Expression and nuclear localization of the TATA-box-binding protein during baculovirus infection

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Received 25 September 2013
Accepted 26 March 2014

The TATA-box-binding protein (TBP) plays a key role in initiating eukaryotic transcription and is used by many viruses for viral transcription. We previously reported increased TBP levels during infection with the baculovirus Autographa californica multicapsid nuclear polyhedrovirus (AcMNPV). The TBP antiserum used in that study, however, cross-reacted with a baculoviral protein. Here, we reported that increased amounts of nuclear TBP were detected upon infection of Spodoptera frugiperda and TN-368 cells with a TBP-specific antiserum. TBP levels increased until 72 h post-infection (p.i.), whilst tbp transcripts decreased by 16 h p.i., which suggested a virus-induced influence on the TBP protein levels. To address a potential modification of the TBP degradation pathway during infection, we investigated the possible role of viral ubiquitin. Infection studies with AcMNPV recombinants carrying a mutated viral ubiquitin gene revealed that the TBP increase during infection was not altered. In addition, pulse–chase experiments indicated a high TBP half-life of ~60 h in uninfected cells, suggesting that a virus-induced increase of TBP stability was unlikely. This increase in TBP correlated with a redistribution to nuclear domains resembling sites of viral DNA synthesis. Furthermore, we observed colocalization of TBP with host RNA polymerase (RNAP) II, but only until 8 h p.i., whilst TBP, but not RNAPII, was present in the enlarged replication domains late during infection. Thus, we suggested that AcMNPV adapted a mechanism to accumulate the highly stable cellular TBP at sites of viral DNA replication and transcription.

INTRODUCTION

The TATA-box-binding protein (TBP) is a key component of the cellular transcription machinery by nucleating the assembly of various transcriptional complexes. All three RNA polymerases (RNAPs) utilize TBP in combination with TBP-associated factors to initiate transcription. The highly conserved C-terminal core domain of TBP binds DNA, and interacts with basal transcription factors and other regulatory proteins, whilst the N terminus of TBP is less well conserved (Burley & Roeder 1996; Davidson, 2003; Hernandez, 1993).

Many viruses use RNAP II and the cellular transcription machinery, often in combination with virus-encoded activators to transcribe their genes. Examples are adenovirus E1A, IE2 of cytomegalovirus (CMV) and papillomavirus type 16 E7, which interact with TBP (Kim et al., 2000; Lee et al., 1991; Phillips & Vousden, 1997). The general assumption is that viruses which depend on RNAP II transcription use TBP as a basal transcription factor. Whether and how viruses interfere with TBP expression and/or regulation is poorly understood (Wang et al., 1997).

A unique feature of the baculovirus Autographa californica multicapsid nuclear polyhedrovirus (AcMNPV) is the sequential involvement of two different RNAPs. Host RNAPII recognizes early viral promoters, whilst late genes and the hyper-expressed very late genes encoding p10 and polyhedrin are transcribed by a viral RNAP (Rohrmann, 2013). This viral polymerase is evolutionarily divergent from cellular polymerases and is composed of four late expression factors (LEFs) (Fuchs et al., 1983; Grula et al., 1981; Guarino et al., 1998b). Late promoter elements recognized by the viral RNAP are distinct from early viral and cellular promoters, and include a 5′-TAAG-3′ motif as...
the transcriptional start site (Lu & Miller, 1997; Chen et al., 2013). Whether basal transcription factors such as TBP contribute to late viral transcription is still open. As most host genes are downregulated gradually during baculovirus infection (Salem et al., 2011), the question arises whether transcription factors of the host cell are able to escape and contribute not only to early but also to late transcription and to the hyper-expression of the very late genes p10 and polyhedrin. Late in infection, viral transcripts compose the majority of the total cellular mRNA population, suggesting a highly efficient transcriptional initiation process (Chen et al., 2013). When we investigated the time course of TBP expression during AcMNPV infection, we observed an increase of TBP levels during the late phases of infection, whilst the TBP mRNAs decreased (Quadt et al., 2002). This observation is in line with the general finding that AcMNPV downregulates host genes at the transcriptional level (Ooi & Miller, 1988; van Oers et al., 2002). To characterize the nuclear distribution of TBP in uninfected cells. During AcMNPV infection, a redistribu-
tion of TBP to nuclear domains takes place (Quadt et al., 2002). Here, we quantified the protein levels of TBP in cells of a different genus such as TN-368. Furthermore, it appears that TBP is ~1–2 kDa smaller in TN-368 than in S. frugiperda cells based on the different migration in SDS-PAGE (Fig. 2a, b). As a control, we also performed the protein analysis with mAb 58C9 directed against the highly conserved C terminus of Drosophila TBP which confirmed the TBP staining pattern obtained with anti-TBP antiserum SA3890 in both cell lines (Fig. 2a, b). To further confirm that anti-TBP antiserum SA3890 did not cross-react with DBP, we stained protein extracts of mammalian MDCKII cells overexpressing DBP under the CMV promoter and did not obtain any signal (data not shown).

In both S. frugiperda and TN-368 cells, the increase in TBP levels during AcMNPV infection was confirmed; however, the increase was less pronounced than estimated previously (Quadt et al., 2002). Here, we quantified the protein levels and showed a 3.5-fold increase in TBP late in infection which was comparable in TN-368 and S. frugiperda cells (Fig. 2c). In addition to the nuclear protein extracts, we also prepared the

### RESULTS

**TBP expression during AcMNPV infection and specificity of the antibody against Spodoptera frugiperda TBP**

To further address the increase of TBP during infection, we initially confirmed the decrease of TBP transcripts by quantitative analysis demonstrating a decline at 16 h post-infection (p.i.) in infected S. frugiperda cells (Fig. 1). Previously, we observed an unexpected increase in the TBP protein level in the two insect cell lines, S. frugiperda and TN-368, both permissive for AcMNPV. In TN-368 cells, multiple TBP bands were detected in protein extracts of cells harvested late in infection (Quadt et al., 2002). Thus, we performed MS analyses to identify the additional TBP bands. Our results revealed that the antiserum against TBP (SA3889) used previously cross-reacted with the viral DNA-binding protein (DBP). The baculovirus DBP is a ssDNA-binding protein expressed late during infection whose sequence is completely unrelated to the TBP sequence (Mikhailov et al., 1998).

Therefore, we tested another TBP antiserum directed against TBP of S. frugiperda cells (SfTBP) for specificity. The previously used TBP antiserum SA3889 recognized both DBP and TBP, whilst the TBP antiserum SA3890 detected only TBP in both TN-368 and S. frugiperda cells (Fig. 2a, b). Both TBP antisera were directed against the less-conserved N terminus of SfTBP, which may explain the lower detection of TBP in cells of a different genus such as TN-368. Furthermore, it appears that TBP is ~1–2 kDa smaller in TN-368 than in S. frugiperda cells based on the different migration in SDS-PAGE (Fig. 2a, b). As a control, we also performed the protein analysis with mAb 58C9 directed against the highly conserved C terminus of Drosophila TBP which confirmed the TBP staining pattern obtained with anti-TBP antiserum SA3890 in both cell lines (Fig. 2a, b). To further confirm that anti-TBP antiserum SA3890 did not cross-react with DBP, we stained protein extracts of mammalian MDCKII cells overexpressing DBP under the CMV promoter and did not obtain any signal (data not shown).

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![Fig. 1. Quantification of tbp transcription. S. frugiperda cells were infected with AcMNPV (10 p.f.u. cell⁻¹). At 0, 4, 16, 24 and 48 h p.i., RNA was extracted and quantitative PCR performed. The relative numbers of transcripts shown were quantified with TBP- and 28S-specific primers from two independent experiments with comparable results.](image-url)
Fig. 2. TBP expression during AcMNPV infection. (a) Nuclear extracts were prepared from uninfected TN-368 cells (un) or cells infected with AcMNPV (10 p.f.u. cell⁻¹) at 4, 8, 16, 24, 48 and 72 h.p.i. Proteins were resolved by SDS-10% PAGE and stained with rabbit anti-TBP (SA3889), with rabbit anti-TBP (SA3890) antiserum or with mouse mAb 58C9 against Drosophila TBP. The arrowheads indicate the TBP band and the asterisks indicate the unspecific staining of DBP or polyhedrin. As a control, staining was performed with rabbit anti-HSP90 antibodies. (b) Nuclear extracts were prepared from uninfected S. frugiperda cells and stained with anti-SfTBP SA3889, anti-SfTBP SA3890, or mAb 58C9. (c) Graph showing relative units of TBP expression in TN-368 and S. frugiperda over time.
cytoplasmic fractions, where less TBP protein was present (Fig. 2d). Increasing TBP levels were still visible, whilst the actin levels were unchanged (Fig. 2d). As a control, we also tested the cellular levels of the heat-shock protein HSP90 and histone H3. HSP90 levels were unchanged in both nuclear (Fig. 2a) and cytoplasmic protein extracts with a decrease at 72 h p.i. (data not shown) confirming recent results (Lyupina et al., 2011). A constant histone H3 level was observed only in nuclear extracts (Fig. 2d), emphasizing that infection did not lead to a generalized increase nor to a cytoplasmic/nuclear redistribution of cellular proteins.

Surprisingly, we observed a decline of TBP in TN-368 cells at 24 until 72 h p.i. suggesting a difference to *S. frugiperda* cells. As the TBP decrease coincided with the hyper-expression of polyhedrin migrating in close proximity to TBP (Fig. 2a), we analysed the TBP expression levels after infection with a polyhedrin-deficient bacmid virus (*AcMPV-CMV/GFP*) and observed a comparable TBP increase late during infection as in WT virus-infected *S. frugiperda* cells (Fig. 3a). As a control of infection, expression of viral DBP was examined in WT- and mutant-infected cells, and the presence and lack of polyhedrin was shown (Fig. 3a). To investigate whether the hyper-expressed polyhedrin masked the detectability of TBP, we incubated nuclear extracts of TN-368 cells harvested from uninfected cells or prepared at early times p.i. with extracts of cells harvested at 48 h p.i. These extracts included high amounts of polyhedrin and TBP was not detected (Fig. 3b). As control, the protein extracts were mixed with extracts prepared 48 h p.i. with the polyhedrin-deficient bacmid virus (*AcMNPV-CMV/GFP*) in which TBP was detectable (Fig. 3b). TBP could only be detected when incubated with polyhedrin-deficient extracts, which supported our conclusion that the presence of polyhedrin indeed masked the detection of TBP in TN-368 cells (Fig. 3b). In addition, when we inhibited protein synthesis with cycloheximide (CHX) at 8 h p.i., TBP was visible late in infection, indicating that the loss of polyhedrin expression allows the detection of TBP (Fig. 3c). The block of protein synthesis by CHX was confirmed by visualizing the lack of polyhedra formation (Fig. 3d). Taken together, we concluded that the increased TBP levels were present in TN-368 cells late in infection, which is comparable with the situation in infected *S. frugiperda* cells.

**Role of viral ubiquitin in regulating TBP levels**

To explore the underlying mechanism of the virus-induced TBP increase, we addressed whether *AcMNPV* potentially interferes with the degradation pathway of TBP during infection. When we initially performed colocalization studies of ubiquitin with TBP during infection, we obtained no evidence for the presence of conjugated ubiquitin in close proximity to TBP (data not shown). Instead, we observed colocalization of IE2 and conjugated ubiquitin in nuclear dots early in infection, and a loss of ubiquitin signals in the nucleus late during infection (Fig. 4). The early viral protein IE2 is suggested to act as a ubiquitin ligase and can itself interfere with the degradation pathway of TBP during infection (Imai et al., 2003, 2005). Thus, our results supported a mechanism of ubiquitin-mediated degradation of IE2 late in infection; however, there was no link to the association of TBP and ubiquitin.

If TBP is not directly modified with ubiquitin, one might envision that *AcMNPV* interferes with the degradation of proteins that contribute to increased TBP levels. v-UBI of *AcMNPV* has been suggested to interfere with the host degradation pathway by acting as a chain terminator of multiubiquitin chain elongation (Haas et al., 1996). When we performed infection studies with an *AcMNPV* recombinant that carried a frameshift mutation in the v-ubi gene (Reilly & Guarino, 1996), we still observed increased TBP levels late in infection (data not shown). To confirm the effect of non-functional v-UBI, we generated a second *AcMNPV* recombinant which carried a replaced RGG motif (v-UBI RGG). This motif is essential for the ligation of ubiquitin to the substrate. Growth rate analysis of the v-UBI RGG mutant in TN-368 cells indicated a slight reduction as compared with WT *AcMNPV*-infected cells (data not shown). This is in line with previous reports demonstrating that deleted v-UBI correlates with decreased virus production; however, v-UBI is not essential for viral replication (Reilly & Guarino, 1996; Katsuma et al., 2011). When TN-368 cells were infected with the v-UBI RGG mutant, the TBP increase during infection was still detected (Fig. 5a). As a control of infection, expression of the early IE2 was investigated. As IE2 was still present during the late phase, infection was probably delayed in mutant-infected cells as compared with WT-infected cells in which IE2 decreased at 16 h p.i. even with a fourfold lower m.o.i.; in contrast, when infection was performed at m.o.i. 0.25, IE2 was detectable until 72 h p.i. (Fig. 5b). The delayed infection is supported by the expression pattern of LEF-3 and DBP which were only detected at 16 h p.i. in mutant-infected cells (Fig. 5c). These results suggested that mutated v-UBI correlated with delayed viral expression; however, v-UBI seemed to play no significant role in the protein turnover of TBP during infection.
Protein stability of TBP

To further address whether the virus potentially interferes with TBP turnover, we determined the protein stability of TBP. When we blocked protein synthesis in uninfected S. asteris (WT AcMNPV) or AcMNPV-CMV/GFP (Δ) at 6, 24 and 48 h p.i. Protein fractions of uninfected or WT-infected cells (6 h p.i.) were mixed with fractions of either WT-infected cells (48 h p.i.) or AcMNPV-CMV/GFP-infected cells (48 h p.i.) prior to SDS-10% PAGE. Staining was performed with rabbit anti-TBP antiserum (SA3890). The corresponding amido black-stained membrane is shown below. (b) Nuclear extracts were prepared from uninfected TN-368 cells (un) or from cells infected with either WT AcMNPV (WT) or AcMNPV-CMV/GFP (Δ) at 6, 24 and 48 h p.i. Protein fractions of uninfected or WT-infected cells (6 h p.i.) were mixed with fractions of either WT-infected cells (48 h p.i.) or AcMNPV-CMV/GFP-infected cells (48 h p.i.) prior to SDS-10% PAGE. Staining was performed with rabbit anti-TBP antiserum (SA3890). The corresponding amido black-stained membrane is shown below. (c) Nuclear extracts were prepared from AcMNPV-infected TN-368 cells at 12, 24, 48 and 72 h p.i. treated with CHX at 8 h p.i. or untreated. Proteins were resolved with SDS-10% PAGE and stained with rabbit anti-TBP antiserum (SA3890). Protein size markers are given on the left. (d) To visualize the formation of polyhedra, AcMNPV-infected cells treated with CHX at 8 h p.i. or untreated are shown by phase-contrast images at 48 h p.i.

**Fig. 3.** TBP expression in TN-368 cells during AcMNPV infection. (a) Nuclear extracts were prepared from uninfected TN-368 cells (un) or from cells infected with either WT AcMNPV (10 p.f.u. cell\(^{-1}\)) or AcMNPV-CMV/GFP (10 p.f.u. cell\(^{-1}\)) at 4, 8, 16, 24, 48 and 72 h p.i. Proteins were resolved by SDS-12% PAGE and stained with rabbit anti-DBP or with rabbit anti-TBP (SA3890) antiserum. The corresponding amido black-stained membranes are shown below. (b) Nuclear extracts were prepared from uninfected TN-368 cells (un) or from cells infected with either WT AcMNPV (WT) or AcMNPV-CMV/GFP (Δ) at 6, 24 and 48 h p.i. Protein fractions of uninfected or WT-infected cells (6 h p.i.) were mixed with fractions of either WT-infected cells (48 h p.i.) or AcMNPV-CMV/GFP-infected cells (48 h p.i.) prior to SDS-10% PAGE. Staining was performed with rabbit anti-TBP antiserum (SA3890). The corresponding amido black-stained membrane is shown below. (c) Nuclear extracts were prepared from AcMNPV-infected TN-368 cells at 12, 24, 48 and 72 h p.i. treated with CHX at 8 h p.i. or untreated. Proteins were resolved with SDS-10% PAGE and stained with rabbit anti-TBP antiserum (SA3890). Protein size markers are given on the left. (d) To visualize the formation of polyhedra, AcMNPV-infected cells treated with CHX at 8 h p.i. or untreated are shown by phase-contrast images at 48 h p.i.

**Fig. 4.** Colocalization of IE2 and ubiquitin. TN-368 cells were infected with AcMNPV (10 p.f.u. cell\(^{-1}\)), and fixed at 4, 8, 16 and 24 h p.i. Cells were stained with rabbit anti-IE2 antiserum (green) and with mAb FK2 (red) against conjugated ubiquitin. Single-staining and merged images of confocal projections are shown. Bar, 9.5 μm.
frugiperda cells with CHX, TBP was still detected 72 h after CHX addition, suggesting a rather high protein stability (Fig. 6a). To further investigate protein stability, we performed pulse–chase experiments in uninfected S. frugiperda cells with [³⁵S]methionine and [³⁵S]cysteine, and determined the half-life of TBP in S. frugiperda cells. At 72 h post-labelling, 60% of the labelled TBP was degraded, indicating a TBP half-life of ~60 h (Fig. 6b). This was the first evidence that TBP represented a highly stable protein. As the high TBP stability was shown in uninfected cells and the TBP increase was already detected at 24 h p.i., a virus-induced impact on even higher protein stability during infection seemed unlikely.

Relocalization of TBP to viral replication sites

To explore the nuclear distribution of TBP in response to infection and to elucidate the underlying mechanism, we performed TBP colocalization studies. After visualizing the sites of viral DNA replication in infected TN-368 cells by 5-bromo-2′-deoxyuridine (BrdU) labelling, we observed colocalization of TBP and BrdU in small and enlarged sites. At 8 h p.i., small replication sites were visible in ~60% of the cells, whilst enlarged sites were detected in ~30% of the cells (Fig. 7a). Further enlargement of the viral replication sites correlated with increasing TBP domains until 24 h p.i. (Fig. 7a). These staining patterns are comparable with the co-staining of the viral DBP and the BrdU-labelled replication sites (Mainz et al., 2002; Quadt et al., 2007). At 48 h p.i., when most cells were already detached, nuclei of adherent cells were filled with polyhedra and showed a diffuse TBP staining (data not shown). To analyse whether the early relocalization of TBP depends on the formation of early replication sites, viral DNA replication was inhibited by treatment of TN-368 cells with aphidicolin. The inhibitor treatment led to a delayed formation of replication sites which coincided with a delayed relocalization of TBP to distinct nuclear domains (data not shown). These results suggested that TBP is not recruited to pre-existing nuclear sites, but that TBP redistribution depended on viral DNA replication. To further investigate whether TBP is recruited to prereplicative sites, we performed colocalization studies of IE2 and TBP. The early viral protein IE2 is localized in nuclear structures prior to viral replication and only partly colocalizes with the replication sites (Murges et al., 2001). Thus, we assume that IE2 initially marks prereplicative sites and later during infection is part of various functional domains in the nucleus that are related to viral DNA replication (Mainz et al., 2002). The co-staining of TBP and IE2 revealed that IE2 dots visible at 4 h p.i. preceded the recruitment of TBP to nuclear domains (Fig. 7b). At
8 h p.i., we observed cells with clusters of IE2 dots where TBP nuclear domains colocalized with the IE2 clusters; however, in cells with a uniform distribution of IE2 dots, no colocalization of IE2 and TBP was observed (Fig. 7b). As soon as IE2 dots form clusters, they colocalize with viral replication sites (Mainz et al., 2002). Thus, we concluded that TBP colocalized only with those IE2 dots that were part of the replication sites. These results strengthen the assumption that nuclear TBP domains assemble independently of IE2 recruitment to early viral replication sites. Furthermore, we assumed that the TBP redistribution to increasing nuclear domains depended on the formation and later enlargement of viral replication sites.

To address whether the expression of viral factors contributed to early TBP relocalization, we blocked protein synthesis with CHX. When TN-368 cells were treated with CHX at 1 h p.i., neither relocalization of TBP nor formation of replication sites was observed at 8 and 24 h p.i. (data not shown). This is in line with the assumption that the formation of TBP sites depends on the onset of viral DNA replication. After treatment with CHX at 4 h p.i., some cells showed TBP domains at 8 h p.i. which did not enlarge at 24 h p.i.; labelling of viral DNA synthesis with BrdU 1 h prior to fixation led to a very weak detection of replication sites at 8 h p.i. and these were not observed at 24 h p.i. as the inhibition of translation correlates with a block of viral DNA synthesis (Fig. 7c). Finally, when cells were treated with CHX at 7 h p.i., cells with enlarged TBP structures were detected at 8 h p.i. The TBP structures disappeared at 24 h p.i. and were replaced by a uniform TBP staining (Fig. 7c). These results indicated that the TBP relocalization and the maintenance of TBP domains correlated with viral DNA replication which in turn depended on viral protein synthesis.

As TBP is a component of the basal transcriptional machinery of the host cell, we hypothesized that TBP localized at sites of transcriptional activity. Thus, we analysed the localization of RNAPII during the course of infection. Whilst RNAPII staining was visible throughout the nucleus in uninfected cells, a redistribution of RNAPII to viral replication sites was observed at 8 h p.i. where RNAPII and TBP colocalized in early and enlarged replication sites (Fig. 7d). At 16 h, and even more obviously at 24 h p.i., RNAPII was again distributed uniformly in the nucleus and did not colocalize with TBP (Fig. 7d). The recruitment of RNAPII and TBP to early replication sites suggests that early viral transcription and viral DNA replication share common nuclear domains. During the late phases of infection RNAPII again localized throughout the nucleus, suggesting that the host RNAP no longer contributed to viral transcription and thus was displaced from the replication/transcription sites. As TBP was still present, we concluded that it also had a functional role at these nuclear sites late during infection.

**DISCUSSION**

In this study, we reinvestigated the expression level and the redistribution of TBP during AcMNPV infection as the antiserum SA3889 used previously cross-reacted with the viral DBP. After demonstrating the TBP specificity of the antiserum SA3890, we confirmed the increased level of TBP during infection, which was, however, less extensive than reported previously, most likely due to the loss of cross-reactivity with DBP (Quadt et al., 2002). In TN-368 cells, the elevated level of TBP in extracts isolated at 48 or 72 h p.i. was not initially detected since the hyperexpression of polyhedrin masked TBP.

As with most other host genes, TBP transcription is downregulated late in infection. Thus, the unexpected increase of the TBP level suggests virus-induced mechanisms that regulate TBP at the protein level. Interestingly, expression of TBP in higher eukaryotes is regulated tightly at the transcriptional level, whilst little is known about mechanisms that regulate TBP turnover and stability (Johnson et al., 2000; Harland et al., 2002). We found no evidence that v-UBI is involved in the protein turnover of TBP during infection, suggesting that the potential interference of v-UBI with the host degradation system does not contribute to increased TBP levels. Surprisingly, we found a rather high stability of TBP in uninfected cells, which makes it unlikely that viral functions affect TBP turnover in the
course of infection. Alternatively, AcMNPV infection might manipulate TBP translation to achieve augmented protein levels late in infection. In general, AcMNPV infection causes a shut-off of host protein synthesis, suggesting that the translational machinery is redirected from host protein synthesis to viral protein synthesis. Interestingly, expression of the SF21 translation initiation factors eIF4E and eIF5A is downregulated at the mRNA level during AcMNPV infection, although the virus relies on the host translation machinery to produce its viral proteins (van Oers et al., 1999, 2001). van Oers et al. (2001) suggest that very late baculovirus mRNAs are less dependent on eIF4E than cellular mRNAs. Thus, the question arises how AcMNPV achieves temporally regulated translation of viral and host proteins. When we blocked protein synthesis with CHX at 8 h p.i., we still observed an increased TBP level, whilst IE2 degradation was delayed (data not shown) as described previously (Ross & Guarino, 1997; Krappa et al., 1995). The presence of the still increased TBP level suggests that the potential upregulation of TBP translation occurred at the transition of early to late phases of infection (6–8 h p.i.) when the TBP transcript was still present.

When we investigated TBP relocalization during infection, we observed colocalization of TBP and BrdU in small replication sites. The localization studies with IE2, however, suggest that TBP was not redistributed to prereplicative sites, which we assume are marked by IE2 (Mainz et al., 2002). The block of protein synthesis strengthens the link between TBP recruitment and viral DNA replication as the loss of replication sites coincided with the lack of TBP redistribution. In contrast, TBP recruitment in herpes simplex virus (HSV)-infected cells appears to be independent of viral DNA replication. We speculated that the high density of TATA sequences on the parental HSV genomes attracts the binding of TBP and further transcription factors which results in the formation of prereplicative sites (Quadt et al., 2006).

During AcMNPV infection, TBP always colocalized with DNA replication sites. The TBP redistribution implies a virus-induced mechanism that leads to enhanced availability of TBP to optimize the infection cycle. One possibility might be a virus-induced interference with DNA-binding or protein–protein interactions that results in increased amounts of free intracellular TBP. Dimerization of TBP is suggested to prevent TBP degradation or auto-regulate its intrinsic DNA binding capacity (Geisberg & Struhl, 2000).

To gain insights into the functional significance of TBP, we performed colocalization experiments with RNAPII. The redistribution of RNAPII and TBP to early replication sites is in line with the assumption that early viral transcription takes place at these sites. In contrast, RNAPII disappeared from enlarged replication sites during the late phases of infection, which correlates with the onset of late transcription by the viral RNAP. LEF-4 is an essential component of the viral RNAP and expression of late structural genes is suppressed upon LEF-4 silencing (Knebel-Mörsdorf et al., 2006). Concomitant with the onset of TBP increase, expression of LEF-4 was detected. Colocalization studies of LEF-4 and TBP would be helpful to further characterize the sites of late and very late transcription; however, the LEF-4 antiserum (Guarino et al., 1998a) is not suitable for immunofluorescence studies. In contrast to AcMNPV infection, the recruitment of TBP to DNA replication sites in HSV-infected cells does not correlate with an increased level of TBP (Quadt et al., 2006). This strengthens the unique feature of baculoviruses to express a viral RNAP that is responsible for the burst of very late transcription. To support our assumption that TBP plays a role during late/very late transcription, we performed TBP silencing experiments. Even at 72 h post-transfection of TBP-specific dsRNA, only minor reduction of TBP was observed, which is in line with the high protein stability of TBP. These minor effects on the TBP level were not sufficient to influence late or very late viral expression (150 TBP and D. Knebel-Mörsdorf, unpublished). Taken together, our results confirm the increase of TBP during AcMNPV infection, and demonstrate the tight link between formation of viral DNA replication sites and the redistribution of TBP during infection. Next to the involvement in early viral transcription, we assume a functional role of TBP in late DNA replication sites and speculate that late/very late transcriptional activity takes place at these sites. It will be of major interest to further elucidate the mechanism of virus-induced upregulation of TBP at the protein level.

**METHODS**

**Cells, viruses, and infection.** *S. frugiperda* IPL-SF21 (Vaughn et al., 1977) and *Trichoplusia ni* TN-368 cells (Hink, 1970) were grown in TC100 medium (Gardiner & Stockdale, 1975) supplemented with 10% FBS. Infections were performed with AcMNPV plaque isolate E (Tija et al., 1979) as WT virus, and with the recombinant viruses AcMNPV-CMV/GFP (van Loo et al., 2001) (provided by Marcel Westenberg (NPO-NRC, The Netherlands)), v-UBI-FS (Reilly & Guarino, 1996) and v-UBI RGG. The recombinant virus v-UBI RGG was constructed using QuikChange site-directed mutagenesis on a plasmid containing the PstI-K fragment of AcMNPV to alter the terminal amino acid sequence of v-UBI from RGG to AAA. The plasmid containing the altered sequence was co-transfected with v-UBI-βgal virus (Reilly & Guarino, 1996) linearized previously with *Bsi36I*. Resulting white plaques were purified and screened by sequencing of PCR fragments to confirm correct insertion of the mutated v-UBI construct.

The time point when the virus inoculum was added to the cells was labelled 0 h p.i. DNA synthesis was visualized by adding 50 μM thymidine analogue BrdU (Sigma) 1 h prior to fixation with 4% paraformaldehyde. After permeabilization with 0.1% Triton X-100, DNA was denatured for 2 min in 0.07 M NaOH. Protein synthesis was blocked by addition of CHX (Sigma) (1 mg ml$^{-1}$).

**Transcriptional analysis.** Total RNA was purified from $2 \times 10^6$ uninfected *S. frugiperda* cells, and from cells at 4, 16, 24 and 48 h p.i. using TRizol reagent followed by a Qiagen RNeasy Mini kit cleanup with on-column DNase digestion. Total RNA (1 μg) was used for cDNA synthesis with Superscript II reverse transcriptase (Invitrogen) following the manufacturer’s protocol in the presence of oligo(dT) primers (20mers) and 285 rRNA reverse primers. Quantitative PCRs were performed using the SYBR GreenER qPCR SuperMix Universal (Invitrogen) on the DNAengine-Opticon 2 System (Bio-Rad).
Fig. 7. Colocalization studies of TBP with BrdU, IE2 and RNAPII in AcMNPV-infected TN-368 cells. Cells were infected with AcMNPV (10 p.f.u. cell−1) and fixed at 4, 8, 16 and 24 h p.i. (a) Uninfected and infected cells were stained with rabbit anti-TBP (SA3890) antiserum (green). To visualize DNA replication, BrdU incorporation was visualized with mouse mAb BrdU (red). Small and enlarged sites are shown at 8 h p.i. (b) Uninfected and infected cells were co-stained with rabbit anti-TBP (SA3890) antiserum (green) and mouse anti-E2 antiserum (red). (c) Infected cells were treated with CHX at 4 or 7 h p.i., fixed at 8 and 24 h p.i., and stained with rabbit anti-TBP (SA3890) antiserum (green). BrdU incorporation was visualized with mouse mAb BrdU (red). (d) Uninfected and infected cells were stained at 8, 16 and 24 h p.i., and co-stained with rabbit anti-TBP (SA3890) antiserum (green) and mouse mAb 7C2 against human RNAPII (red). Single-staining and merged images of confocal projections are shown. Bars, 9.5 μm.

ACKNOWLEDGEMENTS

We thank Marcel Westenberg, Marc Vigneron, Mirka Uhlirova, Ute Schepers, Keiju Okano, George F. Rohrmann and Robert Weinzirl for reagents and protocols. We are grateful to Tobias Steinfeldt for help with the pulse–chase experiments. Additional thanks go to Jürgen Dohmen, Mats Paulsson, Philipp Petermann, Markus Plomann and Renza Roncarati for helpful suggestions and critical comments on the manuscript. This research was supported by the Deutsche Forschungsgemeinschaft (grant KN536/11-1) and the Köln Fortune Program/Faculty of Medicine, University of Cologne.

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