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)-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 hydrolysis. Both gp16 and gp17, which constitute the terminase of bacteriophage T4, are involved in ATP hydrolysis (11). In bacteriophage 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 polysacrylamide 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 RNAase-free H2O was mixed with 10 μl of purified procapsids (0.4 mg/ml) and then dialyzed on a 0.025-ml 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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‡ The abbreviations used are: ds, double-stranded; gp, gene product; wt, wild type.
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 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.

For isocratic elution, a column with a diameter of 0.55 cm was packed with 5 ml ATP-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 was applied to an ATP-affinity column containing 5 mM ATP in TMS and washed with binding buffer. After collecting eight 250-μl fractions, the column was eluted with ATP in the same binding buffer. For isocratic elution, a column 0.8 cm in diameter 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 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-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/mol) of [γ-32P]ATP in the reaction buffer (30). When one or more components were omitted, the components were 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 × 10^{-7} 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 RNA

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**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 (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.

**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 pRNAGconC, 116-base negative control rRNA, aptRNA, and mutant aptRNAGconC 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.
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<sup>com</sup> essential for ATP-binding (Fig. 1), was changed to a C, the resulting mutant pRNA<sup>com</sup>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), ~10<sup>8</sup> 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<sub>wt</sub> and aptRNA Only Bound to ATP Resins with the Attachment Site at the C-8 Position—Seven different affinity resins were tested for pRNA<sub>wt</sub> 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<sub>wt</sub> 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<sub>wt</sub> 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<sub>wt</sub> and aptRNA/ATP interaction require a specific three-dimensional configuration and that such a requirement is similar for both RNAs.

Both aptRNA and pRNA<sub>wt</sub> 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<sub>wt</sub> 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<sub>wt</sub> and aptRNA had a higher affinity for ATP than for ADP (Fig. 4).

Further investigation revealed that aptRNA had a higher affinity than pRNA<sub>wt</sub> 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<sub>wt</sub> but aptRNA Was Inhibited Strongly by AMP and Adenosine—As noted earlier, pRNA<sub>wt</sub> had higher affinity for ATP than for AMP. However, it has been demonstrated previously that the ATP-binding RNA aptamer could bind ATP, ADP, 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<sub>wt</sub>, 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<sub>d</sub> for ATP bound as determined by isocratic elution (26, 34) is 14 μM for ATP-binding RNA aptamer (26) while it is 100 μM (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<sub>wt</sub> 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<sub>wt</sub> 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

| RNAs           | Mutation | ATP-binding | Components added | Virus produced |
|---------------|----------|-------------|------------------|----------------|
| aptRNA        | Chimera  | 80          | ATP, RNase, γ-S-ATP | if/ml          |
| pRNA<sub>wt</sub> | wild type pRNA | 20         | +                | +              |
| aptG<sup>com</sup>C | G<sup>com</sup>–C | 0           | +                | +              |
| pRNA<sub>com</sub>G<sup>com</sup>C | G<sup>com</sup>–C | 0           | +                | +              |
| 116-base rRNA |          | 0           |                  | 0              |
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, ADP, and AMP—To compare the binding affinity for ATP, ADP, and AMP, [3H]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, [3H]aptRNA was applied onto a 0.8-ml ATP-agarose affinity column containing 5 mM ATP, washed with binding buffer, and then 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 µl.

Determination of the Apparent Dissociation Constants for RNA/ATP Complex—The Apparent $K_d$ 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: $\text{Apparent } K_d = \frac{[L]}{V_t - V_e}[V_e - V_0]$, 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 $(0.1 \pm 0.06)$ mM and $(1.3 \pm 0.8)$ 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
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 Kd. 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 Kd s for the complexes of aptRNA/H18528 ATP free and pRNA wt/H18528 ATP free are around 0.004 and 0.04 mM, respectively. The finding of a difference in Apparent Kd 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, I). 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 structural 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 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.

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...
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 \([^{32}P]ATP\) was assayed by thin layer chromatography on a polyethyleneimine-cellulose plate. Components involved in DNA packaging were mixed alone or in combination with \([^{32}P]ATP\). 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). However, 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 pRNAwt 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<sub>wt</sub>, 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<sub>wt</sub> and aptRNA have a higher affinity for ATP than for ADP. 5) All of them contain a conserved G<sub>cons</sub> at a similar position.

The differences include the following: 1) Both pRNA<sub>wt</sub> 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 pocket 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<sub>wt</sub> (Figs. 2 and 4). However, the specific activity of aptRNA in phi29 assembly is lower than that of pRNA<sub>wt</sub> (data not shown). Two possibilities might explain this discrepancy. First, the binding of pRNA<sub>wt</sub> 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<sub>wt</sub>/ATP interaction in phi29 assembly.

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

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REFERENCES
1. Guo, P. (1994) <i>Seminars in Virology</i> 5, 1–3
2. Earnshaw, W. C. and Casiens, S. R. (1980) <i>Cell</i> 21, 319–331
3. Black, L. W. (1988) In <i>The Bacteriophages</i> (Calendar, R., ed) Plenum Publish-