of RNA ligation by wheat germ ligase are summarized in Fig. 5. The cleavage step is a proposed processing event generating molecules with 2',3'-cyclic phosphate ends, as outlined above. At the moment, the requirement of 2',3'-cyclic phosphate termini for ligation is the only, and indirect, evidence we have supporting the existence of this step. 5' termini of molecules to be ligated are activated by phosphorylation. Indeed, we have detected a 5'-hydroxyl polynucleotide kinase in wheat germ extracts, and we have shown that the phosphate from the 5' terminus is incorporated into the newly formed 3',5'-phosphodiester bond of the N<(p)(p)>N structure.

The last step of ligation would involve opening of the 2',3'-cyclic phosphate ring to yield the 2'-phosphomonoester and formation of the new 3',5'-phosphodiester bond. We have identified in wheat germ extracts a 3'-phosphodiesterase activity that transforms the 2',3'-cyclic phosphate-terminated Ω into its 2'-phosphorylated counterpart. Although the evidence that this 3'-phosphodiesterase indeed participates in the ligation process is missing, the enzyme proved to be useful for the preparation of 2'-phosphorylated Ω molecules.

As indicated in Fig. 5, the ligation step in which a 5'-phosphorylated and a 2',3'-cyclic phosphate termini are joined still requires ATP. The function of this ATP remains to be established; it is not needed for hydrolysis of the 2',3'-cyclic phosphate terminus of Ω by the 3'-phosphodiesterase activity (unpublished data). ATP may be used for 5'-end activation via the formation of an $A^{5'}pp^{5'}N$ structure as described for T4 RNA ligase (19).

The 5'-hydroxyl polynucleotide kinase activity detected in wheat germ extracts may be similar to the one found previously in HeLa cell (17) and mouse L cell (18) nuclei. The polynucleotide 2',3'-cyclic phosphate 3'-phosphodiesterase identified in the present work acts on polynucleotides but not on mononucleotides (unpublished data). Consequently, it differs and can be separated from the 3'-phosphodiesterase acting on 2',3'-cyclic mononucleotides also present in wheat germ extracts (unpublished data). The latter phosphodiesterase activity was previously detected in other systems (20, 21).

At present we can only speculate about the biological role of the unconventional 2'-phosphomonoester,3',5'-phosphodiester linkage and about the possible substrates of the ligase described. The function of the 2'-phosphate adjacent to the newly formed bond, which makes this bond insensitive to most RNases (13), could be to render it resistant to further action of putative cleavage enzyme(s) (Fig. 5), unless a specific or nonspecific phosphatase were to remove this 2'-phosphate, generating a classical phosphodiester bond. Finally, this 2'-phosphate could act as a positive signal in other as yet undefined processes.

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1. Abelson, J. (1979) *Annu. Rev. Biochem.* **48**, 1035–1069.
2. Sharp, P. A. (1981) *Cell* **23**, 643–646.
3. Halbreich, A., Pajot, P., Foucher, M., Grandchamp, C. & Slonimski, P. (1980) *Cell* **19**, 321–329.
4. Grabowski, P. J., Zaug, A. J. & Cech, T. R. (1981) *Cell* **23**, 467–476.
5. Sänger, H. L., Klotz, G., Riesner, D., Gross, H. J. & Kleinschmidt, A. K. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3852–3856.
6. Gross, H. J., Domdey, H., Lossow, C., Jank, P., Raba, M., Alberty, H. & Sänger, H. L. (1978) *Nature (London)* **273**, 203–208.
7. Randles, J. W., Davies, C., Hatta, T., Gould, A. R. & Francki, R. I. B. (1981) *Virology* **108**, 111–122.
8. Silber, R., Malathi, V. G. & Hurwitz, J. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3009–3013.
9. Knapp, G., Beckmann, J. S., Johnson, P. F., Fuhrman, S. A. & Abelson, J. (1978) *Cell* **14**, 221–236.
10. O'Farrell, P. Z., Cordell, B., Valenzuela, P., Rutter, W. J. & Goodman, H. M. (1978) *Nature (London)* **274**, 438–445.
11. DeRobertis, E. M. & Olson, M. V. (1979) *Nature (London)* **218**, 137–143.
12. Knapp, G., Ogden, R. C., Peebles, C. L. & Abelson, J. (1979) *Cell* **18**, 37–45.
13. Konarska, M., Filipowicz, W., Domdey, H. & Gross, H. J. (1981) *Nature (London)* **293**, 112–116.
14. Konarska, M., Filipowicz, W., Domdey, H. & Gross, H. J. (1981) *Eur. J. Biochem.* **114**, 221–227.
15. Cameron, V. & Uhlenbeck, O. C. (1977) *Biochemistry* **16**, 5120–5126.
16. Richardson, C. C. (1965) *Proc. Natl. Acad. Sci. USA* **54**, 158–165.
17. Shuman, S. & Hurwitz, J. (1979) *J. Biol. Chem.* **254**, 10396–10404.
18. Winicov, I. (1977) *Biochemistry* **16**, 4233–4236.
19. Ohtsuka, E., Nishikawa, S., Sugira, M. & Ikehara, M. (1976) *Nucleic Acids Res.* **3**, 1613–1623.
20. Drummond, G. I. & Yamamoto, M. (1971) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 4, pp. 355–371.
21. Drummond, R. J., Hamil, E. B. & Guha, A. (1978) *J. Neurochem.* **31**, 871–878.