

A Viral RNA That Binds ATP and Contains a Motif Similar to an ATP-binding Aptamer from SELEX*

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The intriguing process of free energy conversion, ubiquitous in all living organisms, is manifested in ATP binding and hydrolysis. ATPase activity has long been recognized to be a capability limited to proteins. However, the presence of an astonishing variety of unknown RNA species in cells and the finding that RNA has catalytic activity have bred the notion that RNA might not be excluded from the group of ATPases. All DNA-packaging motors of double-stranded DNA phages involve two nonstructural components with certain characteristics typical of ATPases. In bacterial virus phi29, one of these two components is an RNA (pRNA). Here we report that this pRNA is able to bind ATP. A comparison between the chemically selected ATP-binding RNA aptamer and the central region of pRNA reveals similarity in sequence and structure. The replacement of the central region of pRNA with the sequence from ATP-binding RNA aptamer produced chimeric aptRNA that is able to both bind ATP and assemble infectious viruses in the presence of ATP. RNA mutation studies revealed that changing only one base essential for ATP binding caused both ATP binding and viral assembly to cease, suggesting that the ATP binding motif is the vital part of the pRNA that forms a hexamer to drive the phi29 DNA-packaging motor. This is the first demonstration of a natural RNA molecule that binds ATP and the first case to report the presence of a SELEX-derived RNA aptamer in living organisms.

One common feature in the assembly of all linear double-stranded (ds)¹-DNA viruses including herpes virus, pox virus, adenovirus, and all of the linear dsDNA phages is that the lengthy viral genome is translocated with remarkable velocity into a limited space within a preformed protein shell and packaged into crystalline density (1–3). This energetically unfavorable DNA motion process is accomplished by an ATP-driven motor (4–7). Careful scrutiny of the well studied dsDNA viruses reveals a striking commonality: all DNA-packaging motors involve two nonstructural components with certain characteristics typical of ATPases (8). For example, gpA and gpNuI of the DNA-packaging motors of λ-phage (9, 10) contain consensus ATP-binding domains and are involved in ATP hydroly-

ysis. Both gp16 and gp17, which constitute the terminase of bacteriophage T4, are involved in ATP hydrolysis (11). In bacterial virus phi29, one of the nonstructural components for DNA packaging is an RNA molecule called pRNA (Fig. 1) (12). One ATP is used to package two base pairs of phage DNA of the phi29 (8) and the T₃ system (13).

The phi29-encoded 120-base pRNA binds the connector and leaves the DNA-filled capsid after completing the DNA-packaging task (14). Six pRNAs form a hexagonal complex to gear the DNA-translocating machinery (14–18). This DNA-packaging motor has been reported recently (19, 20) to be the strongest nanometer motor with a stalling force of >50 piconewtons. It stuffs the viral procapsid with DNA at an initial speed of 100 base pairs/second under the extra load. The crystal structure of one of the important motor components, the connector where the pRNA binds, has been solved (6, 7). It has been reported that pRNA enhances the ATPase activity of gp16 (21, 22). It would be very intriguing to find out how the pRNA is involved in the transformation of chemical energy from ATP into motion and whether pRNA serves only as a stimulating factor or could interact with ATP directly.

The presence of an astonishing variety of unknown RNA species in cells and the finding that RNA has enzymatic activity (23, 24) have bred the notion that RNA might not be excluded from the group of ATPases that has long been believed to be only proteins. One method for the examination of this idea is the chemical approach using the *in vitro* selection-amplification technique (25, 54) to identify ATP-binding RNA dubbed aptamers (26) from synthesized random RNA pools.

Here we report that the central region of pRNA is similar to the ATP-binding RNA aptamer (26) in sequence and structure. We also demonstrate that phi29 pRNA directly binds ATP.

EXPERIMENTAL PROCEDURES

Preparation of pRNA—RNAs were prepared as described previously (27). DNA oligomers were synthesized with the desired sequences and used to produce dsDNA by PCR. The DNA products containing the T₇ promoter were cloned into plasmids. RNA was synthesized with T₇ RNA polymerase by run-off transcription and then purified from a polyacrylamide gel. The sequences of both plasmids and PCR products were confirmed by DNA sequencing.

***In vitro* Production of Infectious Virions of phi29 Virion Particles with aptRNA and ATP**—The purification of procapsids (28, 29), gp16 (30), and DNA-gp3 (31, 32); the preparation of the tail protein (gp9) (29), neck proteins (gp11, gp12) (29), and the morphogenetic factor (gp13) (29); and the procedure for the assembly of infectious phi29 virion *in vitro* (29) have been described previously. 1 μg of RNA in 1 μl RNase-free H₂O was mixed with 10 μl of purified procapsids (0.4 mg/ml) and then dialyzed on a 0.025-μm-type VS filter membrane against TBE (2 mM EDTA, 89 mM Tris borate, pH 8.0) for 15 min at room temperature. The mixture was subsequently transferred for another dialysis against TMS (100 mM NaCl, 10 mM MgCl₂, 50 mM Tris, pH 7.8) for an additional 30 min. The pRNA-enriched procapsids were then mixed with gp16, DNA-gp3, and ATP (1.4 mM final concentration except when otherwise indicated) to complete the DNA-packaging reaction. After 30 min, gp11, gp12, gp9, and gp13 were added to the DNA-packaging reactions to

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¹ The abbreviations used are: ds, double-stranded; gp, gene product; wt, wild type.

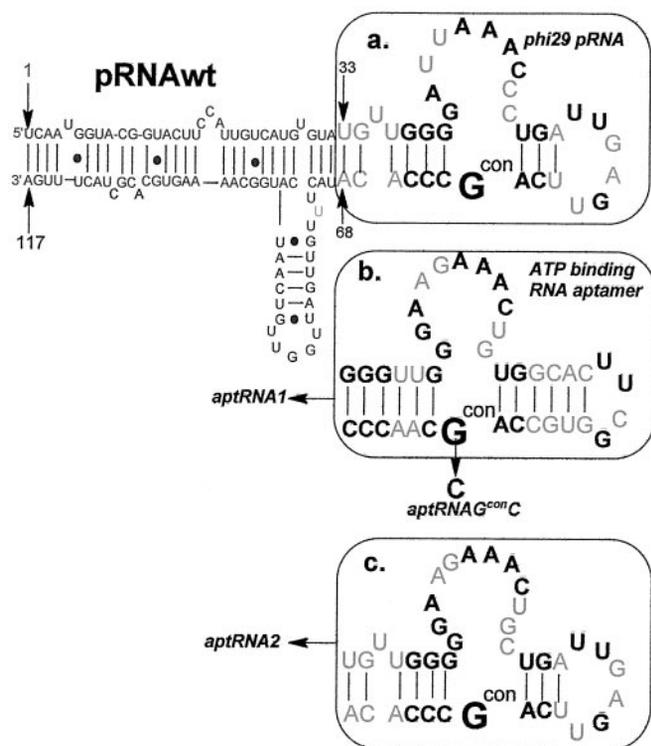


FIG. 1. Comparison of the central region of pRNA_{wt} with the ATP-binding RNA aptamer and construction of chimeric pRNA/ aptamer RNAs. Sequence comparison of the central region of pRNA_{wt} (a) with the 40-base ATP-binding RNA aptamer, ATP-40-1 (b) (36). The similar bases are *highlighted*. G^{con} enlarged is a conserved base essential for ATP binding. b and c, chimeric aptRNAs were constructed by replacing the central region of the phi29 pRNA with the sequence from the aptamer.

complete the assembly of infectious virions, which were assayed by standard plaque formation.

Inhibition of phi29 Assembly by ADP, AMP, and Adenosine—Inhibition assays were performed with the highly sensitive *in vitro* phi29 assembly system described above with the exception that the final concentration of ATP was 0.25 mM. Reaction buffer containing 0.25 mM ATP in TMS was mixed with varied concentrations of ADP, AMP, and adenosine before being added to the *in vitro* phi29 assembly system and assayed for the formation of phi29 plaques.

ATP-binding Assay for pRNA with ATP-Agarose Affinity Column—A column that was 0.55 cm in diameter was packed with affinity-agarose resin immobilized with 5 mM ATP (or higher concentration) and attached through the C-8 position to cyanogen bromide-activated agarose. Lyophilized resin was soaked in distilled water for >30 min before column packing. After washing with 10 ml of distilled water and then with 10 ml of binding buffer (300 mM NaCl, 20 mM Tris, pH 7.6, 5 mM MgCl₂), 1 μg (2.5 × 10⁻⁵ μmol) of ³H-labeled RNA in 100 μl binding buffer was applied to the ATP affinity column. The column was then washed with 3 ml of binding buffer and eluted with the same buffer containing ATP or other nucleotides as indicated. Fractions were collected and subjected to scintillation counting. A 116-base rRNA was used as a negative control.

Determination of Apparent Dissociation Constants for RNA-ATP Complex—The Apparent *K_d*s for RNA/ATP interaction were determined by two methods, isocratic elution (26, 34) and ATP gradient elution.

For isocratic elution, a column with a diameter of 0.55 cm was packed with 2.8 ml of ATP C-8-agarose containing 5 mM ATP. After washing the column with an excess amount of binding buffer, 2.5 × 10⁻⁵ μmol of [³H]pRNA or aptRNA in 100 μl of binding buffer was applied to the column. The RNAs were then eluted with the same binding buffer.

For ATP gradient elution, a column 0.8 cm in diameter was packed with 0.8 ml ATP C-8-agarose immobilized with 5 mM ATP. 1 μg (2.5 × 10⁻⁵ μmol) of [³H]pRNA in 100-μl binding buffer was applied to the column. After washing with 5 ml of binding buffer, RNA was eluted with a 2-ml step-up gradient with increasing concentrations of ATP in the binding buffer.

ATPase Assay by Thin Layer Chromatography—The purified DNA-

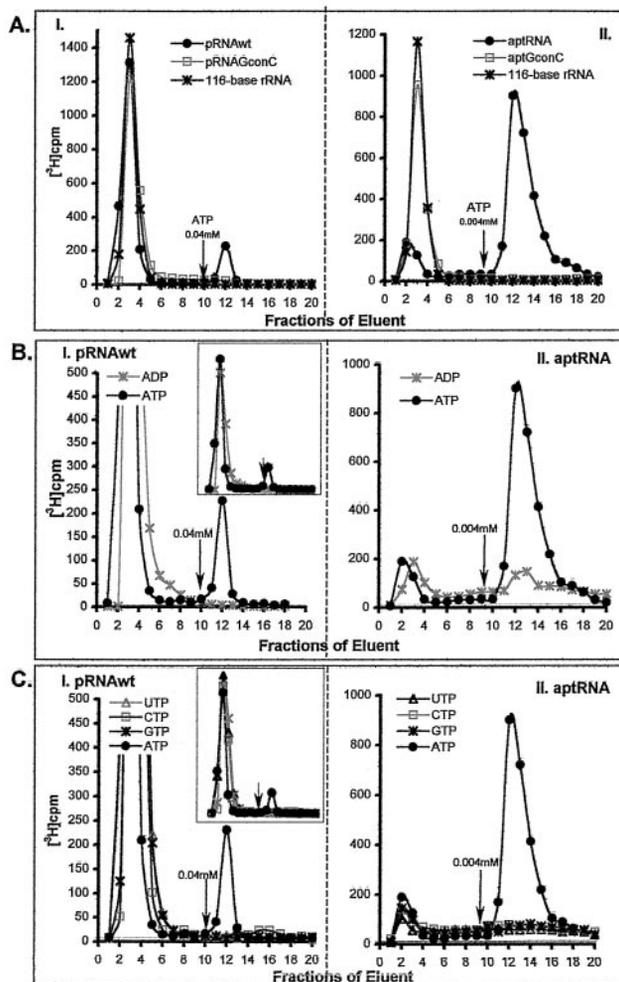


FIG. 2. ATP-binding assay with ATP-agarose affinity column. A, binding of pRNA_{wt} (I) and aptRNA (II) to ATP. [³H]pRNA_{wt}, mutant pRNA^{con}C, 116-base negative control rRNA, aptRNA, and mutant aptRNA^{con}C were applied to an ATP-agarose affinity column containing 5 mM ATP and washed with binding buffer. After collecting eight 250-μl fractions, the column was eluted with ATP in the same binding buffer. B, comparison of pRNA_{wt} (I) and aptRNA (II) binding affinity for ATP and ADP. C, comparison of pRNA_{wt} (I) and aptRNA (II) binding affinity for UTP, CTP, GTP, and ATP.

packaging components, gp16 (0.24 μg), DNA-gp3 (0.1 μg), procapsid (3.2 μg), and RNA (1 μg), were mixed individually or in combination with 0.3 mM of unlabeled ATP and 0.75 μCi (6000 Ci/mmol) of [³²P]ATP in the reaction buffer (30). When one or more components was omitted, the component(s) was replaced with the same volume of TMS. After 30 min of incubation at room temperature, 3 μl of the reaction mixture was spotted onto a polyethyleneimine-cellulose plate (J. T. Chemical Co.) (8) and air-dried. The plate was then soaked in methanol for 5 min, air-dried, and then run in 1 M formic acid and 0.5 M lithium chloride. Autoradiograms were produced with Cyclone Storage PhosphorScreen (Packard Instrument Co.). At the same time, a parallel experiment was conducted with the same components to test the results of phi29 virion assembly. Only those assembly reactions with a yield higher than 5 × 10⁷ plaque-forming units/ml were selected for ATPase assay.

RESULTS

Phi29 pRNA Was Able to Bind ATP—To investigate whether pRNA could interact with ATP directly, an ATP-agarose affinity column containing 5 mM ATP (or higher concentration) was used to detect the binding of pRNA_{wt}, the shortest pRNA with wild type pRNA phenotype, to ATP (Fig. 2A, I). [³H]pRNA_{wt} was applied to an ATP-affinity column and washed with a large volume of binding buffer. [³H]pRNA_{wt} was eluted from the column when 0.04 mM ATP was added to the buffer, suggesting that pRNA_{wt} binds ATP specifically. When the 116-base rRNA

TABLE I
 ATP-binding and viral assembly activities of pRNA and mutants

RNAs	Mutation	ATP-binding	Components added			
			ATP	RNase	γ -S-ATP	Virus produced
		%				<i>pfu/ml</i>
aptRNA	Chimera	80	–	–	–	→0
			–	–	+	→0
			+	+	–	→0
			+	–	+	→0
pRNA _{wt}	wild type pRNA	20	+	–	–	3×10^8
aptG ^{con} C	G ^{con} →C	→0	+	–	–	3×10^8
pRNA _{wt} G ^{con} C	G ^{con} →C	→0	+	–	–	→0
116-base rRNA		→0	+	–	–	→0

served as the negative control, no detectable RNA was eluted by as high as 5 mM ATP buffer, thereby indicating that the pRNA/ATP interaction was specific to pRNA. When the conserved base G^{con}, essential for ATP-binding (Fig. 1), was changed to a C, the resulting mutant pRNA^{G^{con}→C} could not bind ATP (Table I).

The Central Region of phi29 pRNA Is Very Similar to ATP-binding RNA Aptamer in Both Sequence and Predicted Secondary Structure—A chemically selected aptamer RNA has been found to be able to bind ATP (Fig. 1) (26). The structural basis for this ATP-binding RNA aptamer has also been elucidated by multidimensional NMR spectroscopy (35–37). All ATP-binding aptamers contain a consensus sequence embedded in a common secondary structure (26, 35–37). The bases essential for ATP-binding have been identified previously (26, 37). The structure of the phi29 pRNA has been investigated extensively (for review see Ref. 38). We compared the structure of ATP-binding aptamers with phi29 pRNA and found that the aptamers are very similar to the central part of phi29 pRNA (Fig. 1).

Infectious Virus Was Produced in the Presence of the Chimeric aptRNA Harboring the ATP-binding Motif—To further confirm that an ATP-binding motif is present in the pRNA molecule, the pRNA motif with a potential for ATP binding was replaced with a sequence from ATP-binding RNA aptamer, ATP-40-1 (26). When chimeric aptRNA was added to the phi29 *in vitro* assembling mixture (29, 39), $\sim 10^8$ infectious virus particles (plaque-forming unit) per milliliter were produced in the test tube (Table I) (Fig. 3). The omission of ATP or aptRNA or the addition of RNase to the reaction mixture failed to generate a single virus (Table I).

ATP Is Required for the Production of Infectious Virus—To establish that the activity of aptRNA is related to ATP, virus assembly using aptRNA was performed with and without ATP. When ATP was omitted from the reaction, not a single plaque was detected. Virus assembly was also inhibited by the poorly hydrolyzable ATP analogue γ -S-ATP, suggesting that the aptRNA-involved viral assembly process is ATP-related (Table I).

Both pRNA_{wt} and aptRNA Only Bound to ATP Resins with the Attachment Site at the C-8 Position—Seven different affinity resins were tested for pRNA_{wt} and aptRNA binding affinity to ATP. These resins vary in nucleotide composition and in location for nucleotide/agarose linkage. Our results show that both pRNA_{wt} and aptRNA bound only to an agarose resin containing ATP in which no binding to the resin with ADP or adenosine-3',5'-diphosphate was detected. For ATP resin, both pRNA_{wt} and aptRNA bound only to agarose resins with the attachment site at the C-8 position but not at N-6 or the hydroxyl position. These results suggest that both pRNA_{wt} and aptRNA/ATP interaction require a specific three-dimensional configuration and that such a requirement is similar for both RNAs.

Both aptRNA and pRNA_{wt} Had Higher Affinity for ATP Than for ADP and AMP—It has been reported that in the phi29 DNA-packaging system, ATP is hydrolyzed to ADP during packaging (8). It would be interesting to know whether pRNA_{wt} and aptRNA can discriminate ATP from ADP. Both ATP (immobilized by ATP) and an ADP affinity-agarose column (immobilized by ADP) were attached through the C-8 position and used to compare relative binding affinity. It was found that both pRNA_{wt} and aptRNA had a higher affinity for ATP than for ADP (Fig. 4).

Further investigation revealed that aptRNA had a higher affinity than pRNA_{wt} in ADP and AMP binding (Fig. 4). The binding affinity of aptRNA for ADP and AMP was very similar. The data partially agree with previous publications showing that the ATP-binding RNA aptamer binds ATP, ADP, AMP, and adenosine equally (26, 35, 40, 41). Nevertheless, the binding affinity of aptRNA to ADP and AMP was lower than that for ATP (Fig. 4). This suggests that the γ -phosphate was involved in ATP binding in aptRNA but not in the ATP-binding RNA aptamer (Fig. 7).

In Viral Assembly Assay, AMP and Adenosine Had Little Inhibition Effect on pRNA_{wt} but aptRNA Was Inhibited Strongly by AMP and Adenosine—As noted earlier, pRNA_{wt} had higher affinity for ATP than for AMP. However, it has been demonstrated previously that the ATP-binding RNA aptamer could bind ATP, AMP, and adenosine with similar affinity (26, 35, 37, 40–42). We showed that the binding affinity of aptRNA is higher for ATP than for ADP and AMP (Fig. 4). The result suggested that the structure of the RNA aptamer motif remains basically unchanged in the context of the chimeric aptRNA, whereas the global structures of aptRNA, pRNA_{wt}, and apt-binding RNA aptamer for holding ATP and AMP are not identical. The 30 bases at the 5' end and the 52 bases at the 3' end obviously have certain physical obstructive effects on general RNA/nucleotide interaction, since the Apparent K_d for ATP_{bound} as determined by isocratic elution (26, 34) is 14 μ M for ATP-binding RNA aptamer (26) while it is 100 μ M (see below) for aptRNA. Moreover, the bases at the 5' end and 3' end might help to promote the holding for the γ -phosphate, because both pRNA_{wt} and aptRNA had a higher affinity for ATP than for ADP and AMP, whereas RNA aptamer bound ATP and AMP equally.

To test whether the ATP-binding aptamer in aptRNA is truly functioning with an interchangeable ATP binding site and whether the ATP binding is related to DNA-packaging and phi29 assembly function, inhibition studies were conducted by adding ADP, AMP, and adenosine to the phi29 assembly system with pRNA_{wt} and aptRNA. Because aptRNA contains an ATP-binding RNA aptamer that binds adenosine, AMP, and ATP with similar affinity, if ATP-binding is related to biological function, then aptRNA containing such an aptamer could be poisoned by the addition of

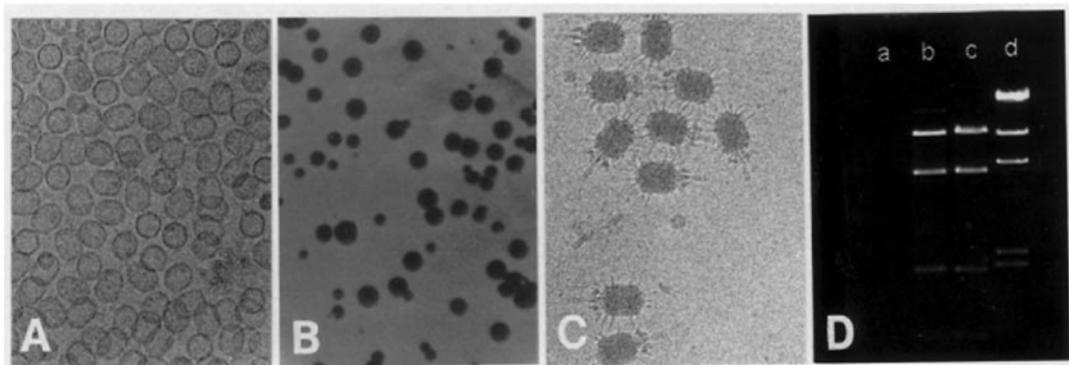
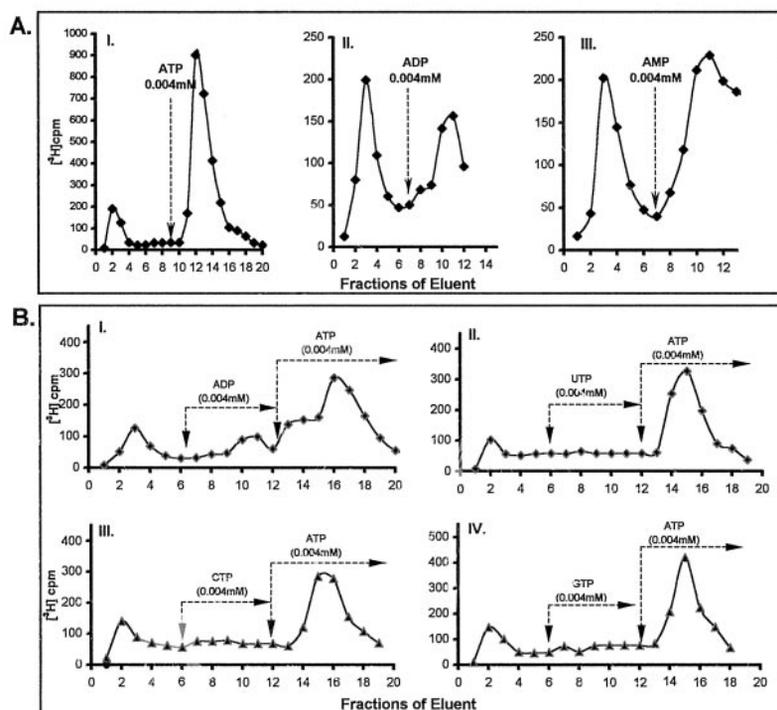


FIG. 3. *In vitro* production of infectious virions of phi29 particles with aptRNA and ATP. A, electron microscopy image of purified phi29 procapsid devoid of genomic DNA. B, plaques formed on a lawn of *Bacillus subtilis* after plating with the infectious virus produced from the reaction with aptRNA and ATP. C, electron microscopy image ($\times 90,000$) of the viral particles purified from the lawn in B. D, agarose gel showing *Eco*RI restriction mapping of genomic DNA from wild type phi29 (lane b) and from the virus assembled with aptRNA (lane c). Lane d shows 1-kilobase ladder, and lane a contains a control sample from procapsid (A) that is devoid of genomic DNA.

FIG. 4. Comparisons of aptRNA-binding affinity for ATP, ADP, and AMP. A, comparison of aptRNA-binding affinity to ATP, ADP, and AMP. $^{[3]H}$ aptRNA was applied onto a 0.8-ml ATP-agarose affinity column immobilized with 5 mM ATP, washed with binding buffer, and then eluted with buffer containing 0.004 mM ATP (I), ADP (II), and AMP (III). B, $^{[3]H}$ aptRNA was applied onto a 0.8-ml ATP-agarose affinity column containing 5 mM ATP, washed with binding buffer, and eluted with buffer containing 0.004 mM ADP (I), UTP (II), CTP (III) or GTP (IV) and then with 0.004 mM ATP. Arrows indicate that the given concentration of specified nucleotides was added to the binding buffer. Each fraction is 250 μ l.



adenosine or AMP, whereas the pRNA_{wt}-packaging system should not be poisoned. The result from phi29 assembly assays revealed that AMP and adenosine inhibited aptRNA activity strongly while the inhibition effect for pRNA_{wt} was not as strong (Figs. 5–7).

Comparison of RNA Binding Affinity for ATP, CTP, GTP, and UTP—To compare the binding affinity for ATP, CTP, GTP, and UTP, pRNA_{wt} (Fig. 2C, I) or aptRNA (Fig. 2C, II) was first attached to the ATP-agarose gel. After washing with an excess amount of binding buffer, the bound RNA was then eluted by the buffers containing ATP, CTP, GTP, and UTP. It was found that ATP buffer could elute the bound aptRNA or pRNA_{wt} effectively while GTP, CTP, and UTP buffer was much less efficient.

Determination of the Apparent Dissociation Constants for RNA-ATP Complex—The Apparent K_d s for RNA/ATP interaction were determined by isocratic elution (26, 34) and ATP gradient elution. The isocratic elution method was used to measure the Apparent K_d for ATP that immobilized on agarose

(ATP_{bound}) while the purpose of the ATP gradient elution was to measure the Apparent K_d for free ATP (ATP_{free}).

With the isocratic elution method (Fig. 8A), Apparent K_d was determined with the equation: Apparent $K_d = [L] \times (V_t - V_0)/(V_e - V_0)$, where [L] is the concentration of ATP immobilized on agarose, V_t is the volume of the column, V_0 is the void volume of the column, and V_e is the volume needed to elute the RNA (Fig. 8). The Apparent K_d for aptRNA and for pRNA_{wt} interacting with the ATP_{bound} was determined to be (0.1 ± 0.06) mM and (1.3 ± 0.8) mM, respectively. The K_d for pRNA_{wt}, which is more than ten times higher than that for aptRNA, indicated that the affinity of pRNA_{wt} for ATP is much lower than that of aptRNA. Therefore, a resin immobilized with higher ATP concentration (≥ 5 mM) was needed to detect the ATP/pRNA interaction through the ATP affinity-agarose method. If the ATP concentration is lower than 5 mM, it is difficult to detect the binding in the elution assay in Fig. 2.

For ATP gradient elution, ATP_{free} will compete with the

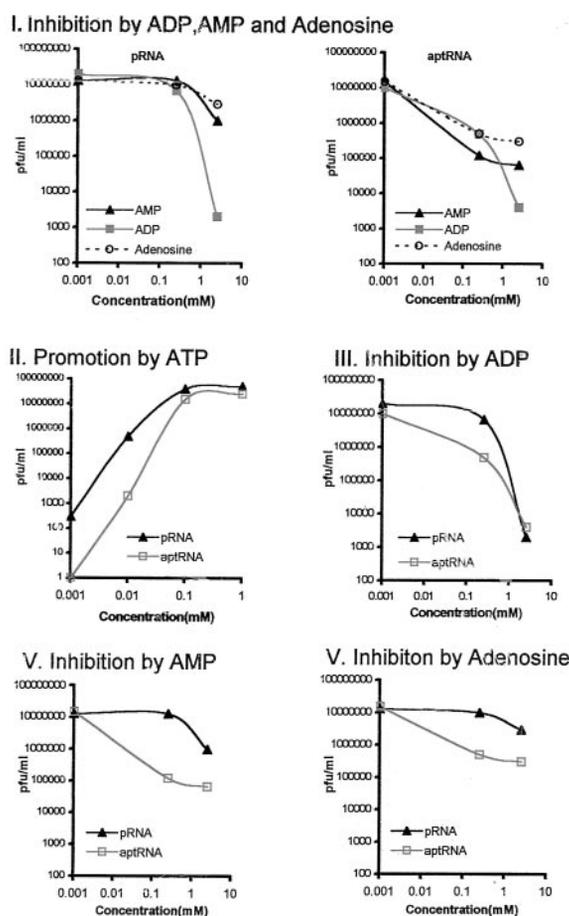


FIG. 5. Comparison of inhibitory effect of pRNA_{wt} and aptRNA by ADP, AMP, and adenosine on phi29 assembly.

ATP_{bound} for binding to aptRNA or pRNA_{wt}. To completely elute the pRNA_{wt} bound to the column, the concentration of ATP_{free} must be close to the Apparent K_d . Otherwise, the ATP_{free} would not be able to snatch the RNA that had been attached to the ATP_{bound} in the column. From the ATP gradient elution (Fig. 8B), it was found that most of the bound aptRNA and pRNA_{wt} was eluted by 0.004 and 0.04 mM ATP_{free}, respectively. This suggests that the Apparent K_d s for the complexes of aptRNA·ATP_{free} and pRNA_{wt}·ATP_{free} are around 0.004 and 0.04 mM, respectively. The finding of a difference in Apparent K_d determined through these two methods is not surprising, because the C-8 linkage of ATP to agarose might hamper the RNA/ATP interaction that involves a three-dimensional contact. Furthermore, it is possible that only a certain fraction of ATP_{bound} in the gel is accessible to aptRNA or pRNA_{wt}.

Changing a Single Base Essential for ATP Binding Caused Both the ATP-binding and Viral Assembly Activities to Cease—Nucleotide G^{con} (Fig. 1) has been shown to be highly conserved in ATP-binding RNA aptamers, and it is the most critical nucleotide for ATP binding (26, 35). One G corresponding to G^{con} of the aptRNA is also conserved in all of the pRNAs of five different bacteriophages (43, 44).

The mutation of G^{con} to C resulted in a mutant aptRNA^{G^{con}C} (Fig. 1) that was not able to bind ATP (Fig. 2A, II). This mutant was also completely inactive in virion assembly (Table I), suggesting that the functions of ATP binding and virion assembly are correlated. When the G^{con} mutation was introduced into the conserved G^{con} of pRNA_{wt}, the ATP-binding activity of the mutant pRNA^{G^{con}C} disappeared (Fig. 2A, I). This mutant was found to be incompetent in phi29 assembly (Table I). By struc-

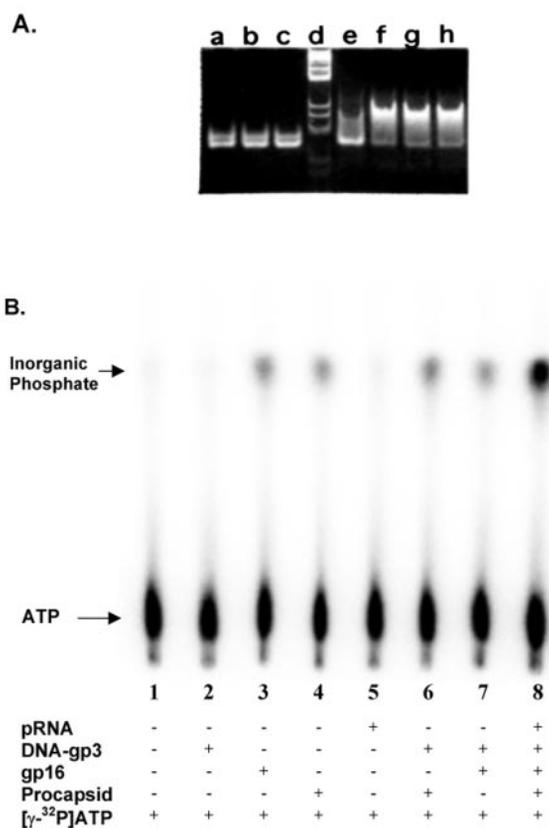


FIG. 6. ATP and RNA interaction. A, ATP-induced RNA conformational change. Purified pRNAs were mixed with different concentrations of ATP and loaded onto 8% native polyacrylamide gel (53). A pRNA bandshift was observed in the presence of ATP (lanes f–h) but not observed in the absence of ATP (lane e), whereas the 5 S rRNA control did not show any migration change either in the presence (lanes b and c) or absence (lane a) of ATP. Lanes b, c, f, g, and h contained 5, 15, 5, 15, and 25 mM ATP, respectively. Lane d is a DNA ladder. B, autoradiogram of ATPase assay by thin layer chromatography showing the hydrolysis of [γ -³²P]ATP in the presence of the ATP-binding pRNA.

tural analysis, in addition to competition and inhibition with binomial distribution analysis (15, 45), it was confirmed that the incompetence of such mutant aptRNA with regard to driving the motor is because of a change in chemistry rather than structure (data not shown).

Conformational Changes of pRNA Induced by ATP—The conformation change of pRNA_{wt} was investigated in the presence and absence of ATP. ATP caused a change in the pRNA_{wt} migration rate in native gels (Fig. 6A). Purified pRNA_{wt} was loaded onto a native gel with different concentrations of ATP. The pRNA_{wt} was observed to migrate more slowly when ATP was present. The band with the slower migration rate was purified and shown to be fully active in DNA packaging. At the same time, the control *Escherichia coli* 5 S rRNA did not show any migration rate change attributed to the presence or absence of ATP (Fig. 6A).

We have reported that magnesium induces a conformational change of pRNA (46, 47). It is possible that the change of pRNA conformation was because of the depletion of ions by ATP. However, we have previously reported that pRNA formed oligomers with a slower migration rate in gel when magnesium is present (46, 47). The formation of a band with a slower migration rate in Fig. 6A suggests that in the presence of ATP, the conformation or oligomerization of pRNA is larger rather than smaller. This phenomenon argues against the possibility that the conformational change is simply the result of the depletion of ions by ATP. If that were true, the RNA would become

FIG. 7. Computer models showing the interaction of ATP-binding RNA aptamer and the central region of pRNA with ATP, ADP, and AMP. **A**, the three-dimensional structure of ATP-binding RNA in backbone format. The aptamer and the central region of pRNA were derived from Refs. 35 and 33, respectively. The ATP-binding RNA aptamer is red, the additional sequence from pRNA is gray, the adenosine is green, and the phosphates are blue. **B**, computer model of the three-dimensional structure of the entire pRNA-ATP complex in Spacefill format.

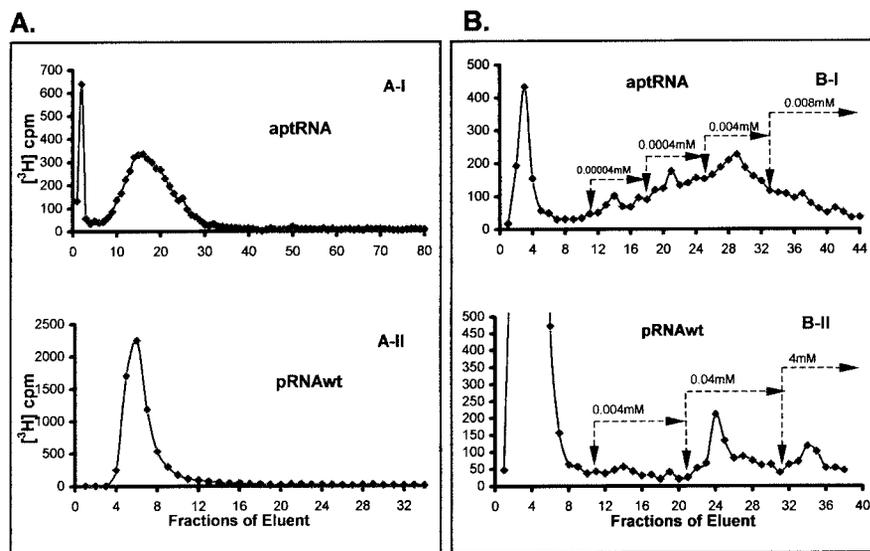
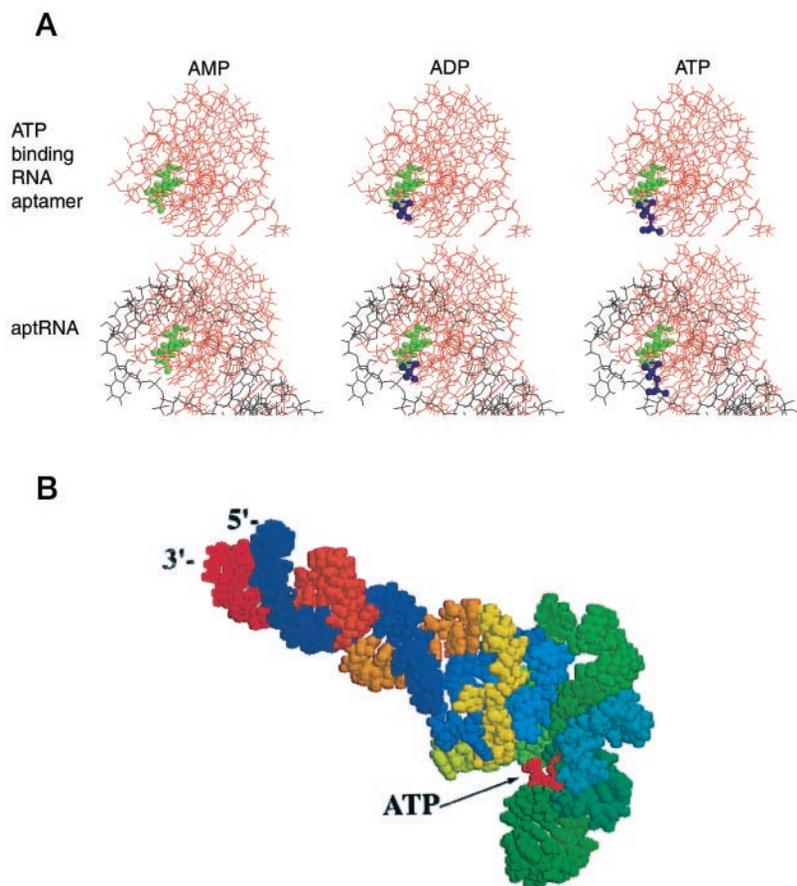


FIG. 8. Determination of the apparent dissociation constants (Apparent K_d) for pRNA and aptRNA:ATP complex. **A**, Apparent K_d determination with isocratic elution. $[^3\text{H}]$ aptRNA (**A-I**) or $[^3\text{H}]$ pRNA_{wt} (**A-II**) was applied to a column packed with ATP C-8 affinity-agarose and eluted with binding buffer (2.7 ml for **A-I** and 2.1 ml for **A-II**). Fractions (2 ml for **A-I** and 0.55 ml for **A-II**) were collected and subjected to scintillation counting. **B**, K_d appraisal with ATP gradient elution. $[^3\text{H}]$ aptRNA (**B-I**) or $[^3\text{H}]$ pRNA_{wt} (**B-II**) was applied to a column packed with ATP C-8 affinity-agarose and eluted with a 2-ml step-up gradient with specified concentration of ATP in binding buffer. Fractions were collected and subjected to scintillation counting.

smaller and run faster in the presence of ATP. The appearance of a broad band representing pRNA with a slower migration rate also suggests that more than one conformation of pRNA may be present.

ATP Was Hydrolyzed to ADP and Inorganic Phosphate in a Reaction Mixture Containing pRNA—Hydrolysis of $[^{32}\text{P}]$ ATP was assayed by thin layer chromatography on a polyethyleneimine-cellulose plate. Components involved in DNA packaging were mixed alone or in combination with $[^{32}\text{P}]$ ATP. After an incubation period of 30 min, the reaction mixture was applied to the polyethyleneimine-plate. Results from thin layer chromatography revealed that the individual component alone exhibited almost undetectable ATPase activity (Fig. 6B). How-

ever, ATP was hydrolyzed to inorganic phosphate in the reaction along with all DNA-packaging components including pRNA.

DISCUSSION

Many RNA aptamers have been isolated and extensively investigated (25, 26, 41, 48–52), but these studies have remained very theoretical since none of these reported aptamers has been identified in RNA molecules in nature. This work demonstrated that the chemically selected ATP-binding RNA aptamer is similar to the central region of pRNA in sequence and structure. The replacement of the central region of pRNA_{wt} with the sequence from the aptamer produced a chimeric aptRNA that was able to

assemble infectious viruses in a test tube containing other purified phi29 structural components. RNA mutation studies revealed that the change of only one base essential for ATP binding caused the activities of ATP binding and viral assembly to cease, confirming that a structure similar to the ATP-binding RNA aptamer is present in bacterial virus phi29.

There are several similarities and differences among pRNA_{wt}, aptRNA, and the ATP-binding RNA aptamer. The similarities include the following. 1) All of them bind ATP. 2) All of them prefer the ATP-resin with a C-8 linkage. 3) All of them have a higher affinity for ATP than for GTP, CTP, and UTP. 4) Both pRNA_{wt} and aptRNA have a higher affinity for ATP than for ADP. 5) All of them contain a conserved G^{con} at a similar position.

The differences include the following. 1) Both pRNA_{wt} and aptRNA have a higher affinity for ATP than for ADP and AMP, whereas the RNA aptamer binds ATP, ADP, AMP, and adenosine equally (26). This is an indication that the 5'- and 3'-extended bases of the aptRNA provide a packet to hold the γ -phosphate of ATP. The preference for ATP over ADP is explainable. Because ATP-binding is related to biological activity, the pRNA must have the ability to trap ATP and to release ADP after ATP hydrolysis. 2) The ATP-binding affinity of aptRNA is higher than that of pRNA_{wt} (Figs. 2 and 4). However, the specific activity of aptRNA in phi29 assembly is lower than that of pRNA_{wt} (data not shown). Two possibilities might explain this discrepancy. First, the binding of pRNA_{wt} to the connector is the rate-determining step in phi29 DNA packaging and assembly. The change of the mutant is at the connector-binding domain. This change might somehow alter its structure and thus hamper the connector binding affinity. Second, although the chemically selected ATP-binding aptamer is an excellent molecule for ATP binding, it might not be after all the best candidate in nature for ATP hydrolysis if such hydrolysis does occur. Too high a binding affinity to the substrate does not signify a good biological component or enzyme, because this component or enzyme will not be dissociated from its substrate easily. Such dissociation might be critical for the turnover in pRNA_{wt}/ATP interaction in phi29 assembly.

Here we found that the putative ATP-binding site in pRNA_{wt} resides within a region interacting with the connector. The significance for such ATP/pRNA_{wt} binding remains to be investigated. One possible implication is that ATP binding to pRNA_{wt} provides a special structure in the assembly of the packaging machinery. Another possible implication is that alternative binding and release of ATP from pRNA_{wt} could induce a conformational change of pRNA_{wt} that in turn rotates the connector and pRNA is part of the ATPase complex.

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REFERENCES

- Guo, P. (1994) *Seminars in Virology* **5**, 1–3
- Earnshaw, W. C., and Casjens, S. R. (1980) *Cell* **21**, 319–331
- Black, L. W. (1988) In *The Bacteriophages* (Calendar, R., ed) Plenum Publishing Corp., New York
- Bazin, C., and King, J. (1985) *Annu. Rev. Microbiol.* **39**, 109–129
- Jimenez, J., Santisteban, A., Carazo, J. M., and Carrascosa, J. L. (1986) *Science* **232**, 1113–1115
- Simpson, A. A., Tao, Y., Leiman, P. G., Badasso, M. O., He, Y., Jardine, P. J., Olson, N. H., Morais, M. C., Grimes, S., Anderson, D. L., Baker, T. S., and Rossmann, M. G. (2000) *Nature* **408**, 745–750
- Guasch, A., Pous, J., Ibarra, B., Gomis-Ruth, F. X., Valpuesta, J. M., Sousa, N., Carrascosa, J. L., and Coll, M. (2002) *J. Mol. Biol.* **315**, 663–676
- Guo, P., Peterson, C., and Anderson, D. (1987) *J. Mol. Biol.* **197**, 229–236
- Parris, W., Davidson, A., Keeler, C. L., Jr., and Gold, M. (1988) *J. Biol. Chem.* **263**, 8413–8419
- Hang, J. Q., Tack, B. F., and Feiss, M. (2000) *J. Mol. Biol.* **302**, 777–795
- Rao, V. B., and Black, L. W. (1988) *J. Mol. Biol.* **200**, 475–488
- Guo, P., Erickson, S., and Anderson, D. (1987) *Science* **236**, 690–694
- Morita, M., Tasaka, M., and Fujisawa, H. (1993) *Virology* **193**, 748–752
- Chen, C., and Guo, P. (1997) *J. Virol.* **71**, 3864–3871
- Trottier, M., and Guo, P. (1997) *J. Virol.* **71**, 487–494
- Guo, P., Zhang, C., Chen, C., Trottier, M., and Garver, K. (1998) *Mol. Cell.* **2**, 149–155
- Zhang, F., Lemieux, S., Wu, X., St.-Arnaud, S., McMurray, C. T., Major, F., and Anderson, D. (1998) *Mol. Cell.* **2**, 141–147
- Hendrix, R. W. (1998) *Cell* **94**, 147–150
- Davenport R. J. (2001) *Science* **291**, 2071–2072
- Smith, D. E., Tans, S. J., Smith, S. B., Grimes, S., Anderson, D. L., and Bustamante, C. (2001) *Nature* **413**, 748–752
- Grimes, S., and Anderson, D. (1990) *Mol. Biol.* **215**, 559–566
- Ibarra, B., Valpuesta, J. M., and Carrascosa, J. L. (2001) *Nucleic Acids Res.* **29**, 4264–4273
- Cech, T. R., Zaug, A. J., and Grabowski, P. J. (1981) *Cell* **27**, 487–496
- Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983) *Cell* **35**, 849–857
- Ellington, A. D., and Szostak, J. W. (1990) *Nature* **346**, 818–822
- Sassanfar, M., and Szostak, J. W. (1993) *Nature* **364**, 550–553
- Zhang, C. L., Lee, C.-S., and Guo, P. (1994) *Virology* **201**, 77–85
- Guo, P., Rajogopal, B., Anderson, D., Erickson, S., and Lee, C.-S. (1991) *Virology* **185**, 395–400
- Lee, C. S., and Guo, P. (1995) *J. Virol.* **69**, 5018–5023
- Guo, P., Grimes, S., and Anderson, D. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 3505–3509
- Anderson, D. L., Hickman, H. H., and Reilly, B. E. (1966) *J. Bacteriol.* **91**, 2081–2089
- Mellado, R. P., Peñalva, M. A., Inciarte, M. R., and Salas, M. (1980) *Virology* **104**, 84–96
- Hoepflich, S., and Guo, P. (2002) *J. Biol. Chem.* **277**, 20794–20803
- Arnold, F. H., Schofield, S. A., and Blanch, H. W. (1986) *J. Chromatogr.* **355**, 1–12
- Dieckmann, T., Suzuki, E., Nakamura, G. K., and Feigon, J. (1996) *RNA* **2**, 628–640
- Cech, T. R., and Szewczak, A. A. (1996) *RNA* **2**, 625–627
- Jiang, F., Kumar, R. A., Jones, R. A., and Patel, D. J. (1996) *Nature* **382**, 183–186
- Guo, P. (2002) *Prog. Nucleic Acids Res. Mol. Biol.* **72**, 415–472
- Lee, C. S., and Guo, P. (1994) *Virology* **202**, 1039–1042
- Nonin, S., Jiang, F., and Patel, D. J. (1997) *J. Mol. Biol.* **268**, 359–374
- Dieckmann, T., Butcher, S. E., Sassanfar, M., Szostak, J. W., and Feigon, J. (1997) *J. Mol. Biol.* **273**, 467–478
- Jiang, F., Patel, D. J., Zhang, X., Zhao, H., and Jones, R. A. (1997) *J. Biomol. NMR* **9**, 55–62
- Bailey, S., Wichtwehkarn, J., Johnson, D., Reilly, B., Anderson, D., and Bodley, J. W. (1990) *J. Biol. Chem.* **265**, 22365–22370
- Chen, C., Zhang, C., and Guo, P. (1999) *RNA* **5**, 805–818
- Chen, C., Trottier, M., and Guo, P. (1997) *Nucleic Acids Symp. Ser.* **36**, 190–193
- Chen, C., and Guo, P. (1997) *J. Virol.* **71**, 495–500
- Trottier, M., Mat-Arip, Y., Zhang, C., Chen, C., Sheng, S., Shao, Z., and Guo, P. (2000) *RNA* **6**, 1–10, 1257–1266
- Ciesiolka, J., Gorski, J., and Yarus, M. (1995) *RNA* **1**, 538–550
- Klug, S. J., and Famulok, M. (1994) *Mol. Biol. Rep.* **20**, 97–107
- Burke, D. H., and Willis, J. H. (1998) *RNA* **4**, 1165–1175
- Wang, C., Xu, F., Jin, Y. X., and Wang, D. B. (1999) *Acta Biochem. Biophys. Sinica* **31**, 504–508
- Szkaradkiewicz, K., Nanninga, M., Nesper-Brock, M., Gerrits, M., Erdmann, V. A., and Sprinzl, M. (2002) *FEBS Lett.* **514**, 90–95
- Chen, C., Sheng, S., Shao, Z., and Guo, P. (2000) *J. Biol. Chem.* **275**, 17510–17516
- Tuerk, G., and Gold, L. (1990) *Science* **249**, 505–510