J Biochem 130, 251–255 (2001)

A Comparative Study on Two GNRA-Tetraloop Receptors: 11-nt and IC3 Motifs[1]

Yoshiya Ikawa, Kenji Nohmi, Shota Atsumi, Hideaki Shiraishi, and Tan Inoue[2]

Graduate School of Biostudies, Kyoto University, Kyoto 606-8502

Received April 23, 2001; accepted May 15, 2001

Natural RNAs often contain terminal loops consisting of GNRA (N=A, G, C, U; R=A, G) and their receptors, which bind to the loops *via* long-range RNA–RNA interactions. Among several known receptors, two characteristic structural elements have been identified that are termed the 11-nt motif (CCUAAG-UAUGG) and IC3 motif (CCCUAAC-GAGGG). These two motifs that share a similar secondary structure have been shown to exhibit distinctively different binding specificities. The 11-nt motif recognizes a GAAA loop with highest specificity among the known receptors, whereas the IC3 motif distinguishes GAAA from other GNRA loops less stringently than any other receptors. To identify the elements in the receptors that determine the binding specificity, a series of chimeric receptors derived from the two motifs were prepared and their properties were examined. We identified characteristic base-pairs and a particular U residue in the receptors as such elements by means of a gel mobility shift assay that evaluates the degree of the tetraloop–receptor interaction. The relationship between the elements and the specificity is discussed together with a model that describes a possible evolutional linkage between the two receptors.

Key words: evolution, GNRA loop, intron, ribozyme, RNA motif.

The long-range tertiary interactions between GNRA (N=A, G, C, U; R=A, G) tetraloops and their receptors are often found in the three-dimensional structures of catalytic RNAs (*1–3*). It has been shown that GUAA, GUGA, and GAAA loops have receptors that are CC-GG pairs, CU-AG pairs and a characteristic motif consisting of an 11 nucleotide sequence (CCUAAG-UAUGG, termed the 11-nt motif; Fig. 1, right), respectively (*1*, *2*). Recently, a new GNRA receptor motif was identified that consists of a 12 nucleotide sequence (CCCUAAC-GAGGG, termed the IC3 motif; Fig. 1, left) (*4*).

The stability of the binding between a loop and its receptor has been elucidated as follows. The binding between the 11-nt motif and a GAAA loop is strongest among the known loop-receptor interactions (*2*, *4*, *5*). The IC3 motif discriminates four tetraloops, GAAA, GUAA, GUGA, and GAGA, less stringently than other known GNRA loop receptors (*4*). The interaction between the IC3 motif and its corresponding tetraloop is weaker than that of the 11-nt/GAAA set, but stronger than that between the CC-GG/GUAA set or CU-AG/GUGA set (*2*, *4*).

As shown in Fig. 1, the 11-nt and IC3 receptors share two CC-GG base-pairs (termed bp-2 and bp-3), a U-A pair (termed bp-4), and bulged AA nucleotides as highly conserved elements (*4*, *5*). However, there are three distinctive differences between the two receptors, as follows. (i) The IC3 motif possesses an additional C-G pair (termed bp-1), (ii) the IC3 motif lacks an unpaired uridine in the 11-nt motif (termed U-3/4), and (iii) the base-pair (termed bp-5) following the AA bulge is G-U in the 11-nt motif but C-G in the IC3 motif (Fig. 1). The element(s) that determine the binding specificity have not been elucidated yet.

The relationship between the structures of the receptors and their binding specificities is interesting from the viewpoint of molecular evolution. Group IC3 introns generally possess an IC3 motif in their P8 regions, which interacts with a GAAA loop in their P2 regions (*6–8*). However, an exception is an intron from the purple bacterium *Azoarcus* sp. HB72 (the *Azoarcus* ribozyme) (*6*). The *Azoarcus* ribozyme, which is distinctly more thermostable than the standard IC3 intron ribozymes, possesses an 11-nt motif instead of an IC3 motif in its P8 region (*9*). This suggests a possible scenario, *i.e.* that the *Azoarcus* ribozyme diverged from the standard IC3 introns by exchanging its IC3 motif for the 11-nt motif and acquired thermostability.

In the present study, we designed and analyzed a series of chimeric receptors based on the 11-nt and IC3 motifs to investigate the relationship of and difference in the mechanisms of the recognition of GNRA tetraloops by the two motifs. A possible scenario for the evolutional linkage between the two receptors is also discussed.

MATERIALS AND METHODS

Mutant Tetrahymena IC1 Ribozyme and P5abc RNA Constructs—Plasmids encoding derivatives of the L-21 form of ΔP5abc mutant ribozyme or P5abc RNAs were prepared from pL-21ΔP5abc (*10*) or pP5abc (*11*) by PCR (*12*), and verified by sequencing. As templates for *in vitro* transcription, derivatives of pL-21ΔP5abc or pP5abc were digested with *Sca*I or *Sma*I, respectively.

[1] This work was supported by Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan

[2] To whom correspondence should be addressed Fax: +81-75-753-3996, E-mail tan@kuchem.kyoto-u.ac.jp

Preparation of RNAs—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 as described (*13*). For the preparation of uniformly ^{32}P-labeled or unlabeled RNAs, *in vitro* transcription was performed in either the presence or absence of [α-^{32}P]GTP, respectively.

Gel Mobility-Shift Assay—Gel mobility-shift assays with mutants of L-21ΔP5abc ribozyme and P5abc RNA were performed as described (*10*, *14*) on 5% polyacrylamide native gels comprising 50 mM Tris-OAc (pH 7.5) and 7.5, 10 or 12.5 mM $Mg(OAc)_2$, with uniformly ^{32}P-labeled P5abc RNA (<10 nM), or its mutants (<10 nM), and 1 μM unlabeled ΔP5abc intron, or its mutants.

RESULTS

Design of Chimeric Receptor Motifs—To investigate the relationship between the 11-nt and IC3 motifs, we designed chimeric receptors based on three distinctive differences. The latter are (i) an additional C-G pair (bp-1) in the IC3 motif, (ii) an unpaired uridine in the 11-nt motif that is missing in the IC3 motif, and (iii) the identity of a base-pair following the AA-bulge (bp-5), which is G-U and C-G in the 11-nt and IC3 motifs, respectively. On the basis of these differences, we designed six chimeric derivatives as intermediary motifs (Fig. 1).

To compare the affinity and specificity of these receptors for GNRA tetraloops, we employed four loops, *i.e.* GUAA, GAAA,GUGA, and GAGA (these four loops can be represented as GWRA loops where W and R stand for A or U, and A or G, respectively)

Comparison of GNRA–Receptor Interactions by RNA-RNA Gel-Mobility Shift Assay Using the Derivatives of the Tetrahymena IC1 Ribozyme—To compare the tetraloop–receptor affinity, we have developed an assay system based on the gel-shift assay involving a bimolecular ribozyme derived from the *Tetrahymena* ribozyme (Refs. *4* and *10* and Fig 2). The *Tetrahymena* ribozyme has a large P5 extension (P5abc) consisting of P5a, P5b, and P5c regions (Fig. 2, center). A GAAA loop in its P5b region (L5b loop) binds to the 11-nt receptor motif in the P6 domain *via* a long-range interaction that plays a significant role in the constitution of the active form of the ribozyme (gray box in Fig. 2, right) (*10*, *15*). It was demonstrated that a separately prepared P5abc domain RNA and the mutant intron lacking the P5abc domain (ΔP5abc intron, abbreviated as ΔE, Fig. 2, left) can form a stable RNA-RNA complex that functions as a catalytically active ribozyme (Fig. 2, right).

The stability of the P5abc RNA-ΔE complex depends on the GNRA-receptor interaction that involves the loop in the L5b region and the receptor in the P6a region (*4*). Previously we showed that, although complete disruption of the GNRA-receptor interaction inhibits the formation of the RNA-RNA complex in the presence of a low concentration of magnesium ions (5–7.5 mM), a defective or weak interaction replacing the original one can be employed for fixing the complex by increasing the concentration of magnesium ions (Refs. *4* and *10* and see also Fig. 3, A, E, and I). In other words, we found that the minimal concentration of magnesium ions required for the formation of a stable RNA-RNA complex is in inverse proportion to the binding affinity between the loop and the receptor. To evaluate the stability of the tetraloop-receptor interaction, we performed RNA-RNA gel-mobility shift assaying of the derivatives of the bimolecular ribozyme by varying the concentration of magnesium ions.

The gel-shift assays were performed with derivatives of ΔE and P5abc RNA (Fig. 3). The 11-nt motif in the ΔP5abc intron was replaced with the IC3 motif, or the chimeric motifs (Fig. 2, left) A GAAA loop in P5abc RNA was replaced with GUAA, GUGA, or GAGA (Fig. 2, center). We prepared 32 possible combinations of the P5abc RNA-ΔE

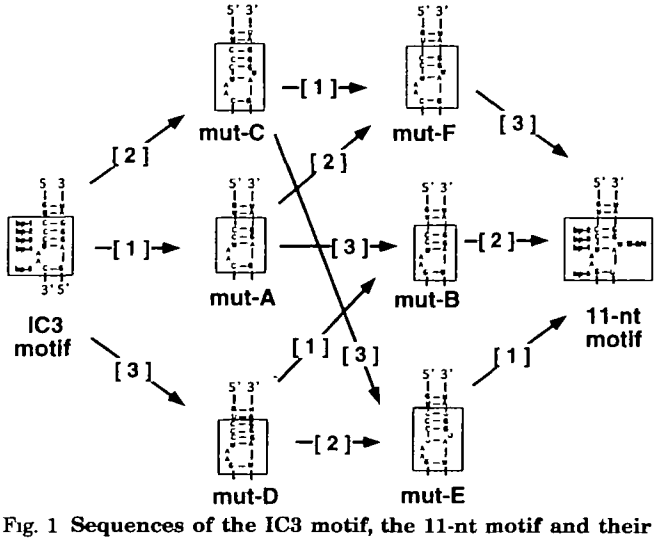

Fig. 1 **Sequences of the IC3 motif, the 11-nt motif and their chimeric motifs.** Schematically, the IC3 motif can be converted to the 11-nt motif *via* three mutations as follows [1] deletion of a C-G pair at bp-1, [2] insertion of a uridine at position-3/4, and [3] replacement of a C-G pair at bp-5 with a G-U pair.

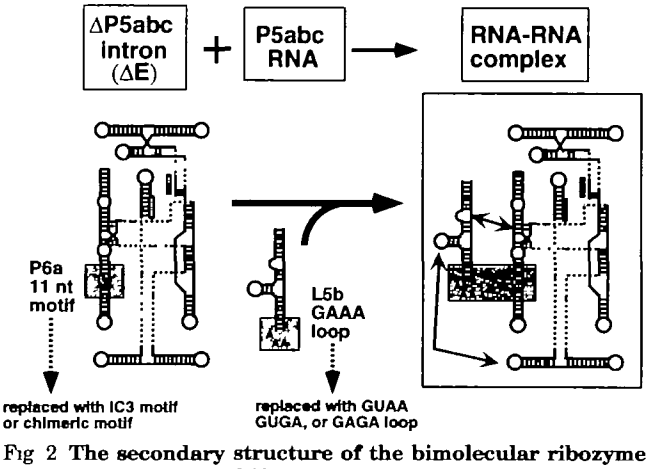

Fig 2 **The secondary structure of the bimolecular ribozyme employed for the gel-shift assay.** L-21 ribozyme derived from the group IC1 intron from *Tetrahymena thermophila* was dissected into two pieces, the ΔP5abc intron (left) and P5abc RNA (center) The two RNAs from a stable complex *via* multiple tertiary interactions (right) A bold line with two black arrowheads indicates the interaction between the L5b region in the P5abc RNA and the P6a region in the ΔP5abc intron (*10*) Gray boxes indicate the 11-nt receptor motif in the P6a region (left), a GAAA loop in the L5b region (center), or their complex (right). The L5b or P6a region was replaced with three tetraloops (GUAA, GUGA, or GAGA) or their receptors, respectively, as shown in Fig. 1

complex that are represented as a matrix of eight ΔEs and four P5abc RNAs.

In the presence of 5 mM Mg^{2+}, no complex formation was observed except for the combination of GAAA in P5b [represented as P5(GAAA)] and the 11-nt motif [represented as ΔE(11nt)] (data not shown). This indicates that the affinity between the GAAA loop and the 11-nt motif is strongest among the combinations employed in this study. In the presence of 10 mM Mg^{2+}, the 11-nt motif also formed a complex with GAGA or GUAA in P5b (Fig. 3, H and F). When the relative amount of unbound P5abc RNA was compared with that contained in the bimolecular complex, it was found that the 11-nt motif binds to a GAGA loop more strongly than a GUAA loop (Fig. 3, H and F). On the other hand, it formed no complex with GUGA in P5b even in the presence of 12.5 mM Mg^{2+} (Fig. 3K) Consistent with the previous results of kinetic analysis, the present results show that the 11-nt motif recognizes the tetraloops in the order GAAA>GAGA>GUAA, but it cannot recognize a GUGA loop under the same conditions (*5*, *16*, *17*)

A mutant ΔE having the IC3 motif [ΔE(IC3)] forms a stable RNA-RNA complex with P5(GAAA) or P5(GUAA) in the presence of 7.5 mM Mg^{2+} (Fig. 3, A and B). ΔE(IC3) also forms a complex with P5(GUGA) or P5(GAGA) in the presence of 10 mM Mg^{2+} (Fig. 3, G and H).The results indicate that the IC3 motif discriminates these four GWRA loops less stringently than the 11-nt motif, as previously reported (*4*).

Among the mutant ΔEs bearing six chimeric motifs, two variants having the mut-F motif [ΔE(m-F)] and the mut-D

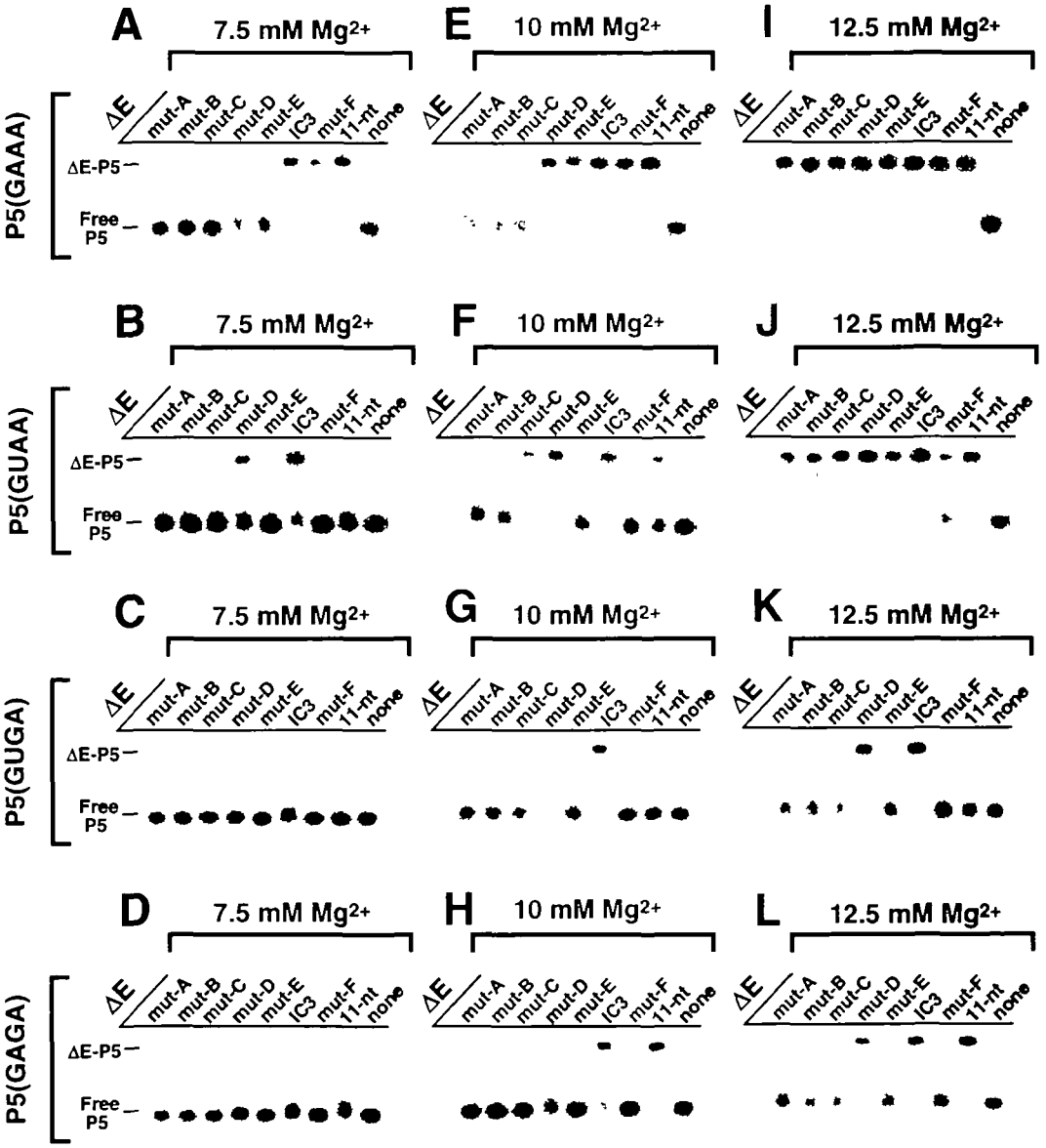

Fig. 3 **RNA-RNA gel-mobility shift assaying of ^{32}P-labeled P5abc RNAs (<10 nM, abbreviated as P5) and the unlabeled L-21 form of ΔP5abc intron ribozymes (1 μM, abbreviated as ΔE).** In the figure, "free P5" and "ΔE-P5" indicate P5abc RNA not associated to the ΔP5abc intron and the bimolecular complex consisting of P5abc RNA and the ΔP5abc intron, respectively. A–D Gel-mobility shift assay in the presence of 7.5 mM Mg^{2+} E–H: Gel-mobility shift assay in the presence of 10 mM Mg^{2+}. I–L: Gel-mobility shift assay in the presence of 12 5 mM Mg^{2+}

motif [ΔE(m-D)] exhibited considerable affinity to a GAAA or GWAA loop, respectively. ΔE(m-F) formed a complex with P5(GAAA) in the presence of 7.5 mM Mg^{2+}, albeit that the complex formation was somewhat less than that of ΔE(IC3) or ΔE(11-nt) (Fig. 3A). On the other hand, ΔE(m-F) formed a very small amount of complex or no complex with P5(GUAA) or P5(GAGA), respectively, even in the presence of 10 mM Mg^{2+}, with which the 11-nt motif can form a complex with P5(GUAA) or P5(GAGA) (Fig. 3, F and H). Moreover, even in the presence of 12.5 mM Mg^{2+}, ΔE(m-F) forms a complex with P5(GUAA) only modestly (Fig. 3J), or no complex with P5(GUGA) or P5(GAGA) (Fig. 3, K and L). The results suggest that the mut-F motif recognizes a GAAA loop in a highly specific manner. The strong preference for a GAAA loop is similar to the feature of the 11-nt receptor motif, but the mut-F motif is unable to recognize a GAGA loop, which can be done moderately by the 11-nt motif (Fig. 3, H and L).

ΔE(m-D) formed a complex with P5(GAAA) or P5(GUAA) in the presence of 7.5 mM Mg^{2+} (Fig. 3, A and B), indicating that the mut-D/GAAA combination is as efficient as the mut-F/GAAA one (Fig. 3A), and the mut-D/GUAA one was also as efficient as the IC3/GUAA one (Fig. 3B). A complex of ΔE(m-D) with P5(GUGA) or P5(GAGA) became detectable with 10 mM Mg^{2+} (Fig. 3, G and H), and became stable on an increase in the concentration of Mg^{2+} to 12.5 mM (Fig. 3, K and L). This indicates that the mut-D motif acts as a receptor for GUGA and GAGA loops more effectively than other chimeric motifs. This is similar to the specificity of the IC3 motif, which discriminates the four GWRA tetraloops weakly, although the affinity of the mut-D motif to the four tetraloops is slightly weaker than that of the IC3 motif.

The remaining four chimeric motifs (the mut-A, mut-B, mut-C, or, mut-E motifs) were less efficient tetraloop receptors than the mut-F or mut-D motif. They are incapable of forming stable complexes in the presence of 7.5 mM Mg^{2+}, with which mut-D motif/GWAA or mut-F motif/GAAA can form a stable complex (Fig. 3, A–D). Among the four ineffective motifs, mut-E functions moderately better than the rest. The mut-E motif/GAAA combination forms a complex in the presence of 10 mM Mg^{2+} (Fig. 3E). However, the affinity of mut-E motif/GAAA is weaker than that of mut-F/GAAA, mut-D/GAAA or mut-D/GUAA (Fig. 3, A and B).

DISCUSSION

The Nucleotides That Determine the Nature of the 11-nt and IC3 Motifs—The RNA-RNA gel-mobility shift assay showed that, among the six chimeric variants, only two (mut-F and mut-D) exhibited considerable affinity to GAAA or GWAA loops, respectively (Fig. 3, A and B). In general, the natures of the mut-F and mut-D motifs resemble those of the 11-nt and IC3 motifs, respectively, indicating that alternation of bp-5 (C-G or G-U) does not significantly modify the nature of the parental motifs (Fig. 1).

However, alternation for the binding specificity was observed for mut-F with a mutation at bp-5. The 11-nt motif, which is known to prefer a GAAA loop, can also bind to a GUAA or GAGA loop with modest affinity (*5*). However, the mut-F motif clearly prefers to bind to a GAAA loop compared with a GUAA, GUGA, or GAGA loop. The motif recognizes the loop with strong affinity comparable to that of the IC3 motif, although it is weaker than that between the 11-nt motif and GAAA.

The secondary structure of the mut-F motif is closely related to that of the 11-nt motif. The difference between the two motifs is attributed to a fifth base-pair (bp-5), which is a G-U and C-G pair in the 11-nt motif and mut-F, respectively (Fig. 1). Previous analysis indicated that bp-5 in the 11-nt motif should preferably be a non Watson-Crick base-pair such as G-U, A-C, or Y-Y (where Y indicates a pyrimidine nucleotide) for recognizing the GAAA loop (*5*). This suggests that the local rearrangement caused by the replacement of a G-U to C-G pair at bp-5 is responsible for the specificity of mut-F and also for weakening of the affinity between the receptor and the loop.

In contrast to bp-5, the first base-pair (bp-1) in the IC3 motif and the unpaired U at position-3/4 in the 11-nt motif play critical roles in determining the nature of the receptors. Deletion of bp-1 from the IC3 motif (the resulting mutant being the mut-A motif) reduces the capability of the receptor (Fig. 3). This result indicates that the base-moieties and/or the ribose-phosphate backbones of bp-1 are important for the function of the IC3 motif.

A mutant with a C-G to G-C base-substitution at bp-1 was examined previously to investigate the role of the base-moieties of bp-1 in the IC3 motif (*4*). The mutant modestly lost the affinity to the GUGA loop (*ca.* 60% activity compared to that of the IC3 motif) but was still capable of interacting with either a GAAA or GUAA loop with affinity comparable to that of IC3, suggesting that the identity of the base-moiety of bp-1 is not critical for the IC3 motif (*4*). Compared with the result for mut-A in this study, the data suggest that the ribose-phosphate backbones of bp-1 are more important, although it remains to be determined whether the backbones act passively as a spacer, or positively as an element interacting directly with the tetraloop.

Likewise, deletion of unpaired U-3/4 from the 11-nt motif (the resulting mutant being the mut-B motif) significantly abolished the receptor function of the 11-nt motif (Fig. 3). In the 11-nt motif, bp-2, -3, and -4 form a helical structure, whose conformation is slightly distorted from a canonical A-type RNA duplex because the U-A pair at bp-4 is of a non Watson-Crick type due to a U inserted between bp-3 and bp-4 (*3*). Such a distorted duplex having a non-Watson-Crick U-A pair appears to force the bulged AA nucleotides to form a A-A platform structure, which is a critical element for specific and strong recognition of a GAAA loop (*3*). We think that the deletion of U-3/4 could reduce the ability of the receptor due to this effect.

In the crystal structure of the 11-nt motif complexed with a GAAA loop, U-3/4 is flipped out from the stem structure consisting of bp-2, -3, and -4 (*3*). U-3/4 appears not to participate in a direct interaction between the motif and the GAAA loop, suggesting that the base identity of U-3/4 is not critical. The base substitution at U-3/4 has no significant influence on the nature of the receptor, according to the results of a mutagenic and phylogenetic study (*5*), suggesting that a ribose-phosphate backbone at U-3/4 affects the local conformation at the stem structure.

It is interesting to build a molecular model of the IC3 motif complexed with a GWRA loop. However, such construction is difficult because the conformation of the receptor motif presumably changes on binding of the tetraloop in an unpredictable manner (*18*)

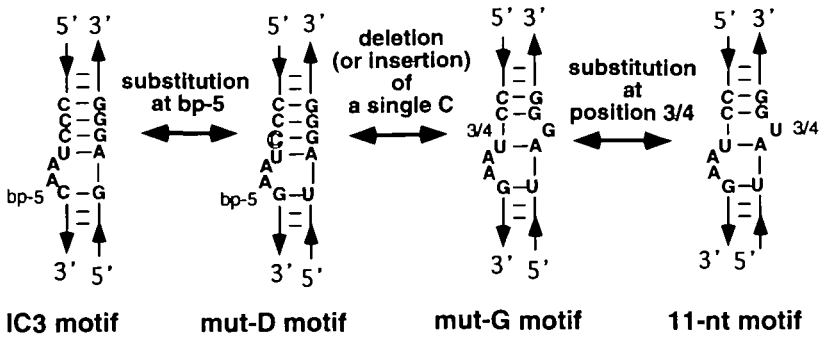

Fig 4 **The structure of the mut-G motif, and a possible relationship between the IC3 motif and the 11-nt motif as to molecular evolution.**

To elucidate the three-dimensional structure of the IC3 motif complexed with GAAA or other tetraloops, we are currently attempting the crystallization of the loop-IC3 receptor complex as a part of P4-P6 RNA. The P4-P6 RNA, which is a portion of the *Tetrahymena* intron, has been crystalized and is known to form a defined tertiary structure including the GAAA loop X 11-nt motif interaction (*3, 19, 20*). We have now prepared a variant P4-P6 RNA whose 11-nt motif is replaced with the IC3 motif.

A Hypothetical Evolutional Pathway—The structural resemblance between the IC3 and 11-nt motifs implies their evolutional relationship. If the IC3 motif was converted to the 11-nt motif (or *vise versa*) during the evolution of a functional RNA, it seems reasonable to assume that its intermediate receptors possess similar affinity when compared with the original receptor. mut-F and mut-D can be regarded as such candidates for the intermediates.

The structures of mut-F and mut-D, however, are not closely related enough for their conversion into each other with a single mutation (see Fig. 1), suggesting that another intermediate motif is needed to explain the evolutionary pathway. As a candidate of such an intermediate, we found a variant form of the 11-nt motif (termed the mut-G motif) based on the results of phylogenetical and structural analysis of the 11-nt motif (Ref. *5*, Fig. 4) The X-ray crystal structure of the 11-nt motif suggests that the base-moiety of U-3/4 does not play a critical role (*3*). Consistent with this observation, phylogenetic comparison indicated the presence of at least seven examples of variant 11-nt motifs whose U-3/4 is substituted by a guanosine (*5*), suggesting that the nature of mut-G is similar to that of the 11-nt motif. Because a single deletion of cytidine at bp-3 can convert the mut-D motif into the mut-G motif in this model (Fig. 4), it is tempting to propose that the two motifs were converted *via* mut-D and mut-G, as shown in Fig 4.

We wish to thank members of Tan Inoue Laboratory for critical reading of the manuscript.

REFERENCES

1 Jaeger, L, Michel, F, and Westhof, E. (1994) Involvement of a GNRA tetraloop in long-range RNA tertiary interactions. *J. Mol. Biol.* **236**, 1271–1276
2 Costa, M and Michel, F (1995) Frequent use of the same tertiary motif by self-folding RNAs. *EMBO J* **14**, 1276–1285
3. 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
4 Ikawa, Y, Naito, D, Aono, N, Shiraishi, H, and Inoue, T (1999) A conserved motif in group IC3 introns is a new class of GNRA receptor *Nucleic Acids Res.* **27**, 1859–1865
5. Costa, M and Michel, F (1997) Rules for RNA recognition of GNRA tetraloops deduced by *in vitro* selection comparison with *in vivo* evolution *EMBO J* **16**, 3289–3302
6 Reinhold-Hurek, B and Shub, D.A. (1992) Self-splicing introns in tRNA genes of widely divergent bacteria *Nature* **357**, 173–176
7 Sugita, M, Luo, L, Ohta, M, Itadani, H., Matsubayashi, T, and Sugiura, M (1995) Genes encoding the group I intron-containing $tRNA^{Leu}$ and Subunit L of NADH dehydrogenase from the cyanobacterium *Synechococcus* PCC6301 *DNA Res.* **2**, 71–76
8. Paquin, B, Kathe, S T, Nierzwicki-Bauer, S.A., and Shub, D.A. (1997) Origin and evolution of group I introns in cyanobacterial tRNA genes *J Bacteriol.* **179**, 6798–6806
9 Tanner, M. and Cech, T (1996) Activity and thermostability of the small self-splicing group I intron in the pre-$tRNA^{Ile}$ of the purple bacterium *Azoarcus RNA* **2**, 74–83
10 Naito, Y, Shiraishi, H, and Inoue, T (1998) P5abc of the *Tetrahymena* ribozyme consists of three functionally independent elements *RNA* **4**, 837–846
11 Williams, K.P., Fujimoto, D N, and Inoue, T (1992) A region of group I introns that contains universally conserved residues but is not essential for self-splicing. *Proc. Natl Acad Sci. USA* **89**, 10400–10404
12 Imai, Y, Matsushima, Y., Sugimura, T., and Terada, M. (1991) A simple and rapid method for generating a deletion by PCR. *Nucleic Acids Res.* **19**, 2785
13 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
14 Ikawa, Y, Shiraishi, H., and Inoue, T. (1996) Characterization of the newly constructed domains that replace P5abc within the *Tetrahymena* ribozyme. *FEBS Lett* **394**, 5–8
15 Cate, J H, Gooding, A.R., Podell, E, Zhou, K., Golden, B L., Szewczak, A.A., Kundrot, C.E., Cech, T.R., and Doudna, J.A. (1996) RNA tertiary structure mediation by adenosine platforms *Science* **273**, 1696–1699
16. Murphy, F.L and Cech, T R. (1994) GAAA tetraloop and conserved bulge stabilize tertiary structure of a group I intron domain. *J Mol Biol.* **236**, 49–63
17. Treiber, D.K. and Williamson, J R (2001) Concerted kinetic folding of a multidomain ribozyme with a disrupted loop-receptor interaction. *J. Mol. Biol.* **305**, 11–21
18 Butcher, S.E, Dieckmann, T, and Feigon, J. (1997) Solution structure of a GAAA tetraloop receptor RNA. *EMBO J* **16**, 7490–7499
19 Juneau, K. and Cech, T.R. (1999) In vitro selection of RNAs with increased tertiary structure stability. *RNA* **5**, 1119–1129
20. Juneau, K., Podell, E., Harrington, D.J., and Cech, T.R. (2001) Structural basis of the enhanced stability of a mutant ribozyme domain and a detailed view of RNA-solvent interactions. *Structure* **9**, 221–231