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NOTES

Baculovirus Infection Raises the Level of TATA-Binding Protein That Colocalizes with Viral DNA Replication Sites

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During the infection cycle of *Autographa californica* multicapsid nuclear polyhedrosis virus, the TATA-binding protein (TBP) of the insect host cell likely participates in early viral transcription, which is mediated by the host RNA polymerase II. However, the role of TBP in late and very late viral transcription, which is accomplished by an alpha-amanitin-resistant RNA polymerase, is unclear. We observed a dramatic increase of TBP protein during the late phases of infection. TBP mRNA levels, however, were not coordinately increased. Indirect-immunofluorescence studies revealed a nuclear redistribution of TBP during infection. After labeling of viral replication centers with bromodeoxyuridine (BrdU), costaining of TBP and BrdU showed that TBP localized to viral DNA replication centers. These results suggest a putative role of TBP during late viral transcription, which may occur in close proximity to viral DNA replication.

The TATA-binding protein (TBP) is a universal transcription factor that is required for initiation by all three eukaryotic RNA polymerases. TBP was identified as a subunit of TFIID, a multisubunit complex composed of TBP and TBP-associated factors (for a review, see reference 7). As part of the preinitiation complex at both TATA box-containing and TATA-less promoters, TBP resembles a target for transcriptional activators and repressors (for a review, see reference 19). One unique feature of TBP is its high level of conservation among all eukaryotes and archaea. In most cases, TBP is encoded by a single gene. The carboxy-terminal core domains of all characterized TBPs are more than 75% identical to the human TBP, while the amino-terminal domain is poorly conserved (7). In insects, TBP-encoding cDNAs have been cloned from *Drosophila melanogaster* cells (8) and from *Spodoptera frugiperda* cells, which resemble a permissive cell line of the baculovirus *Autographa californica* multicapsid nuclear polyhedrosis virus (Ac*M*NPV) (20). An amino acid sequence comparison reveals that the C terminus of *S. frugiperda* TBP is 93% identical to that of *Drosophila* TBP and about 75% identical to the C termini of all other known TBPs (20).

The gene expression cascade of Ac*M*NPV is temporally regulated and characterized by the sequential involvement of two different RNA polymerases. Host RNA polymerase II recognizes early viral, TATA box-containing promoters; thus, TBP is thought to participate in early viral transcription. Late genes and the hyperexpressed very late genes encoding p10 and polyhedrin are transcribed by an alpha-amanitin-resistant RNA polymerase that has been reported to be a complex of four virus-encoded proteins (3, 4). The purified RNA polymerase recognizes late and very late viral promoters, although the burst of very late transcription was not observed by in vitro experiments, suggesting that factors contributing to the hyperexpression of the very late promoters are still unknown (4). Only when in vitro transcription assays were performed with protein extracts of purified cell nuclei was the burst in very late transcription observed (14). Transcriptional initiation at late and very late promoters occurs within a conserved TAAG sequence motif, and promoter activity is independent of upstream sequence elements (18, 22). Mutagenesis studies reveal that the transcriptional burst of the polyhedrin promoter depends on only part of the 5′ untranslated region (14). Viral factors have been reported to participate in the regulation of late and very late promoters (23, 24); however, little is known concerning the possible involvement of host cell factors in late transcription. Since the baculovirus expression system is based on the hyperexpressed polyhedrin and p10 promoters, their transcriptional regulation is of considerable interest.

Expression of TBP during the course of infection. If TBP plays a role in the regulation of late and very late viral transcription, one precondition is the presence of TBP during the late phases of infection. In general, host protein synthesis is gradually shut down during infection (1, 25). Hence, we investigated the time course of TBP expression during Ac*M*NPV infection. The insect cell lines *Trichoplusia ni* TN-368 and *S. frugiperda* IPLB21 were infected with Ac*M*NPV plaque isolate E at a multiplicity of 10 to 20 PFU per cell, and detergent-based nuclear protein extracts were prepared as previously

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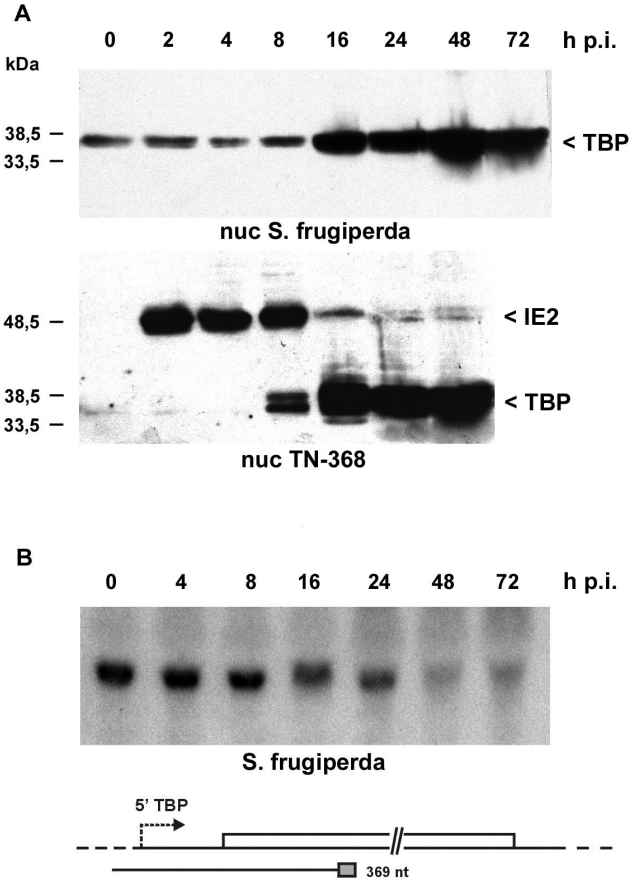

FIG. 1. Expression of TBP in Ac*M*NPV-infected TN-368 and *S. frugiperda* cells. (A) Detergent-based nuclear extracts were prepared from uninfected *S. frugiperda* (lane 0, nuc S. frugiperda) or TN-368 cells (lane 0, nuc TN-368) or from cells infected at 2, 4, 8, 16, 24, 48, and 72 h p.i. Proteins were resolved on sodium dodecyl sulfate–10% polyacrylamide gels and stained with the polyclonal anti-Sf/TBP antiserum raised in rabbits. As control, the Western blot with the protein extracts of TN-368 cells was also stained with the anti-IE2 antiserum (11). The arrowheads on the right indicate the TBP protein of about 36 kDa and the IE2 protein of 49 kDa. Protein size markers are given on the left. (B) Total RNA (100 μg) isolated from uninfected *S. frugiperda* cells (lane 0) or from cells at 4, 8, 16, 24, 48, and 72 h p.i. (lanes 4, 8, 16, 24, 48, and 72) was analyzed on an 1.2% agarose gel containing 2.2 M formaldehyde. The Northern blot was hybridized to a TBP-specific RNA probe of 369 nucleotides. The schematic representation of the TBP open reading frame, with the putative transcriptional start site, and the position of the RNA probe are given below. The box indicates the T7 promoter.

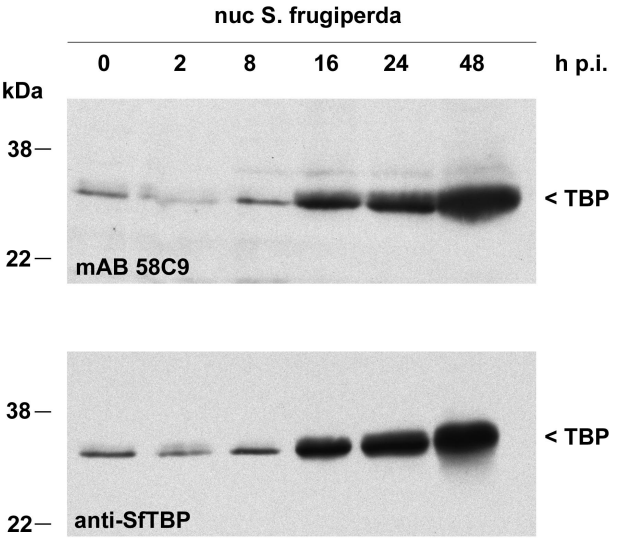

FIG. 2. Specificity of the TBP protein in insect cells. Detergent-based nuclear extracts were prepared from uninfected *S. frugiperda* (lane 0) or from cells infected at 2, 8, 16, 24, and 48 h p.i. Proteins were resolved on sodium dodecyl sulfate–10% polyacrylamide gels and stained with rabbit anti-TBP antiserum directed against the N terminus of Sf/TBP or with MAb 58C9 directed against the C terminus of *Drosophila* TBP. The arrowheads indicate the TBP protein band, and protein size markers are given on the left.

described (15). The analysis was performed by Western blotting with a polyclonal antibody directed against *S. frugiperda* TBP. The polyclonal antiserum was generated by immunizing rabbits with denatured TBP purified from bacteria which overexpressed the N-terminal 25-kDa fragment of TBP. In nuclear extracts of uninfected and infected *S. frugiperda* cells, the anti-TBP antiserum recognized a polypeptide of about 36 kDa which matches the predicted size of the TBP open reading frame product (Fig. 1A). Interestingly, a significant increase of TBP protein was observed in extracts prepared between 16 and 72 h postinfection (p.i.), which represents the late and very late phases of infection (Fig. 1A).

In TN-368 cells, TBP was barely detectable in extracts of uninfected cells and of infected cells harvested during the early phase. Since TN-368 cells are derived from a different genus than *S. frugiperda* cells, this result is likely related to the fact that the anti-TBP antiserum is directed against the less conserved N terminus of *S. frugiperda* TBP (Fig. 1A). For TN-368 cells, multiple specific bands were detected, which might indicate posttranslational modifications of TBP. As in *S. frugiperda* cells, a dramatic increase in TBP was observed during the late phases of infection in TN-368 cells, suggesting a general phenomenon in Ac*M*NPV-permissive insect cells (Fig. 1A).

Transcription of TBP during infection. One possible explanation of the TBP increase during the late phases of infection could be the transcriptional activation of the TBP gene during the course of infection. However, Northern blot analysis revealed a steady-state level of the TBP transcript until 24 h p.i. followed by a decreasing but still detectable level of TBP transcript until 72 h p.i. (Fig. 1B). The analysis was performed with total RNA prepared from uninfected and Ac*M*NPV-infected *S. frugiperda* cells. The RNA was hybridized to an in vitro-transcribed RNA probe of 369 nucleotides which was synthesized from a PCR fragment (Fig. 1B). The fragment was generated with a T7 promoter containing primer 5′-GGGTAATACGACTCACTATAGGGCATAGCAT TTGGCAAAAT-3′ from a *Not*I-linearized cDNA clone, pSf-TBP, which was identified and characterized by Rasmussen and Rohrmann (20).

The time course of TBP transcription in *S. frugiperda* cells correlates well with previous observations showing a decline in TBP transcript levels by 24 h p.i. (20). In addition, decreases of several other host transcripts, such as actin, histones, and heat shock protein 70, have been observed, which led to the assump-

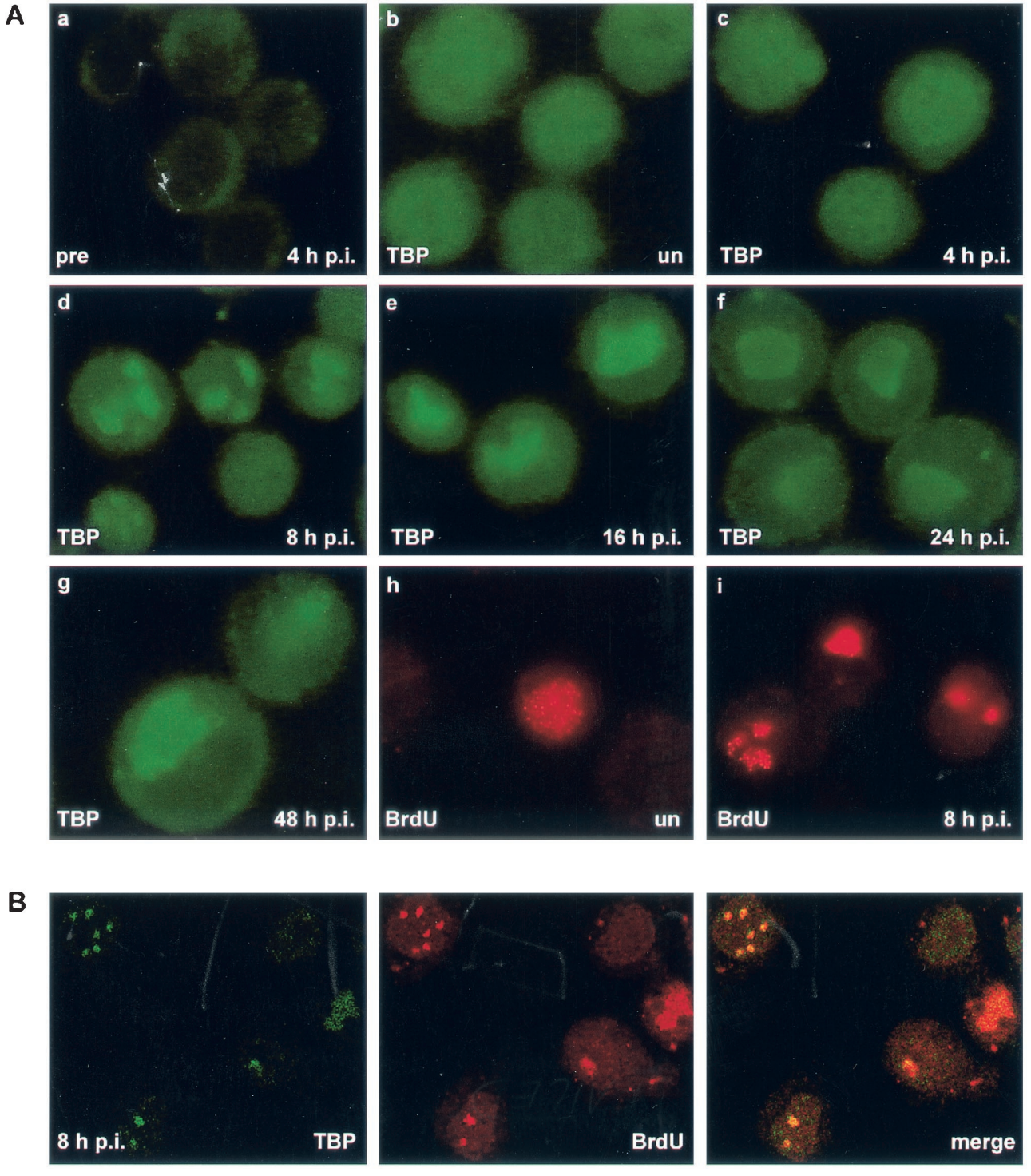

FIG. 3. Localization of TBP and colocalization with viral DNA replication sites after Ac*M*NPV infection of *S. frugiperda* cells. (A) Uninfected and Ac*M*NPV-infected *S. frugiperda* cells were fixed in 2% paraformaldehyde and permeabilized in 0.1% Triton X-100 at 4, 8, 16, 24, and 48 h p.i. Staining was performed with the anti-Sf/TBP antiserum and fluorescein isothiocyanate-conjugated anti-rabbit immunoglobulin (Jackson Laboratory) (a to g). The specimens were viewed using a Zeiss Axiovert 135 microscope linked to the INTAS digital camera system (a to i). As a control, cells at 4 h p.i. were stained with rabbit preimmune serum (a). To visualize DNA replication, uninfected *S. frugiperda* cells and cells at 8 h p.i. were stained first with BrdU and then with mouse MAb BrdU (red) as previously described (13) (h and i). (B) Cells at 8 h p.i. were costained with rabbit anti-Sf/TBP antiserum (green) and mouse MAb BrdU (red). For confocal imaging, a Leica DM IRBE microscope linked to Leica TCS-SP was used. Confocal images and the merged image are shown.

tion that host RNA synthesis is inhibited during the late phase in Ac*M*NPV-infected *S. frugiperda* cells (16).

The transcriptional analysis demonstrated that the high level of TBP protein did not correlate with increased transcription of the TBP gene during the late phases of infection. Thus, it is conceivable that virus infection influences the turnover of the TBP protein. One might speculate that viral factors interfere with cellular degradation pathways. Little is known regarding cellular events that might regulate the TBP level; most knowledge concentrates on the role of TBP in the formation of transcription initiation complexes. To date, studies on the transcriptional regulation of TBP expression are limited (5), yet regulation on the translational level and protein stability are still an enigma. Virus-induced increase of TBP expression has been described only for the hepatitis B virus X protein, which leads to the upregulation of TBP transcription (9).

Specificity of the TBP protein. Our finding of increased expression of TBP protein conflicts with previous studies performed with *S. frugiperda* cells. Rasmussen and Rohrmann (20) analyzed total protein extracts and found constant levels of TBP protein up to 72 h p.i. The data presented in Fig. 1 also conflict with previous results showing a decrease in TBP in salt-extracted nuclear protein extracts prepared at 24 h p.i. (11). In both of the previous reports, protein analysis was performed using the monoclonal antibody 58C9 directed against the highly conserved C terminus of *Drosophila* TBP. Therefore, we questioned whether the increase in TBP was related to the antiserum directed against *S. frugiperda* TBP. To explain our finding, we directly compared the TBP staining pattern of anti-Sf/TBP antiserum with that of the monoclonal antibody (MAb) 58C9. In detergent-based nuclear protein extracts of Ac*M*NPV-infected *S. frugiperda* cells, staining with both antibodies demonstrated increasing levels of TBP at 16 h p.i. (Fig. 2). It is worth mentioning that the exposure time of the Western blot stained with MAb 58C9 was much longer than with the anti-Sf/TBP antiserum (Fig. 2). Thus, we conclude that the antibodies directed against either the N terminus of *S. frugiperda* TBP or the C terminus of *Drosophila* TBP are able to recognize the same protein.

The protein extract preparations differed from our previous studies; hence, we stained TBP with the anti-Sf/TBP antiserum in salt-extracted nuclear protein extracts of Ac*M*NPV-infected *S. frugiperda* cells which were prepared as previously described (10, 11). As in previous studies with the MAb 58C9, no increase in the TBP level was detectable in salt-extracted nuclear protein extracts during the late phase of infection (data not shown). One possible explanation is that TBP may vary in its intranuclear localization in the course of infection. It is conceivable that TBP becomes associated with nuclear structures late in infection, which may contribute to its extractability.

Intranuclear localization of TBP. In order to gain insights into the nuclear distribution of TBP in response to Ac*M*NPV infection, we performed indirect immunofluorescence studies. In uninfected *S. frugiperda* cells, TBP staining with the anti-Sf/TBP antiserum was visible throughout the nucleus with no significant change early in infection (Fig. 3A, panels b and c). Upon close examination using confocal microscopy, we observed cells with a granular staining of TBP in the nucleoplasm (data not shown). By 8 h p.i., the nuclear staining was increased with intense TBP staining of discrete nuclear compartments that fused to larger structures as infection proceeded (Fig. 3A, panels d, e, f, and g). The nucleus was visualized by counterstaining with propidium iodine, whose staining pattern of DNA was reminiscent of the staining pattern of TBP (data not shown). Staining of viral replication proteins, such as the DNA binding protein, showed a comparable localization in large nuclear structures, while the IE2 protein was found in distinct nuclear structures during the late phases of infection (data not shown) (13). To address the question of whether the nuclear TBP structures late in infection point to a colocalization of TBP and DNA, replicating DNA was visualized. The thymidine analogue 5-bromo-2′deoxyuridine (BrdU) (Sigma) was added to the cell medium 1 h prior to fixation, and the cells were stained with an anti-BrdU antibody (clone B44; Becton Dickinson) as previously described (13). In uninfected *S. frugiperda* cells, cellular DNA replication was visible throughout the nucleus as tiny dots that were detectable in fewer than 20% of the cells (Fig. 3A, panel h). In contrast, viral DNA replication centers were visible as distinct nuclear domains by 8 h p.i. (Fig. 3A, panel i) and enlarged to foci late in infection as previously described (13). After costaining with anti-TBP antiserum and the MAb clone B44 directed against BrdU, confocal imaging revealed the colocalization of TBP and viral DNA replication centers (Fig. 3B). As soon as viral DNA replication was visible in distinct compartments, colocalization with TBP was observed (Fig. 3B). The enlargement of the viral DNA replication sites coincided with the growing nuclear TBP structures (Fig. 3B). At 16, 24, and 48 h p.i., enlarged viral DNA replication sites, which covered most of the nucleus, showed the same staining pattern as the TBP structures (data not shown). Both TBP structures and DNA replication sites exhibited a granular staining (Fig. 3B). Whether the granular staining indicates the direct association of TBP and DNA within the DNA replication sites still has to be elucidated. In mammalian cells, direct visualization of green fluorescent protein-TBP by immunofluorescence in live cells suggests that TBP binds not only to transcriptionally active chromatin in the interphase nucleus but also to highly condensed and transcriptionally inactive chromatin during mitosis (2).

The observation that TBP is localized to baculoviral DNA replication sites provides the first evidence that late viral transcription and DNA synthesis may take place at common structures. However, we cannot exclude a potential role of TBP in the regulation of viral DNA replication, as has been proposed for DNA replication of human papillomaviruses (6). For other large DNA viruses, such as herpes simplex virus type 1 (HSV-1), it has been suggested that both viral DNA synthesis and late transcription occur within replication compartments. This assumption is based on colocalization studies of the host RNA polymerase and viral DNA replication sites (12, 21) as well as on localization studies of DNA and RNA synthesis with use of direct labeling methods (17).

In contrast to herpesviruses, Ac*M*NPV induces its own RNA polymerase and accomplishes very high-level and efficient late transcription in a cellular environment whose functions appear to be downregulated. Hence, it will be of great interest to elucidate the mechanism by which Ac*M*NPV succeeds in maintaining the high level of the cellular protein TBP and to reveal its functional significance. Since little is known regarding TBP stability and regulation of its protein level, studies of Ac*M*NPV

infection may provide the first insights into the maintenance of this cellular key transcription factor.

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