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Characterization of a cyclin homolog from Bombyx mori nucleopolyhedrovirus

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Abstract

We have identified and characterized a *cyclin* homolog from Bombyx mori nucleopolyhedrovirus (BmNPV), encoding a 34 kDa protein (ORF 120) with 48% homology to the host *Bombyx mori* cellular cyclin B. The expression of the *viral cyclin* (*v-cyc*) was detected from 12 h following virus infection and the maximum transcript levels were seen at 24–36 h. The transcription start site mapping of *v-cyc* revealed the presence of a transcript initiating from a TAAG motif located 13 nucleotide (nt) upstream of the ORF as well as longer transcripts initiating from farther upstream region and encompassing the preceding ORF 119. The transcription was terminated at 15 nt downstream of the ORF 120. The expression of the host cellular *cyclin* B declined following virus infection and the transcript disappeared almost completely by 24 h even as the expression of *v-cyc* reached high levels. The synthesis of the viral cyclin was detected at 36–48 h post-infection. The viral cyclin in association with other host or viral proteins catalysed phosphorylation of histone H1. The host cells were arrested in G2/M phase following virus infection and thus, the virus cyclin in association with other proteins maintains the host cells at the G2/M phase while permitting the virus DNA replication.

Keywords: BmNPV; *Bombyx mori*; Cyclin; Cell cycle regulation; Silkworm; Transcriptional analysis

1. Introduction

Bombyx mori nucleopolyhedrovirus (BmNPV) belongs to the family Baculoviridae and infects the mulberry silkworm larvae, a major economic problem in tropical sericulture. The complete nucleotide sequence of the genomic DNA (128 kb) of BmNPV-T3 strain is currently available (accession no. L33180) (Gomi et al., 1999). BmNPV-BGL, a local isolate of BmNPV, nearly identical in restriction pattern to the T3 strain except for a few polymorphic sites for restriction enzymes *Eco* RI and *Xho* I (Palhan and Gopinathan, 1996), has been exploited by our group for high level expression of cloned foreign genes in *B. mori* derived cell lines as well as in the silkworm larvae (Palhan et al., 1995; Sumathy et al., 1996; Sriram et al., 1997; Sehgal and Gopinathan, 1998). Compared to the prototype baculovirus Autographa californica multinucleocapsid nucleopolyhedrovirus (AcMNPV), the information available on the BmNPV as a high level expression system or on the genetic and molecular aspects of viral replication and transcription is very limited.

AcMNPV infection leads to the arrest of host cell cycle progression at the G2/M boundary but the viral DNA replication continues in such cells (Braunagel et al., 1998). Normal eukaryotic cell cycle is regulated by a family of cyclins which are activated by complex formation with cyclin dependent kinases (cdks) accompanied by phosphorylation. Together, the cyclins and cdks are responsible for cell cycle progression. Cyclin B-Cdk2 complex plays an important role in G2/M stage of the cell cycle and cyclin B is degraded in mid-

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metaphase of the cell cycle following ubiquitination (Hunter and Pines, 1994; Morgan, 1995; Peter and Herskowitz, 1994; Sherr and Roberts, 1995; Reed, 1992). On the contrary, Cdk2 is present all through the cell cycle phases, and interacts with different cyclin partners depending on the stages of cell cycle (Nurse, 1994; Pagano et al., 1993). In yeast, several cyclins have been characterized (designated as Cln1-3 and Clb1-6), but only a single type of cdk (Cdc28) is known. In higher eukaryotes, there are several cyclins (in human, designated as cyclins A–H) as well as associated kinases (cdks) dedicated to distinct cyclins (Hunter and Pines, 1994; Morgan, 1995; Nurse, 1994; Pagano et al., 1993). The *cyclin* B (full length) and *cyclin* E (cyclin box) homologs from *B. mori* have been cloned and characterized (Takahashi et al., 1996; Baluchamy and Gopinathan, 2000).

Some of the viruses like AcMNPV, Kaposi's sarcoma herpes virus (KSHV), Herpes simplex virus (HSV) and Walleye Dermal Sarcoma Virus also encode cyclin homologs (Belyavskyi et al., 1998; Mengtao et al., 1997; Jung et al., 1994; Rovnak and Quackenbush, 2002). We report here the characterization of the cyclin homolog with an associated histone kinase activity encoded in the BmNPV genome, that shared about 48% homology to the host cyclin B. Further, we demonstrate the G2/M arrest in cell cycle and the degradation of the cellular cyclin B in the host cell line BmN, following BmNPV infection.

2. Materials and methods

2.1. Cell line and virus infection

Bombyx mori derived cell line, BmN, originally obtained from Dr. S. Maeda (University of California, Davis, CA, USA) was routinely maintained at 27 °C in TC100 medium (GIBCO-BRL) supplemented with 10% fetal bovine serum (FBS) and 50 μg/ml of gentamycin. The cells (1×10^6 per 35 mm culture dish) were infected with BmNPV-BGL (Palhan and Gopinathan, 1996) at a multiplicity of infection (moi) of 1–10 for different experiments. Maintenance of virus stocks and determination of viral titers were done as described by Maeda (1989).

2.2. Cloning of cyclin homolog from BmNPV

High titer stocks (10^8 pfu/ml) of the budded virus form of BmNPV were prepared by infection of BmN cells in T 75 bottles and the virus was concentrated from the cell culture supernatants by a series of low and high speed centrifugations and banding on sucrose by ultracentrifugation (Palhan and Gopinathan, 1996).

Forward and reverse primers, P1 and P2 (see Table 1) for PCR were designed based on the genomic sequence of BmNPV ORF 120 (nt 115154–116024), the region corresponding to the cyclin reported from AcMNPV. BmNPV genomic DNA was amplified by PCR using these primers and *Taq* DNA polymerase, the ends were polished with *Vent* (exo+) DNA polymerase and the blunt-ended products were cloned into plasmid vector pBS SK^+ at the *Eco* RV site for sequencing and probe preparations, or at the *Pvu* II site in the expression vector pRSET C (Invitrogen) for protein isolation. Protocols for cloning, labeled DNA probe preparation by random priming and Southern and Northern blots were performed as described in Sambrook et al. (1989). The location of the cloned DNA fragment on the viral genomic DNA was verified by Southern blots. Genomic DNA (5 μg) was digested with the restriction enzymes *Eco* RI and *Xho* I, and subjected to electrophoresis on 0.8% agarose gel, transferred onto nylon membrane (Amersham) and hybridized with ^{32}P labeled DNA probe corresponding to BmNPV cyclin region.

2.3. Sequence analysis

DNA sequencing was carried out manually by the dideoxy chain termination method (Sambrook et al., 1989) using Sequenase Version 2.0 DNA sequencing kit (Amersham) or at the automated DNA sequencing facility. Sequence data were analysed using the GCG package (Devereux), BLAST, BLAST N, Clustal W and GAP alignment.

2.4. RNA isolation and slot blot- and Northern hybridizations

Total RNA from BmN cells, uninfected or infected with BmNPV was isolated by the guanidium isothiocyanate

Table 1
Oligonucleotides used in PCR amplifications

	Sequence	Primer	Amplification
P1	5′-atgaaacgtgtcaaatgcaac-3′	Forward	BmNPV ORF 120 (115154–116027)
P2	5′-ttatttattaaaattata-3′	Reverse	
P3	5′-aaccagtttcctgtcatg-3′	Forward	BmNPV ORF 120 upstream (114423–115165)
P4	5′-gacacgtttcatttttata-3′	Reverse	
P5	5′-gtctaatttactgttaagc-3′	Forward primer	BmNPV ORF 120, 3′ region for transcription termination site mapping (115921–115940)
P6	5′-gcctgaggatattgaag-3′	Forward	*B. mori* cyclin B (cyclin box)
P7	5′-gatagcaaatgaagtgagagg-3′	Reverse	
P8	5′-atgtcaaagcctaacgtt-3′	Forward	BmNPV very late gene, p10 (108497–108706)
P9	5′-gagtctggaggatccgg-3′	Reverse	

The nucleotide co-ordinates on BMNPV genome (Gomi et al., 1999) for the amplified regions are indicated in parentheses. The oligodeoxy nucleotide primers as well as the DNA sequencing primers were synthesized at GIBCO-BRL or Bangalore Genei.