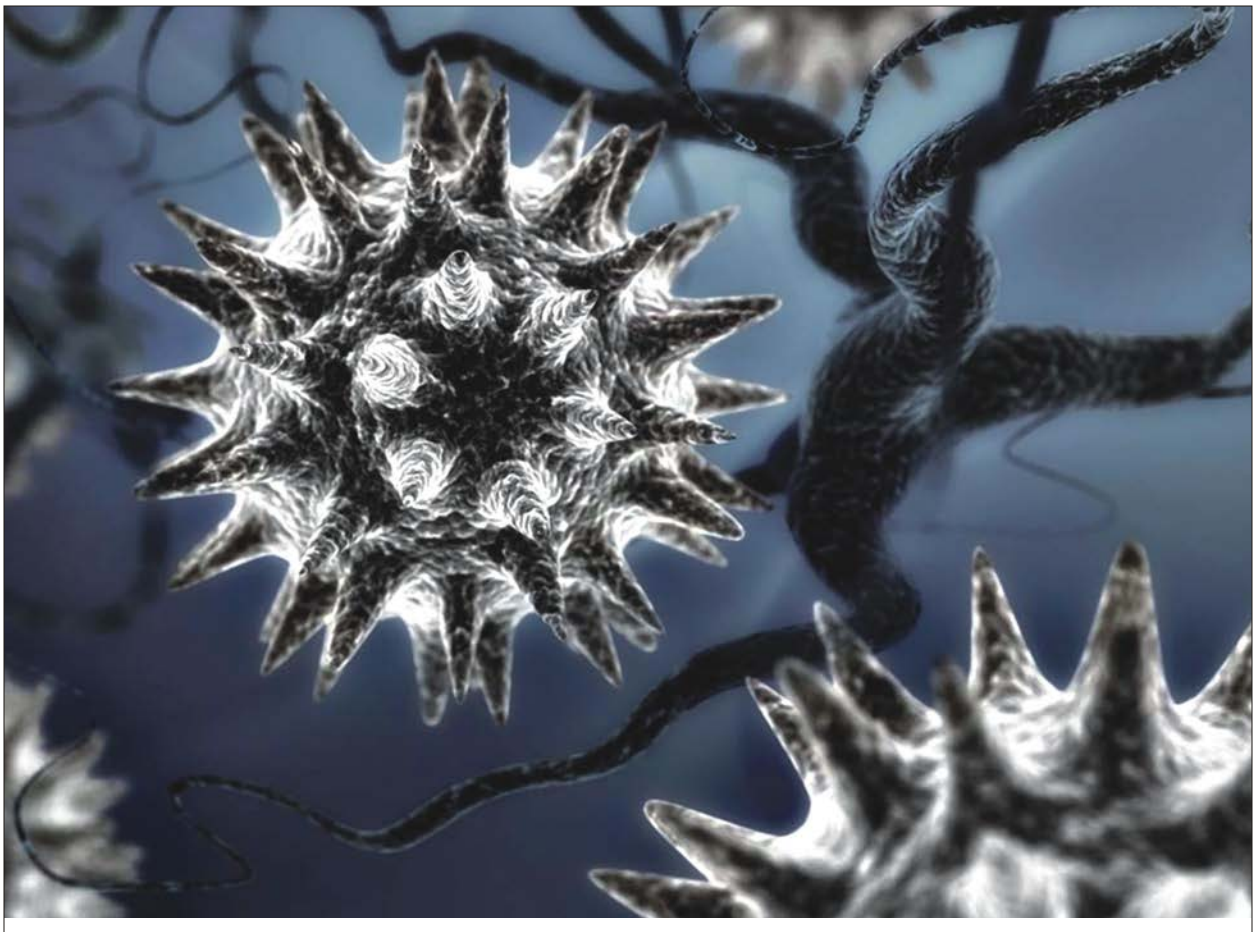

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Molecular Cloning, Expression and Analysis of *Antheraea mylitta* Cypovirus Genome Segments 8 and 11

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Abstract: The genome segments 8 (S8) and 11 (S11) of the 11 double-stranded segmented RNA genome of *Antheraea mylitta* cypovirus (AmCPV) were converted to cDNA, cloned and sequenced. S8 consisted of 1677 nucleotides with a long ORF of 527 amino acids and could encode a protein of approximately 60 kDa, termed p60. AmCPV segment 11 consisted of 390 nucleotides and did not show presence of any ORF.No sequence similarity of S8 or S11 was found by searching nucleic acid and protein sequence databases using BLAST. Secondary structure prediction showed that p60 contained 13 α-helices and 18 extended β-sheets along the entire length of the protein. S8 ORF was expressed as His-tagged fusion proteins in *E. coli*, purified through Ni-NTA chromatography and polyclonal antibody was raised indicating that p60 is strongly immunogenic. Immunoblot analysis of polyhedra, infected and uninfected cells with anti-p60 antibody showed that p60 is a viral structural protein. Motif scan search showed some similarity of p60 with inosine 5'-monophosphate dehydrogenase (IMPDH) containing a pair of Cystathionine Beta Synthase (CBS) domains and it was hypothesized that p60 may be involved in nucleotide biosynthesis and viral RNA replication. Due to absence of any ORF in S11 its function remained unclear.

Key words: *Antheraea mylitta*, cypovirus, genome segment 8, genome segment 11, gene cloning, expression

INTRODUCTION

Cypoviruses (CPVs) belong to the genus *Cypovirus* in the family *Reoviridae* (Payne and Martin, 1983; *van* Regenmortel *et al*., 2000). These viruses infect midgut cells of a wide range of insects belonging to the order Diptera, Hymenoptera and Lepidoptera (Belloncik, 1989; Fouilland and Morel, 1994; Belloncik and Mori, 1998). Viral infection is characterized by the production of large numbers of occlusion bodies called polyhedra in the cytoplasm of infected cells. The genomes of CPVs like those of other members of the *Reoviridae* are usually composed of 10 double stranded (ds) RNA segments (S1-S10) (Payne and Martin, 1983) although, in some cases, a small, eleventh segment (S11) has been reported (Arella *et al*., 1988). Each dsRNA is composed of a plus-stranded mRNA and its complementary minus strand in an end to end base paired configuration except for a protruding 5' cap on the plus strand. Among the family *Reoviridae*, complete sequences of dsRNA genomes have been reported for members of the genera *Orthoreovirus*, *Rotavirus*, *Orbivirus*, *Phytoreovirus*, *Coltivirus*, *Seadornavirus, Dinovernavirus* and putative members of *Fijivirus* and *Cypovirus,* (Duncan, 1999; Estes and Cohen, 1989; Roy *et al*., 1990; Suzuki, 1995; Attoui *et al*., 2000a; Attoui *et al*., 2000b; Attoui *et al*., 2005; Nakashima *et al*., 1996).

From the *Cypovirus*, complete nucleotide sequence of type 14 *Lymnatria dispar* CPV-14 (Rao *et al*., 2001), type 1 *Dendrolimus punctatus* CPV (DpCPV-1) (Zhao *et al*., 2003a; b),

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type 15 *Trichoplusia ni* CPV (Rao *et al*., 2000) and type 1 *Bombyx mori* CPV (BmCPV-1) (Hagiwara *et al*., 1998a, b, 2001, 2002; Hagiwara and Matsumoto, 2000; Ikeda *et al*., 1998) have been reported and deposited in the Genbank. The elucidation of these cypovirus sequences has led to a better understanding of the possible functions that each dsRNA segment may play a role in viral pathogenesis. In case of BmCPV, segment 1, 3, 4, 6, 7, 10 encodes structural proteins VP1, VP2, VP3, VP4, VP5, polyhedrin and segment 2, 5, 8, 9 encodes viral non-structural proteins RNA dependent RNA polymerase, p101, p44, NS5, respectively (Hagiwara *et al*., 1998a, b, 2002; Hagiwara and Matsumoto, 2000; Ikeda *et al*., 1998). Segments 1, 3, 4, 6, 7 and 10 of DpCPV encode viral structural proteins while segments 2, 5, 8 and 9 encode non-structural proteins (Zhao *et al*., 2003a, b). Besides these, polyhedrin genes of several other cypoviruses such as *Euxoa scandens* CPV (Fossiez *et al*., 1989) *Orgyia pseudotsugata* CPV, *Heliothis armigera* CPV (Galinski *et al*., 1994), *Choristoneura fumiferana* CPV (Echeverry *et al*., 1997) and *Uranotaenia sapphirina* CPV (Shapiro *et al*., 2005) have been molecularly characterized but no sequence homology has been found among them.

The Indian non-mulberry saturniidae silkworm, *Antheraea mylitta*, produces an exotic variety of silk called tasar silk. Being wild in nature, a major population of these silkworms is getting destroyed by viral infection (Jolly *et al*., 1974) and we have reported earlier that a type IV CPV called AmCPV containing 11 dsRNA segments is the causative agent (Qanungo *et al*., 2000). AmCPV belongs to genus *Cypovirus* of the family *Reoviridae* (Qanungo *et al*., 2000). Molecular cloning and characterization of AmCPV genome segment 9 and 10 of this CPV show that segment 9 codes for viral non-structural protein NSP38 having RNA binding property (Qanungo *et al*., 2002) and segment 10 codes for polyhedrin (Sinha-Datta *et al*., 2005), respectively, but no sequence similarity has been found with any other sequences in the public databases. To understand the role of each genome segment of AmCPV in virus replication and pathogenesis complete characterization of all its genome segments is necessary but the structure and functions of no other genome segments except 9 and 10 have been reported. Here we report molecular cloning, sequencing and characterization of another two genome segments (S8 and S11) of AmCPV and show by immunoblot analysis that segment 8 encodes a 60 kDa viral structural protein whereas due to absence of any ORF in S11 its function could not be ascertained.

MATERIALS AND METHODS

Silkworm and Virus

CPV-infected Indian non-mulberry silkworm *A. mylitta* was collected from the tasar silk farms of West Bengal and Jharkhand states of India.

Purification of Polyhedral Bodies, Isolation of Total Genomic RNA and Extraction of Genome Segments 8 and 11 RNA

Polyhedra were purified from the midguts of infected silkworm larvae by sucrose density gradient centrifugation according to the method of Hayashi and Bird (1970) with some modification (Qanungo *et al*., 2000). Genomic RNA was extracted from the purified polyhedra by the standard guanidinium isothiocyanate method (Ausubel *et al*., 1995) and fractionated in 1% agarose gel. The genome segments 8 and 11 were excised from ethidium bromide-stained gel and eluted using RNaid kit (Bio 101).

Molecular Cloning and Sequencing of Genome Segments 8 and 11

S8 and S11 genomic RNA of AmCPV were converted to cDNA as described by a sequence independent RT method (Lambden *et al*., 1992) by using two primers (AG1 and AG2). The 3'-end of 5'-phosphorylated primer, AG1, (5' PO_4-CCCGGATCCGTCGACGAATTCTTT-NH_2 3') was blocked by NH_2 to prevent its concatenation in subsequent dsRNA/DNA ligation reactions.

Approximately 200 ng of purified segments 8 and 11 RNA were taken and in each case primer AG1 was ligated to both 3'ends of dsRNA by using T4 RNA ligase for one hour at 37°C. The tailed RNA was denatured by heating, annealed to primer AG2 (5'AAAGAATTCGTCGACGGATCCGGG 3'), which is complementary to AG1 and reverse transcribed at 55°C for 50 min by using Thermoscript reverse transcriptase (Invitrogen). The template RNA from RNA/cDNA hybrid was removed by digestion with RnaseH and reannealing of the cDNA strands was done by incubating at 65°C for 2 h. The cDNA ends were repaired by incubation with *Taq* DNA polymerase (Bioline) at 72°C for 20 min and cloned into pCR2.1-TOPO vector (Invitrogen) to make plasmid pCR2.1 TOPO/AmCPV8 and pCR2.1 TOPO/AmCPV11. After transforming in *E. coli* TOP 10 cells, plasmids were isolated and characterized by *Eco*RI digestion. Recombinant plasmids containing proper size insert were then sequenced by using an ABI 3100 automated DNA sequencer with M13 forward and reverse primers as well as internal primers designed from deduced sequences.

Sequence Analysis

Genome Sequence of AmCPV S8 and S11 were analyzed by Sequencher program and homology searches were done using BLAST (Altschul *et al*., 1997). Conserved motifs were identified using MotifScan program (http://myhits.isb-sib.ch/cgi-bin/motif_scan). The molecular weight of the deduced protein, isoelectric point and composition were calculated using protein calculator program (http://www.scripps.edu/~cdputnam/protcalc. html) (Zhang *et al*., 2001). Secondary structure was predicted using PHD (Rost and Sander, 1994) and GOR4 program (Garnier *et al*., 1996).

Northern Hybridization

In order to verify cloning of the S8 and S11 cDNA from the corresponding RNA of AmCPV, all the genomic dsRNA segments were separated in an agarose gel and observed by staining with ethidium bromide. The RNA segments in the gel were then denatured by brief treatment with 0.1 M NaOH, neutralized and blotted onto nitrocellulose membrane (Sambrook et al., 1989). The membrane was then hybridized with ^{32}P-labelled cloned S8 ans S11 cDNA from AmCPV, washed and autoradiographed (Qanungo *et al*., 2002; Feinberg and Vogelstein, 1983).

Expression of AmCPV S8 in *E. coli*

The entire 528 amino acid protein coding region of AmCPV8 cDNA (from nucleotide 17 to 1584) was amplified by PCR from plasmid pCR2.1TOPO / AmCPV8 by using *Pow* polymerase (Roche) and two synthetic primers, AGCPV 49F (5' ATCGACGGATCCATGGCGTACCCAG 3': forward primer) and AGCPV 51R (5' TGATACTCGAGAGCAAACATACAC 3': reverse primer), complementary to bases 5-29 and bases 1584-1608, respectively and containing *Bam*HI (in the forward primer) and *Pst*I (in the reverse primer) restriction enzyme sites (underlined). The amplified PCR product (1.5 kb) was digested with *Bam*HI and *Pst*I, separated on a 1% agarose gel and purified from the gel by using a Qiaquick gel extraction kit (Qiagen). The purified DNA was ligated to *Bam*HI/*Pst*I digested pQE-30 vector (Qiagen) in-frame with a sequence encoding six histidine residues at the N-terminus. The recombinant plasmid, pQE-30/AmCPV8, was then transformed into *E. coli* M15 cells and colonies were screened following *Bam*HI and *Pst*I digestion.

For protein expression, recombinant bacteria were grown in 5 mL LB medium containing ampicillin (100 μg mL^{-1}) and kanamycin (25 μg mL^{-1}) for 3 h at 37°C and then induced with 1 mM IPTG for an additional 4 h at the same temperature. Bacteria were harvested by centrifugation, lysed by boiling with sample loading buffer (60 mM Tris-HCl, pH 6-8; 10% glycerol ; 2% SDS; 5% β-mercaptoethanol and 1 μg mL^{-1} bromophenol blue) for 3 min and then loaded onto a 5% stacking gel cast above a 10% resolving SDS-polyacrylamide gel (Laemmli 1970). After electrophoresis, the protein bands in the gel were stained with Coomassie brilliant blue (Ausubel *et al*., 1995). The molecular mass of the expressed recombinant protein was determined by comparison to standard protein molecular mass markers and by using Quantity One software in Gel-Doc 2000 (Bio-Rad).

Purification of His-Tagged Protein

Recombinant bacteria containing pQE-30/AmCPV8 were grown in 1L LB medium and induced with IPTG as described above. The insoluble His-tagged fusion protein (p60) was first purified as inclusion bodies (Caligan *et al*., 1995). After solubilizing the inclusion bodies in 6 M guanidine hydrochloride, further purification of protein was carried out using a Ni-NTA agarose kit (Qiagen) according to the manufacturer's protocol. The total amount of purified protein was quantitated by Bradford method (1976) using BSA as the standard and purity was checked by SDS-10% PAGE (Laemmli, 1970).

Rabbit Immunization and Production of Polyclonal Antibodies

One rabbit was immunized with bacterially expressed, purified, recombinant His-tagged protein (p60) by standard methods (Qanungo *et al*., 2002; Harlow and Lane, 1988). In brief, purified protein (600 mg) was mixed with Freund's complete adjuvant and injected subcutaneously at multiple sites. Three booster doses with Freund's incomplete adjuvant and the same amount of protein were administered via the same route at 4-week intervals. Twelve days after the final booster, blood was collected, serum prepared and the antibody titer was determined by ELISA (Harlow and Lane, 1988).

Western Blot Analysis

For detecting the expression of S8 encoded P60 in infected cells, protein samples from dissected midgut of AmCPV infected and uninfected 5th instar larvae were prepared by homogenizing the tissue in PBS followed by centrifugation at 10,000 g for 10 min (Qanungo *et al*., 2002). Each protein sample in the supernatant was boiled in sample loading buffer and run on SDS-10% PAGE under reducing conditions. After electrophoresis, proteins from the gel were transferred onto a duralose membrane (Stratagene) using a transblot cell (Bio-Rad) according to manufacturer's protocol. The membrane was then blocked in PBS containing 3% BSA for 1 h at room temperature followed by incubation with 200 fold diluted affinity purified anti-p61 polyclonal antibody for 1 h. After washing with PBS the membrane was incubated with 200-fold diluted protein A-conjugated horse raddish peroxidase for 1 h and then washed and color development was done by using HPO color development kit (Bio-Rad).

Nucleotide Sequence Accession Number

The nucleotide sequence data reported here for segments 8 and 11 of AmCPV have been deposited in Genbank database and have been assigned the Accession No. DQ975382 and DQ975383, respectively.

RESULTS

Molecular Cloning of AmCPV Genome Segments 8 and 11

AmCPV genome segments 8 and 11 were purified from the genomic RNA of AmCPV after separating by 1% agarose gel electrophoresis, converted to cDNA and cloned into pCR 2.1 TOPO vector to create plasmid pCR 2.1 TOPO/AmCPV8 and pCR2.1 TOPO/AmCPV11, respectively. The plasmids isolated from the transformed *E.coli* were digested with *Eco*R1 and analyzed by agarose gel electrophoresis. Digestion of recombinant pCR2.1TOPO/ AmCPV8 on digestion yielded a single band of about 1.6kb indicating full length cloning of AmCPV8 (Fig. 1A, lane b). Digestion of recombinant plasmid pCR2.1TOPO/AmCPV11 yielded two fragments of 264 and 125 bp in size (Fig. 1B, lane b) indicating full length cloning of 0.39 kb AmCPV11 with an internal *Eco*R1 site.

Northern Analysis

Among the 11 dsRNA segments present in AmCPV the cDNA of S8 and S11 specifically hybridized with S8 and S11 genomic RNA, confirming the cloning of S8 (Fig. 2A, lane b) and S11 genomic RNA from AmCPV (Fig. 2B, lane b).

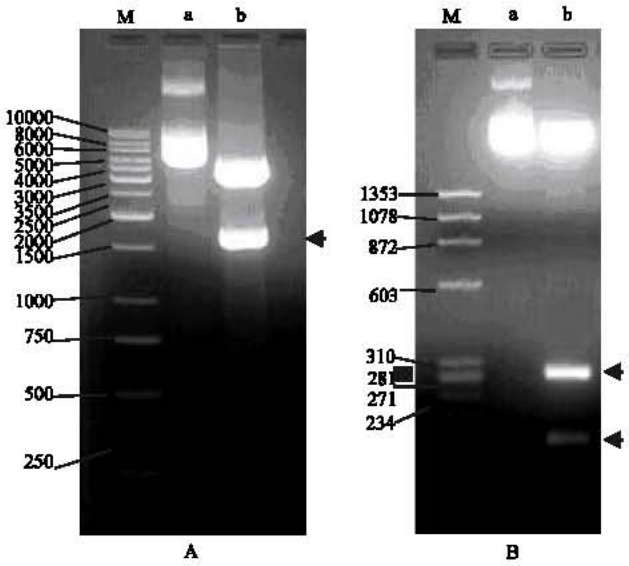

Fig. 1: Analysis of (A) pCR2.1 TOPO/AmCPV8 and (B) pCR2.1 TOPO/AmCPV11 by agarose gel electrophoresis. lane M, Molecular weight marker; lane a, Undigested and lane b, *Eco*RI digested plasmids. Arrows indicate the position of insert DNA

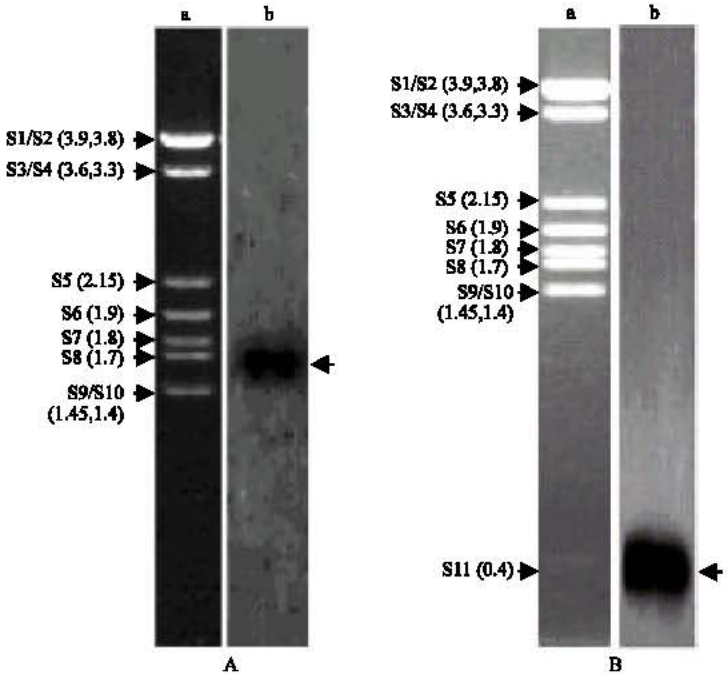

Fig. 2: Northern blot analysis of AmCPV genome RNA with cloned S8 (A) and S11 (B) cDNA. AmCPV genomic RNA segments were resolved by 1% agarose gel electrophoresis as discrete bands (lane a) and hybridized with ^{32}P-labelled cloned cDNA (lane b). Arrow in the right indicates hybridization of S8 or S11 cDNA to S8 or S11 genomic RNA. The number and sizes (in parenthesis) of different RNA segments of AmCPV in kb are shown by arrows in the left

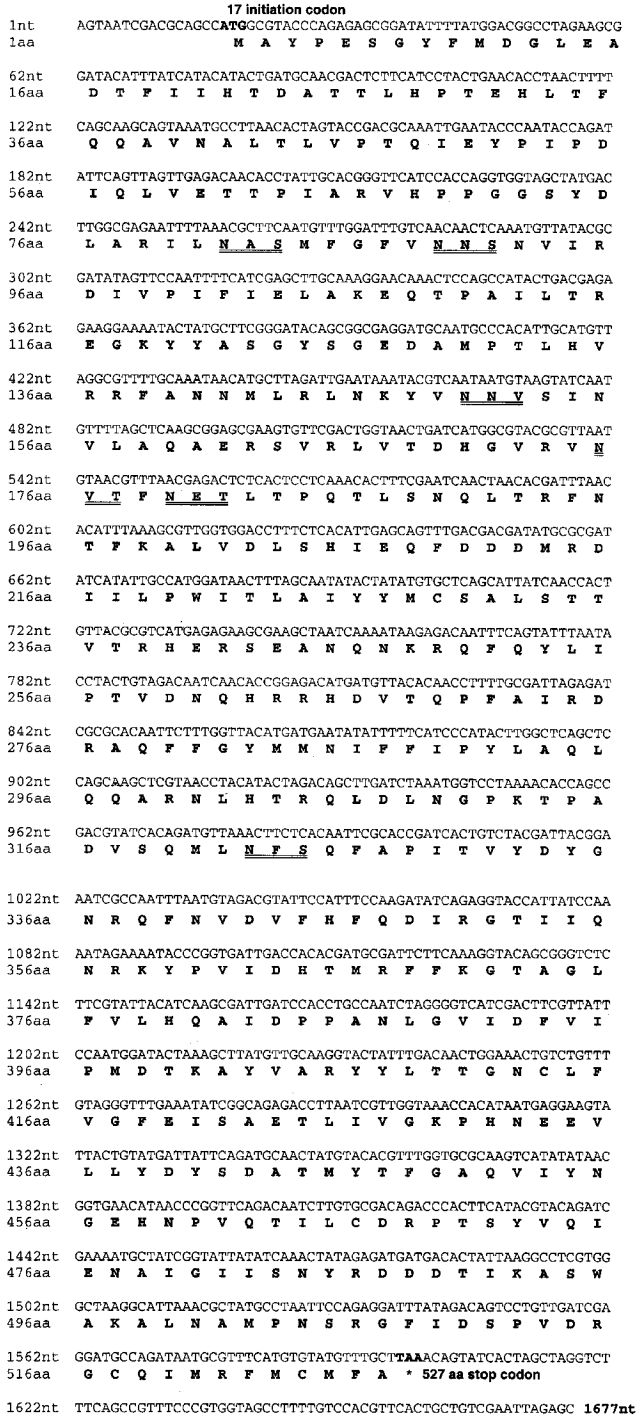

Fig. 3a: Complete nucleotide (nt) and deduced amino acid (aa) sequences of AmCPV genome segment 8. The initiation and termination codons are shown in bold. Six potential N-linked glycosylation sites are double underlined

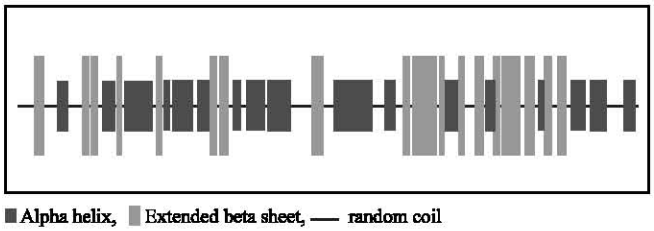

Fig. 3b: Secondary structure predicted of AmCPV p60, predicted according to the PHD method of Rost and Sander (1994)

Molecular Analysis of Genome Segments 8 and 11

The cDNA of S8 and S11 were sequenced and analyzed using bioinformatics tools. S8 cDNA consisted of 1677 nucleotides and contained a single long ORF of 527 amino acids starting with the ATG codon (at base 17) and ending with the TAA stop codon (at base 1599). Sixteen nucleotides upstream of the start codon and seventy seven nucleotides downstream of the stop codon were present as 5' and 3' untranslated regions (Fig. 3a). The molecular weight of the encoded protein was deduced as 60 kDa and we termed the protein as p60. No significant homology was detected with any nucleotide or protein sequences available in the public databases using BLAST. The deduced amino acid composition resulted in an isoelectric point of 5.88 and showed that protein is rich in alanine (7.3%), Arginine (6.0%), asparagine (6.1%), isoleucine and threonine (7.6%), leucine (7.4%), phenylalanine (6%) and valine (6.7%) residues. Six potential N-linked glycosylation sites (double underlined), two myristoylation sites, two sulfation sites and several phosphorylation sites (not marked) are found in the protein coding region. Secondary structure prediction with PHD and GOR4 showed that 42.88% of the residues are likely to form random coils, 36.62% would form α-helices and 20.49% would form extended sheets. A total of 13 α-helices and 18 extended β-sheets were found along the entire length of protein (Fig. 3b). MotifScan search showed significant similarity of p60 with inosine monophosphate dehydrogenase (IMPDH) enzyme containing two cystathionine beta synthase (CBS) domains at amino acid residues 16-69 and 116-168 (Fig. 4) with the characteristic of sheet/helix/sheet/sheet (beta-alpha-beta-beta) topology (Fig. 5).

Segment 11 cDNA consisted of 390 nucleotides and did not presence of any significant ORF (Fig. 6). BLAST searches yielded no similarities with protein and nucleic acid sequences available in the public databases.

Expression and Purification of Genome S8 Encoded Protein

To express S8 encoded protein in *E. coli*, the ORF was cloned in pQE30 vector, expressed in *E. coli* and purified as 6X His-tag fusion protein through Ni-NTA affinity chromatography. Analysis of induced bacterial lysate and purified protein through SDS 10%- PAGE showed the production of ~60 kDa protein (Fig. 7, lanes a and c) but, no such protein band was found in uninduced bacterial lysate (Fig. 7, lane b).

Detection of P60 Expression in Virions and Viral Infected Cells

To demonstrate the expression of p60 in virions and virus infected cells, CPV infected *A. mylitta* midgut cells and purified polyhedral bodies were analyzed through SDS-10% PAGE and subjected to immunoblot analysis using raised anti-p60 polyclonal antibodies. A major immunoreactive protein band of approximately 60 kDa was found in lane containing lysate of infected midgut cell (Fig. 8, lane c) and polyhedral bodies (Fig. 8, lane a) but not in lane containing lysate of uninfected cell (Fig. 8, lane b).

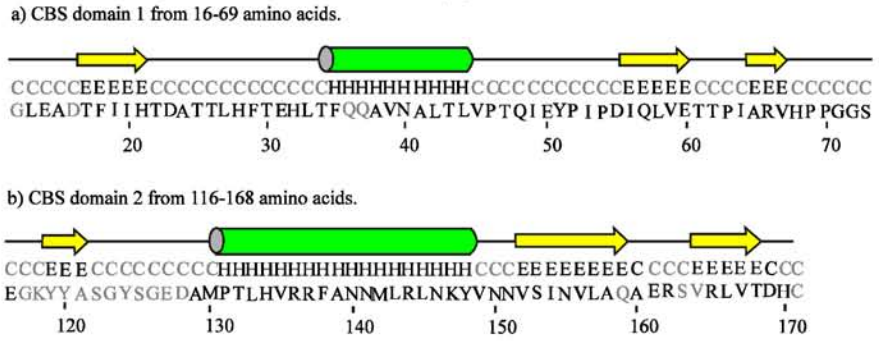

Fig. 4: Location of CBS domains containing β-α-β-β motif in p60. The cylinders ( ) represent alpha helix and arrows ( ) represent the beta strands

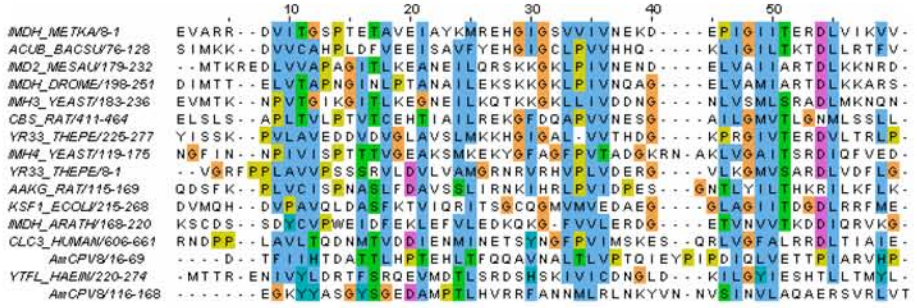

Fig. 5: Alignment of two CBS domains found in AmCPV segment 8 with that of enzymes, channel and few hypothetical proteins. The sequences are annotated with their SWISS-PROT identifier followed by the start point and end point of each domain. AmCPV8/16-69 and AmCPV8/116-168 denotes two CBS domains found in AmCPV segment 8, from 16-69 and 116-168 amino acid residues, respectively. IMDH_ARATH, IMDH_METKA, IMDH_DROME, IMD2_MESAU, IMH3_YEAST and IMH4_YEAST are inosine monophosphate dehydrogenases from *Arabidopsis thaliana*, *Methanopyrus kandleri*, *Drosophila melanogaster*, *Mesocricetus aurateus* and *Saccharomyces cervisiceae*, respectively. YR33_THEPE is a hypothetical protein from *Thermophilum pendens.* YTFL_HAEIN is a hypothetical protein from *Haemophilus influenza.* CLC3_HUMAN is a chloride channel protein from *Homo sapiens.* AAKG_RAT is a 5' AMP activated protein kinase from *Rattus norvegicus.* KSF1_ECOLI is a polysialic acid capsule expression protein from *E. coli.* CBS_RAT is a cystathionine beta synthase H-450 protein from *Rattus norvegicus.* ACUB_BACSU is acetoin utilization protein B from *Bascillus subtilis.* The shaded boxes indicate the conserved residues in the CBS domains from different species

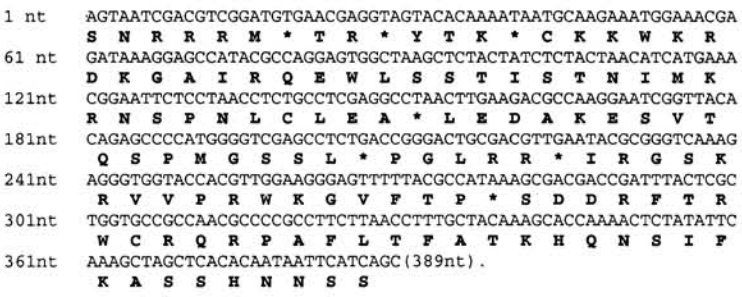

Fig. 6: Complete nucleotide (nt) and deduced amino acid (aa) sequences of AmCPV genome segment 11

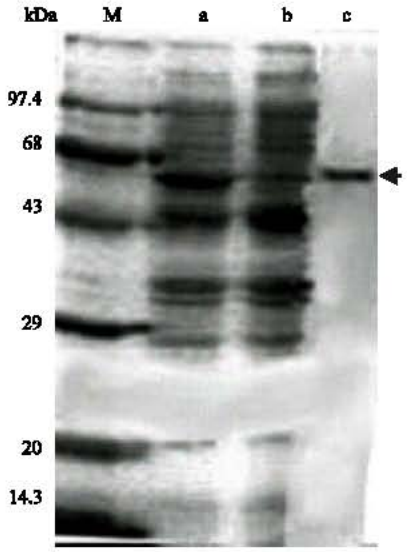

Fig. 7: Analysis of recombinant *E. coli* lysate and purified p60 by SDS-10% PAGE. Lane M, molecualr weight marker; lane a, IPTG induced *E. coli* lysate; lane b, uninduced *E. coli* lysate; lane c, Ni-NTA purified p60. Arrow indicates the position of expressed protein. Molecular size markers (in kDa) are indicated in the left

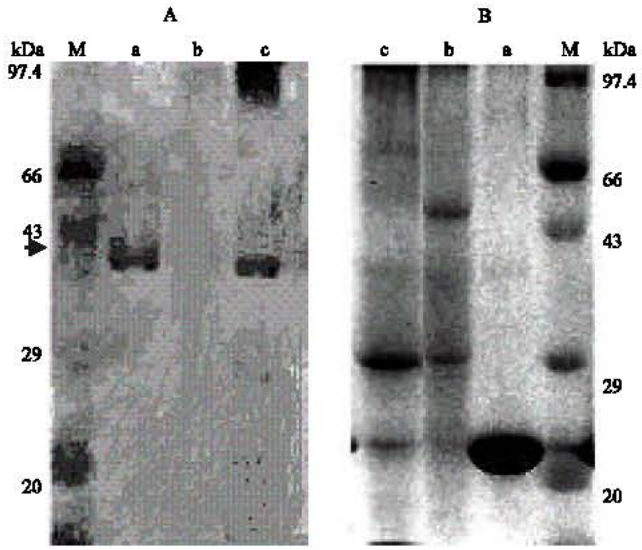

Fig. 8: SDS-PAGE and western blot analysis of p60. Samples were separated by SDS-10% PAGE and stained with Coomassie brilliant blue (A) or transblotted onto nitrocellulose membrane and reacted with anti-p60 polyclonal antibody (B). lane M, molecular weight markers; lane a, Purified AmCPV polyhedra; lane b, Uninfected and lane c, CPV-infected midgut cell lysate of *A. mylitta* larva. Arrow indicates the position of immunoreactive protein. Molecular size markers (kDa) are shown in the right

DISCUSSION

AmCPV although infects and destroys an economically important insect species, *Antheraea mylitta*, which produces tasar silk, but except segments 9 and 10 none of its other genome segments have been fully characterized at the molecular level. But full molecular characterization of this CPV is very much needed to understand the viral life cycle, pathogenesis and to find target sites for the development of effective anti-viral compound. Here we describe molecular analysis of another two genome segments of this CPV, S8 and S11, by cloning, sequencing and expressing in *E. coli*. The 1677 nucleotides long cloned S8 cDNA consists of an ORF of 527 amino acids and its molecular mass is calculated to be approximately 60 kDa. AmCPV S11 cDNA consists of 390 nucleotides and does not show presence of any significant ORF to encode a protein. Presence of conserved terminal sequences at 5' and 3' ends is a characteristic feature found in different genome segments of most of the virions of the *Reoviridae* family (Patton and Spencer, 2000). At the 5' and 3' end of AmCPV S8, AGTAAT and AGAGC sequences and at the 5' and 3' end AGTAT and TCAGC (instead of AGAGC) were found as observed at the 5' and 3' ends of AmCPV genome segment 10 encoding polyhedrin (Sinha-Datta *et al*., 2005) indicating the genome structure of this CPV may follow the same pattern as observed in other CPVs but cloning and sequencing of other genome segments of AmCPV are required to confirm it. Absence of any significant sequence similarity of S8 and S11 either at nucleotide or amino acid level with the available database indicates that segment 8 of AmCPV encodes a novel protein and further confirms that a new type of CPV infects tasar silk worm, *A. mylitta* which has been reported earlier (Qanungo *et al*., 2000, 2002; Sinha-Datta *et al*., 2005). Although most CPVs contain 10 segmented RNA in their genome, but some CPV like BmCPV contains an eleventh segment (a deletion of segment 10) in its genome (Arella *et al*., 1988). Here, we have also observed the presence of an eleventh genome segment in AmCPV but it is not the deletion of segment 10 or any of its other genome segment as shown by hybridization of S8 cDNA only with S8 genomic RNA and not with any other segments. But due to absence of any significant ORF its function could not be ascertained.

Cloning of S8 ORF in bacterial expression vector and analysis of bacterially expressed recombinant His-tagged fusion protein showed the production of a protein of 60 kDa, Six potential N-linked glycosylation sites have been found in ORF of p61. When expression of this protein was analyzed in AmCPV infected midgut cells or in purified polyhedra by immunoblot analysis using anti-p60 polyclonal antibodies, an immunoreactive band of approximately 60 kDa was observed. This indicates that the protein has not undergone glycosylation during expression in eukaryotic cells. Production of high titer antibodies (10^{-5}) in rabbit as shown by ELISA (data not shown) and western blot indicate that p60 is highly antigenic to induce strong immune response. The antibodies generated in this study could aid in the development of a rapid and simple diagnostic test to detect AmCPV. Further work is required to demonstrate if this antibody possesses any neutralizing activity to this CPV. Detection of p60 protein expression in virus infected midgut cells and in polyhedra by Immunoblot analysis using anti-p60 antibody indicate that p60 codes for a viral structural protein.

Since a significant similarity of p60 was found with IMPDH enzyme containing two CBS domains by motif scan analysis it may be assumed that p60 may possess IMPDH like activity. It has been shown that IMPDH of human, *T. foetus* and *E. coli* can bind to single stranded nucleic acids with nanomolar affinity through its CBS domains (not by its catalytic domain) (Mc Lean *et al*., 2004). In eukaryotes, CBS domains appear to mediate cytoplasmic targeting, protein-protein interaction (Bateman, 1997) and proteins with two CBS domains are part of a family called Bateman domain (Kemp, 2004). CBS domains have been shown to bind ligands with an adenosyl group such as AMP, ATP and S-adenosyl- methionine and may regulate the activity of the protein (Bateman, 1997). In *S.cerevisiae*, IMPDH binds to telomeric sequences through its Bateman domain (Cornuel *et al*., 2002). Perhaps nucleic acid binding is a general property of Bateman domains and p60 encoded by AmCPV S8, by binding to viral RNA through its Bateman domain may help in viral transcription or replication.

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