13. Based on the t test or Wilcoxon signed ranks test ($\alpha < 0.05$).
14. M. G. Hadfield, thesis, Stanford University (1967). Hadfield studied *Serpulorbis squamigerous* at HMS during the early 1960s but relied on collections from southern California to obtain specimens.
15. Least squares linear regression of the change in abundance (y = 1993 abundance − 1932 abundance, x = tidal height in feet) of *Calliostoma ligatum* indicated a significant shift toward lower tidal zones ($y = 3.4 - 3.7x$; $r^2 = 0.48$; $P < 0.0001$).
16. The logarithm of the ratio of mean abundance between 1993 and 1932 for species grouped by range categories differed significantly among range categories [one-way ANOVA; $F = 6.8$, df = 2, $P < 0.005$ (all species); $F = 6.2$, df = 2, $P < 0.01$ (only species with significant abundance changes)]. Results of multiple comparisons (with the Bonferroni probability level adjustment) indicated that the mean ratio of abundance for southern species was greater ($P < 0.025$) than that for cosmopolitan and northern species (Fig. 1B), either for comparisons with all species included or only for species exhibiting a significant change in abundance.
17. P. W. Glynn, *Beaufortia* **12**, 1 (1965); thesis, Stanford University (1963).
18. Shoreline temperatures were determined each morning (0600 to 0900 hours) at HMS from a bucket sample dipped from the shore.
19. Slopes for minimum, mean, and maximum ST for the period 1921 to 1993 are 0.011°, 0.013°, and 0.021°C per year, respectively. A test of homogeneity of slopes between minimum and maximum ST was marginally insignificant ($F = 2.59$, $P \approx 0.11$).
20. A. Bakun, *Science* **247**, 198 (1990); L. K. Rosenfeld, F. B. Schwing, N. Garfield, D. E. Tracy, *Cont. Shelf Res.* **14**, 931 (1994).
21. P. D. Jones, T. M. L. Widley, P. B. Wright, *Nature* **322**, 430 (1986); P. D. Jones *et al.*, *J. Climate Appl. Meteorol.* **25**, 161 (1986).
22. G. A. Jacobs *et al.*, *Nature* **370**, 363 (1994); M. J. McPhaden, *ibid.*, p. 326.
23. W. H. Quinn, V. T. Neal, S. E. Antunez de Mayolo, *J. Geophys. Res.* **92**, 14449 (1987). Strong to very strong ENSO events occurred in 1925 to 1927, 1933, and 1982 to 1983. Weak to moderate events occurred in 1930 to 1932 and 1986 to 1987. A moderate ENSO event also occurred in 1991 to 1993 (F. Chavez, personal communication).
24. J. A. Estes and J. F. Palmisano, *Science* **185**, 1058 (1974); P. K. Dayton, *U.S. Dep. Commer. Fish. Bull.* **73**, 230 (1975).
25. J. H. McLean, *Biol. Bull. (Woods Hole, Mass.)* **122**, 95 (1962).
26. L. F. Lowry and J. S. Pearse, *Mar. Biol.* **23**, 213 (1973).
27. The numerical density of *Mytilus californianus* in mussel beds exceeds 1900 m^{-2} (*7*) but averaged 6.2 (SD = 11.7) per plot in resurveyed plots during 1931 to 1933 and decreased to 1.5 (SD = 6.2) in 1993 to 1994 ($P < 0.02$). Thus, mussels were, and remain, a minor component of resurveyed plots.
28. A. W. Ebeling, D. R. Laur, R. J. Rowley, *Mar. Biol.* **84**, 287 (1985); H. L. Andrews, *Ecology* **26**, 24 (1945); P. K. Dayton, M. J. Tegner, P. E. Parnell, P. B. Edwards, *Ecol. Monogr.* **62**, 421 (1992); J. Watanabe, in preparation. Percentage cover of a 1-ha section of the HMS kelp bed varied from 4 to 78% over a 6-year period.
29. T. Hahn and M. Denny, *Mar. Ecol. Prog. Ser.* **53**, 1 (1989); D. R. Lindberg, K. I. Warheit, J. A. Estes, *ibid.* **39**, 105 (1987).
30. Human predation effects on intertidal biota are known from Chile; C. A. Moreno, J. P. Sutherland, H. F. Jara, *Oikos* **42**, 155 (1984).
31. M. M. Littler, in *The California Islands: Proceedings of a Multidisciplinary Symposium*, D. M. Power, Ed. (Santa Barbara Museum of Natural History, Santa Barbara, CA, 1980), pp. 265–306; R. R. Seapy and M. M. Littler, *ibid.*, pp. 307–324; S. N. Murray, M. M. Littler, I. A. Abbott, *ibid.*, pp. 325–340; C. D. McQuaid and G. M. Branch, *Mar. Ecol. Prog. Ser.* **19**, 145 (1984). See also R. F. Scagel in (*11*).
32. S. E. Emerson and J. B. Zedler, *Mar. Biol.* **44**, 315 (1978); F. C. Gunnill, *Mar. Ecol. Prog. Ser.* **3**, 231 (1980).
33. We thank J. T. Harvey, D. C. Reed, J. Watanabe, M. Yoklavich, and four reviewers for advice and comments on the manuscript.

28 February 1994; accepted 1 November 1994

Minor Groove Recognition of the Conserved G·U Pair at the *Tetrahymena* Ribozyme Reaction Site

Scott A. Strobel and Thomas R. Cech*

The guanine·uracil (G·U) base pair that helps to define the 5′-splice site of group I introns is phylogenetically highly conserved. In such a wobble base pair, G makes two hydrogen bonds with U in a geometry shifted from that of a canonical Watson-Crick pair. The contribution made by individual functional groups of the G·U pair in the context of the *Tetrahymena* ribozyme was examined by replacement of the G·U pair with synthetic base pairs that maintain a wobble configuration, but that systematically alter functional groups in the major and minor grooves of the duplex. The substitutions demonstrate that the exocyclic amine of G, when presented on the minor groove surface by the wobble base pair conformation, contributes substantially (2 kilocalories·mole^{-1}) to binding by making a tertiary interaction with the ribozyme active site. It contributes additionally to transition state stabilization. The ribozyme active site also makes tertiary contacts with a tripod of 2′-hydroxyls on the minor groove surface of the splice site helix. This suggests that the ribozyme binds the duplex primarily in the minor groove. The alanyl aminoacyl transfer RNA (tRNA) synthetase recognizes the exocyclic amine of an invariant G·U pair and contacts a similar array of 2′-hydroxyls when binding the $tRNA^{Ala}$ acceptor stem, providing an unanticipated parallel between protein-RNA and RNA-RNA interactions.

Docking of the splice site helix of the *Tetrahymena* group I intron into the RNA active site is a model system for studying helix packing in the formation of RNA tertiary structure. The splice site helix (also called the P1 helix) contains the 5′-exon paired to the internal guide sequence (IGS) of the intron (*1*, *2*) (Fig. 1). Following duplex formation, the helix is docked into the ribozyme active site (*3*, *4*) where the 5′-exon–intron boundary is cleaved by nucleophilic attack by exogenous guanosine (*5*). The 5′-splice site is defined by a U at the end of the 5′-exon; the U is paired with a G in the IGS (Fig. 1). While there is a general requirement for sequence complementarity between the 5′-exon and the IGS (*1*, *2*, *6*, *7*), the only specific sequence requirement is at the 5′-splice site where the G·U pair is highly conserved (*7*). The evolutionary conservation of this pair provided the first indication that it plays an important functional role for the ribozyme.

Replacement of the G·U pair with other combinations of natural bases has demonstrated that no other pair is as reactive (*8*, *9*), though an A·C pair retains a portion of the wild-type activity (*8*). Because a protonated form of the A·C pair can be drawn in a wobble configuration, it was postulated that the shape rather than specific functional groups of the bases is important for ribozyme activity (*8*). To further address the contribution of the G·U pair to RNA folding, we have undertaken a systematic examination of the functional groups within the G·U pair. We substituted synthetic bases for the G·U pair (Fig. 2) in the context of the well-defined *Tetrahymena* L-21 *ScaI* ribozyme (*3*, *10*–*13*), a form of the group I intron that cleaves

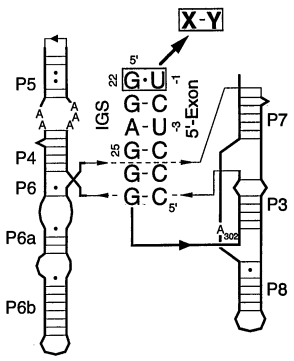

Fig. 1. Diagram of the splice site helix (capital letters) and catalytic core (solid lines) of the group I intron including the position numbers of specific bases within the helix (*31*). The phylogenetically conserved G·U base pair (box) is the site of base substitution, X-Y.

Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309–0215, USA.

*To whom correspondence should be addressed.

RNA oligonucleotide substrates (5′-splice site analogs) in trans.

Full-length ribozymes were generated with diaminopurine riboside, inosine, purine riboside, adenosine (A), or 2′-deoxy-7-deazaguanosine (d7dG) in place of the conserved G at position 22 (X in Fig. 1) (*14–16*). A truncated T7 RNA polymerase transcript (L-38 *ScaI*) was joined to a synthetic IGS oligonucleotide (17-nucleotide oligomer) containing the nucleoside substitution with the use of T4 DNA ligase (*13, 17, 18*). Analogs of the 5′-exon (5′-GGCCCUCY-3′) with C, 5-methyl-uridine or 5-methyl-isocytidine in place of the conserved U (Y in Fig. 1) were prepared by solid-phase oligoribonucleotide synthesis (*16*). Previous work has demonstrated that a ligated ribozyme containing the conserved G was as active as a ribozyme made entirely by transcription (*13*). Therefore, any difference in binding can be attributed to the modifications at the G·U pair.

The stability of the interaction between the 5′-exon and the ribozyme was measured by a gel mobility–shift assay with a radiolabeled 5′-exon analog (*11, 19*). Two factors contribute to binding: (i) the strength of the base pairing interaction between the 5′-exon and the IGS, and (ii) the formation of tertiary contacts between the splice site helix and the ribozyme active site. Modification of the G·U pair affects both of these terms. To assess the contributions of the two effects, we measured duplex stabilities (which are independent of the ribozyme) for pairing of the 5′-exon (GGCCCUCY) with an oligonucleotide IGS analog (XGAGGG) (*14*). By subtracting the base pairing contribution from the total effect on 5′-exon binding to the ribozyme, we calculated modulations in tertiary interaction energy (Table 1).

Substitution of the critical G with inosine (I), a guanosine analog lacking the N_2 exocyclic amine, reduced the equilibrium dissociation constant for binding of the 5′-exon analog GGCCCUCU (Table 1) by a factor of 28 (2.0 kcal·mol^{-1}). In a G·U wobble base pair, the exocyclic amine of G does not participate in a direct hydrogen bonding interaction with U (Fig. 2) (*20, 21*), and the base-pairing stabilities of the G·U and I·U pairs are equivalent in the sequence context of the splice site duplex (Table 1). Thus, we suggest that the 2.0 kcal·mol^{-1} reduction in binding energy is due to a loss of a tertiary interaction between the exocyclic amine and the catalytic core.

We tested the assignment of this energy to a specific contact rather than a global conformational change, such as undocking of the splice site helix from the ribozyme core, by monitoring the energetic contribution of the 2′-OH at position −3U (Fig. 3). When the duplex is docked into the active site, the 2′-OH of −3U forms a tertiary interaction with a conserved base in the catalytic core of the ribozyme (A_{302} in Fig. 1) (*12*). This interaction contributes ~1.4 kcal·mol^{-1} to binding (see the G·U column in Fig. 3). No favorable contribution is observed if the docking equilibrium is shifted toward an open complex (*13, 19*). The observation that the 2′-OH at −3U continues to make a strong tertiary contact in the context of an I·U pair (Fig. 3) suggests that the splice site helix is in a closed complex that has been weakened by the loss of a tertiary contact to the exocyclic amine.

While the effect of the I·U substitution is suggestive of a direct contact with the N_2 exocyclic amine, we wanted to test the contribution of this functional group in the context of other wobble base pairs. A wobble pair has been proposed to form between A and C (*8*) (Fig. 2). Compared to the G·U pair, an A·C wobble pair presents a different array of functional groups in the major groove, forms a weaker base pair at neutral pH, and lacks the minor groove N_2 exocyclic amine (*8, 22*). As might be expected considering these changes, binding of the 5′-exon analog GGCCCUCC by a ribozyme with A at position 22 (*A22*) was weaker (3.7 kcal·mol^{-1}) than that observed for molecules that form the wild-type G·U pair. Correction for the reduced base-pairing stability of the A·C pair (0.7 kcal·mol^{-1}) revealed that 3.0 kcal·mol^{-1} of tertiary interaction energy was lost by making this geometrically conservative substitution. The A·C mutation appears to shift the equilibrium toward

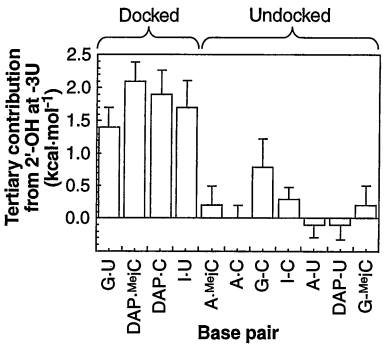

Fig. 3. Tertiary contribution of the 2′-OH at position −3U in the context of each base pair. Loss of a tertiary contribution is indicative of undocking of the splice site helix. Values represent the free energy of binding the −3U 2′-deoxy analog GGCCCdUCY minus that of the all-ribose analog. In each case the relative energetic contribution has been corrected by subtracting 1.1 kcal·mol^{-1} to compensate for the reduction in duplex stability resulting from the single deoxy substitution (*19*). Although the 2′-OH makes an intermediate contribution in the context of a G-C pair, a more complete characterization of the effect of this mutation shows the helix to be undocked (*19*). The assignment of the helix as docked or undocked is supported by measurement of the equilibrium dissociation constant for GMP binding in the context of each base pair (*13, 19, 32*).

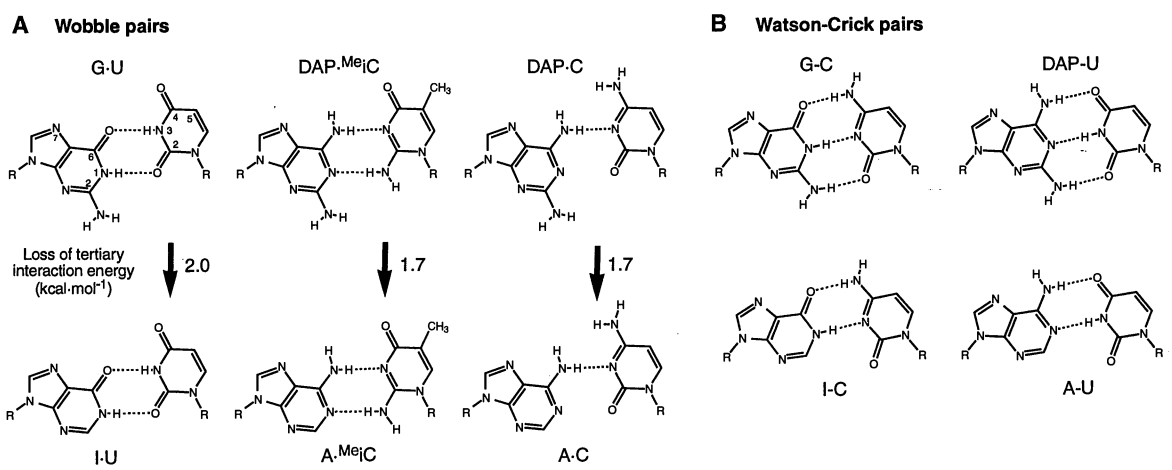

Fig. 2. (**A**) Proposed hydrogen bonding pattern and base configuration of wobble pairs. The upper and lower rows include wobble pairs with and without an N_2 exocyclic amine, respectively. Tertiary interaction energy is defined in Table 1. (**B**) Hydrogen bonding pattern and configuration of base pairs with a Watson-Crick pairing scheme. The upper and lower rows include pairs with and without an N_2 exocyclic amine, respectively.

an undocked complex as evidenced by the failure of the 2′-OH at −3U to make a tertiary interaction (Fig. 3). These data imply that more than just a wobble configuration is important for folding of the splice site helix into the ribozyme active site.

To generate a more stable wobble pairing partner for A, we synthesized the nucleoside 5-methyl-isocytidine (^{Me}iC) and incorporated it in place of U in the oligonucleotide GGCCCUC^{Me}iC (*14*). Transposition of the carbonyl and the amine of C allows ^{Me}iC to form two hydrogen bonds with A (Fig. 2), which results in a more stable wobble pair than A·C (Table 1). This improved base pairing stability is reflected in tighter binding of the ^{Me}iC oligonucleotide by the *A22* ribozyme. However, the stabilization is at the level of base pairing rather than the recovery of a tertiary contact. The A·^{Me}iC duplex remains in an open complex (Fig. 3), having lost ~2.8 kcal·mol^{-1} in tertiary stabilization compared to the wild-type G·U pair (Table 1).

To further assess the contribution of the amine to splice site helix docking, we reintroduced the N_2 exocyclic amine into the A·C and A·^{Me}iC pairs, using 2,6-diaminopurine riboside (DAP), an A analog with an N_2 exocyclic amine similar to G (Fig. 2). If the N_2 amino group forms a tertiary contact with the ribozyme active site as suggested by the I·U pair, then substitution of A with DAP should rescue the loss of tertiary binding energy observed for the A·C and A·^{Me}iC pairs.

Ribozyme *DAP22* bound the C and ^{Me}iC 5′-exon analogs 20-fold tighter than did the *A22* ribozyme (Table 1). This 1.8 kcal·mol^{-1} enhancement in binding is not due to improved base pairing stability. In the context of this duplex, A·^{Me}iC and DAP·^{Me}iC pairs are equally stable, as are A·C and DAP·C pairs. Therefore, restoration of the N_2 amino in the context of both the DAP·C and DAP·^{Me}iC wobble pairs restores almost 2 kcal·mol^{-1} of tertiary binding energy. Furthermore, on the basis of the tertiary contribution of the 2′-OH at −3U, the DAP substitution restores docking of the splice site helix. Thus, in three different sequence contexts—G·U, DAP·C, and DAP·^{Me}iC—the N_2 amino contributes appreciably (2 kcal·mol^{-1}) to docking of the splice site duplex, strongly implicating it in a tertiary interaction with the active site. Although the hydrogen bonding partner for the exocyclic amine is not known, photocrosslinking experiments (*23*) and molecular modeling (*7*) have shown that the G at position 22 is located proximal to the internal loop between helices P4 and P5 (Fig. 1) (*23*). This loop contains several invariant adenosines that might be the phylogenetically conserved hydrogen bonding partner for the exocyclic amine (Fig. 1).

In contrast to the minor groove, substitutions in the major groove had little effect on docking (Fig. 4). Introduction of a methyl group at the 5-position of U (^{Me}U) or substitution of the N_7 imino group of G with a carbon did not substantially affect the energetics of splice site helix docking (*24*). Furthermore, equivalence in tertiary binding energy between the DAP·C and DAP·^{Me}iC pairs suggests that the O_4 of the pyrimidine ring does not contribute to tertiary stabilization. ^{Me}iC and C differ at this position in having a carbonyl and an amine, respectively. Therefore, neither the major groove side of the purine nor any of the functional groups of the pyrimidine are likely to make energetically significant tertiary contacts

Table 1. Stability of 5′-exon analog binding calculated relative to the G·U wobble pair.

(X, Y) base pair*	K_d (nM)†	Loss in free energy (kcal·mol^{-1})		
		Total‡	Base pairing§	Tertiary interactions‖
Wild-type G·U wobble pair				
G·U	0.05 ± 0.007	(0)	(0)	(0)
Wobble pairs with exocyclic amine				
G·^{Me}U	0.05 ± 0.01	0.0	−0.3	0.3
DAP·^{Me}iC	0.5 ± 0.1	1.3	0.2	1.1
DAP·C	1.1 ± 0.4	1.9	0.6	1.3
dG·U	10 ± 5	3.2	0.3	2.9
d7dG·U	52 ± 4	4.2	0.7	3.5
Wobble pairs without exocyclic amine				
I·U	1.4 ± 0.5	2.0	0.0	2.0
A·^{Me}iC	9 ± 3	-3.1	0.3	2.8
A·C	25 ± 4	3.7	0.7	3.0
Watson-Crick pairs				
G-C	0.4 ± 0.2	1.3	−1.3	2.6
I-C	15 ± 2	3.4	0.0	3.4
DAP-U	15 ± 4	3.4	−0.4	3.8
A-U	16 ± 3	3.4	0.0	3.4
Other pairs				
G-^{Me}iC	17 ± 5	3.5	−0.4	3.9
I-^{Me}iC	54 ± 9	4.2	0.4	3.8
Pur-U	47 ± 4	4.1	0.4	3.7
Pur-C	130 ± 30	4.7	0.8	3.9
Pur-^{Me}iC	100 ± 40	4.5	0.5	4.0

*Base pairs represent the 5′-terminal base X (position 22 in Fig. 1) of the L-21 *ScaI* ribozyme pairing with the 3′-terminal base Y (position −1) of the 5′-exon analog GGCCCUCY. †Equilibrium dissociation constants (K_d) for the ribozyme–5′-exon analog complex were measured at 30°C by native gel mobility–shift under conditions of excess ribozyme and a trace concentration ($\leq K_d/10$) of 5′-^{32}P-labeled 5′-exon analog (*11, 19*). Each ribozyme was prefolded at 50°C for 20 min in 50 mM tris (pH 7.5), 10 mM NaCl, 0.1 mM EDTA, 10 mM $MgCl_2$ and incubated with 5′-exon analog for 30 min to 24 hours depending on the magnitude of the K_d being measured (*19*). In all cases doubling the incubation time led to no change in binding, ensuring that the ribozyme–5′-exon analog complex was at equilibrium. The fraction of 5′-exon analog bound (θ) at each ribozyme concentration ([*E*]) was quantitated by PhosphorImager analysis. The K_d was calculated by a nonlinear least squares fit of the equation $\theta = [E]/([E] + K_d)$. Each value represents the average of at least three independent measurements with errors reported as standard deviations. ‡Total free energy loss at 30°C [calculated as $RT\ln(K_d^{XY}/K_d^{GU})$ where *R* and *T* equal 0.00198 kcal·mol^{-1}·K^{-1} and 303.15 K, respectively] relative to the total free energy for formation of a G·U pair. Positive numbers indicate overall binding is weaker than the G·U pair. Errors in this column are usually less than 0.1 kcal·mol^{-1} and do not exceed 0.3 kcal·mol^{-1}. §Free energy loss (or gain) attributable to differences in duplex stability between G·U and the other pairs. Duplex stabilities were measured by thermal denaturation analysis with the IGS analog XGAGGG and the 5′-exon analog GGCCCUCY (*14, 19*). Free energy values were calculated at 30°C with the values from Table 2 in (*14*). Positive numbers indicate that the duplex is weaker than the G·U pair. Errors in this column are usually less than 0.1 kcal·mol^{-1} and do not exceed 0.2 kcal·mol^{-1} (*14*). ‖Free energy attributable to loss of tertiary interactions between the splice site duplex and the ribozyme active site, calculated by subtracting the free energy of base pairing from the total relative free energy. Positive numbers indicate the extent to which tertiary interaction energy has been lost relative to a G·U pair. Errors in this column are usually less than 0.2 kcal·mol^{-1} and do not exceed 0.5 kcal·mol^{-1}.

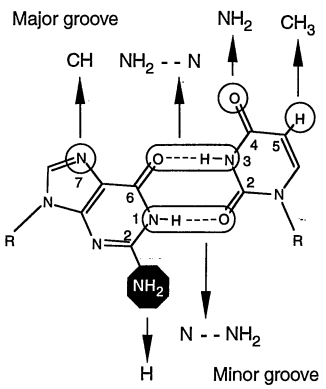

Fig. 4. The G·U pair with the functional group substitutions characterized in this study (arrows). Among the functional groups modified, only the N_2 amino of G (octagon) appears to make a direct contribution that is energetically significant. The U does not appear to interact directly with the ribozyme.

with the ribozyme (*25*) (Fig. 4).

The wobble configuration is important for presentation of the exocyclic amine on the minor groove surface. A considerable loss of tertiary binding energy is observed for the G-C (*19*) and DAP-U pairs (Table 1 and Fig. 2B). Both pairs have an exocyclic amine, but it is hydrogen-bonded in a Watson-Crick configuration. Deletion of the amine in these contexts does not result in a substantial loss of tertiary binding energy (compare G-C to I-C and DAP-U to A-U, Table 1), which suggests that the amine cannot make a strong tertiary interaction if it is presented in a Watson-Crick geometry. Furthermore, these Watson-Crick pairs fail to dock into the active site, as measured by the nearly complete loss of tertiary interaction energy ($\geq$3.5 kcal·mol^{-1}, Table 1) and minimal contribution of the 2′-OH at −3U (Fig. 3). Similar effects are observed for base pairs with purine riboside (Pur) that form at most one hydrogen bond, including Pur·U, Pur·C, and Pur·^{Me}iC, and for non–wobble pairs with ^{Me}iC, including G-^{Me}iC and I-^{Me}iC.

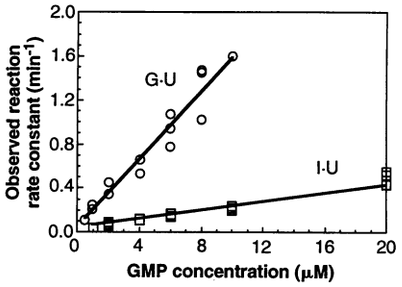

Fig. 5. Reaction rate constants for cleavage of 5′-GGCCCUCUAAAAA-3′ by the *G22* and *I22* ribozymes as a function of GMP concentration at 30°C. The slopes are equal to 15 × 10^4 and 1.9 × 10^4 M^{-1}min^{-1} for *G22* and *I22*, respectively, and represent the second order rate constant $(k_{cat}/K_m)^G$. Single-turnover reactions were carried out at 30°C as described (*13*) with saturating ribozyme concentration (200 nM such that $[E] \gg K_d^S$) and trace ^{32}P-labeled oligonucleotide substrate. Under these conditions the second order rate constant $(k_{cat}/K_m)^G$ measures the rate of the reaction E·S + G → [E·S·G]‡, the transition state for the chemical step (*33*).

We conclude that the G·U pair is highly conserved because it is the only combination of natural bases in a wobble geometry with an N_2 exocyclic amine that is available for bonding in the minor groove. Although the U defines the 5′-splice site, none of its functional groups appears to be directly recognized by the ribozyme (Fig. 4). Instead, its role is to hold the G in a wobble geometry, thus presenting the exocyclic amine of G for recognition. Considering that highly conserved G·U pairs are present in a diversity of RNAs (*26*), the exocyclic amine of G might commonly serve as a minor groove contact for RNA folding and for duplex recognition by macromolecular RNA and protein ligands.

In addition to a ground state tertiary interaction, the exocyclic amine also contributes to transition state stabilization. The *I22* ribozyme cleaved the oligonucleotide substrate GGCCCUCUAAAAA at one-eighth the rate of the wild-type *G22* ribozyme (Fig. 5) (*27*). Extension of these measurements to high guanosine monophosphate (GMP) concentration revealed that both ribozymes bound GMP with equal affinity [equilibrium dissociation constant for GMP (K_d^G) ≈ 80 μM], but *I22* reacted at one-sixth the rate of *G22* in the chemical step (k_c = 13 min^{-1} for *G22* and 2.3 min^{-1} for *I22*) (*28*). On the basis of the structure of a G·U wobble pair (*21*), the scissile phosphate is blocked from access to the N_2 amino by the intervening ribose, so the small transition state stabilization provided by the amine is likely to be indirect. For example, the stabilization could be mediated by its hydrogen bonding partner within the ribozyme, an ordered water molecule (*21*), or a bound Mg^{2+} ion.

Studies have implicated three specific 2′-OH groups at nucleotide positions −3U, G22, and G25 as important tertiary contacts for splice site helix recognition by the ribozyme (*12*, *13*). The N_2 exocyclic amine and the 2′-OH groups are all located on the surface of the minor groove (Fig. 6A). Thus, the ribozyme uses molecular handles similar to those used by tRNA^{Ala} synthetase in binding its cognate tRNA acceptor stem (*29*) (Fig. 6B). It is especially notable that the catalytic centers of the RNA and the protein both bind double-stranded RNA by recognizing an amino group presented on the minor groove surface by a G·U wobble pair and a subset of the surrounding 2′-OH groups. The exocyclic amine provides specificity, whereas the 2′-OH groups increase binding energy. A similar structural convergence between protein and RNA binding was demonstrated when the P5abc RNA subdomain and the tyrosyl-tRNA synthetase were both shown to recognize the core of the group I intron (*30*). These examples suggest that proteins and RNA, two structurally dissimilar molecules, can adopt three-dimensional surfaces that function similarly in ligand binding.

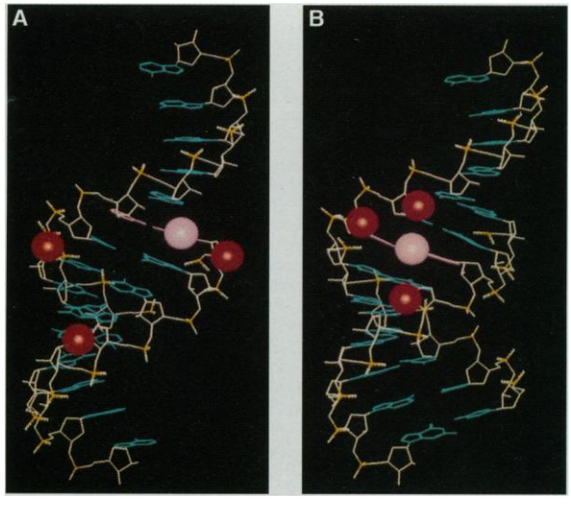

Fig. 6. Minor groove determinants for RNA duplex binding by a ribozyme and a protein enzyme. (**A**) Model of the splice site helix. The functional groups implicated in tertiary interactions with the catalytic core are shown as spheres. The critical 2′-hydroxyls are shown in red and the N_2 amino of G is shown in purple. The G·U base pair is also purple. The single-stranded extension above the duplex region is the 3′-terminal A_5 tail of the substrate strand. The IGS strand is to the right and below. (**B**) Model of the tRNA^{Ala} amino acyl acceptor stem helix with the functional groups important for recognition by its cognate synthetase (*29*). The color scheme is the same as that in (A). The single-stranded extension above the acceptor stem is the 3′-terminal CCA of the tRNA. A second exocyclic amine at G-C pair 2-71 (immediately above the G·U pair 3-70 shown in purple) also contributes to synthetase binding. It is not highlighted because the contribution is small compared to that made by the amine of the G·U pair (*29*).

REFERENCES AND NOTES

1. R. B. Waring, P. Towner, S. J. Minter, R. W. Davies, *Nature* **321**, 133 (1986).
2. M. D. Been and T. R. Cech, *Cell* **47**, 207 (1986); A. J. Zaug, M. D. Been, T. R. Cech, *Nature* **324**, 429 (1986).
3. P. C. Bevilacqua, R. Kierzek, K. A. Johnson, D. H. Turner, *Science* **258**, 1355 (1992).
4. D. Herschlag, *Biochemistry* **31**, 1386 (1992).
5. T. R. Cech, A. J. Zaug, P. J. Grabowski, *Cell* **27**, 487 (1981); T. R. Cech, D. Herschlag, J. A. Piccirilli, A. M. Pyle, *J. Biol. Chem.* **267**, 17479 (1992).
6. F. L. Murphy and T. R. Cech, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9218 (1989); D. Herschlag and T. R. Cech, *Biochemistry* **29**, 10172 (1990).
7. F. Michel and E. Westhof, *J. Mol. Biol.* **216**, 585 (1990).
8. J. A. Doudna, B. P. Cormack, J. W. Szostak, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7402 (1989).
9. E. T. Barfod and T. R. Cech, *Mol. Cell. Biol.* **9**, 3657 (1989).
10. A. J. Zaug, C. A. Grosshans, T. R. Cech, *Biochemistry* **27**, 8924 (1988); D. Herschlag and T. R. Cech, *ibid.* **29**, 10159 (1990).
11. A. M. Pyle, J. S. McSwiggen, T. R. Cech, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8187 (1990).
12. A. M. Pyle and T. R. Cech, *Nature* **350**, 628 (1991); P. C. Bevilacqua and D. H. Turner, *Biochemistry* **30**, 10632 (1991); A. M. Pyle, F. L. Murphy, T. R. Cech, *Nature* **358**, 123 (1992); D. Herschlag, F. Eckstein, T.

R. Cech, *Biochemistry* **32**, 8299 (1993).
13. S. A. Strobel and T. R. Cech, *Biochemistry* **32**, 13593 (1993).
14. S. A. Strobel, T. R. Cech, N. Usman, L. Beigelman, *ibid.* **33**, 13824 (1994).
15. R. Green, J. W. Szostak, S. A. Benner, A. Rich, N. Usman, *Nucleic Acids Res.* **19**, 4161 (1991).
16. Oligoribonucleotide synthesis followed the general procedure described by N. Usman, K. K. Ogilvie, M. Jiang, and R. J. Cedergren [*J. Am. Chem. Soc.* **109**, 7845 (1987)] and S. A. Scaringe, C. Francklyn, and N. Usman [*Nucleic Acids Res.* **18**, 5433 (1990)]. Syntheses of the DAP phosphoramidite and the ^{Me}iC and ^{Me}U oligoribonucleotide solid supports are described in (*14*). The Pur phosphoramidite synthesis is described by D. Fu, S. B. Rajur, and L. W. McLaughlin [*Biochemistry* **32**, 10629 (1993)]. The I and d7dG phosphoramidites were purchased from Glen Research and ChemGenes, respectively.
17. M. J. Moore and P. A. Sharp, *Science* **256**, 992 (1992).
18. The gene for T4 DNA ligase containing a COOH-terminal His_6 tag was cloned into a plasmid with a bacteriophage T7 RNA polymerase promoter to allow overexpression. The enzyme was purified to near homogeneity with a Ni^{2+}–nitrilotriacetic acid–agarose resin (Qiagen) with imidazole as eluent. The ligase was as active and pure as commercially available enzyme preparations.
19. A. M. Pyle *et al.*, *Biochemistry* **33**, 13856 (1994); D. S. Knitt, G. J. Narlikar, D. Herschlag, *ibid.*, p. 13864.
20. S. R. Holbrook, J. L. Sussman, R. W. Warrant, S.-H. Kim, *J. Mol. Biol.* **123**, 631 (1978).
21. Determination of an x-ray crystal structure of a G·U pair by S. R. Holbrook, C. Cheong, I. Tinoco Jr., and S.-H. Kim [*Nature* **353**, 579 (1991)] revealed a highly ordered water molecule between the exocyclic amine of G and the 2′-OH of U. They proposed that the water might contribute to transition state stabilization of the group I ribozyme.
22. D. R. Hickey and D. H. Turner, *Biochemistry* **24**, 3987 (1985); S. M. Freier *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9373 (1986).
23. J.-F. Wang, W. D. Downs, T. R. Cech, *Science* **260**, 504 (1993).
24. A thymidine substitution at position −1 has been reported to have no appreciable effect on binding [D. Herschlag, F. Eckstein, T. R. Cech, *Biochemistry* **32**, 8312 (1993)]. The CH substitution at the N_7 of G (*d7dG22*) was made in a 2′-deoxy background. Therefore, the comparison must be made to *dG22*, which also has the 2′-deoxy substitution. Interpretation of the absence of an appreciable effect at this position is somewhat hindered by the fact the 2′-deoxy substitution results in significant undocking of the splice site helix (*13*).
25. The contribution made by the purine O_6 and pyrimidine N_3 positions remains ambiguous. Neither the DAP·C nor the DAP·^{Me}iC pair restores binding completely to the level of the G·U pair. DAP·C and DAP·^{Me}iC are ~1.0 kcal·mol^{-1} less stable than G·U and G·^{Me}U, respectively. This might be interpreted as a major groove contact to the O_6 carbonyl of G that is disrupted upon substitution with an amine. Although we cannot completely rule out this possibility, we note that functional groups neighboring the O_6 carbonyl do not influence tertiary interaction energy, so it seems more likely that the energetic difference is derived from a less specific effect. It might result from a negative steric interaction due to the shape of the DAP wobble pairs not being completely isomorphic with G·U, or an indirect electrostatic effect in the minor groove because the hydrogen bond formed adjacent to the exocyclic amine differs between the G·U and the DAP wobble pairs (Fig. 2A). Thus, while the G·U and DAP·^{Me}iC pairs are energetically equivalent at the terminus of an isolated helix, it appears that the active site of the ribozyme is more sensitive to the subtleties of bond polarity and base shape.
26. Hepatitis delta virus ribozyme: M. D. Been, *Trends Biol. Sci.* **19**, 251 (1994); group II self-splicing introns: F. Michel, K. Umesono, H. Ozeki, *Gene* **82**, 5 (1989); spliceosome: M. J. Moore, C. C. Query, P. A. Sharp, in *The RNA World*, R. F. Gesteland and J. F. Atkins, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993), pp. 303–357; 16S and 23S ribosomal RNAs: R. R. Gutell, N. Larsen, C. R. Woese, *Microbiol. Rev.* **58**, 10 (1994); RNA selected for valine binding: I. Majerfeld and M. Yarus, *Nature Struct. Biol.* **1**, 287 (1994). Although not within a G·U pair, the exocyclic amine of a G is important for catalysis within the hairpin ribozyme [B. M. Chowrira, A. Berzal-Herranz, J. M. Burke, *Nature* **354**, 320 (1991)].
27. A somewhat smaller effect on reactivity was reported for a G to I substitution in a different *Tetrahymena* intron construct (*15*). The correlation of observed rates with individual steps in the reaction has not been established for that construct.
28. S. A. Strobel and T. R. Cech, data not shown.
29. K. Musier-Forsyth *et al.*, *Science* **253**, 784 (1991); K. Musier-Forsyth and P. Schimmel, *Nature* **357**, 513 (1992).
30. G. Mohr, M. G. Caprara, Q. Guo, A. M. Lambowitz, *Nature* **370**, 147 (1994). In this case it has yet to be determined if the RNA and protein recognize the same RNA functional groups.
31. T. R. Cech, S. H. Damberger, R. R. Gutell, *Nature Struct. Biol.* **1**, 273 (1994); J. L. Salvo and M. Belfort, *J. Biol. Chem.* **267**, 2845 (1992); R. Saldanha, G. Mohr, M. Belfort, A. M. Lambowitz, *FASEB J.* **7**, 15 (1993).
32. T. S. McConnell, T. R. Cech, D. Herschlag, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8362 (1993); P. C. Bevilacqua, K. A. Johnson, D. H. Turner, *ibid.*, p. 8357; S. A. Strobel and T. R. Cech, unpublished results.
33. D. Herschlag, J. A. Piccirilli, T. R. Cech, *Biochemistry* **30**, 4844 (1991).
34. We thank J. A. Piccirilli for assistance with the experimental design and L. Beigelman and N. Usman for advice with oligoribonucleotide synthesis. Supported by a Life Sciences Research Foundation fellowship sponsored by the Howard Hughes Medical Institute to S.A.S. T.R.C. is an Investigator of the Howard Hughes Medical Institute and an American Cancer Society Professor. We thank the W. M. Keck Foundation for the support of RNA science on the Boulder campus.

8 September 1994; accepted 16 November 1994

Genetic Decreases in Atrial Natriuretic Peptide and Salt-Sensitive Hypertension

Simon W. M. John, John H. Krege, Paula M. Oliver, John R. Hagaman, Jeffrey B. Hodgin, Stephen C. Pang, T. Geoffrey Flynn, Oliver Smithies*

To determine if defects in the atrial natriuretic peptide (ANP) system can cause hypertension, mice were generated with a disruption of the *proANP* gene. Homozygous mutants had no circulating or atrial ANP, and their blood pressures were elevated by 8 to 23 millimeters of mercury when they were fed standard (0.5 percent sodium chloride) and intermediate (2 percent sodium chloride) salt diets. On standard salt diets, heterozygotes had normal amounts of circulating ANP and normal blood pressures. However, on high (8 percent sodium chloride) salt diets they were hypertensive, with blood pressures elevated by 27 millimeters of mercury. These results demonstrate that genetically reduced production of ANP can lead to salt-sensitive hypertension.

Essential hypertension is a heterogeneous disease in which blood pressures are harmfully high without overt cause; it affects a large number of individuals in many populations (*1*). Both genetic and environmental factors have been implicated in its etiology. Several complex physiological systems affect blood pressure, including one that is mediated by the 28–amino acid atrial natriuretic peptide (ANP) (*1*, *2*). ANP is produced mainly in the cardiac atria, where it is stored in dense granules as a large precursor (proANP), which is the major component of the granules (*2*). ANP is the COOH-terminal portion of the precursor. In response to an increase in atrial distension, ANP is released into the circulation and interacts with receptors in the vasculature, kidney, and adrenal glands. Although in pharmacological doses ANP is known to lower blood pressure and promote salt excretion, its physiologic functions have not been clearly defined and have not been proven (*3*).

Because of the actions of ANP, efforts have been made to determine whether genetic variants of the ANP system are involved in the etiology of essential hypertension. For example, plasma ANP concentrations in the children of two normotensive parents are higher than in children with a hypertensive parent, especially when the children are on high salt intakes (*4*). Several polymorphisms have been identified in the human *proANP* gene (*5*). Nevertheless, no association between two *proANP* markers and hypertension has been found in patients although they were not evaluated for their responses to dietary salt (*6*). These results indicate that the

S. W. M. John, P. M. Oliver, J. R. Hagaman, J. B. Hodgin, O. Smithies, Department of Pathology, University of North Carolina, Chapel Hill, NC 27599, USA.
J. H. Krege, Departments of Internal Medicine and Pathology, University of North Carolina, Chapel Hill, NC 27599, USA.
S. C. Pang, Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada.
T. G. Flynn, Department of Biochemistry, Queen's University, Kingston, Ontario K7L 3N6, Canada.
*To whom correspondence should be addressed.