Stereochemical Course of Catalysis by the Tetrahymena Ribozyme

Jayaraj Rajagopal, Jennifer A. Doudna, Jack W. Szostak


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the kernels were classified into types by a
cluster analysis method and use of nearest
centroid sorting. To select the best number
of groups, the cluster analysis was run four
times specifying different numbers of clusters
(6, 8, 10, and 12). Ten clusters corre-
sponded most closely to the types perceived
subjectively. The attributes of the ten types
are given in Table 1. These archæological
types should not be understood as repre-
sentating actual races of maize; they are rather
an initial classification for assessing the rela-
tive variation through time.

Types C, F, and G are small, mostly
beaked kernels that fall in the size range of
the modern Andean popcorn races (2, 3).
Type I is a short, thick type with a low row
number (8-row). Types A, D, and E are
larger, long in proportion to their width, with
quite high row (12- to 18-row) num-
bers and many square-capped kernels. Types
H and I are large broad kernels, with round-
cap ed caps, flat cross sections, and low row (8-
and 10-row) numbers.

The relation among the ten types can be
seen in a plot of the first and second canon-
ical variables, which together account for
86% of the variability in the assemblage
(Fig. 2). The first canonical variable (in-
creasing width, length, and relative flatness)
is associated with time, as is shown in the
bar graphs below the scatterplot in Fig. 1.
The two earliest assemblages are dominated
by small type F. The Pancán 2 assemblage
appears to be more varied, although the
sample size is small. Greater variation and
domination by a new type (type C, a high
row number beaked type) is evident in level
I of Pancán. Diversity in types is greatest in
Wanka II and Wanka III, each phase with
kernels of all ten types, though in different
proportions. Two unusually large type H
kernels found in Wanka II contexts ap-
proach the size and shape of the Cuzco
races, suggesting that large-kerned, low-
row number varieties were grown in the
Mantarao before Inca times, rather than be-
ing spread with Inca hegemony as has been
suggested (2).

On the coast of Peru researchers have
noted, after a long and rather stable period
dominated by popcorns, a sudden change
toward new flint and flour maize types from the
highlands (2, 3). They postulate that this
change is associated with the Wari expan-
sion out of the Ayacucho region (Middle
Horizon, about A.D. 550 to 850). The
Mantarao Valley data indicate that an in-
flux of new flint and flour types there is not
associated with Middle Horizon, but rather
with the Late Intermediate (after A.D.
1000). This maize evidence parallels the lack
of Middle Horizon Wari architecture and the
scarcity of Wari sherds in the area in
indicating that the people of the upper Man-
taro received little direct Wari influence.

At the close of the Middle Horizon in the
Mantarao area a number of changes in
way of growing, preparing, and eating food are
associated with the new use of large-kerned
maize types. Stone hoes become more
common (15), as do the actual remains of
corn and other crops (15, 21). Large grind-
ing stones and manos become more common
in relation to mortars and pestles (15),
perhaps reflecting grinding of the new
flour maize types. Bowls become larger in
Wanka I times, with new vessel types ap-
pearing in Wanka II and Inca times (15).
This may reflect new foods suitable to floury
maize, such as chicha (maize beer), mote
(hominy), kancha (toasted maize), and sanco
(fine cornmeal cakes). Maize in Inca times,
and probably earlier, was a high-prestige
and ceremonial food (22).

Comparison of charred maize kernels from
two time periods has helped in under-
standing changes in maize use and associat-
ed culture in the Mantarao Valley over a
millennium. The data show (i) a continuity in
use of small-sized maize into and through
the Middle Horizon, indicating a lack of
Wari influence there as compared to the
coast, and (ii) a florescence of large-kerned
flint and flour types occurring in the Late
Intermediate, associated with new cul-
tural dimensions in farming, crops, and eat-
ing.

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Stereochemo Course of Catalysis by the
Tetrathymena Ribozyme

JAYARAJ RAGJAGOPAL, JENNIFER A. DOUDNA, JACK W. SZOSTAK

The group I intron from Tetrathymena catalyzes phosphodiester transfer reactions on various RNA substrates. A modified RNA substrate with a phosphorothioate group in one stereoisomeric form at the site of reaction was synthesized in order to determine the stereochemo course of an RNA-catalyzed reaction. The reaction product was digested with a stereospecific nuclease to determine the configuration of the product phosphorothioate. The reaction occurs with inversion of configuration at phosphorus, implying an in-line pathway for the reaction.

The group I self-splicing ribo-
somal RNA intron from Tetrathymena catalyzes its excision from the pri-
mary RNA transcript and exon ligation to form the mature transcript (1, 2). In vitro, the free intron can function as a site-specific ribonuclease, a terminal transferase, a phos-
phortransferase, and an acid phosphatase, depending on the RNA substrate provided

and the conditions of the reaction (3–5). This RNA is a true catalyst in that it can
mediate multiple turnovers of some reac-
tions (3, 6).

Whether the ribozyme catalyzes transes-
terification reactions stereospecifically has

Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.
not been determined even though the stereochemical course of such reactions can provide evidence for particular catalytic mechanisms. Many protein enzymes that catalyze similar reactions of nucleic acid substrates have been studied with phosphorothioate analogs (one of the peripheral oxygen of a phosphate ester is replaced by sulfur) that allow the stereochemical course of the reaction to be determined. All protein enzymes that have been investigated stereochemically catalyze nucleophile displacements at phosphodiester bonds with inversion of the configuration at phosphorus (7, 8).

We initially attempted to determine the stereochemistry of the RNA-catalyzed reaction with our previously described substrate cleavage assay (9, 10). However, the phosphorothioate analog turned out to be an extremely poor substrate for the cleavage reaction, and we were unable to obtain a large enough sample for analysis. We have found that a template-directed oligonucleotide ligation reaction occurs with much greater efficiency than the cleavage reaction, with up to several hundred turnovers per enzyme molecule (11). We therefore decided to use this system to examine reaction stereospecificity.

A combination of primer, template, and ligation oligonucleotides was designed such that the reactive phosphate could be specifically substituted with a phosphorothioate. This phosphate is the 5' phosphate of the single adenosine residue in the ligator oligonucleotide (Fig. 1). The RNA's were synthesized by T7 RNA polymerase transcription of synthetic deoxyoligonucleotides (12). For a control experiment with no phosphorothioate, the primer was labeled with [α-32P]GTP (ribose) guanosine triphosphate), and the purified RNA's were tested for ligation by the intron. After incubation, the primer was almost completely incorporated into a 20-nucleotide product, which comigrated with a synthetic RNA of the same sequence. The identity of the ligated product was confirmed by RNase (ribonuclease) T1 digestion; the digestion pattern was identical to that of the control RNA.

The ligator RNA was then synthesized with rCTP, rGTP, rUTP (ribose cytidine, guanosine and uridine triphosphate), and αS-ATP (adenosine triphosphate) (Sₜ iso-

Fig. 2. Autoradiogram of a denaturing 20 percent polyacrylamide gel on which products of the snake venom digest were isolated. Control and ligated product RNA's were purified on denaturing 20 percent polyacrylamide gels, eluted in 0.3M NaCl, precipitated, and resuspended in distilled water. Portions of each were digested to completion with RNase T1, resulting in a single 32P-labeled tetranucleotide with the sequence CUAG (Fig. 1). The tetramers were gel-purified, concentrated on DEAE columns, eluted in TEAB buffer, dried at reduced pressure, and resuspended in distilled water. The tetramers were then digested with snake venom phosphodiesterase, and the products were separated by electrophoresis on a 20 percent denaturing acrylamide gel. (Lane 1) Control RNA, undigested; (lane 2) ligated product RNA, undigested; (lanes 3 and 8) RNase T1-digested control RNA (uniformly [α-32P]GTP-labeled) for size markers; (lane 4) tetramer isolated from RNase T1-digested control RNA; (lane 5) snake venom digest of the control tetramer; (lane 6) tetramer isolated from RNase T1-digested product RNA; (lane 7) snake venom digest of the product tetramer.

Fig. 1. Scheme for determining the stereospecificity of phosphodiester bond transfer. The ribozyme-catalyzed ligation reaction was carried out in 10 mM NH₄Cl, 20 mM MgCl₂, 30 mM tri-HCl, pH 7.5, 1 mM aurin tricarboxylic acid, and 10 μM primer, ligator, template, and enzyme RNA's in a 50-μl reaction volume. The reaction was incubated for 3 hours at 38°C, and then stopped by the addition of an equal volume of 90 percent formamide containing 1 mM EDTA. The product was purified and processed as described for Fig. 2.
labeled with $^{32}$P and the second is a phosphorothioate. The sequence of the tetramer from the control RNA was confirmed by nearest neighbor analysis. Each tetranucleotide was digested with snake venom phosphodiesterase (SVPDE), which has a 1700-fold greater activity on $R_p$ isomers (8, 13), and the products were separated by gel electrophoresis (Fig. 2). The control RNA ($R_p$ isomer) was digested to mononucleotides, with less than 1 percent of the $^{32}$P in the dinucleotide region. The ligated product RNA was only digested to the labeled dinucleotide pUAp. Quantitation of the gel on a $\beta$-scanner (Betagen) showed that less than 2 percent of the radioactivity was in mononucleotides. The inability of the phosphodiesterase to cleave the dinucleotide to mononucleotides indicates that the phosphorothioate product is S and therefore that the ribosome reaction proceeds with inversion of configuration. The experiment was repeated with ($\alpha$-$^{32}$S)-labeled rATP to label the ligator and control RNA’s, and the same result was obtained: the phosphorothioate in the ribosome-generated RNA was resistant to phosphodiesterase cleavage, while the control RNA was digested to mononucleotides.

Replacing the pro-$R$ oxygen of the reactive phosphate with sulfur decreases the rate of reactions by a factor of about 1000. One possible explanation for this effect is that the larger sulfur interferes with the coordination of a Mg$^{2+}$ ion that is normally bound to the two phosphate oxygens, where it functions either in substrate binding, in stabilizing the transition state, or in charge neutralization to facilitate the nucleophilic attack. This rate decrease notwithstanding, it is clear from a number of cases where the stereochemical course of enzymatic reactions has been studied both by the use of $^{18}$O, $^{15}$O, or $^{18}$O phosphodiester groups and by the use of a phosphorothioester, that the elemental substitution does not affect the stereochemical consequence at phosphorus (8, 14).

Any odd number of nucleophilic substitution reactions at phosphorus can result in inversion of configuration of the phosphorothioate (7, 8). In the simplest scheme, a single displacement reaction occurs in which the enzyme catalyzes the nucleophilic attack of the 3' hydroxyl of the primer on the first internal phosphate of the ligator, resulting in product formation. The 5'-guanosine residue of the ligator is released. In the transition state of such a reaction the phosphorus has trigonal bipyramidal geometry, with incoming and leaving groups in the axial positions.

This mechanism implies an in-line orientation of the incoming and leaving hydroxyl groups. It seems likely that this mechanism will apply to all of the phosphotransfer reactions catalyzed by the ribosome. If so, the guanosine that attacks the P1 stem in the first step of self-splicing must be oriented by the enzyme so that its O3' is opposite the O3' of the leaving exon. This is an important constraint in attempts to model the active site of the enzyme.

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Identification of the Fusion Peptide of Primate Immunodeficiency Viruses

MARNIX L. BOSCH, PATRICIA L. EARL, KATHLEEN FARGNOLI, SILVIA PICCIAUFOCO, FABRIZIO GIOMBINI, FLOSSIE WONG-STAAAL, GENOVEFFA FRANCHINI

Membrane fusion induced by the envelope glycoproteins of human and simian immunodeficiency viruses (HIV and SIVmac) is a necessary step for the infection of CD4 cells and for the formation of syncytia after infection. Identification of the region in these molecules that mediates the fusion events is important for understanding and possibly interfering with HIV/SIVmac infection and pathogenesis. Amino acid substitutions were made in the 15 NH2-terminal residues of the SIVmac gp32 transmembrane glycoprotein, and the mutants were expressed in recombinant vaccinia viruses, which were then used to infect CD4-expressing T cell lines. Mutations that increased the overall hydrophobicity of the gp32 NH2-terminus increased the ability of the viral envelope to induce syncytia formation, whereas introduction of polar or charged amino acids in the same region abolished the fusogenic function of the viral envelope. Hydrophobicity in the NH2-terminal region of gp32 may therefore be an important correlate of viral virulence in vivo and could perhaps be exploited to generate a more effective animal model for the study of acquired immunodeficiency syndrome.

The envelope glycoproteins of SIVmac (gp120 and gp32) are derived from a gp160 precursor through endoproteolytic cleavage and contain all determinants necessary for host cell infection and syncytium formation (1). The gp32, like the gp41 of HIV-1 (2), is the transmembrane glycoprotein (3) that is expressed on the surface of infected cells and incorporated in the viral membrane; gp32 anchors gp120, which contains the binding site for the CD4 antigen (4, 5). The smaller size of the SIVmac transmembrane glycoprotein (gp32 as opposed to HIV-1 gp41) is due to the presence of a premature termination codon in the SIVmac env gene (6); some data indicate that the region after the termination codon is expressed in vivo in SIVmac-infected animals (7).

Several studies indicate that the hydrophobic NH2-terminus of the transmembrane glycoprotein is primarily responsible for the membrane fusion events involved with HIV and SIVmac infection and with syncytium formation. This region has amino acid sequence similarity to the fusion peptides of ortho- and paramyxoviruses (8), and it has been shown, as for other enveloped viruses, that the cleavage of the env precursor resulting in exposure of the gp41 NH2-terminus is a necessary event for HIV infectivity (9). Furthermore, insertion mutagenesis performed on this region of the HIV-1 gp41 (inserting four to six amino acids after position 6 of the gp41) abolishes the capacity of the envelope glycoproteins to induce syncytia in a heterologous expression system (4).

The fusion peptides of ortho- and para-