This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.

Author(s): Ihalainen, Teemu; Willman, Sami; Niskanen, Einari; Paloheimo, Outi; Smolander, Hanna; Laurila, Juha; Kaikkonen, Minna; Vihinen-Ranta, Maija

Title: Distribution and dynamics of transcription-associated proteins during parvovirus infection.

Year: 2012

Version:

Please cite the original version:

All material supplied via JYX is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.
Distribution and Dynamics of Transcription-Associated Proteins during Parvovirus Infection

Teemu O. Ihalainen, a,b Sami F. Willman, a Einar A. Niskanen, a,c Outi Paloheimo, a Hanna Smolander, a,d Juha P. Laurila, a,e Minna U. Kaikkonen, f and Maija Vihinen-Ranta a

Nanoscience Center, Department of Biological and Environmental Science, University of Jyväskylä, Finland; a; Department of Health Sciences and Technology, Laboratory for Biologically Oriented Materials, ETH Zurich, Zurich, Switzerland; b; Department of Biochemistry, Medical Genetics Cluster, Erasmus University Medical Center, Rotterdam, The Netherlands; c; Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland; d; University of Turku, Turku, Finland; and e; AI Virtanen Institute, Department of Biotechnology and Molecular Medicine, University of Eastern Finland, Kuopio, Finland

Canine parvovirus (CPV) infection leads to reorganization of nuclear proteinaceous subcompartments. Our studies showed that virus infection causes a time-dependent increase in the amount of viral nonstructural protein NS1 mRNA. Fluorescence recovery after photobleaching showed that the recovery kinetics of nuclear transcription-associated proteins, TATA binding protein (TBP), transcription factor IIB (TFIIB), and poly(A) binding protein nuclear 1 (PABPN1) were different in infected and noninfected cells, pointing to virus-induced alterations in binding dynamics of these proteins.

In animals, several DNA viruses depend on host cell nuclear replication and transcription machinery (52). TATA binding protein (TBP) and transcription factor IIB (TFIIB) are key constituents of assembly of the host cell transcription initiation complex. Previous studies have shown that TBP interacts with viral transcription activators, including adenovirus EIA (21, 22, 32), hepatitis B virus pX and NS5A (38), herpes simplex virus 1 (HSV-1) VP16 (24, 34), human cytomegalovirus IE2 (23, 31, 49), and human immunodeficiency virus (HIV) Tat (30, 39, 48). The poly(A) binding protein nuclear 1 (PABPN1) accumulates to splicing speckles. It binds with high affinity to nascent poly(A) tails, thus stimulating their extension and controlling their length (27). Interaction of viral components with PABPN1 can lead to stimulated transcription (HSV-1 ICP27) (18, 19) or reduced host cell mRNA maturation and export (influenza A virus NS1) (9, 10). TAP and CRM1, essential export factors of nuclear mRNA, are also responsible for the nuclear export of HSV and influenza A virus mRNAs (8, 28, 40, 41). Moreover, promyelocytic leukemia (PML) nuclear bodies, involved in a wide variety of cellular processes, including regulation of transcription, interact with nuclear components of HSV-1 (35), polyomaviruses (29, 45), and parvoviruses (adeno-associated virus [AAV], minute virus of mice [MVM]) (20, 51).

Canine parvovirus (CPV) is a single-stranded DNA virus (46). Its nonstructural protein NS1 serves as an initiator and a helicase in viral DNA replication and as an activator of the viral promoters during diversion of the cellular machinery toward viral protein expression (11, 13, 36, 37).

We examined CPV infection-induced alterations in distrib-
tion and dynamics of nuclear proteins associated with the transcription (TBP, TFIIB, PML), processing (PABPN1), and nuclear export (TAP) of mRNAs.

Synthesis of cellular mRNA and total RNA in infected cells was spectrophotometrically quantified by the use of purified mRNA and by image analysis of SYBR green II-labeled cytosolic RNA (Molecular Probes, Eugene, OR). The amount of cellular mRNA of infected cells at 24 h postinfection (p.i.) was similar to that of noninfected cells ($n = 16$, $P > 0.05$; Fig. 1B). Simultaneously, the amount of cytoplasmic total RNA was decreased in infected cells ($n = 9$, $P < 0.01$; Fig. 1C). In contrast, reverse transcription (RT)-PCR studies showed that the emergence of NS1 mRNA at 4 h p.i. was followed by its significantly increased synthesis at 12 h p.i. (Fig. 1D).

Our previous studies indicated that in CPV infection, part of the enhanced cyan fluorescent protein (ECFP)-PML foci were situated in close proximity to or colocalized with the CPV NS1 protein in infected cells. Here, we examined the virus-induced change

**FIG 2** PML-EYFP binding and organization of PML bodies in nuclei. Qualitative FRAP experiments were performed in cells stably expressing H2B-EYFP and transiently expressing PML(IV)-EYFP. (A) Distributions of PML bodies [PML(IV)-EYFP, yellow] and chromatin [H2B-ECFP, cyan] in living noninfected and infected cells at 24 h p.i. (B) FRAP of PML(IV)-EYFP (yellow) in noninfected cells. Chromatin is visualized with H2B-ECFP (cyan). The red box denotes the bleach area of the FRAP experiment. Bars, 5 μm. (C) PML(IV)-EYFP fluorescence recovery in infected (red) and noninfected (black) cells. Error bars indicate standard deviations. The FRAP bleaching and imaging parameters were as follows: rectangular region bleaching of interest (ROI), 1.28 μm by 24.4 μm; bleaching iterations, 10; frame interval, 1,000 ms.

**FIG 3** TBP-EGFP and TFIIB-EGFP dynamics are altered in virus infection. The results of confocal imaging and quantitative FRAP studies in cells stably expressing TBP-EGFP or TFIIB-EGFP fusion proteins are shown. (A and D) Live cell images of (A) TBP-EGFP and (D) TFIIB-EGFP distribution in the nuclei of noninfected and infected cells at 24 h p.i. Bars, 5 μm. (B) FRAP curves of TBP-EGFP in infected (red) and noninfected (black) cells. (E) FRAP curves of TFIIB-EGFP in infected (red) and noninfected (black) cells and EGFP in noninfected (blue) cells. (C, D, and F) TBP-EGFP and TFIIB-EGFP fluorescence recovery (green) data from noninfected and infected cells fitted into the FRAP model (blue). Errors bars indicate standard deviations. The FRAP bleaching and imaging parameters for TBP-EGFP/TFIIB-EGFP were as follows: circular ROIs with a radius of 1.34 ± 0.2 and 0.76 ± 0.15 μm; iterations, 10/8; frame intervals, 1,000/65 ms.
in the distribution and dynamics of PML(IV)-EYFP (42). In the noninfected cells, the nuclear PML(IV)-EYFP foci were distributed evenly, whereas in infected cells, they were confined to the nuclear or nucleolar periphery (Fig. 2A). The times of fluorescence recovery after photobleaching (FRAP) in infected and noninfected cells were almost identical, suggesting that the PML(IV)-EYFP binding properties were not affected at late stages of infection (Fig. 2B and C). Detailed descriptions of the FRAP protocol employed in this study are provided by Ihalainen et al. (25).

The effect of infection on nuclear dynamics of TBP and TFIIB was studied by FRAP analysis. In the nuclei of noninfected cells, TBP-enhanced green fluorescent protein (EGFP) and TFIIB-EGFP (7) were distributed relatively homogeneously, with some nucleolar enrichment of TFIIB-EGFP. At 24 h p.i., both proteins accumulated in the replication body area (Fig. 3A, B, and D). The replication body area contains viral NS1 and cellular proliferating cell nuclear antigen (PCNA). This virus-induced nuclear structure covers the entire nucleus at 24 h p.i., with the exclusion of the nucleolus (14, 25, 26, 50). However, TBP-EGFP and TFIIB-EGFP showed different FRAP behavior characteristics. The recovery of TBP-EGFP was slower (Fig. 3B) than that of freely diffusing EGFP or TFIIB-EGFP (Fig. 3E). The diffusion coefficients of TBP-EGFP and TFIIB-EGFP were calculated using simple mass scaling of the EGFP diffusion coefficient. The TBP-EGFP free diffusion times in

FIG 4 PABPN1-EGFP binding is increased and TAP-EGFP binding is unaltered in infection. Cells transiently expressing PABN1-EGFP or TAP-EGFP fusion proteins were examined using confocal imaging and FRAP analysis. (A and D) Confocal images of (A) PABN1-EGFP and (D) TAP-EGFP distribution in living noninfected and infected cells at 24 h p.i. Photobleached areas are denoted by red boxes and circles. (B) PABPN1-EGFP recoveries in noninfected cells. Levels of measured (black) and simulated (green) PABPN1-EGFP FRAP in noninfected cells are shown in comparison to those of free EGFP (blue). (C) PABPN1-EGFP recoveries in infected cells. Simulated slow diffusion of mRNA (blue) and higher mRNA binding on-rate (green) compared to PABPN1-EGFP recovery (red) in infected cells are shown. (E) Fluorescence recovery of TAP-EGFP in noninfected (black) and infected (red) cells. The recovery (green) in the noninfected cell was fitted into the free diffusion model, yielding a good fit (blue). Error bars indicate standard deviations. FRAP bleaching and imaging parameters for PABPN1-EGFP/TAP-EGFP were as follows: circular ROIs with a radius of 0.7 ± 0.3 and 1.34 ± 0.2 μm; iterations, 8/10; frame intervals, 250/200 ms. (G) PABN1-EGFP distribution in various noninfected and infected cells at 24 h p.i. Bars, 5 μm.
noninfected and infected cells were 442 s and 147 s and the binding times 167 s and 58.8 s, respectively (Fig. 3C). The TFIIB-EGFP free diffusion times in noninfected and infected cells were 4.5 s and 3.0 s and the binding times 0.56 s and 0.63 s, respectively (Fig. 3F). These data indicate that TBP and TFIIB bind more frequently and are released faster in infected than noninfected cells.

Next, FRAP experiments were performed in PABPN1-EGFP (3)- or TAP-GFP (4)-expressing cells with or without infection. In noninfected cells, PABPN1-EGFP-containing nuclear speckles were distributed randomly, whereas in infected cells at 24 h p.i., they localized close to the nuclear membrane (Fig. 4A and G). TAP-EGFP, distributed homogeneously in nuclei of noninfected cells, localized in infected cells at the viral replication compartment (Fig. 4D). The FRAP of PABPN1-EGFP was slower in infected than noninfected cells (Fig. 4B and C), suggesting either slow diffusion or more-frequent binding to mRNA poly(A) tails. In Virtual Cell simulations, the theoretical diffusion constants of PABPN1-EGFP and mRNA were set at 22 and 0.04 μm²/s, with partial binding of PABN1-EGFP to immobile speckle domains. The best fit yielded PABPN1-EGFP free diffusion, mRNA binding, and speckle binding times of 0.1, 2.5, and 50 s, respectively. The fit of PABPN1-EGFP recovery simulation in infected cells was good, with 10-times-higher mRNA binding on-rate and adjusted speckle binding parameters (Fig. 4C). In this case, the free diffusion time for PABPN1-EGFP, mRNA binding time, and speckle binding time were 0.01, 2.5, and 1,000 s, respectively. These models suggest that, during infection, PABPN1-EGFP diffuses freely for only a short time and binds to mRNA or speckle domains faster (10 times higher on-rate) and more strongly.

Interestingly, the rates of TAP-GFP fluorescence recovery were identical in noninfected and infected cells (Fig. 4E). The recovery fitted to the free diffusion model, yielding a diffusion coefficient of 2.2 ± 0.3 μm² s⁻¹. Mass scaling of the EGFP diffusion coefficient suggested that TAP-EGFP showed effective diffusion behavior (43), with 83% of TAP-EGFP bound and the rest freely diffusing (Fig. 4F).

Our previous studies demonstrated accumulation of CPV NS1 and viral DNA into the nuclear replication body and marginalization of host cell chromatin to the nuclear periphery (24, 25). Here we observed the time-dependent increase in NS1 mRNA production. At the same time, the amount of cellular mRNA was unaltered and that of cytoplasmic nascent RNA decreased. These findings imply that viral gene expression and virus-induced chromatin marginalization do affect cellular RNA dynamics.

In this study, photobleaching techniques were used to assess interactions between nuclear transcription-related proteins and viral components. FRAP studies exploring the dynamics of TBP, a protein essential for transcription initiation (2), demonstrated faster binding and release in infected than in noninfected cells. TBP interacts with TBP-associated factors to form the TFIID complex, which binds to chromosomal histone H3 (47). At late stages of infection, histone H3 is concentrated at the nuclear periphery, which could explain the weaker binding and the decreased binding time of TBP in the viral replication body area (E. A. Niskanen, O. Kalliolinna, T. O. Ihalainen, M. Vuokko, and M. Vihinen-Ranta, et al., unpublished data). The shorter free diffusion time, in turn, may be due to the relative increase in TBP binding sites in the replication body area. In parallel, infection did not affect PML recovery kinetics. These results suggested that, in contrast to some other viruses (1, 16, 17), PML bodies are not affected in CPV infection.

As in previous studies (6), TFIIB, also required in transcription initiation, showed shorter binding times than TBP in noninfected cells. The measured binding time reflects the time of preinitiation complex binding with TFIIB and polymerase II association with the promoter (15). In infected cells, TFIIB accumulated into the replication body area and the binding time was similar to that in control cells, although the association rate was higher. The amount of TFIIB-EGFP was not affected by infection (our unpublished result), and the faster association indicates that transcription initiations are more frequent in infected cells. Based on these data, together with our previous studies, we propose a model for CPV infection progression in which the replication body grows and fills the nucleus. In parallel, the host cell chromatin is marginalized along with PML bodies and speckle domains. Inside the replication body, transcription is initiated more often than in noninfected cells, leading to a accumulation of the viral NS1 mRNA (Fig. 5).

In infected cells at 24 h p.i., PABPN1-EGFP localized to the splicing speckle domains (4, 5) and diffusively to the replication body area. FRAP experiments with virtual cell simulations in infected cells pointed to faster mRNA binding, however, with a longer residence time in the speckle domains.

TAP, an mRNA export protein interacting with nucleoporins and polyadenylated mRNA (12, 44), accumulated in the viral repl-
lication body area. The FRAP of TAP in noninfected cells was similar to that in infected cells. The diffusion coefficient of 2.2 ± 0.3 μm²/s of TAP (70 kDa) (33) was 6.5 times too small to correspond to free diffusion. However, the recovery data fitted well with the free diffusion model and collectively imply that the binding/unbinding reactions are extremely fast and that the recovery resembles that seen with slow diffusion. It previously has been reported that inside the nucleus, TAP diffused freely, with a diffusion coefficient of 1.2 ± 0.07 μm²/s (5). Together, these results suggest that binding of TAP to mRNA is transient.

ACKNOWLEDGMENTS

We thank Colin Parrish for the infectious CPV clone and antibodies, R. Everett for PML(IV)-EYFP, J. Langowski for H2B-ECFP, S. Huang for TBP-EGFP, M. Carmo-Fonseca for PABN1-EGFP and TAP-EGFP constructs, and Klaus Hedman for comments on the manuscript. The first-class experimental support of Milla Häkkinnen, Jenni Reimari, and Irene Helkala is gratefully acknowledged.

The work was supported by grants from the Academy of Finland (Contracts 129774 and 138388), National Graduate School in NanoScience, and National Graduate School in Informational and Structural Biology.

REFERENCES


52. Zubierowski SE, Deluca NA. 2008. Stabilized binding of TBP to the TATA box of herpes simplex virus type 1 early (tk) and late (gC) promoters by TFIIA and ICP4. J. Virol. 82:3546–3554.