Turkish Journal of Biology

Volume 22 | Number 2 Article 2

1-1-1998

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DEMİRBAĞ, Zihni (1998) "Transcription of the Autographa californicaNuclear Polyhedrosis Virus p39 Gene in Bombyxmori Cell Culture," *Turkish Journal of Biology*: Vol. 22: No. 2, Article 2. Available at: https://journals.tubitak.gov.tr/biology/vol22/iss2/2

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Tr. J. of Biology
22 (1998) 153-159
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Transcription of the *Autographa californica* Nuclear Polyhedrosis Virus *p39* Gene in *Bombyx mori* Cell Culture

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Received: 21.05.1996

Abstract: Transcription of the *Autographa californica* nuclear polynedrosis virüs (AcNPV; Family: Baculoviridae) *p39* gene (delayed-early gene) has been studied extensively in the productive system of *Spodoptera frugiperda* cells. This is the first transcriptional study of this gene in the *Bombyx mori* cell line. In this study, comparative transcription of the AcNPV p39 gene was examined in *S. frugiperda* and *B. mori* cell cultures. Northern blot hybridization of totoal RNA samples, isolated from mock-infected (O and 48 hour post infection, h.p.i.) and AcNPV-infected (0, 2, 6, 12, 24, 48 h.p.i.) *S. frugiperda* and *B. mori* cell lines, was analyzed. Results indicates that the 1.08 kb RNA fragment, specific to the *p39* gene, is present in AcNPV-infected *S. frugiperda* and *Bombyx mori* cell lines. Moreover, the amount of transcript is higher and transcription occurs later in the cell line than in the *S. frugiperda* cell line.

Key Words: AcNPV, Baculovirus, *p39* gene, transcription, *Bombyx mori*

Autographa californica Nüklear Polinedrozis Virüs'ün p39 Geninin *Bombyx mori* Hücre Kültüründe Transkripsiyonu

Özet: *Autographa californica* nüklear polihedrozis virüs (AcNPV, Familya: Baculovirus)'ün *p39* geninin (erken gen) transkripsiyonu, *Spodoptera frugiperda* üretken sisteminde yoğun bir şekilde çalışılmıştır. Bu, *Bomyx mori* hücre kültüründe yapılan *p39* genine ait ilk transkripsiyonel calışmadır. Bu çalışmada, AcNPV' *p39* geninin *S. frugiperda* ve *B. mori* hücrelerindeki karşılaştırmalı transkripsiyonu araştırıldı. AcNPV ile enfekte olmamış (0 ve 48 saat sonra) ve enfekte olmuş (0, 2, 6, 12, 24 ve 48 saat enfeksiyon sonrası) *S. frugiperda* ve *B. mori* hücre suşlarından izole edilen toplam RNA örnekleri northern blot hibridizasyonuyla analiz edildi. Sonuçlar, *p39* geni için özel olan 1.08 kb RNA fragmentinin hem *S. frugiperda* hem de *B. mori* hücre suşlarında mevcut olduğunu gösterdi. Aynı zamanda, *S. frugiperda* hücreleriyle karşılaştırıldığında, bu transkriptin *B. mori* hücrelerinde yüksek oranda mevcut olduğu ve transkripsiyonunun geciktiği tespit edildii.

Anahtar Sözcükler : AcNPV, Baculovirus, *p39* geni, transkripsiyon, *B. mori*

Introduction

The *Autographa californica* nuclear polyhedrosis virus (AcNPV) is an important gene expression vector (16). In addition, AcNPV has been developed as a biopesticide and used in agriculture for many years (11, 5). Ideal AcNPV should have high virulence for a broad range of hosts and should not harm beneficial insects, and it should be an efficient expression vector. It is therefore necessary to understand the molecular mecanism of the host specificity of AcNPV.

Gene expression in AcNPV-infected cells is coordinately regulated and sequentially ordered (8). Four phases of transcriptional activation–immediate early (alpha), early (beta), late (gamma) and very late (delta)–can be distinguished during the replication cycle of AcNPV (18). The transcripts of the first two groups of genes are synthesized by an α-amanitin-sensitive RNA polymerase (9,15). The products of early genes are expressed from parental DNA, and include enzymes involved in DNA replication and factors which modulate viral gene expression. Activation of late and very late gene transcription is dependent on both early-gene expression (4) and DNA replication (4, 17), and involves the induction of a virus-induced RNA polymerase activity that is insensitive to α-amanitin (9,15). Late and very late genes are expressed from progeny DNA and their products provide structural proteins necessary for budded virus production and occluded virus production at very late stages of infection (22, 23, 24).

ie-1 is an immediate early gene and the only known *trans*-regulator gene of AcNPV. Guarino and Summers (12) determined that an immediate-early gene *(ie-1)* *trans*-activates transcription of delayed-early gene (*p39*). They (13) also found that expresion of the p39 gene product (39K) was enhanced 1000 fold when a baculovirus repeated element was *cis*-linked to the p39 gene.

A host ragne system model, developed earlier, that AcNPV replicaton is productive in *Spodoptera frugiperda* cells; however, it is abortive in *Bombyx mori* cells (2). One earlier study indicated that specific transcript of *ie-1* gene is present in AcNPV-infected *B. mori* cells at higher levels than in AcNPV-infected productive *S. frugiperda* cells (3).

In this first transcriptional study of a delayed-early gene (the *p39*) of AcNPV in abortive infection, the transcription of the *p39* gene was analyzed at 0, 2, 6, 12, 24, and 48 hours post infection (0 and 48 hour mock-infected samples were used as controls) to find out if transcription levels of this gene are also higher in AcNPV-infected *B. mori* cells because of the high level of expression of the *ie-1* gene in AcNPV-infected *B. mori* cells investigated earlier (3).

Materials and Methods

Cell: A monolayer culture of cell line BM-5 from *Bombyx mori* (10) was grown in 25 cm^2 plastic flasks (Corning). The flasks were seeded with $1x10^6$ cells in Hink's TNMFH cul-

ture medium (14) supplemented with 10% fetal bovine serum, and cells were grown at 28°C. Routine subculture was done at 5-day intervals at a 1:5 ratio.

Virus: Budded virions (BV) of *Autographa californica* nuclear polyhedrosis virus (AcNPV) were obtained from the supernatant of infected *S. frugiperda* cell cultures at 48-60-hour post-infection (h.p.i.). The first passage of the virus was prepared by a plaque assay experiment (20). The final plaque-purified virus was used to inoculate *S. frugiperda* and *B. mori* cells for northern blot analysis.

Preparation of DNA probe: For preparation of the *p39* gene-specific fragment, two oligonucleotides (a:5'CTC CGG AGA CTG CGG CCG TGT GCA 3', b: 5'TAC GCG CTA TTC GAA ATA AGC CTC3') were synthesized. These oligonucleotides, template DNA and Taq DNA polymerase were used for amplification of the p39 gene fragment in a DNA thermaCycler (Erkin Elmer,480; 3 cycles X 95°C for 1 min, 50°C for 1 min to prevert non-specific hybridization and 72°C for 1 min; 35 cycles X 95°C for 1 min, 42°C for 1 min, 72°C for 2 min). A 500 bp-amplified fragment of the p39 gene was radiolabeled with ^{32}P-dCTP according to the random primed labeling procedure (Boehringer Mannheim Random Primer Labeling Kit, 6, 7).

Cell and virus preparation for RNA isolation: Cells were seeded at 1 x10^6 per flask (Corning, 25 cm^2) and incubated overnight at 28°C. The cells were then inoculated with AcNPV (multiplicity of infection, m.o.i.= 50); adsorption occurred for 2 hours at room temperature. The infected cell cultures were incubated at 28°C, and at various times post-infection, flasks were placed on an aluminum plate over an ice bed. The medium was removed by aspiration. Each monolayer was washed twice with 5ml of ice-cold PBS free of calcium and magnesium ions. Total cellular RNA was isolated by a differential phenol extraction method (19) from 0 and 48-hours mock-infected *S. frugiperda* and *B. mori* cells and from AcNPV-infected cells at 0, 2, 6, 12, 24 and 48 hours post-infection.

Northern Blot Analysis: RNA samples were electrophoresized in horizontal 1% agarose gels containing 37% formaldehyde (in water) and 1 x MOPS according to the procedure of Berger and Kimmel (1). The northern blot procedure was carried out as described by Sambrook et al. (19). A high-stringency state (50% formamide, 5 x SSPE, 0.1 mg/ml salmon DNA, 5 x Denhard's, 0.1% SDS, 42°C overnight)z was chosen, and 100% homology was given between the probe and respective transcript. After hybridization, the nitrocellulose membrane was washed with 0.1% x SSPE, 0.1 SDS at 65°C for 15 min, air dried and exposed to Kodak XAR-5 X-Omat AR film at-70°C with a Dupont *Cornex Lightening Plus* intensifying screen.

Results

The northern blot of the *p39* gene was analyzed at 0, 2, 6, 12, 24 and 48 hours post-infection (h.p.i.) to find out if transcripton of this gene is also higher in AcNPV infected *B. mori* cells. A five hundred base of the *p39* gene -specific fragment was used as a

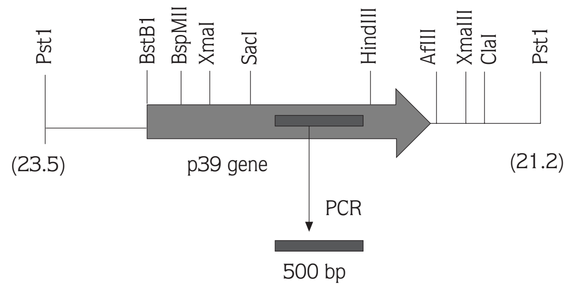
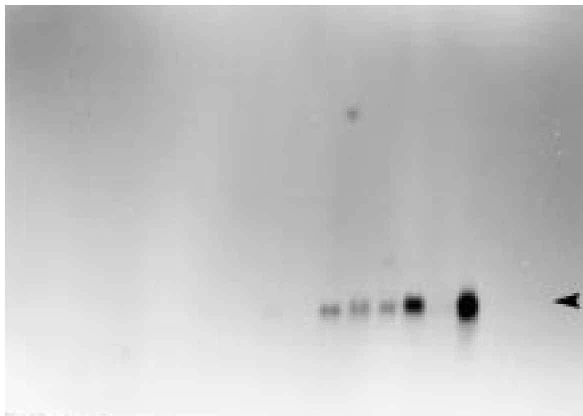


Figure1. Generation of a probe specific for *p39* gene of *Autographa californica* nuclear polyhedrosis virus. The PstK fragment of AcNPV contains the p39 gene. A 500 bp subfragment of this gene was amplified by polymerase chain reaction (PCR) in a DNA thermoCycler. This fragment was radiolabeled with ^{32}-P dCTP and used as a probe to detect mRNA specific to the *p39* gene.

0 0 0 0 2 2 6 6 12 12 24 24 48 48 48 48

s b s b s b s b s b s b s b s b



1.08 kb

Figure 2. Transcriptional analysis of the *p39* gene of *Autographa californica* nuclear polyhedrosis virus in abortive and productive infections. Total RNA was extracted at various times (indicated by hour, top row) after infection, fractionated by agarose gel electrophoresis and detected by northern blot hybridization. ^{32}-P radiolabeled *p39* gene-specific probe was used. s, b: infected *S. frugiperda*, *B.mori* cells; s, b: mock infected *S. frugiperda*, *B.mori* cells. The arrow indicates the 1.08 kb mRNA specific for the p39 gene.

probe (Figure 1). Culture of *S. frugiperda* and *B. mori* cells were infected with AcNPV at an m.o.i. of 50, and total cellular RNA was extracted at various times of post-infection. These RNA samples were fractionated by agarose gel electrophoresis, blotted onto nitrocellulose membranes and probed with a ^{32}P–labeled internal fragment of the *p39* gene under high stringency conditions (see Materials and Methods for details of procedures).

A 1.08 kb transcript, specific to the *p39* gene, was detected in abortive infection of *B. mori* cells as well as in productive infections of *S. frugiperda* cells (Figure 2). The data show that this transcript is present between 6-48 h.p.i. in productive AcNPV-*S. frugiperda* infection and 12-48 h.p.i. in abortive infection. It reached maximum levels at around 24 h.p.i. in productive infection and at 48 h.p.i. in abortive infection. At 12 h.p.i. the level of this transcript was similar in both cell lines. However, the levels of this transcript were higher at 24 and 48 h.p.i. in abortive *B. mori* cells. The differences in the level of the

transcript were 20 fold at 24 h.p.i. and 100 fold at 48 h.p.i. greater than productive infection. Infection beyond 48 h.p.i. was not carried out because of the lysis of the majority of cells due to virus production.

Discussion

In order to understand the restriction of AcNPV replication in *B. mori* cells and to learn more about the level at which critical blocks occur, the two different infections for transcription of the *p39* gene (a delayed-early gene) were compared. An earlier study (3) showed that *ie-1* gene of AcNPV is transcribed at higher levels in abortive ihfection of *B. mori* cells than in productive infection of *S. frugiperda* cells. In another study, Guarino and Summers (12) determined that an immediate-early gene (*ie-1*) *trans*-activates transcription of *p39*, and they also found that expression of the *p39* gene product (39K) was enhanced 1000 fold when a baculovirus repeated element was cis-linked to the p39 gene (13). In this study, the *p39* gene was utilized to find out if similar *trans*-activation occurs in abortive infection, and also to learn the kinetics of a delayed-early gene transcription in abortive infection.

As a result of the present sutdy (Figure 2), transcription of a delayed-early (*p39*) gene occurs in AcNPV-infected *B. mori* cells and the levels of this transcripts are 20-100 fold higher in *B. mori* cells than in AcNPV-infected *S. frugiperda* cells. However, the increase in *p39* gene transcription was lower than that observed for the *ie-1* gene in abortive cells in a previous study (3). Since the increase in *p39* gene transcription observed in *S. frugiperda* cells in this study was similar to that observed previously in *B. mori* cells, concluded that *trans*-activation of the *p39* gene by *ie-1* also occurs in *B. mori* cells. However, this *trans*-activation is not as efficient as that observed in productive infection (20-100 fold versus 1000 fold). The lower level of transcription probably results from incompatibility of the host factors required for transcription of the delayed-early gene in *B.mori* cells. The higher level of *p39* transcript at a late time of infection (24 h.p.i. and 48 h.p.i.) is due probably to absence of late viral factor(s) and the consequent absence of inhibition of the *p39* gene.

Replication in *B. mori* cells was controlled at the transcriptional level, and the critical control occurs most probably before or during delayed-early gene expression. Therefore, the activation of the viral factor for virus replication is still effective in abortive infection; however, the abortive replication in *B. mori* cells is primarily due to inefficient activation of host factor(s).

Characterization of these factors and elucidation of their mechanisms of action in both homologous and heterologous cell lines will improve our understanding of the molecular mecnanisms of baculovirus host specificity and regulation of gene expression in productive infections. The identification of genes for regulatory polypeptides of host origin or of viral DNA elements governing host specificity will allow engineering of desirable hybrid viruses, as demonstrated very recently by the replication of a recombinant AcNPV in nonpermissive

cells (21). It will also be useful in pest control and in the design of gene expression vectors that are more efficient for industrial scale-up.

Acknowledgements

This study was funded by TÜBİTAK (TBAG-1291) and the Research Council of Karardeniz Technical University (94.11.002.15).

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