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A Small Structural Element, Pc-J5/5a, Plays Dual Roles in a Group IC1 Intron RNA

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The P4-P6 domain of group IC1 intron ribozymes such as that of the *Tetrahymena* autonomously folds into a hairpin-shaped structure in which the J5/5a region serves as a hinge. Phylogenetic comparisons of these IC1 introns suggested that the J5/5a region (termed Pc-J5/5a motif) in a subclass of IC1 introns such as the one from *Pneumocystis carinii* functions not only as a hinge but also as a receptor for a GAAA-tetraloop. We investigated the role of this motif by transplanting the structural unit, Pc-J5/5a motif, of *Pneumocystis carinii* into the P4-P6 domain of the *Tetrahymena* intron. The results showed that the Pc-J5/5a motif binds to a GAAA loop with high affinity and also facilitates the bending of the *Tetrahymena* P4-P6 domain more positively than the original J5/5a region.

P5abc, a large peripheral domain of the group IC1 intron ribozyme from *Tetrahymena thermophila* that is responsible for the enhancement of its catalytic activity (1–4) is involved in a hairpin-shaped structure together with the P4-P6 helical domain (Figs. 1A and 2B, left, the domain is termed P5abc-P4-P6) (5–7). Long-range interactions between the L5b region in P5abc and P6a internal-loop in P4-P6 helix (denoted as L5b X P6a) (5, 7) and those between the A-rich bulge in P5abc and P4 stem in P4-P6 helix (denoted as A-rich bulge X P4) (7, 8) are critical for the folding of the RNA (Figs. 1 and 2A). The folded structure of P5abc-P4-P6 domain bends sharply at the J5/5a region (Figs. 1A and 2A, left) (7). To examine whether the J5/5a internal loop positively directs the bending of the P5abc-P4-P6 domain RNA or serves passively as a flexible hinge, various mutant RNAs having mutations in the J5/5a region were prepared and tested for their folding ability (9). The analysis revealed that the noncanonical base pairings and specific stacking of the base moieties in the J5/5a region positively direct the folding, although their contribution is less than that of the long-range interactions (9).

A group I intron from *Pneumocystis carinii* (Fig. 1B) that belongs to a subclass of the IC1 introns possesses P5abc and a GAAA tetraloop in its L9 region (10, 11). The introns of this subclass (as well as some from IC2) often have CC-GG base-pairs and an additional stem-loop in their J5/5a region (denoted as P5.1, Fig. 1B and Table 1) whereas the *Tetrahymena* intron (and its close homologue) having a UAAU loop in L9 does not possess CC-GG pairs at the corresponding site (Fig. 1A).

This sequence covariation is observed between the structure of J5/5a containing the CC-GG base-pairs and the L9 GAAA loop in the *Pneumocystis carinii*-type IC1 introns. The receptor motifs for GWAA tetraloops (where W stands for A or U) contain CC-GG base-pairs that specifically recognize the last two adenines in the tetraloops (12, 13). The J5/5a region of the *Pneumocystis carinii* IC1 intron (denoted as Pc-J5/5a motif) shares nine nucleotides with the 11 nt GAAA receptor motif (the gray shaded area within J5/5a region in Fig. 1B, see also Table 1), suggesting that the Pc-J5/5a motif is a variant form of the 11 nt receptor motif fused with the hinge region of P5abc-P4-P6 domain (13). These imply that the J5/5a region in *Pneumocystis carinii*-type IC1 introns serve as both a hinge in the bent RNA and a receptor for the GAAA tetraloop at L9. We examined this hypothesis by employing the J5/5a region of the *Pneumocystis carinii* group IC1 intron (Fig. 1B) and an RNA molecule consisting of the P5abc-P4-P6 domain from the *Tetrahymena* intron (denoted as the P4-P6 RNA, Fig. 2B).

MATERIALS AND METHODS

Constructs of P4-P6, Z4-6, Y4-6 and their mutants. Template DNAs for *in vitro* transcription of P4-P6, P4-P6mL5b, P4-P6(IC3) and P4-P6(UU-AA)RNA were generated by PCR. In each reaction, 1 ng of pTZIVSU (14) or its derivative possessing an appropriate mutation (15) was used as a template. Sequences of primers for preparing P4-P6 RNA and its mL5c mutant were 5′-TAA TAC GAC TCA CTA TAG GAA TTG CGG GAA AGG GGT CAA-3′ (denoted as p46-1, the promoter sequence for T7 RNA polymerase is underlined) and 5′-TGA ACT GCA TCC ATA TCA A-3′ (p46-2). Primers used for

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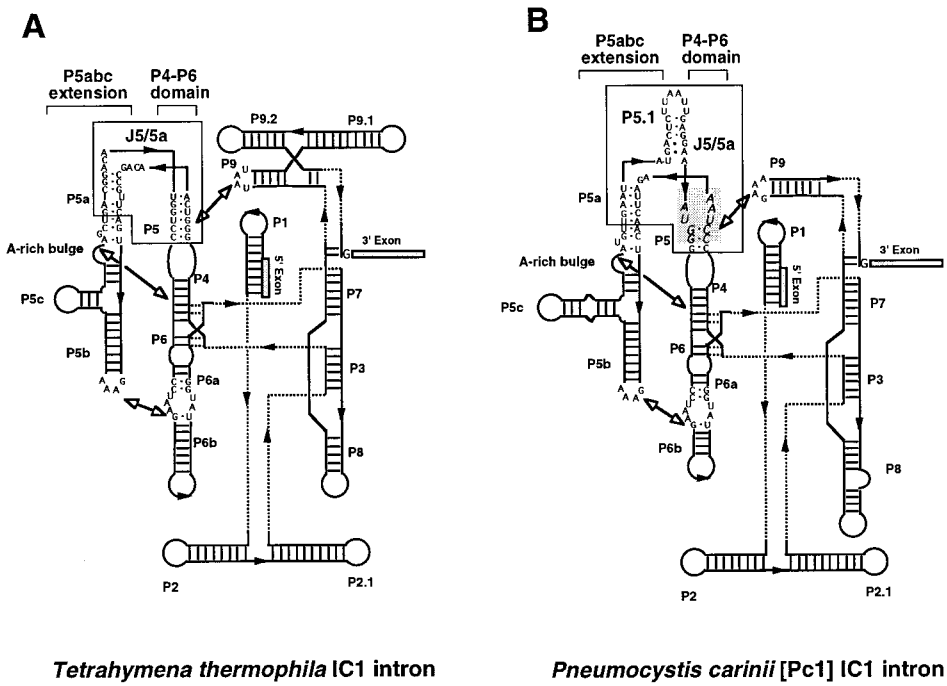

***Tetrahymena thermophila* IC1 intron** ***Pneumocystis carinii* [Pc1] IC1 intron**

FIG. 1. The secondary structures of the two group IC1 introns from *Tetrahymena* and *Pneumocystis*. Arrowheads superimposed on lines indicate 5′-to-3′ polarity. Lines with two white arrowheads indicated the long-range tertiary interactions discussed in the text. 5′ and 3′ exons are indicated as gray bars. Structural elements except J5/5a, L5b, L9 and the 11 nt GAAA receptors in P6a are indicated by solid lines. Sequences of J5/5a, L5b, L9, and the 11 nt GAAA receptors in P6a regions are shown. J5/5a regions discussed in the text are boxed. (A) The secondary structure of the group IC1 intron from *Tetrahymena thermophila* possessing a UAAU loop at L9. (B) The secondary structure of the group IC1 intron from *Pneumocystis carinii* possessing a GAAA loop at L9. The gray area within the J5/5a region shares the identical sequence with the 11 nt GAAA receptor motif.

preparing P4-P6(IC3) were p46-1 and 5′-TGA ACT GCA TCC CTC GCA-3′. P4-P6(UU-AA) was prepared with p46-1 and 5′-TGA ACT GCA TTT TGC AAC A-3′ primers. A template DNA for *in vitro* transcription of P4-P6J5/5aBP was generated through PCR by using pTZIVSU as a template. Primers for preparing P4-P6J5/5aBP were 5′-TAA TAC GAC TCA CTA TAG GAA TTG CGG GAA AGG GGT CAT GTC CGT TCA GTA-3′ (denoted as p46BP, the promoter sequence for T7 RNA polymerase is underlined) and p46-2.

Plasmid DNA encoding a mutant *Tetrahymena* ribozyme possessing a Pc-5/5a motif in P6a (denoted as pTZ-Z46) or a J5/5a (denoted as pTZ-Y46) region was prepared from pTZIVSU or its derivative (16). In these mutant *Tetrahymena* ribozymes possessing Pc-5/5a motifs, the terminal loop in the J5/5a motif which was originally 5′-UUAAUU-3′ (Fig. 1B) was replaced with a stable tetraloop (5′-UUCG-3′) to improve its stability (Figs. 2C and 5B). Template DNAs for *in vitro* transcriptions of Z4-6, Z4-6mL5b were generated by PCR. For each PCR, 1 ng of a plasmid pTZ-Z46 or its derivative possessing an UUCG tetraloop in L5b was used as a template. Primers for preparing Z4-6 RNA and its mL5b mutant were p46-1 and 5′-TGA ACT GCA TCC ATT TCC TC-3′ (pZ46). A template DNA for *in vitro* transcriptions of Z4-6J5/5aBP was generated through PCR by using pTZ-Z46 as a template. Primers for preparing Z4-6J5/5aBP were p46BP and pZ46. Template DNAs for *in vitro* transcriptions of Y4-6 and Y4-6mL5b were generated by PCR. For each PCR, 1 ng of a plasmid pTZ-Y46 or its derivative possessing an UUCG tetraloop in L5b was used as template. Primers for preparing Y4-6 RNA and its mL5b mutant were 5′-TAA TAC GAC TCA CTA TAG GAA TTG CGG GAA AGC CCT AAA GAT-3′ (the T7 RNA polymerase promoter sequence is underlined) and p46-2.

All RNAs employed in this study were prepared by transcription *in vitro* with T7 RNA polymerase and purified by electrophoresis on 5% polyacrylamide denaturing gels.

Native polyacrylamide gel electrophoresis. Native polyacrylamide gel electrophoresis were performed as described (17, 18). Native 5% polyacrylamide gels (39:1 acrylamide: bisacrylamide) and running buffer for gel electrophoresis containing 50 mM Tris-OAc, pH 7.5 and various concentrations of Mg(OAc)2 were used. P4-P6, Z4-6, Y4-6 and their mutants were incubated in distilled water at 90°C for 1.5 min. 10× folding buffer [final: 50 mM Tris-OAc, pH 7.5 and appropriate concentrations of Mg(OAc)2] was added to the RNA solution and incubated for 10 min at 50°C and then for 10 min at 30°C. 5% glycerol and 0.25% BPB were added to the samples and loaded on the gel. Gels were run at constant voltage (3 V/cm) for 4–6 h at 30°C.

DMS modification and reverse transcription. DMS modifications were performed as described (5) with some modifications. A 50 μl solution containing 15 pmol of an RNA, 50 mM Tris-Cl (pH 7.5), 10 mM $MgCl_2$ was preincubated as described above. After the mixture was cooled to 30°C, 0.5 μl DMS-ethanol (1:9) solution was added and the mixture was incubated for 15 min at 30°C. To detect the modified adenines and cytidines, reverse transcription with Superscript II (Gibco BRL) was performed by using a [5′-^{32}P]-labeled DNA primer complementary to G179-U206.

RESULTS AND DISCUSSION

J5/5a Motif of P. carinii Group IC1 Intron as a GAAA Tetraloop Receptor

First, we attempted to see whether the J5/5a region of the *P. carinii* group IC1 intron (Pc-J5/5a motif) is a GAAA tetraloop-receptor. A derivative of the P4-P6 RNA of the *Tetrahymena* intron in which the 11 nt

TABLE 1

Alignment of J5/5a Region of the Group IC1 and IC2 Introns Possessing P5.1 as Well as GAAA Loops at Their L9

	P5 →	P5a →	P5a′ ←	P5.1 →	P5.1′ ←	P5′ ←
Pc 1:	CCCUAAAG-	AUU--(P5abc)---	AAUA-	UGACUC----	UUAAUU---	GAGGAAAUGGG
Pc S:	CCCUAAAG-	AUU--(P5abc)---	AAUA-	UGACUC----	UUAAUU---	GAGGAAAUGGG
Pc 2:	CCCUAAAG-	AUU--(P5abc)---	AAUA-	UGAAUCUU--GAUUG--		AAGAUGAAAUGGG
Ce S:	UCCUAAGAG	CUA--(P5abc)---	UAGA-	UGAAGCUUCC-6nt-		GGAGGCUGAAAUGGA
Cm S:	CCCUAACAG	CUC--(P5abc)---	GAGA-	UGAGCCGUUC-27nt-		GGCGCGGAAAUGGG
PaND12	UCCUAAAG-	CUU--(P5abc)---	AAGA-	UGAUCAGAG--15nt-		CUCUGAGAAAUGGA
Ag S:	CCCUAAAG-	CCU--(P5abc)---	AGGA-	UGACCUU----29nt-		AAGGUCGCAAUGGG
PaND13	CCUUAAAG-	CUA--(P5abc)---	UAGA-	UGCAC------GAAA------		GGAAAUAGG
NcND5:	UCCUAAAG-	CUU--(P5abc)---	AAGA-	UGAGU------GAAA-----		ACAAAAUGGA
PaATP6	CCCUAAAG-	CUC--(P5abc)---	GAGA-	UUCUA------GUAA-----		UAGAAAUGGG
PaOx1:	CCCUAAAG-	CUU--(P5abc)---	AAGA-	UGAUU------GAAA-----		AAAAAAUGGG
Ss S:	UCCUAAAG-	CUU--(P5abc)---	AAGA-	UGAUC------GAAA-----		GAGAAAUGGA
PaND11	CCUUAAAG-	CUU--(P5abc)---	AAGA-	UAUAC------GAAA-----		GUAUAAUAGG
C1 S:	UCCUAUAG-	CUC--(P5abc)---	GAGA-	UG---------CGA---------		CAAUGGA
Cs S:	CCCUAAAG-	CUU--(P5abc)---	AAGA-	UG---------CAC---------		CAAUGGG
Dp S:	CCCUAAAG-	CUU--(P5abc)---	AAGA-	UG---------CAA---------		CAAUGGG
Ds S:	CCCUAAAG-	CUU--(P5abc)---	AAGA-	UG---------CAA---------		CAAUGGG
Mc S:	CCCUAAAG-	CUU--(P5abc)---	AAGA-	UG---------CAA---------		CAAUGGG
Ps S:	CCCUAAAGAG	CCC--(P5abc)---	GGGAUUGUCUCUAG--AGA-----			CUUGCAAUGGG
11nt:	XCCUAAGY-	----------	----------	----------	----------	yUAUGGx

Note. Underlined nucleotides have been implicated to form base-pairs involving a noncanonical G–A pair (10, 11). Nucleotides represented as bold letters are identical to those of the 11 nt motif. The last line indicates the sequence of 11 nt motif in that X-x and Y-y form Watson-Crick base-pairs, respectively. The following abbreviations were used (10, 11); Pc 1, *Pneumocystis carinii* LSU rRNA-i1 (IC1); Pc S, *Pneumocystis carinii* SSU rRNA (IC1); Pc 2, *Pneumocystis carinii* LSU rRNA-i2 (IC1); Ce S, *Chorella ellipsoidea* SSU rRNA (IC1); Cm S, *Chorella mirabilis* SSU rRNA (IC1); PaND12, *Podospora anserina* mt. ND1-i2 (IC2); Ag S, *Acanthamoeba griffini* SSU rRNA (IC1); PaND13; *Podospora anserina* mt. ND1-i3 (IC1); NcND5, *Neurospora crassa* mt. ND5 (IC2); PaATP6, *Podospora anserina* mt. ATP6 (IC2); PaOX1, *Podospora anserina* mt. OX1 (IC2); Ss S, *Scleotinia scleotiorum* mt. SSU rRNA (IC2); PaND11, *Podospora anserina* mt. ND1-i1 (IC1); C1 S, *Chorella luteoviridis* SSU rRNA-2 (IC1); Cs S, *Chorella sorokiniana* SSU rRNA-i1 (IC1); Dp S, *Dunaliella parva* SSU rRNA-1 (IC1); Ds S, *Dunaliella parva* SSU rRNA (IC1); Mc S, *Mesotaenium caldariorum* SSU rRNA (IC1); Ps S, *Porphyra spiralis* SSU rRNA (IC1).

GAAA receptor in its P6a region is replaced with the Pc-J5/5a motif (resulting RNA is termed Z4-6RNA) was constructed. In this construct, the relative positions of the CC-GG pairs which are conserved between the two motifs are fixed. If the Pc-J5/5a motif acts as a GAAA receptor in the construct, a folded structure can be observed by employing native gel electrophoresis or chemical modification techniques (5, 19).

The stability of the folded P4-P6 RNA has been shown to correlate with their mobilities on the native-polyacrylamide gel (9, 19). We employed the P4-P6 RNA as a positive control representing the folded structure (Fig. 2A, left) and its mutant termed P4-P6 J5/5aBP in which the original J5/5a loop is replaced with consecutive Watson-Crick base-pairs as a negative control representing an extended state (Fig. 2A, right) (9, 19). In addition to these control molecules, three P4-P6 RNA mutants were prepared, P4-P6mL5b, P4-P6(UU-AA) and P4-P6(IC3) (Fig. 2B). P4-P6mL5b and P4-P6(UU-AA) are variants in which the L5b X P6a interaction is disrupted. P4-P6(IC3) is a variant in which the 11 nt GAAA receptor in P6a region has been replaced with another GAAA receptor termed IC3 motif which recognizes the GAAA receptor with less affinity than the 11 nt receptor; thus this RNA is likely to form a less stable structure than that of the P4-P6 RNA (20). In addition to these P4-P6 RNA mutants, two mutant Z4-6 RNAs were also prepared (Fig. 2C), Z4-6 J5/5aBP and Z4-6mL5b RNA. Z4-6 J5/5aBP possesses Watson-Crick base-pairs in its J5/5a region in place of the internal loop so that the variant cannot be in a folded state. Z4-6mL5b possesses a UUCG loop in L5b region so that possible interaction between the GAAA loop in L5b and the Pc-5/5a motif in the Z4-6 RNA would be disrupted in this variant.

To determine the affinity of the Pc-J5/5a motif to the GAAA loop, Z4-6 RNA, P4-P6 RNA and their mutants were subjected to native-polyacrylamide gel-electrophoresis by varying the concentration of magnesium ions in the gel and the running buffer (Fig. 3). In the presence of 5 mM magnesium ions where P4-P6 RNA has been shown to fold stably (5, 21), P4-P6 RNA migrated distinctively faster than its J5/5aBP variant (Fig. 3D) (9, 19). Two variant P4-P6 RNAs lacking P6a X L5b interaction exhibited identical mobility and migrated faster than the J5/5aBP variant but slower than the wild type P4-P6 RNA, suggesting that the mutant P4-P6 RNAs lacking P6a × L5b interactions can still form a folded state with help from the remaining interactions, albeit with less stability than that of P4-P6

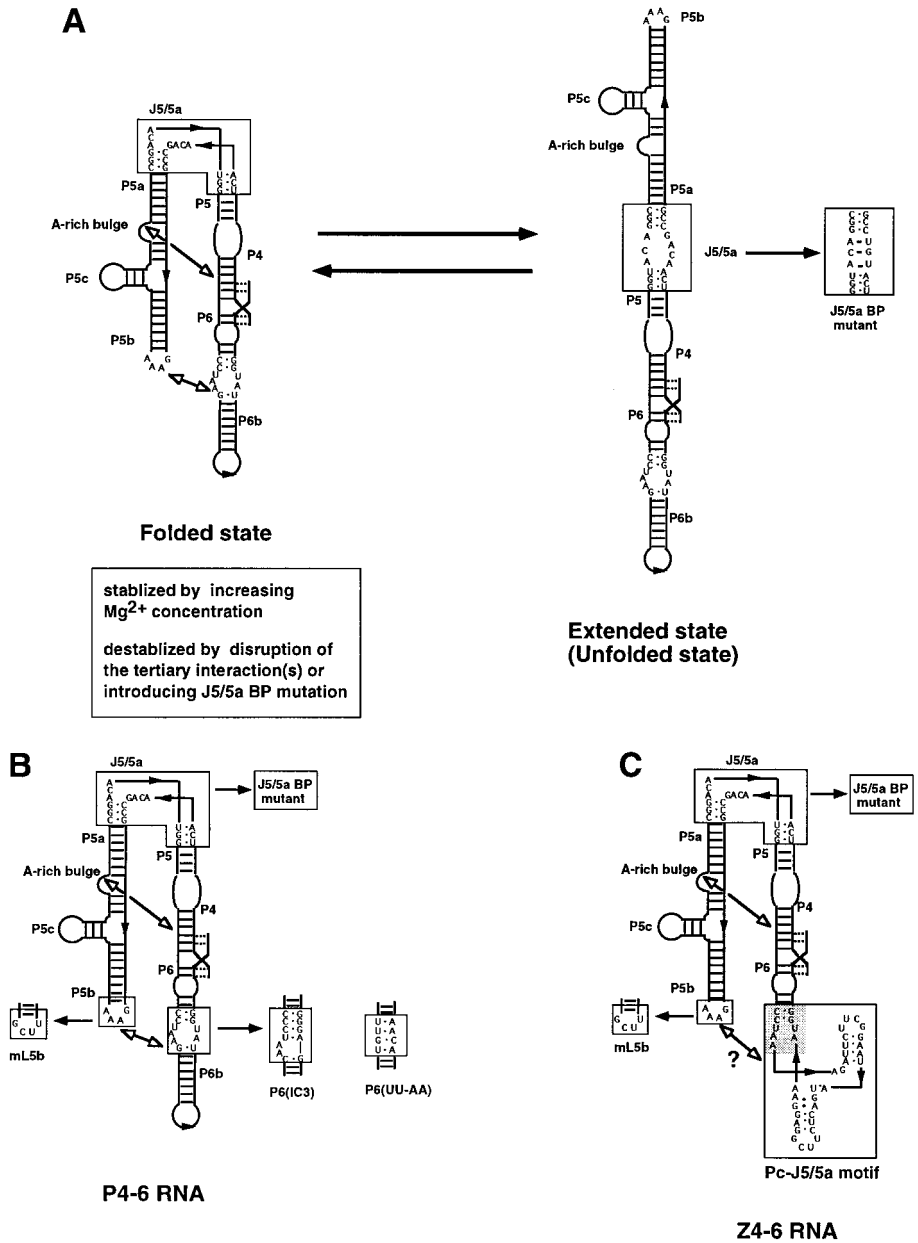

FIG. 2. Structures of P4-P6 RNA, Z4-6 RNA and their mutants. (A) Schematic representation of equilibrium between the folded and extended (or unfolded) states of P4-P6 RNA. Mutant P4-P6 RNA whose J5/5a region was replaced with Watson–Crick base-pairs (P4-P6J5/5aBP mutant) can form only the extended state due to the loss of the hinge region (9). (B) The secondary structure of P4-P6 RNA and its mutants. P4-P6 RNA consists of P4-P6 domains (positions A104-A261) of the *Tetrahymena* IC1 intron ribozyme and possesses two guanosines at its 5′ terminal for *in vitro* transcription (21). (C) The secondary structure of Z4-6 RNA and its mutants. Z4-6 RNA is derived from P4-P6 RNA by replacing the 11 nt GAAA receptor motif in the P6a region with Pc-J5/5a motif. The gray area within the Pc-J5/5a motif shares identical sequence with the 11 nt GAAA receptor motif.

RNA. The mobilities of P4-P6(IC3) and Z4-6 RNA were similar to that of P4-P6 RNA and distinctively faster than those of other variant RNAs, indicating that these two RNAs can fold stably in the presence of 5 mM of magnesium ions (Fig. 3D). This indicates that the Pc-J5/5a motif recognizes a GAAA tetraloop in L5b as effectively as the 11 nt receptor or the IC3 receptor in the presence of 5 mM of magnesium ions.

To confirm that the Pc-J5/5a motif interacts with the GAAA loop, we attempted chemical modification with dimethyl sulfate (DMS) which methylates the N-1 position of adenine residues (Fig. 4). Three adenosines in a free GAAA loop were modified by DMS but two of them were protected when the tetraloop was associated with the 11 nt receptor (5). We examined the modification patterns of the GAAA loop in Z4-6 RNA by

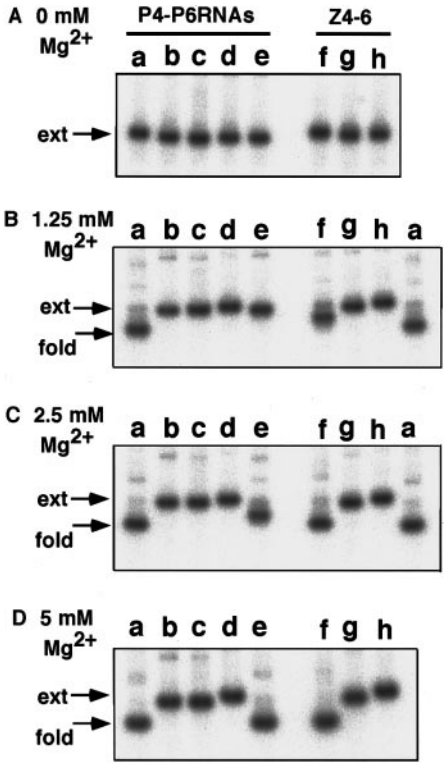

FIG. 3. Native polyacrylamide gel electrophoresis of P4-P6 RNA, Z4-6 RNA and their mutants. 'Ext' and 'fold' indicate the extended and folded states of the RNAs, respectively. Lane a, P4-P6 RNA; lane b, P4-P6mL5b; lane c, P4-P6(UU-AA); lane d, P4-P6J5/5aBP; lane e, P4-P6(IC3); lane f, Z4-6 RNA; lane g, Z4-6mL5b; lane h, Z4-6J5/5aBP. (A) Native gel mobility in the absence of magnesium ion. (B) 1.25 mM magnesium ions. (C) 2.5 mM magnesium ions. (D) 5 mM magnesium ions.

means of DMS modification of Z4-6 RNA in the presence of 10 mM of magnesium ions (Fig. 4). Consistent with previous reports (5), the adenosines in the GAAA loop of P4-P6 RNA (positions A151-A153) were protected (lane 2) whereas those in P4-P6 J5/5aBP RNA were modified (lane 3). Similar protection patterns were observed in both Z4-6 RNA (lanes 4 and 5) and a Z4-6 J5/5aBP variant (lane 6), indicating that the Pc-J5/5a motif recognizes the GAAA loop in the folded state of Z4-6 RNA (Fig. 4). The results from the native-gel analysis and this assay indicate that the J5/5a motif from *P. carinii* acts as a tetraloop receptor which recognizes a GAAA loop in a manner similar to that of the 11 nt receptor.

Relative Affinities of 11 nt, IC3 and J5/5a Motif Receptors to a GAAA Loop

We attempted to determine the relative affinities of the 11 nt, IC3 and Pc-J5/5a motifs for a GAAA loop. Because it was previously demonstrated that the wild type P4-P6 RNA can stably form a folded state in the presence of more than 1 mM of magnesium ions (9), we performed native-gel analysis varying the magnesium concentration from zero to 5 mM (Fig. 3). In the presence of 2.5 mM of magnesium ions, P4-P6 RNA and Z4-6 RNA exhibited similar mobilities (Fig. 3C). However, the mobility of the P4-P6(IC3) variant became slower than that of P4-P6 or Z4-6 RNAs, indicating that the folded structure of P4-P6(IC3) was less stable than those of P4-P6 or Z4-6 RNA (Fig. 3C). In the presence of 1.25 mM of magnesium ions, the mobility of Z4-6 RNA became more retarded than that of P4-P6 RNA, but faster than P4-P6(IC3) (Fig. 3B), indicating that the rank order for recognition of the GAAA loop is as follows; 11 nt motif followed by Pc-J5/5a motif and then the IC3 motif.

Pc-J5/5a Motif as a Hinge in the Hairpin-Shaped RNA

In addition to its function as a GAAA loop receptor, sequence comparison of group IC1 introns predicted that the J5/5a region of *P. carinii* group IC1 introns acts as a hinge for facilitating correct folding of the P5abc-P4-P6 domain. Mutational analyses of the J5/5a region of the *Tetrahymena* ribozyme demonstrated that the region contributes positively for folding of the

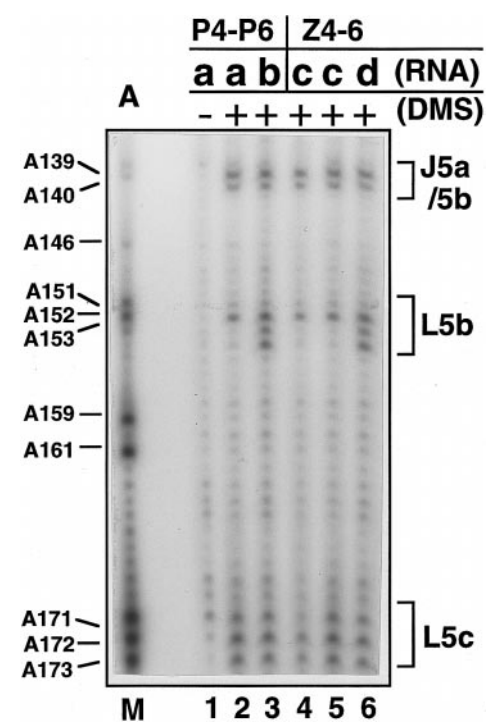

FIG. 4. Chemical modification of GAAA tetraloop in L5b region of P4-P6, Z4-6 RNA or their J5/5aBP mutants. Lane M: Positions of adenines in P4-P6 RNA detected by reverse transcription in the presence of dideoxy thymidine triphosphate. Lanes 1–6: DMS modifications of P4-P6 RNA (a), P4-P6J5/5aBP (b), Z4-6 RNA (c) and Z4-6J5/5aBP (d) RNAs were incubated in the presence (+) or absence (−) of DMS. Positions of methylation were then determined by reverse transcription with a [5′-^{32}P]-labeled primer. Reverse transcription terminated one nucleotide before the residues modified by DMS (22).

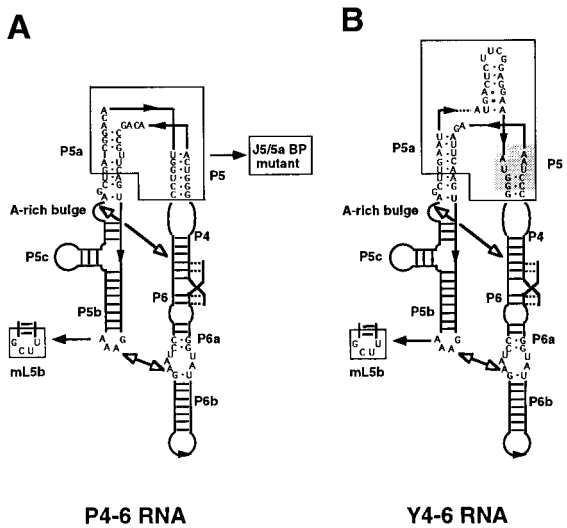

FIG. 5. Structure and folding of Y4-6 RNA possessing Pc-J5/5a motif as the hinge region. (A) The secondary structure of P4-P6 RNA and its mutants. (B) The secondary structure of Y4-6 RNA and its mutants. Y4-6 RNA is derived from P4-P6 RNA by replacing its J5/5a region with Pc-J5/5a motif. The gray area within the Pc-J5/5a motif shares identical sequence with the 11 nt GAAA receptor motif.

P5abc-P4-P6 domain (9). The predicted secondary structure of the J5/5a region of the *P. carini* group I intron (Pc-J5/5a motif) is more complicated than the J5/5a region of the *Tetrahymena* intron because Pc-J5/5a motif possesses an extra stem-loop structure P5.1 at the hinge region. To see whether the Pc-J5/5a motif functions as a hinge, we replaced the J5/5a region of the P4-P6 RNA with the Pc-J5/5a motif (the resulting RNA is termed Y4-6 RNA, Fig. 5B) and examined its folding ability (Fig. 6).

To precisely detect the effect of the J5/5a region, we used a mutant P4-P6 RNA (P4-P6mL5b RNA) and Y4-6 RNA (Y4-6mL5b RNA) in which the long-range L5b X P6a interaction is disrupted by replacing the GAAA loop at L5b with a UUCG loop (Fig. 5). The folding ability of the two RNAs were examined by native gel electrophoresis in the presence of various concentrations of magnesium ions (Fig. 6). In the absence of magnesium, the wild type P4-P6 and P4-P6mL5b and P4-P6J5/5aBP showed the same mobility on a native-gel (Fig. 6A). Y4-6 and Y4-6mL5b exhibited the same mobility which was slightly slower than P4-P6 RNA and its variants (Fig. 6A), presumably because the J5/5a region of Y4-6RNA is bulkier than that of P4-P6 RNA (Fig. 5). In the presence of 2.5–10 mM magnesium ions where P4-P6 RNA can fold stably, Y4-6 RNA migrated slightly slower than P4-P6 RNA likely due to the mass of the Pc-J5/5a motif (Figs. 6B–6D). Under the same conditions, P4-P6mL5b and Y4-6mL5b variant RNAs migrated slower than P4-P6 and Y4-6 RNAs, respectively, but faster than P4-P6J5/5aBP, indicating that the folded forms of P4-P6mL5b and Y4-6mL5b are less stable than the corresponding P4-P6 and Y4-6, respectively. In the presence of magnesium, the mobility of Y4-6mL5b RNA is faster than that of P4-P6mL5b RNA despite the mass of J5/5a region of Y4-6mL5b (Figs. 6B–6D). These results suggest that Y4-6mL5b RNA folds more stably than P4-P6mL5b RNA in the presence of magnesium ions, implying that the Pc-J5/5a motif facilitates bending of the P5abc-P4-P6 domain more efficiently than the J5/5a region of the *Tetrahymena* intron. It should be noted that some retarded bands were observed weakly in lane d (Y4-6 RNA) of Figs. 6B–6D. This might suggest that these were due to aberratly folded Y4-6 RNA and/or its dimeric form which can be formed by the non-native interaction between Pc-J5/5a motif at J5/5a region and GAAA loop in L5b.

In this study, we have shown independently that the J5/5a region of the *P. carinii* IC1 intron (denoted as Pc-J5/5a motif) functions as a GAAA loop receptor (Figs. 3 and 4) and also facilitates the formation of a sharp bend in the P4-P6 RNA consisting of the P5abc-P4-P6 domain of the *Tetrahymena* intron (Fig. 6). In our assay system, it appears that the conformation of

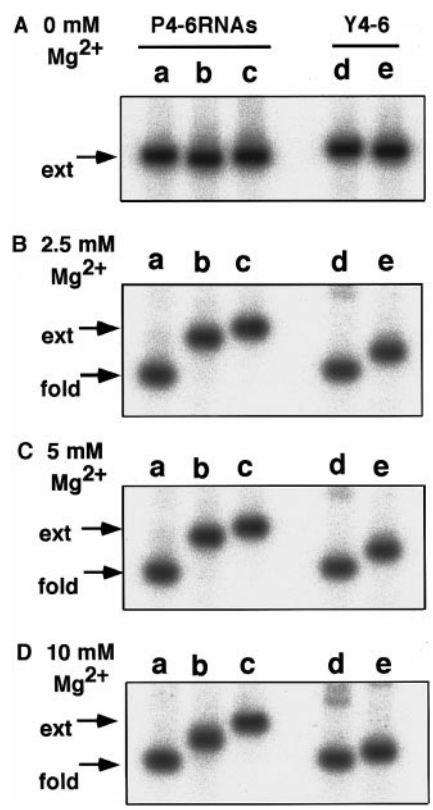

FIG. 6. Native polyacrylamide gel electrophoresis of P4-P6 RNA, Y4-6 RNA and their mutants. "Ext" and "fold" indicate the extended and folded state of P4-P6RNA, respectively. Lane a, P4-P6 RNA; lane b, P4-P6mL5b; lane c, P4-P6J5/5aBP; lane d, Y4-6 RNA; lane e, Y4-6mL5b. (A) Native gel mobility in the absence of magnesium ion. (B) 2.5 mM of magnesium ions. (C) 5 mM of magnesium ions. (D) 10 mM of magnesium ions.

the Pc-J5/5a motif is not fixed in the bent structure of Z4-6 RNA (Fig. 2C). It also appears that the Pc-J5/5a motif transplanted into the hinge region of P4-P6 RNA is unable to bind to the GAAA loop in its L5b region when Y4-6 RNA folds into the correct hairpin-shaped structure as shown in Fig. 5B. However, in the correctly folded *P. Carinii* IC1 intron ribozyme, the GAAA loop in L9 is recognized by Pc-J5/5a motif whose conformation is strictly fixed in the bent structure with help from L5b X P6a and A-rich bulge X P4 tertiary interactions (Fig. 1B). Because the motif is a fusion of a GAAA receptor and the hinge region of the hairpin shaped RNA, it is possible to imagine that its receptor- and hinge-function work synergistically. The binding of the GAAA loop to the Pc-J5/5a motif should enhance the stability of the bent conformation of the motif so that the folded structure of P5abc-P4-P6 domain in the *P. Carinii* intron could be further stabilized when its J5/5a motif binds to L9 GAAA loop. Likewise, the conformationally fixed Pc-J5/5a motif in P5abc-P4-P6 domain of the *P. carinii* intron might bind to the GAAA loop more strongly than a motif whose conformation is not fixed, such as that in Z4-6 RNA.

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