Genomes of complex viruses have been demonstrated, in many cases, to be packaged into preformed empty capsids (procapsids). This reaction is performed by molecular motors translocating nucleic acid against the concentration gradient at the expense of NTP hydrolysis. At present, the molecular mechanisms of packaging remain elusive due to the complex nature of packaging motors. In the case of the double-stranded RNA bacteriophage $\phi 6$ from the Cystoviridae family, packaging of single-stranded genomic precursors requires a hexameric NTPase, P4. In the present study, the purified P4 proteins from two other cystoviruses, $\phi 8$ and $\phi 13$, were characterized and compared with $\phi 6$ P4. All three proteins are hexameric, single-stranded RNA-stimulated NTPases with $\alpha\beta$ folds. Using a direct motor assay, we found that $\phi 8$ and $\phi 13$ P4 hexamers translocate 5' to 3' along ssRNA, whereas the analogous activity of $\phi 6$ P4 requires association with the procapsid. This difference is explained by the intrinsically high affinity of $\phi 8$ and $\phi 13$ P4s for nucleic acids. The unidirectional translocation results in RNA helicase activity. Thus, P4 proteins of Cystoviridae exhibit extensive similarity to hexameric helicases and are simple models for studying viral packaging motor mechanisms.

A number of biological systems rely on active transport of nucleic acids (NA) powered by the hydrolysis of NTPs. This is observed in prokaryotic organisms, for example, during cell division, conjugation, and sporulation (1). NTP-dependent translocation is also utilized by many DNA viruses for genome encapsidation, or “packaging,” into preformed capsids, examples including herpesviruses and adenoviruses, as well as tailed bacteriophages, such as $\phi 29$, $\lambda$, P22, and T4 (2–7).

Genome packaging is best understood for the dsDNA bacteriophage $\phi 29$, whose portal complex serves as a packaging engine (8). The complex contains three components: 1) the head-tail connector, a dodecamer of protein p10, attached to a unique 5-fold prohead vertex; 2) a multimeric ring of packaging RNAs; and 3) a homomultimeric ATPase composed of protein p16 (6, 8). The portal of $\phi 29$ can translocate DNA against loads up to 57 piconewtons and therefore is one of the most powerful molecular motors in nature (9).

Porta complexes of many other DNA bacteriophages are organized similarly to $\phi 29$, although the packaging RNA requirement is limited to $\phi 29$-related phages (10). In addition, DNA translocation in these systems is often accompanied by the processing of genomic DNA concatemers (terminase activity) (3, 11). Because the $\phi 29$ p16 and terminases of other bacteriophages have been shown to catalyze NTP hydrolysis in vitro, these proteins are thought to generate the energy for the packaging reaction (12). It has been suggested that the energy is somehow transmitted from the transiently associated terminase to the packaging RNA ring and connector, which in turn effects unidirectional DNA translocation (6, 13). However, the complexity of the portal structures in DNA viruses has made it difficult to elucidate the packaging mechanism experimentally.

In vitro packaging of dsRNA viruses has been accomplished only for bacteriophages $\phi 6$ and $\phi 8$ of the Cystoviridae family, which infect the plant pathogenic bacterium Pseudomonas syringae (14, 15). All cystoviruses contain a lipid envelope and a tripartite dsRNA genome composed of S, M, and L segments. Similarly to $\phi 29$, $\phi 6$ packaging can be assayed in vitro using recombinant empty particles, called procapsids (PCs) and (+) sense ssRNA genome precursors $s^+$, $m^+$, and $l^+$, respectively (14). Segment $s^+$ is packaged first, followed by $m^+$ and $l^+$ (16). The specificity of packaging is due to the $pac$ sites located within the 5′-terminal ~300-nt regions of all three RNA precursors (14, 17).

The $\phi 6$ PC consists of four virus-encoded proteins, P1, P2, P4, and P7. P1 forms the structural framework of the PC (18). P2 is an RNA-dependent RNA polymerase that replicates and transcribes the packaged RNA (19, 20). P7 is important for PC stability and proper functioning (21, 22). Comparison between the wild-type and protein-deficient $\phi 6$ PC has shown that P4 is essential for RNA packaging (23, 24). P4 sequence contains characteristic NTPase motifs, which are conserved among the Cystoviridae (Fig. 1), although there is no other detectable sequence identity. Indeed, the purified $\phi 6$ P4 is an unspecific NTPase, hydrolyzing ribo-, deoxyribo-, and deoxyribonucleoside triphosphates (25, 26).

The $\phi 6$ P4 forms doughnut-shaped hexamers in the presence of divalent cations and ATP or ADP, and the NTPase activity is
associated only with the multimeric form. The NTPase activity is weakly stimulated by ssRNA (27). Cryo-electron microscopy reconstruction of d6 PC has localized P4 hexamers to the particle 5-fold vertices, thus constituting a symmetry mismatch (28). Our recent micrographs of d8 subviral particles show that the localization of P4 is likely to be similar for other cystoviruses (29). The symmetry mismatch is also typical for packaging motors of tailed bacteriophages, and it has been proposed to facilitate rotation of the portal during nuclear acid translocation (11).

Although P4 NTPase activity is essential for RNA packaging (24, 27), it is still unknown whether P4 alone constitutes the packaging motor or if other PC proteins are also involved. In addition, the details of the mechanochemical coupling during packaging motor or if other PC proteins are also involved. In addition, the details of the mec

P4 Expression and Purification—d6 and d8 P4s were purified as described (27, 33). To construct a d13 P4 expression plasmid, pDK3, gene 4 was PCR-amplified from the d13 L segment cDNA (plasmid pLM2200 (32) using recombinant Pfu DNA polymerase (Stratagene) and the oligonucleotides 5′-AGGGATGTCATAGATGTCGAAAG-T-A′ and 5′-GGGGAATCCTTACATATACACGATCT-3′. The PCR fragment was inserted into the vector pETT–3 (34) at the Ndel-HindIII sites. Soluble d13 P4 was produced in Escherichia coli strain BL21(DE3) transformed with pDK3 as described for d8 P4 (33).

**NTPase Assays**—The NTPase activity of the purified P4 proteins was assayed in 20 mM Tris (pH 8.0), 75 mM NaCl, 7.5 mM MgCl₂, 4 mM unlabeled NTP, and 0.5 μCi of the relevant [α-32P]NTP (3000 Ci/mmol; Amersham Biosciences). The final P4 concentration was 0.1 mg/ml. Reactions were incubated for 1 h at 37 °C, and 1 μl of the reactions were analyzed on polyethyleneimine-cellulose TLC plates (Merck). Chromatograms were developed as described (26). The amount of radioactivity in the nucleotide spots was measured using a phosphor imager (Fuji BAS 1500).

The steady-state rate of NTP hydrolysis was studied as the rate of phosphate release using the EnzChek phosphate assay kit (Molecular Probes, Inc., Eugene, OR). The concentration of P, was proportional to the absorbance at 360 nm. The calibration was done using standard KH₂PO₄ solutions. Steady-state kinetics of phosphate release was measured using a Victor² plate reader (Wallac-PerkinElmer Life Sciences). ATP was purchased from Amersham Biosciences, ribonucleotides (poly(A), poly(U)), and poly(G)) were from Sigma (concentrations reported in moles of nucleotide), and E. coli rRNA (16 and 25S) was from Roche Applied Science.

The rate of NTP hydrolysis (measured by phosphate release), τ, was described by Michaelis-Menten kinetics as follows,

\[
\nu = \frac{v_0[S]}{[P] + K_m + [S]}
\]

where \(K_m\) represents the Mila chief constant and \(k_{cat}\) is the enzyme turnover number.

**RNA Substrates**—RNA oligonucleotides RNA2 (5′-GACACCUAGCCAUUCCUCC-3′) and RNA3 (5′-GCACAGUGGAAUUGGAGGAG-3′) were labeled by T4 polynucleotide kinase in the presence of [γ-32P]ATP (35) and purified through 68% P-25 spin columns (Bio-Rad) equilibrated with 10 mM Tris-HCl, pH 7.4. Synthetic ssRNAs were produced by run-off transcription in vitro with T7 RNA polymerase (19). RNAs were 5′-S, 5′-20 R5, 5′-11 R5, and 5′-20 R5 were transcribed from the EcoR-cut plasmids pLM659, pLM717, pLM1772, and pLM1773, respectively (16, 26). RNAs (residues 72–136) was transcribed from a plasmid P4 CRF fragment amplified using oligonucleotides 5′-GCGAATACGCTACTATAGGGAGGATAGGCT-3′ (37); (promoter sequence italicized and 5′-CGACTCTAGGATTCGAGGAGAGGAC-3′ as up- and downstream primers, respectively.

To produce the RNA1 probe, plasmid pEM21 was constructed by inserting the duplex of two complementary oligonucleotides, 5′-CAGTCGTCTCCATCTACATATAGGGAGGAGGAGGAGGAGGAGGATAGGCT-3′ (37); (promoter sequence italicized and 5′-CGACTCTAGGATTCGAGGAGAGGAC-3′ as up- and downstream primers, respectively.

The resultant plasmid was linearized with HindIII and transcribed with T7 RNA polymerase in the presence of [α-32P]UTP. All transcripts were precipitated with 3 M LiCl, precipitated with ethanol, and dissolved in sterile water.

**COD Assays**—COD activity of PCs was assayed under the conditions optimized for RNA packaging (37). Complete and protein-deficient PCs were produced as described (24). To prepare RNA duplexes, unlabeled RNAs were mixed with 32P-labeled RNA probes in hybridization buffer containing 15 mM HEPES-KOH, pH 7.5, 150 mM NaCl, and 0.75 mM EDTA. The solution was boiled for 2 min and slowly cooled to room temperature. If necessary, residual nonannealed probe was removed by passing the mixture through a Sephacryl S-300 spin column (Amersham Biosciences) equilibrated with 10 mM Tris-HCl (pH 7.5) 0.25 M NaCl, and 0.75 mM EDTA. COD assays of RNA eluates were performed as described (27) with 50 mM Tris-HCl (pH 8.9), 2 mM dithiothreitol, 0.15 mM EDTA, 5 mM MgCl₂, 80 mM NH₄Ac, 0.6% polyethylene glycol 4000, 1 μM ATP or its nonhydrolyzable analog AMP-PCP, 1 unit/μl RNaseA (Promega), 1.5 μg/μl of RNase, and 0.2 μg of an RNA duplex. The reactions were usually incubated for 1–2 h at 30 °C and transferred to ice. The volume was then brought to 0.1 μl with 0.1 M Tris (pH 8.0), 1 mg/ml yeast tRNA (Sigma) in water followed by phenol-chloroform extraction and ethanol precipitation. The samples were dissolved in Tris-EDTA and analyzed by native 7% PAGE (5 mg/ml yeast tRNA (Sigma) in water, followed by phenol-chloroform extraction and ethanol precipitation. The samples were dissolved in Tris-EDTA and analyzed by native 7% PAGE (in standard Tris-borate-EDTA buffer) at room temperature. The gels were dried and analyzed by autoradiography and/or phosphorimaging (Fuji BAS 1500).
For P4 proteins, the COD assay was performed in 10-μl reaction mixtures containing 20 mM Tris-HCl (pH 7.5), 7.5 mM MgCl₂, 75 mM NaCl, 5 mM nucleotide (NTP, NDP, or AMP-NPN), 0.1 μg of RNA duplex, and 0.05 μg of P4. The reactions were incubated at 23 °C for 1 h; stopped by adding 2 μl of 15% glycerol, 20 mM EDTA, 3% SDS, and analyzed by native PAGE as above.

Dynamic Light Scattering—The hydrodynamic properties of individual proteins were studied using a batch dynamic light scattering instrument (Precision Detectors) equipped with deconvolution software for correlation function analysis (33).

Raman Spectroscopy—Protein solutions were concentrated (to 20 mg/ml each), sealed in sterile capillaries (Kimax 34504), and thermostated at 5 °C. Raman spectra were recorded on a Spex 500M spectograph equipped with a notch filter and charge-coupled device detector (SpectrumOne; Instruments S.A., Edison, NJ) using a spectral slit width of 4 cm⁻¹. A solid state, diode-pumped, frequency-doubled Nd:YVO₄ laser (model Verdi 522 nm; Coherent, Santa Clara, CA) was used to obtain 150 milliwatts at the sample. Each spectrum represents the accumulation of 15 exposures of 2-min duration on each of two independently prepared protein solutions. Spectra were corrected for buffer and NTP contributions and normalized for difference computations as described previously (38). The positions of strong bands are reported with ±1 cm⁻¹ accuracy, whereas those of weak and broad bands are reported with ±2 cm⁻¹ accuracy.

Small Angle Neutron Scattering—48 P4 was concentrated to 4 mg/ml and dialyzed against D₂O buffer solution (20 mM Tris-DCI, 50 mM NaCl, 7.5 mM MgCl₂). The sample was filtered and centrifuged (Beckman Airfuge; rotor A-95, 90,000 rpm, 10 min), and supernatant was transferred into a thermostatted quartz sample cell (2-mm path, 20 °C). The SANS data were collected on beam line D22 (Institute Laue Langevin; Grenoble, France) using a collimation distance of 4.4 m, neutron wavelength of 0.6 nm, and a 4-m sample-detector distance. The data were processed as described (39) to obtain an averaged and corrected scattering curve. The curve was modeled by scattering from a set of dummy atoms using the DAMMIN program (40) and imposing 6-fold symmetry.

Cryoelectron Microscopy—For cryoelectron microscopy, samples of 0.1 mg/ml 48 P4 and 0.25 mg/ml 413 P4 in buffer (20 mM Tris-HCl (pH 8.0), 50 mM NaCl, 7.5 mM MgCl₂) were vitrified as described elsewhere (41) and analyzed using the Spider program package (42). Reference-free alignment was used for the averaging (43). No symmetry constraints were applied.

RESULTS

Table I: Properties of purified P4 proteins

|        | d8 P4 | d13 P4 | d66 P4 |
|--------|-------|--------|--------|
| Subunit mass (kDa)² | 34.1  | 37.6   | 35.0   |
| Multimeric status* | Hexamer | Hexamer | Hexamer (unstable in the absence of nucleotides) |
| Hydrodynamic radius (nm) and shape | r₁ = 5.1; ring | r₂ = 5.9; ring | r₁ = 5.9; ring |
| RNA stimulation of NTPase activity | Strong | Strong | Weak |
| Nucleic acid binding | Tight | Tight | Undetectable |
| COD activity | Strong | Moderate | Undetectable |

* Deduced from the cDNA sequence assuming that the N-terminal Met is removed.

Table II: Properties of purified P4 proteins

|        | d8 P4 | d13 P4 | d66 P4 |
|--------|-------|--------|--------|
| Subunit mass (kDa)² | 34.1  | 37.6   | 35.0   |
| Multimeric status* | Hexamer | Hexamer | Hexamer (unstable in the absence of nucleotides) |
| Hydrodynamic radius (nm) and shape | r₁ = 5.1; ring | r₂ = 5.9; ring | r₁ = 5.9; ring |
| RNA stimulation of NTPase activity | Strong | Strong | Weak |
| Nucleic acid binding | Tight | Tight | Undetectable |
| COD activity | Strong | Moderate | Undetectable |

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In order to establish nucleotide substrate specificity, we have determined the $k_{\text{cat}}$ and $K_m$ for the P4 proteins and nucleotides ATP and UTP (Fig. 3B and Table II). Notably, no hydrolysis was detected for $\phi 8$ P4; therefore, $K_m$ constants have not been determined. The $K_m$ value for ATP and UTP hydrolysis is similar for $\phi 6$ P4, whereas $\phi 13$ P4 exhibits higher affinity for UTP (Table II and Fig. 3B). However, judged by $k_{\text{cat}}/K_m$, both enzymes prefer the UTP substrate (Table II).

It has been reported that the NTPase activity of $\phi 6$ P4 was enhanced 1.6-fold by the addition of ssRNA (27). We therefore examined the effect of ssRNA on the NTPase activities ($k_{\text{cat}}$) of the P4 proteins (Fig. 3C). A weak stimulation of the $\phi 13$ P4 NTPase activity, comparable with that of the $\phi 6$ P4, was observed in the presence of ribosomal RNA. Interestingly, an NTPase rate comparable with that of the $\phi 6$ and $\phi 13$ proteins was detected for $\phi 8$ P4 in the presence of rRNA. This suggests that the $\phi 8$ NTPase is strongly coupled to the presence of ssRNA. NTPase activity also became cooperative in the presence of RNA (data not shown).

To find the best ssRNA cofactor for each P4 NTPase, we analyzed the effect of synthetic polyribonucleotides on $k_{\text{cat}}$ (Fig. 3C). Poly(C) and poly(A) acted as the best cofactors for the $\phi 8$ P4, $\phi 6$ P4 was also stimulated by poly(C) and poly(A) with a preference for poly(A). On the other hand, all polynucleotides except poly(G) stimulated $\phi 13$ P4. Poly(G) had an inhibitory effect.

**Nucleic Acid Binding Properties of P4 Proteins—**NA binding to P4 proteins was assessed by an electrophoretic mobility shift assay (gel shift), which detects stable nucleoprotein complexes (nanomolar affinity). P4 proteins from $\phi 6$, $\phi 8$, and $\phi 13$ were incubated with long ssRNA, dsRNA, ssDNA, or dsDNA and analyzed by nondenaturing agarose gel electrophoresis (Fig. 4A). No stable complexes were detected for $\phi 6$ P4. In the case of $\phi 8$ and $\phi 13$ proteins, slowly migrating complexes were formed with all four types of NA. The extent of the retardation increased with the P4 amount added. Notably, the ssRNA probe used in this assay was the circular genome of bacteriophage M13, which indicated that free NA ends were dispensable for P4 binding.

Since $\phi 8$ and $\phi 13$ P4s bind all types of NA, we studied the possible effect of dsRNA, ssDNA, and dsDNA on the NTPase activity of these proteins. Of these, only dsRNA slightly stimulated the $\phi 8$ P4 NTPase, whereas no activation was detected in other combinations (Fig. 4B).

**COD Assay Strategy—**The current $\phi 8$ packaging assays require the presence of intact PC particles. We sought to develop a more direct method for detecting RNA translocation activity of isolated as well as procapsid-associated P4 proteins. Electron microscopy reconstructions indicated that the genomic RNA might be packaged via a narrow central opening in the P4 protein (28). Given the extensive secondary structure of ssRNA precursors (17), it is likely that packaging would lead to separation of short complementary regions.

Accordingly, an RNA substrate was prepared by annealing an unlabeled 700-nt-long 5'-terminal fragment of the s5 segment (s5 RNA) with the 32P-labeled 66-nt-long RNA oligonucleotide (RNA1). RNA1 was designed to target the 3'-proximal region of s5, −300 nt downstream of the pac site. Only the middle 18 nt of RNA1 can form a duplex with the corresponding sequence (590–607 nt) of s5, the 5’ and 3’ termini forming single-stranded overhangs (Fig. 5A). If the packaging apparatus recognizes s5/RNA1, RNA1 may be displaced in the course of s5 translocation. The liberated RNA1 probe can be separated from the input duplex in PAGE analysis under native conditions (30).

**P4 Proteins of $\phi 8$ and $\phi 13$ But Not $\phi 6$ Can Translocate RNA Substrates—**We examined the COD activity of isolated P4 proteins. For this purpose, P4 enzymes were incubated with the s5/RNA1 duplex under various conditions. In contrast to $\phi 6$ P4 (Fig. 5B, lane 1), both $\phi 8$ and $\phi 13$ proteins displaced the RNA1 probe from the duplex in the presence of ATP (Fig. 5B, lanes 4 and 7), $\phi 6$ P4 showing noticeably higher activity. No COD activity was detected when ATP was omitted or substituted with either ADP or AMP-PNP (Fig. 5B, lanes 6, 8, and 9, and data not shown). Thus, isolated $\phi 8$ and $\phi 13$ P4 can translocate RNA.
Fig. 3. NTPase activity of P4 proteins. A, P4s from ϕ6, ϕ8, or ϕ13 were incubated at 37 °C for 1 h with [α-32P]ATP or UTP, as indicated, and the reaction mixtures were analyzed by TLC. Migration of ATP, ADP, UTP, and UDP is indicated. B, concentration dependence of phosphate release kinetics in the presence of 0.1 μM P4 proteins at 25 °C for ATP and UTP substrates. C, the effect of different ssRNA (nucleotide unit concentration was 1 mM) on the ATPase activity of the three P4 proteins was measured using steady state kinetics (left panel) and kcat determination (right panel, ATP concentration 1 mM). The analysis was repeated three times, and error bars show the S.E. The color scheme employed in the left panel corresponds to that in the right panel and designates the type of RNA used.
when ATP was replaced by a nonhydrolyzable analog AMP-PCP (Fig. 5D, lanes 1 and 2). Prolonged incubations in the presence of ATP but not AMP-PCP led to the displacement of >40% of RNA1 (Fig. 5E).

To confirm that the RNA1 displacement was due to sR5 packaging, three control substrates were assayed with the WT PCs. These contained small deletions in the 5′ region, s(Δ11–23)R5, s(Δ11–32)R5, or s(Δ11–43)R5 annealed with RNA1. Both Δ11–32 nt and Δ11–43 nt deletions are known to disrupt the s+ pact activity, whereas the Δ11–23 nt deletion is neutral (16). In line with these results, RNA1 was efficiently displaced from the s(Δ11–23)R5/RNA1 duplex, but not from s(Δ11–32)R5/RNA1 or s(Δ11–43)R5/RNA1 (Fig. 5D, lanes 4–9).

**RNA Translocation by PC Requires the Presence of P4** — Previous experiments with mutated φ6 PC have shown that P4 is indispensable for RNA packaging (24). We therefore tested the COD activities of several recombinant PC variants. PC containing −10% of the normal P4 amount due to the point mutation S250Q in P4 (P1P2P4*P7) displaced the RNA1 probe with an efficiency close to that of the WT PC (Fig. 6A). Furthermore, PC containing the normal amount of P4 but missing protein P2 (P1P4P7) also supported oligonucleotide displacement, albeit with 10-fold reduced efficiency compared with the WT particles. As expected, PCs missing P4 (P1P2P7) did not displace RNA1.

We also assayed the COD activity of PCs assembled in vitro (22). For this purpose, different combinations of individual PC proteins were incubated with sR5/RNA1 under conditions promoting PC assembly (22). The RNA1 probe was efficiently displaced in the mixture containing all four PC proteins, whereas other mixtures showed very little COD activity (Fig. 6D).

**DISCUSSION**

Many viruses encapsidate their genomes through NTP-dependent packaging into preformed capsids. This process requires a portal complex that operates as a molecular motor converting chemical energy into mechanical work. Although DNA packaging in tailed bacteriophages has been studied to a great extent, the molecular basis of mechanochemical coupling is far from being understood. This may reflect the complexity of the packaging motor in which the terminase only transiently associates with the portal protein and the consequent need to study translocation in the context of assembled capsids.

RNA translocation of dsRNA bacteriophages (*Cystoviridae*) seems to be a much simpler system. As demonstrated here, it can be assayed without the need to monitor RNA inclusion to the PC particles using the COD technique (Figs. 5 and 6), which can also be applied to purified packaging proteins.

Only two short amino acid spans, apparently important for NTP binding and catalysis, are noticeably conserved between φ6, φ8, and φ13 P4s by different nucleic acids; poly(C) was used as ssRNA, and dsRNA, ssDNA, and dsDNA were as described for A.
proteins have nanomolar affinity for nucleic acids (Figs. 3 and 4). The high affinity for nucleic acids correlates with the ability of these proteins to translocate along RNA (Fig. 5 and Table I).

The results of this study indicate that RNA packaging in Cystoviridae is catalyzed by a single protein species, P4, which therefore represents one of the simplest nucleic acid pumps described so far. The isolated φ8 P4 exhibits the properties of a tightly coupled motor hydrolyzing NTP only in the presence of RNA, whereas φ6 P4 may need to be attached to the PC particle to become an RNA-dependent translocase. In line with this idea, previous Raman spectroscopy studies have shown that φ6 P4 undergoes extensive conformational changes upon its binding to the PC particle (38).
RNA Packaging in dsRNA Viruses

Although all or most of the PC 5-fold vertices seem to be occupied by P4 hexamers (18, 28), a model has been suggested, where only one of the 12 vertices operates as a packaging portal (24). Consistently, particles containing only ~10% of P4 display complementary oligonucleotides almost as efficiently as WT PCs (Fig. 2D). It is also notable that no specific sequence requirements were observed in the COD assays for purified δδ and δδδ P4s. Because packaging in Cystoviridae requires the presence of pac sites, located at the 5’ termini of genome precursors (14), other PC proteins (e.g. P1) are likely to be responsible for the RNA selection.

Our data suggest that cystoviral P4s are structurally and functionally related to other hexameric enzymes that can couple NTP hydrolysis with unidirectional translocation along nucleic acids and other types of mechanical work. These include replicative helicases (e.g. bacterial DnaB and phage T4 gp41), transcription termination factor Rho, cell-encoded DNA pumps, and the AAA family ATPases (47). For some of these proteins, such as the DNA helicase gp4 of bacteriophage T7, a high resolution structure is available, offering a model for translocation (48). Interestingly, gp4 translocates DNA in the 5’ to 3’ direction, similarly to the polarity of RNA translocation by δδ P4 (Fig. 5C). Furthermore, cystoviral P4 proteins show substantial sequence homology with T7 gp4 in both of the two conserved regions (Fig. 1). This encourages us to propose that the molecular basis of P4 catalyzed RNA translocation could be similar to that suggested for T7 gp4 (48).

In conclusion, isolated P4 proteins of cystoviruses δδδ and δδδδ function as NTP dependent RNA translocases in vitro, which argues that the packaging device of at least some viruses can be exceptionally simple and perhaps mechanistically similar to the hexameric helicases. Notably, a helicase-like protein has been implicated in genome encapsidation of the adenovirus-associated virus 2 (49), indicating a wider spread of this paradigm. P4 hexamers of δδ and δδδ can bind all types of nucleic acids, but only ssRNA stimulates the NTPase activity and supports translocation. Thus, the nucleic acid is likely to play an active role in the coupling between NTPase activity and translocation.