J. Biochem. **133**, 665–670 (2003)
DOI: 10.1093/jb/mvg085

The P5 Activator of a Group IC Ribozyme Can Replace the P7.1/7.2 Activator of a Group IA Ribozyme

Yoshiya Ikawa, Kenta Sasaki, Hiroyuki Tominaga and Tan Inoue*
Graduate School of Biostudies, Kyoto University, Kyoto 606-8502

Received January 20, 2003; accepted March 5, 2003

The P5 or P7 extensions in the group I intron ribozyme serve as "modular activator units" by stabilizing the conserved core of the ribozyme. The P5 extension of a group IC1 intron was introduced to a barely active group IA2 intron lacking its original P7 extension. The inserted P5 extension significantly activated the chimeric construct. Because the CYT-18 protein factor is also known to activate mutant group IA2 and IC1 introns lacking their P7 and P5 extensions, respectively, the RNA and protein activator units function in an analogous manner.

Key words: group I intron, nrdB, ribozyme, RNA, Tetrahymena.

A group I self-splicing intron is a unimolecular catalytic RNA that possesses a conserved core structure within which the catalytic center is organized (*1*). Introns are classified into several subgroups based on the structural features of their peripheral extensions located outside the core (*1*, *2*). Two large extensions, the P5 extension attached to the P5 region (Fig. 1A) and the P7 extension inserted between the P7 and P3 regions (Fig. 1B), are known to play crucial roles in improving ribozyme activity. The P5 extension, possessing a phylogenetically well-conserved adenosine-rich (A-rich) bulge, is present in almost one-half of the known introns, including subgroups IB1, IB2, IB4, IC1, and IC2 (Fig. 1A) (*1*, *3*). The P7 extension, consisting of one (P7.1) or two (P7.1–7.2) stem-loop structure(s), is observed widely in the remaining subgroups that lack P5 extensions (Fig. 1B) (*1*).

The role of the P5 extension was investigated by employing the *Tetrahymena* group IC1 intron (*4*, *5*). The IC1 intron mutant possessing an A-rich bulge but lacking the P5b and P5c elements in the P5 extension (ΔP5bc mutant, Fig. 1A) is still active, whereas another mutant lacking the A-rich bulge and the P5b and P5c elements (ΔP5a2bc mutant) is only slightly active (*5*). However, the ΔP5a2bc mutant is active if its RNA structure is stabilized by high concentrations of Mg^{2+}, demonstrating that the bulge is primarily responsible for activation (*5*).

The role of the P7 extension was investigated by employing the *sunY* group IA2 intron, which has a P7 extension consisting of a P7.1 and P7.2 hairpin structure (*6*). Under conditions where the *sunY* intron self-splices efficiently, the derivative lacking the P7 extension (ΔP7.1/7.2 mutant) is inactive. However, the ΔP7.1/7.2 mutant is active if its RNA structure is stabilized by high concentrations of Mg^{2+} and monovalent ions, demonstrating that P7.1 and P7.2 are also responsible for activation (*6*). The secondary structures of the two mutant introns, ΔP5a2bc and ΔP7.1/7.2, closely resemble each other.

These observations suggest that P5 and P7 extensions are "modular activator units" that play the same role in activating group I introns (*7*). This hypothesis is supported by the fact that an inactive intron lacking a P5 or P7 extension can be activated by an activator protein called CYT-18, which can act as a functional alternative to P5 and P7 extensions (*7–9*).

The relationship between the CYT-18 protein and the P5 extension is interesting because both CYT-18 and the P5 extension bind to the P4–P6 region in the conserved core when activating the intron (*10*, *11*). The P5 extension can be converted to a *trans*-acting factor like CYT-18 (*12*). The CYT-18 protein and a separately prepared P5 extension RNA can activate a hardly active *Tetrahymena* intron ribozyme whose P5 extension has been removed (the *Tetrahymena* ΔP5abc intron) *in trans* by forming an RNA-Protein (RNP) complex (*9*) and an RNA-RNA complex (*12*), respectively.

In the present study, we investigated the modularity of these RNA activator units. We tested whether, as in the case of CYT-18, the P5 extension activates a group IA intron lacking a P7 extension. In the investigation, the phylogenetically conserved and functionally important regions of the *Tetrahymena* P5 extension were inserted into the P5 region of the mutant *nrdB* group IA2 intron whose P7 extension had been removed.

MATERIALS AND METHODS

Ribozymes—Plasmids encoding a ΔP5bc mutant of the *Tetrahymena* IC1 intron were described previously (*5*). Plasmids encoding derivatives of the *nrdB* IA2 intron and NT-chimeric ribozymes were prepared by PCR from pnrdB, which was constructed by subcloning the *nrdB* intron into a pTZ18U vector and then deleting the open reading frame in the terminal loop of P6 (*13*). All precursor ribozymes employed in this study possess the 5′ exon (29 nucleotides) and lack the last five nucleotides of the introns. The template DNAs for the *in vitro* transcription of these precursor RNAs were generated by 20 cycles of PCR (94°C for 1 minute, 55°C for 1 min, 72°C for 2 minutes) using *Ex Taq* DNA polymerase (Takara Shuzo, Tokyo). For PCR, 1 ng of template plasmid (an appropriate derivative of pnrdB) was used along with the

*To whom correspondence should be addressed. E-mail: tan@kuchem.kyoto-u.ac.jp

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on September 29, 2012

© 2003 The Japanese Biochemical Society.

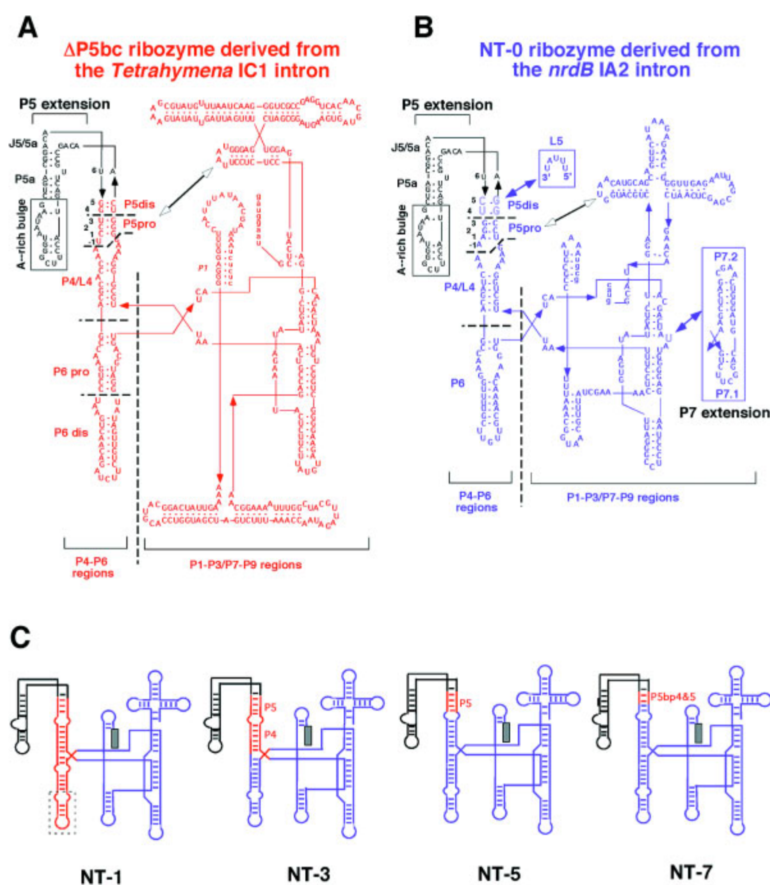

Fig. 1. **Secondary structures of the derivatives of the Tetrahymena group IC1 (A) and nrdB group IA2 (B) introns used in this study.** Dotted lines indicate boundaries used to construct chimeric NT ribozymes. Filled arrowheads indicate 5′-3′ polarity. Solid lines with two open arrowheads indicate the interaction between P5 and L9. In ΔP5bc variants of the *Tetrahymena* ribozyme, P5b and P5c regions (positions 139–179) of the wild-type ribozyme were replaced with a 5′-UUCG-3′ tetraloop. In the NT-0 derivative of the *nrdB* intron, the P7 extension was replaced with a single uridine and the L5 loop was replaced with three base-pairs (P5bp4-6) and the P5 extension. The parenatal *nrdB* intron possesses L5 and P7 extensions and its ΔP7.1/7.2 mutant possesses the L5 but lacks the P7 extension. In the Δ-forms of the NT chimeric ribozymes and the ΔP5a2bc variant of the *Tetrahymena* ribozyme, the A-rich bulge regions were replaced with 5′-UUCG-3′ tetraloops. (C) Schematic representation of the secondary structures of NT-1, NT-3, NT-5 and NT-7 chimeric ribozymes. Gray boxes indicate 5′ exons. In NT-1, dotted box indicates a distal part of P6 deleted to construct the NT-1′ mutant.

following primers: NB5 [5′-TAATACGACTCACTATA-GGCTATTCGTTTTTATGTATCTTTTGCGT-3′ (the promoter sequence for T7 RNA polymerase is underlined)] and NR4 (5′-GTTCGATTGAGCTCGCTAATT-3′).

Preparation of RNAs—All RNAs employed in this study were prepared by transcription *in vitro* with T7 RNA polymerase in the presence of [α-^{32}P]ATP and purified by electrophoresis on 5% polyacrylamide denaturing gels as described (*14*).

Assay of the Guanosine-Dependent 5′ Splice Site Cleavage Reaction—Uniformly ^{32}P-labeled precursor ribozymes possessing their 5′ exons were dissolved in distilled water and then incubated at 80°C for 3 min. After cooling to 50°C, a 5× concentrated reaction buffer (200 mM Tris-Cl, pH 7.5, 50 mM KCl, 50 mM NH_4Cl, and an appropriate concentration of $MgCl_2$) was added. The solution was kept at 50°C for 10 min and then cooled to 37°C. The guanosine-dependent 5′ splice site cleavage reactions were started by adding GTP. The final conditions of the reaction buffer were 40 mM Tris-Cl, pH 7.5, 10 mM KCl, 10 mM NH_4Cl, an appropriate concentration of $MgCl_2$, and 500 μM GTP. The mixture was incubated at 37°C for 1 h and quenched on ice by the addition of an equal volume of stop solution (150 mM EDTA, 70% formamide, and 0.25% of xylene cyanol). For measurement of the time course of ribozyme reactions, aliquots removed at specified times were treated with an equal volume of stop solution, and the products were electrophoresed on 5% polyacrylamide denaturing gels. The gels were dried and the relative intensities of each band were quantitated by using a BioImage Analyzer BAS-2500 (Fuji Film).

RESULTS

Activity of the nrdB Intron without a P7 Extension—A derivative of the *nrdB* group IA2 ribozyme lacking a P7 extension (ΔP7.1/7.2 mutant) (Fig. 1B) was designed on the basis of the previous deletion experiments using its closest homologue, the *sunY* intron (*6*, *13*, *15*). Derivatives of the wild type and the ΔP7.1/7.2 mutant introns containing short 5′ exons and lacking the last five nucleotides of the intron were subjected to the first step of the self-splicing reaction (guanosine dependent 5′ splice site cleavage reaction). The wild-type intron was active (Fig. 2A), whereas the ΔP7.1/7.2 mutant was inactive or hardly active at 5 or 10 mM Mg^{2+}, respectively (Fig. 2A).

The minimal (or phylogenetically conserved) activator elements derived from the P5 extension of the *Tetrahymena* IC1 intron were inserted into the ΔP7.1/7.2 mutant (Fig. 1B). We previously showed that these elements effectively activate the *Tetrahymena* ΔP5abc intron ribozyme as a *cis*-element (the resulting mutant was ΔP5bc, Fig. 1A), but only slightly serve as its *trans*-activator (*12*). Therefore, the effect on the ΔP7.1–7.2 *nrdB* mutant was investigated by employing the elements as a *cis*-element.

To insert the elements, the length of the P5 stem was adjusted to that of the *Tetrahymena* ribozyme because the length of the P5 stem is phylogenetically conserved in

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on September 29, 2012

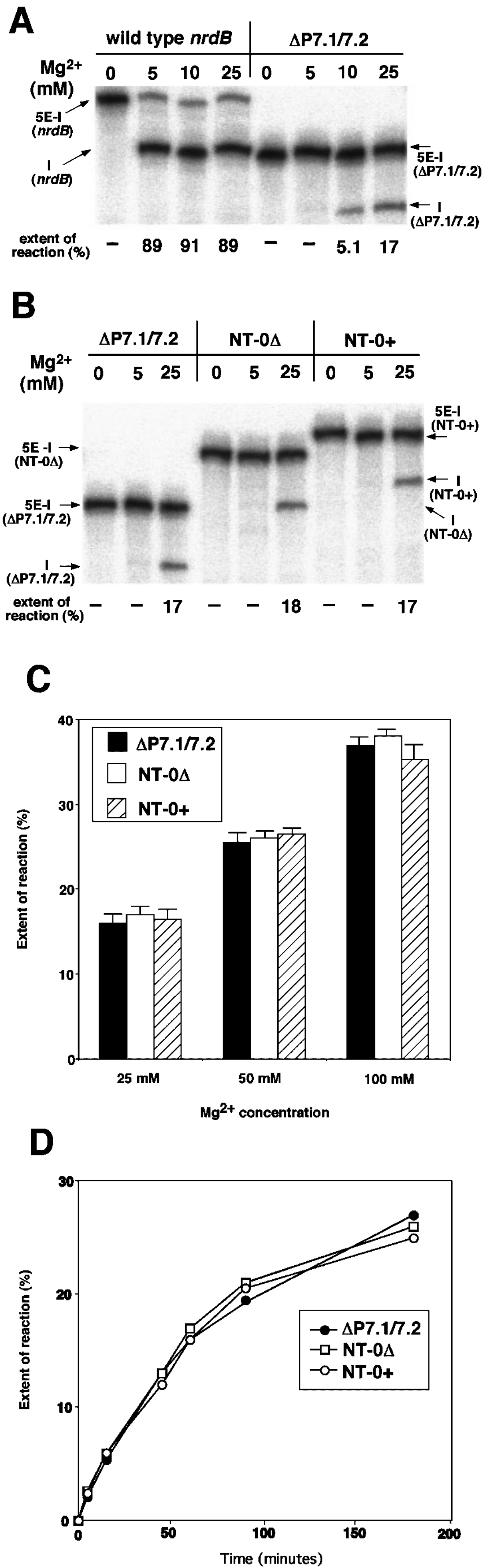

Fig. 2. **Assay of the 5′ splice site cleavage activity of the wild-type nrdB intron, its ΔP7.1/7.2 mutant, and NT-0 chimeric ribozymes.** 5E and I indicate the 5′ exon and intron, respectively. (A) Effects of deletion of the P7 extension from the *nrdB* intron. (B) Effects of insertion of the P5 extension into the mutant *nrdB* intron whose P7 extension was deleted. (C) Effects of Mg^{2+} concentration on the 5′ splice site cleavage activity of ΔP7.1/7.2, NT-0Δ and NT-0+ ribozymes. (D) Time courses of the 5′ splice site cleavage reactions of ΔP7.1/7.2, NT-0Δ and NT-0+ ribozymes in the presence of 25 mM Mg^{2+}.

the group IB and IC introns that naturally possess the P5 extension (*3*). Two base-pairs, 3′U-5′G and 3′C-5′G, were inserted as the fourth and fifth base-pairs of P5 in order to adjust the thermodynamic stability to equal that of the corresponding region of the *Tetrahymena* intron and also in order to avoid setting the consecutive pyrimidines or purines in the P5′s 3′ or 5′ strand. (Note: Since the A and U at P5bp6 are unpaired in the crystal structure of the *Tetrahymena* P4-P6 RNA (*10*), they were employed without alteration.) The resulting mutant is called NT-0+ (Fig. 1B). (hereafter '+' denotes a construct possessing the A-rich bulge). The mutant intron lacking the A-rich bulge is called NT-0Δ. (hereafter 'Δ' denotes a construct lacking the A-rich bulge). NT-0Δ was prepared as the control.

The activities of the two constructs were compared with that of the ΔP7.1/7.2 mutant (Fig. 2). The activities of the three RNAs were almost equal at 25–100 mM Mg^{2+} (Fig. 2, B–D).

Required Elements for Activation by the P5 Extension—Extra-elements in the *Tetrahymena* ribozyme that are required for activation by the P5 extension were added to the NT-0 ribozyme and the activity of the resulting RNA was examined. Accordingly, a chimeric intron with a core composed of the P4–P6 regions of the *Tetrahymena* riboyzme and the P3–P7 regions of the *nrdB* ribozyme was first prepared (Table, see also Fig. 1C) (*16*, *17*). In this construct, the P5 extension was presumed to bind to the P4 region because it is known to interact with this region in the P5 extension-P4–P6 domain RNA isolated from the *Tetrahymena* intron (Fig. 1C). This construct (NT-1+) was active at 25 mM Mg^{2+}, whereas the construct lacking the bulge (NT-1Δ) was inactive at 25 mM Mg^{2+} (Fig. 3A) and also at 50–100 mM Mg^{2+} (data not shown). The activity of NT-1Δ was low presumably because the chimeric core structure was inappropriately tuned compared with the parental intron. However, NT-1+ was active, indicating that it was activated due to the interaction between the two elements from the *Tetrahymena* intron, that is, the P4–P6 domain and the A-rich bulge of the P5 extension.

The distal part of the P6 region (P6dis), which does not participate in the interaction between the A-rich bulge and P4 stem of the *Tetrahymena* intron (*10*), was truncated from NT-1. The resulting derivative (NT-1′) was active in the presence of the bulge (NT-1′+) but inactive without the bulge (NT-1Δ), as anticipated, demonstrating that P6dis is nonessential for activation (Fig. 3A).

Next, the P6 or P4/L4/P5 of NT-1 was replaced with the corresponding region from the *nrdB* intron to construct the chimeric mutants NT-2 and NT-3 (Table 1 and Fig. 1C). NT-2 containing the P4/L4/P5 from *nrdB* and the P6 from *Tetrahymena* was inactive, whereas NT-3 contain-

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on September 29, 2012

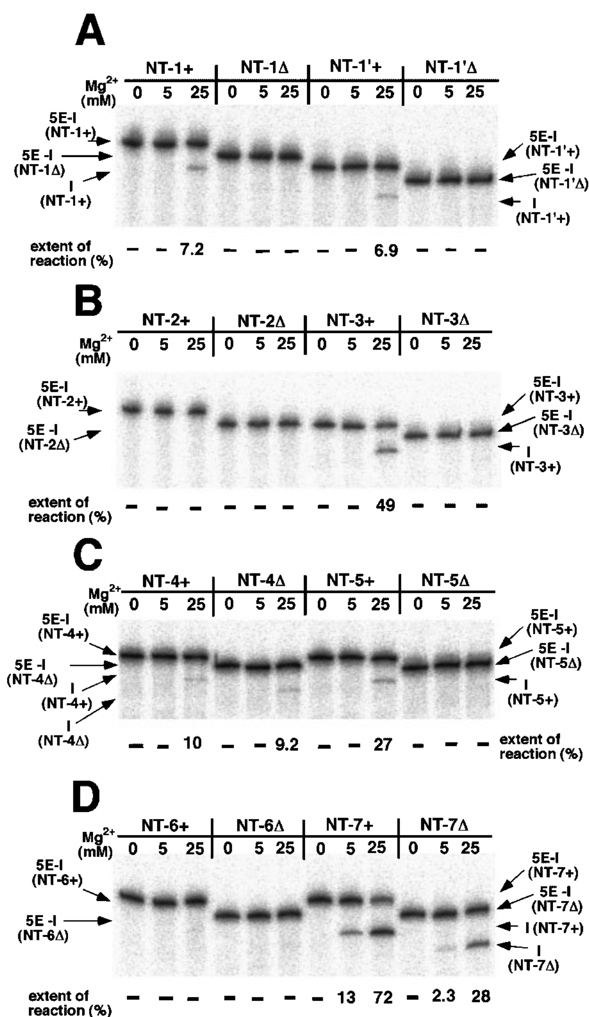

Fig. 3. **Assay of the 5′ splice site cleavage activity of a series of chimeric NT ribozymes.** 5E and I indicate the 5′ exon and intron, respectively. (A) NT-1 and NT-1′, (B) NT-2 and NT-3, (C) NT-4 and NT-5, (D) NT-6 and NT-7.

ing the P4/L4/P5 from *Tetrahymena* and the P6 from *nrdB* was active in the presence of the bulge (Fig. 3B), indicating that P4/L4/P5 is responsible for activation. NT-3+ was more active than NT-1+ according to the quantitative analysis, suggesting that the core of the NT-3 chimera was more optimized for catalysis than that of NT-1. (Note: NT-3Δ was inactive while both NT-0Δ and ΔP7.1/7.2 were active, indicating that the core region of NT-3Δ was less well tuned than those of NT-0Δ and ΔP7.1/7.2.)

Two chimeric introns, NT-4 and NT-5 (Table, Fig. 1C), were designed and constructed from the NT-0 ribozymes. P4/L4 of NT-4 and P5 of NT-5 were derived from the *Tetrahymena* intron, respectively. The molecular design was based on the observations described above (Table). The modest activity of NT-4Δ was not improved by the presence of the bulge (NT-4+) (Fig. 3C, 4, A and B). The activity of NT-5Δ became detectable with the the bulge (NT-5+) (Fig. 3C), demonstrating that P5 from the *Tetrahymena* intron is responsible for activation.

The P5 regions in the chimeric ribozymes can be dissected into distal (P5dis) and proximal (P5pro) halves

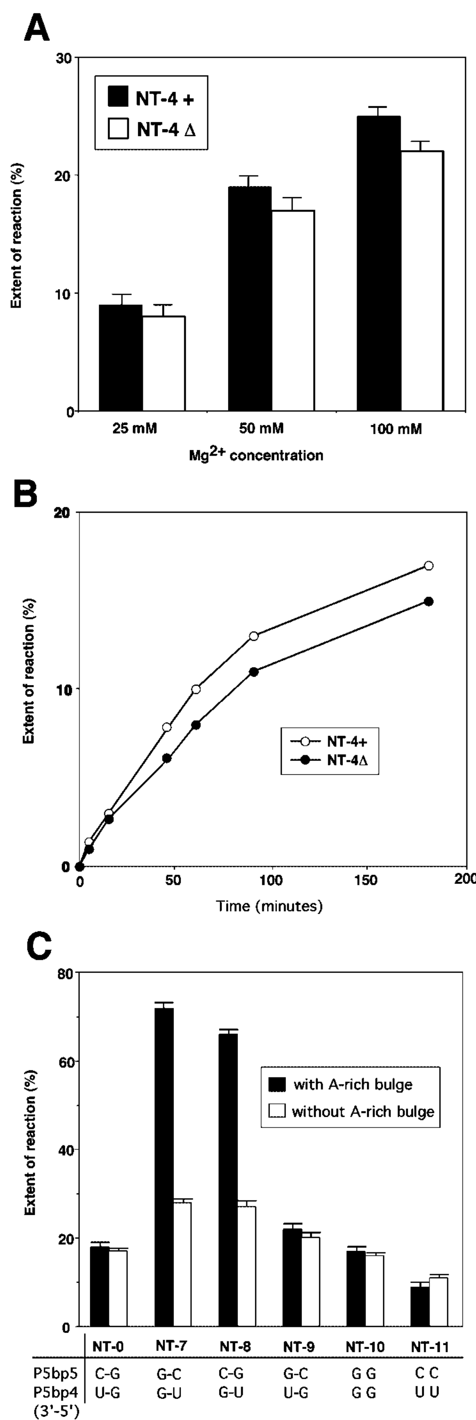

Fig. 4. **P5 region is responsible for the A-rich bulge-dependent activation.** (A) Effects of Mg^{2+} concentration on the 5′ splice site cleavage activity of the NT-4+ and NT-4Δ ribozymes. (B) Time courses of the 5′ splice site cleavage reactions of the NT-4+ and NT-4Δ ribozymes in the presence of 25 mM Mg^{2+}. (C) Effects of base identity at the distal part of P5.

(Fig. 1). No specific sequence requirement or tertiary interaction involving P5dis is known (Fig. 1B). The *nrdB* and *Tetrahymena* introns contain an L9 loop consisting of a GUAA and UAAU tetraloop whose canonical receptors are CC:GG and GG:UC, corresponding to the second and

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on September 29, 2012

Table 1. **Chimeric group I intron ribozymes.**

	P1-P3/P7-P9	P6	P4/L4	P5pro	P5dis	Activation[a]
TetΔP5bc[b]	Tet	Tet	Tet	Tet	Tet	+
NT-0	nrdB	nrdB	nrdB	nrdB	nrdB[c]	–[d]
NT-1	nrdB	Tet	Tet	Tet	Tet	+
NT-1′	nrdB	Tet[e]	Tet	Tet	Tet	+
NT-2	nrdB	Tet	nrdB	nrdB	nrdB[c]	–[d]
NT-3	nrdB	nrdB	Tet	Tet	Tet	+
NT-4	nrdB	nrdB	Tet	nrdB	nrdB[c]	–[d]
NT-5	nrdB	nrdB	nrdB	Tet	Tet	+
NT-6	nrdB	nrdB	nrdB	Tet	nrdB[c]	–[d]
NT-7	nrdB	nrdB	nrdB	nrdB	Tet	+

"Tet" or "nrdB" indicates that the corresponding region was derived from the *Tetrahymena* IC1 (Fig. 1A) or *nrdB* IA2 intron (Fig. 1B), respectively. [a]In the presence of the A-rich bulge activation was observed (+)/not observed (–). [b]Reference *5*. [c]The region is not present in the wild-type *nrdB* intron and 3′U-5′G and 3′C-5′G pairs were introduced artificially as the fourth and fifth base-pairs of P5 to insert the P5 extension (see description of the design of NT-0 in Results). [d]No or very weak activation was observed even by varying the incubation time (15–180 min) and $MgCl_2$ concentration (5–100 mM). [e]A truncated form of the *Tetrahymena* P6 region lacking the distal portion (Fig. 1B).

third base-pairs of P5pro, respectively (Fig. 1). However, the crystal structure of a mutant *Tetrahymen*a ribozyme that lacks the P2/2.1 and P9.1/9.2 regions and possessses a GAAA L9 tetraloop in place of the UAAU loop indicate that the GG:UC receptor also recognizes a GNAA-type tetraloop (*18*). Consistent with the structural data, replacement of the L9 GUAA loop of NT-3+ with a UUCG loop abolished its activity (data not shown), suggesting that the L9 GUAA loop of NT-3+ interacts with the GG: UC pairs in its P5pro region. The identity of a nucleotide at position (-1) of P5pro is phylogenetically variable (*1*).

NT-6+ and -6Δ with the P5pro from *Tetrahymena* and NT-7+ and -7Δ with the P5dis from *Tetrahymena* were prepared to investigate the role of the P5dis and P5pro. (Note: The P5dis of NT-6 consists of non-*Tetrahymena*-type base-pairs; see the description of the design of NT-0.) Both NT-6+ and NT-6Δ were inactive (Fig. 3D). However, NT-7Δ, which was more active than the construct ΔP7.1/7.2, was dramatically improved by the bulge, as seen for NT-7+ (Fig. 3D). The data demonstrate that *Tetrahymena*-type P5dis is responsible for the A-rich bulge dependent activation although its functional importance has not been demonstrated.

To identify the nucleotides required for activation by the A-rich bulge, four chimeric variants (NT-8 , -9, -10, and -11) with a variety of P5bp4 and P5bp5 combinations were prepared and their activities were compared with those of NT-0+ and NT-7+ (Fig. 4C). Only NT-7 and NT-8 containing 3′G-5′U as P5bp4 were activated by the bulge, indicating that bp4 is critical for activation (see table and Fig. 4C).

DISCUSSION

The essential structural elements in the P5 extension that are required for the activation of group I introns are the A-rich bulge, J5/5a, and P5bp4 in the distal part of P5 (P5dis). The importance of P5bp4 was unexpected because no previous analyses had indicated its involvement. In the crystal structure of the P4–P6 RNA of *Tetrahymena*, the stem structure is highly distorted presumably due to the sharp bend on the J5/5a region, although the Watson-Crick (P5bp5) and 3′G-5′U wobble (P5bp4) base-pair are maintained (*10*). Although the mechanism by which P5bp4 contributes to the function of the P5 extension remains to be determined, it seems conceivable that identity P5bp4 is critical for the proper association between the P5 extension and P4–P6 regions. This is because P5bp4 is very close to the hinge region (J5/5a) connecting the P4–P6 regions and the P5 extension. This idea is also supported by the observation that the hairpin structure of the correctly folded *Tetrahymena* P4–P6 domain RNA is severely destablized when its P5dis region is replaced with that of the NT-0 ribozyme (Y. Ikawa, unpublished data). Together with this observation, our results suggest that P5dis has to fulfill a specific function in order to enable the correct association of the P4–P6 regions and the P5 extension. It is proposed that P5dis should be distorted not only to facilitate folding, but also to enable the formation of the fourth and fifth base-pairs. This is suggested by the fact that NT-10 and -11 lacking these pairs are inert to activation. If so, it would appear that a 3′G-5′U (but not 3′U-5′G) wobble pair at P5bp4 plays a key role in activation (Fig. 4). The importance of 3′G-5′U may due to the different twist angles between a 3′G-5′U and its neighbors from that of a 3′U-5′G (*19*) that would lead to a misorientation of J5/5a and the A-rich bulge.

A weakly active *nrdB* group IA2 intron, whose P7 extension was deleted, was activated by inserting the P5 extension from the *Tetrahymena* group IC1 intron, which consists of an A-rich bulge, J5/5a, and P5dis. This result indicates that the A-rich bulge can activate a variety of group I intron subclasses including IA, IB, and IC. A comparison of the results of the present study with those of the previous study on the P5 extension and CYT-18 protein indicates that the functional properties of the P5 extension and the CYT-18 protein closely resemble each other in that they can interact with the conserved P4–P6 region (*10*, *11*), act as *trans*-factors (*9*, *12*), and activate two representative subgroups, IA and IC [(*8*, *9*) and this study].

In summary, the commonality between the RNA and protein component can be regarded as an example of "molecular mimicry" (*20*, *21*) which may reflect the

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on September 29, 2012

molecular evolution from RNAs to RNPs (RNA-protein complexes) (*22–26*).

We thank the members of the Inoue Laboratory for critical reading of the manuscript. This work was supported by Grants-in-Aid for Scientific Research on Priority Areas (T. Inoue) and the Encouragement of Young Scientists (No. 13780554, Y. Ikawa) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Michel, F. and Westhof, E. (1990) Modelling of the three-demensional architecture of group I catalytic introns based on comparative sequence analysis. *J. Mol. Biol.* **216**, 585–610
2. Lehnert, V., Jaeger, L., Michel, F., and Westhof, E. (1996) New loop-loop interactions in self-splicing introns of subgroup IC and ID. *Chem. Biol.* **3**, 993–1009
3. Collins, R.A. (1988) Evidence of natural selection to maintain a functional domain outside of the 'core' in a large subclass of Group I introns. *Nucleic Acids Res.* **16**, 2705–2715
4. Joyce, G.F., van der Horst, G., and Inoue, T. (1989) Catalytic activity is retained in the *Tetrahymena* group I intron despite removal of the large extension of element P5. *Nucleic Acids Res.* **17**, 7879–7889
5. Ikawa, Y., Yoshimura, T., Hara, H., Shiraishi, H., and Inoue, T. (2002) Two conserved structural components, A-rich bulge and P4 X J6/7 base-triples, in activating the group I ribozymes. *Genes. Cells* **7**, 1205–215
6. Doudna, J.A. and Szostak, J.W. (1989) Miniribozymes, small derivatives of the *sunY* intron, are catalytically active. *Mol. Cell. Biol.* **9**, 5480–483
7. Inoue, T. (1994) Time to change partners. *Nature* **370**, 99–100
8. Mohr, G., Zhang, A., Gianelos, J.A., Belfort, M., and Lambowitz, A.M. (1992) The *neurospora* CYT-18 protein suppresses defects in the phage T4 *td* intron by stabilizing the catalytically active structure of the intron core. *Cell* **69**, 483–494
9. Mohr, G., Caprara, M.G., Guo, Q., and Lambowitz, A.M. (1994) A tyrosyl-tRNA synthetase can function similarly to an RNA structure in the *Tetrahymena* ribozyme. *Nature* **370**, 147–150
10. Cate, J.H., Gooding, A.R., Podell, E., Zhou, K., Golden, B.L., Kundrot, C.E., Cech, T.R., and Doudna, J.A. (1996) Crystal structure of a group I ribozyme domain: Principles of RNA packing. *Science* **273**, 1678–1685
11. Caprara, M.G., Myers, C.A., and Lambowitz, A.M. (2001) Interaction of the *Neurospora crassa* mitochondrial tyrosyl-tRNA synthetase (CYT-18 protein) with the group I intron P4–P6 domain. Thermodynamic analysis and the role of metal ions. *J. Mol. Biol.* **308**, 165–190
12. van der Horst, G., Christian, A., and Inoue, T. (1991) Reconstitution of a group I intron self-splicing reaction with an activator RNA. *Proc. Natl Acad. Sci. USA* **88**, 184–188
13. Shub, D.A., Gott, J.M., Xu, M.Q., Lang, B.F., Michel, F., Tomaschewski, J., J.Pedersen-Lane, and Belfort, M. (1988) Structural conservation among three homologous introns of bacteriophage T4 and the group I introns of eukaryotes. *Proc. Natl Acad. Sci. USA* **85**, 1151–1155
14. Milligan, J.F., Groebe, D.R., Witherell, G.W., and Uhlenbeck, O.C. (1987) Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. *Nucleic Acids Res.* **15**, 8783–8798
15. Green, R. and Szostak, J.W. (1992) Selection of a ribozyme that functions as a superior template in a self-copying reaction. *Science* **258**, 1910–1915
16. Tanner, N.K. and Sargueil, B. (1995) Dissecting and analyzing the secondary structure domains of group I introns through the use of chimeric intron constructs. *J. Mol. Biol.* **252**, 583–595
17. Ikawa, Y., Naito, D., Shiraishi, H., and Inoue, T. (2000) Structure-function relationships of two closely related group IC3 intron ribozymes from *Azoarcus* and *Synechococcus* pre-tRNA. *Nucleic Acids Res.* **28**, 3269–3277
18. Golden, B.L., Gooding, A.R., Podell, E.R., and Cech, T.R. (1998) A preorganized active site in the crystal structure of the *Tetrahymena* ribozyme. *Science*, **282**, 259–264
19. Masquida, B. and Westhof, E. (2000) On the wobble G-U and related pairs. *RNA* **6**, 9–15
20. Pedersen, G.N., Nyborg, J., and Clark, B.F. (1999) Macromolecular mimicry of nucleic acid and protein. *IUBMB Life* **48**, 13–18
21. Nakamura, Y. (2001) Molecular mimicry between protein and tRNA. *J. Mol. Evol.* **53**, 282–289
22. Lambowitz, A.M. and Perlman, P.S. (1990) Involvement of aminoacyl-tRNA synthetases and other proteins in group I and group II intron splicing. *Trends. Biochem. Sci.* **15**, 440–444
23. Herschlag, D. (1995) RNA chaperons and the RNA folding problem. *J. Biol. Chem.* **270**, 20871–20874
24. Cech, T.R. and Golden, B.L. (1998) Building a catalytic active site using only RNA. in: The RNA world 2nd edition (Gesteland, G.F., Cech, T.R., and Atkins, J.F., eds) pp. 321–349, Cold Spring Harbor Laboratory Press, New York.
25. Lambowitz, A.M., Caparara, M.G., Zimmerly, S., and Perlman, P.S. (1998) Group I and group II ribozymes as RNPs: Clies to the past to the guides to the future. in: The RNA world 2nd edition (Gesteland, G.F., Cech, T.R., and Atkins, J.F., eds) pp. 451–485, Cold Spring Harbor Laboratory Press, New York.
26. Schimmel, P. and Kelley, S.O. (2000) Exiting an RNA world. *Nat. Struct. Biol.* **7**, 5–7

Downloaded from http://jb.oxfordjournals.org/ at Islamic Azad University on September 29, 2012