Host Cell Nuclear Proteins Are Recruited to Cytoplasmic Vaccinia Virus Replication Complexes

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Received 13 October 2004/Accepted 1 August 2005

Vaccinia virus is a member of the poxvirus family, and accordingly has a very large DNA genome that is replicated and assembled into progeny solely within the cytoplasm of the cell (reviewed in reference 12). Poxviruses are unique among DNA genome viruses because their genome never crosses the nuclear membrane where nucleic acid synthesis otherwise occurs. Nucleic acid biosynthesis in the cytoplasm of the cell has long encouraged the concept that the virus likely encodes all of the proteins required for DNA and mRNA synthesis since virus replication complexes may not have access to proteins normally targeted to the nucleus. Indeed, there is no evidence for participation of host proteins in vaccinia virus DNA replication or early gene transcription. All proteins implicated in these processes to date are virus encoded. In addition, most proteins known to be required for intermediate and late gene transcription are virus encoded (reviewed in reference 2). Three different viral transcriptional activator proteins, including the viral capping enzyme, the E4L protein, and VITF-3, are required for intermediate gene transcription initiation. The viral A1L, A2L, and G8R gene products are essential for late transcription. The vaccinia virus A18R, G2R, and J3R proteins have been implicated in intermediate and late gene transcription elongation and termination (4). Nevertheless, there is also evidence for participation of host cell proteins in vaccinia virus transcription. Reconstitution of intermediate transcription in vitro was reported to require an unidentified protein, called VITF-2 (13). This activity was detected in the cytoplasmic fraction of virus-infected cells but was predominantly in the nuclear fraction of uninfected cells. In addition, the heterogeneous nuclear ribonucleoproteins A2/B1 and RBM3 were shown to activate transcription from a late promoter in vitro (18). Finally, intermediate and late transcription termination and transcript release in an in vitro system were shown to require a cellular protein fraction (9). Whether the termination factor is a nuclear protein has not been reported.

Two nuclear proteins have been reported to be localized in the cytoplasm of vaccinia virus-infected cells. RNA polymerase II (PolII) was reported to be located in the cytoplasm (11, 16) in a manner that was blocked by inhibitors of viral DNA synthesis (16). Also, the nuclear transcription factor YY1 was reported to be localized with viral replication complexes in the cytoplasm (3). Taken together, these observations suggest that targeting of nuclear proteins may be altered by vaccinia virus infection.

The import and export of proteins to and from the nucleus occurs through the nuclear pore complex (reviewed in reference 15). Proteins smaller than 40 to 50 kDa diffuse passively through the pore. Larger proteins require active transport processes that involve peptide signal sequences in the transported proteins that are specific for different transport pathways. Ran proteins serve as molecular escorts for the transported protein on both sides of the complex and are regenerated by a GDP-GTP exchange reaction. At the nuclear pore, the transported protein-Ran complexes dock with importin and exportin proteins that impart specificity to the transport process. While the specific details of gating at the nuclear pore are not clear, a group of proteins referred to as nucleoporins are believed to participate in gating of transported proteins.

In this study, the effect of vaccinia virus infection on the intracellular targeting of several nuclear proteins was determined. Six different nuclear proteins with diverse functions were shown to be located in the cytoplasm of vaccinia virus-
infected cells. These results suggest that vaccinia virus can recruit nuclear proteins to replication complexes for use in viral processes.

MATERIALS AND METHODS

Cells, viruses, plasmids, and DNA transfections. All experiments performed here utilized BSC40 African green monkey kidney cells. Cells were plated at less than 50% confluence and routinely infected with 10 PFU/cell of vaccinia virus Western Reserve strain. Infection proceeded in a volume of Dulbecco modified essential medium supplemented with 10% fetal calf serum sufficient to just cover the cell monolayer for 1 h, after which threefold more medium was added. YY1 full-length cDNA and deletion constructs were from Bernhard Lüscher, Medizinische Hochschule Hannover, Germany. Where indicated, YY1 and its variants were expressed from a cytomegalovirus (CMV) promoter with an influenza virus hemagglutinin (HA) tag at the proteins’ amino termini. The red fluorescent protein (RFP)-YY1 fusion protein was expressed from a plasmid designed to fuse RFP to the amino terminus of YY1. The RFP gene was from plasmid pDsRed2-C1 (Clontech). For expression in bacteria, the YY1 gene was inserted into plasmid pET-28a (Novagen), and the protein was purified by nickel-agarose (QIAGEN) chromatography. Plasmid pXRGG, encoding the nucleocytoplasmic shuttling protein, was from John Hanover, National Institutes of Health.

Plasmid DNA was purified on QIAGEN columns. Transfections were performed with 4 μg of DNA using Lipofectamine transfection reagent (Invitrogen Life Technologies) according to the manufacturer’s instructions.

Antibodies. Primary antibodies used in this study were directed against the whole molecule of YY1, the N-terminal 12 amino acids of TATA binding protein (TBP), an internal peptide of SP1, an internal peptide of histone deacetylase 8 (HDAC8), the N-terminal 16 amino acids of TAF II p38, the C-terminal 18 amino acids of the largest subunit of PolII, and the whole lamin B protein (all from Santa Cruz Biotechnology).

Indirect, direct, and confocal immunofluorescence. Cells grown on coverslips were washed with phosphate-buffered saline (PBS; 0.15 M NaCl, 15 mM NaPO₄, pH 7.4) and fixed with 4% paraformaldehyde for 10 min. Cells were then
permeabilized with 0.5% Triton X-100 in PBS for 20 min and blocked with 4% bovine serum albumin in PBS (BSA-PBS) for 30 min. Primary antibody was diluted in 200 μl BSA-PBS and overlaid onto coverslips for 1 h in a humidified container. Optimal primary antibody dilutions were determined to be those which stained the nuclei of uninfected cells with minimal staining of the cytoplasm. Coverslips were washed three times in PBS and overlaid with fluorescein 5(6)-isothiocyanate (FITC)-conjugated second antibody for 40 min. Second antibody dilutions were determined by lack of significant staining of virus-infected cells. Some secondary antibodies required blocking with serum from the animal species from which the antibody was derived. Second antibodies were from Covance and Santa Cruz Biotechnology. Coverslips were washed again three times in PBS and stained for 1 min with 4',6-diamidino-2-phenylindole (DAPI) and washed briefly in PBS before mounting on a slide with Vectashield mounting medium (Vector Laboratories, Burlingame, CA). Fluorescence microscopy was performed on an Olympus FX60 microscope.

Red and green fluorescent protein expression vectors were transfected into cells as described above. At the indicated times, cells were washed with PBS and fixed with paraformaldehyde as described above for immunofluorescence prior to fluorescence microscopy.

Confocal microscope images were acquired using an MRC-1024 confocal laser scanning microscope (Bio-Rad, Hemel Hempstead, England) on a Diaphot 300 (Nikon, Tokyo, Japan) inverted microscope using a 40×1.4 numerical-aperture lens. The 488-nm line of the krypton-argon laser (Ion Laser Technology, Salt Lake City, Utah) was used to excite the FITC, and the emission was collected using a 522/35-nm band-pass filter. The 363-nm line of the argon ion laser (Coherent, Santa Clara, CA) was used to excite the DAPI, and the emission was collected with a 455/30-nm band-pass filter. A transmission image using the 488-nm line was also collected.

Nucleocytoplasmic shuttling assays. BSC40 cells were transfected with plasmid pXRGG (10). After 40 h, cells were treated with 5 μM dexamethasone (Dex; Sigma) or infected with vaccinia virus for 2 h and then treated with Dex. To reverse the nuclear location of the protein, cells were washed three times with medium lacking serum and incubated for 2 h in the same medium. Cells were harvested at the indicated points in the procedure and treated with 4% paraformaldehyde. Green fluorescence was followed by microscopy.

Subcellular fractionation and immunoblotting. BSC40 (2 × 10⁶) cells were transfected with RFP-YY1 fusion protein was expressed from a CMV promoter for 24 h prior to infection with vaccinia virus. Cells were fixed with paraformaldehyde, stained with DAPI, and visualized by fluorescence microscopy. Red fluorescence (RFP) and DAPI fluorescence are merged in the third column. Phase indicates phase-contrast images. Rows of pictures are uninfected cells (Un) and cells infected for 1 and 4 h.

FIG. 2. Effect of vaccinia virus infection on localization of a RFP-YY1 fusion protein. The RFP-YY1 fusion protein was expressed from a CMV promoter for 24 h prior to infection with vaccinia virus. Cells were fixed with paraformaldehyde, stained with DAPI, and visualized by fluorescence microscopy. Red fluorescence (RFP) and DAPI fluorescence are merged in the third column. Phase indicates phase-contrast images. Rows of pictures are uninfected cells (Un) and cells infected for 1 and 4 h.

FIG. 3. Effect of macromolecular synthesis inhibitor on the localization of RFP-YY1 in vaccinia virus-infected cells. The RFP-YY1 fusion protein was expressed in BSC40 cells which were infected with vaccinia virus in the absence (Inf) or presence of HU (Inf + HU) for 3 h. At that time, HU-containing medium was replace with medium containing cycloheximide and incubated another 2 h (Inf + HU + CH). Uninf, uninfected cells. Pictures depict red fluorescence (RFP) and DNA (DAPI), and Merged is the overlapping FITC and DAPI fluorescence.
proteins, and the cytoplasmic fraction was subjected to centrifugation at 10,000 × g for 5 min to pellet residual nuclei. Protein concentrations were determined with the Bradford assay. Equal masses of total protein from the cytoplasmic fractions in uninfected versus virus-infected cell extracts as well as equal masses of nuclear proteins from uninfected and virus-infected cell extracts were loaded onto a sodium dodecyl sulfate-polyacrylamide gel and subjected to electrophoresis. The contents of the gel were transferred to nitrocellulose electrophoretically and blocked with 5% nonfat dry milk and probed with primary antibody, followed by secondary antibody conjugated to horseradish peroxidase. Proteins were visualized with ECL (Amersham) chemiluminescent detection on X-ray film.

RESULTS

Localization of nuclear transcription factors in vaccinia virus-infected cells. The nuclear transcription factor YY1 was previously described as accumulating in the cytoplasm of vaccinia virus-infected cells (3). In order to characterize the behavior of YY1 in more detail, a time course experiment was performed as the virus progressed through the infectious cycle. As described previously (1, 3), immunofluorescence microscopy using anti-YY1 antibody and FITC-conjugated secondary antibody showed that YY1 was predominantly located in the nuclei of uninfected BSC40 cells (Fig. 1). Evidence for viral
DNA replication in the cytoplasm, characterized by DAPI staining, could be observed as early as 1 h postinfection. No cytoplasmic FITC or DAPI staining was identifiable prior to this time (data not shown). As the infection proceeded over subsequent hours, the size of the cytoplasmic structures staining with DAPI grew larger. Staining these same cells with anti-YY1 antibody showed that the antibody staining and DAPI staining were localized to the same regions of the cytoplasm. While some YY1 was identifiable in the cytoplasm of infected cells, the majority of antibody staining remained nuclear. This conclusion was confirmed by immunoblotting of nuclear and cytoplasmic subcellular fractions in which most of the YY1 remained in the nuclear fraction of virus-infected cells (see below).

Other nuclear transcription factors were examined for their response to vaccinia virus infection. TBP was chosen for its preference for A-T rich sequences in DNA because the vaccinia virus genome is 66% A-T in base composition and contains hundreds of potential TBP binding sites. As an alternative factor, SP1 was selected because there are only two of its high-affinity recognition sequences (GGGCGG) in the vaccinia virus genome. Immunofluorescence microscopy was performed using antibodies directed against TBP and SP1. Both proteins were localized in the nuclei of uninfected cells (Fig. 1). Both proteins also colocalized with virus replication complexes identifiable by DNA staining in the cytoplasm of virus-infected cells in a manner similar to what was observed with YY1. Therefore, colocalization of nuclear transcription factors with vaccinia virus replication complexes is not unique to YY1.

The localization of YY1 was addressed through an alternative approach by constructing a fusion of RFP to the amino terminus of YY1 that was expressed from a nuclear CMV promoter. Electrophoretic gel shift experiments showed that the fusion protein was capable of binding DNA with a canonical YY1 binding site (data not shown). BSC40 cells were transfected with the fusion protein expression construct for 24 h, and red fluorescence was followed as a function of time after infection by vaccinia virus. The RFP was located almost exclusively in the nuclei of uninfected cells (Fig. 2). After 1 to 4 h of infection with vaccinia virus, red fluorescence was observed at perinuclear locations in the cytoplasm of the cell. The fluorescent structures colocalized with DAPI staining in the cytoplasm. The similarity of results obtained with antibody staining of YY1 and the RFP fusion protein show that the cytoplasmic location of YY1 is not an antibody-dependent observation.
that cytoplasmic targeting of nuclear transcription factors requires viral DNA synthesis and not postreplicative protein synthesis.

A possible means for colocalization of nuclear transcription factors with viral replication complexes is binding to the viral genome. As a test of this notion, HA-tagged YY1 deletion mutants were analyzed for their ability to colocalize with the viral replication complexes. YY1 is a modular-structured zinc finger transcription factor of 414 amino acids in length (1). The four zinc fingers are located at the protein’s carboxy terminus in amino acids 295 to 414. The isolated zinc finger domain was shown previously to interact with the YY1 recognition sequence in DNA with affinity comparable to the intact protein (8). While the nuclear localization signal in YY1 has not been identified precisely, deletion of zinc fingers 2 and 3 was shown previously to result in cytoplasmatic location of the protein, while deletion of fingers 1 and 4 did not alter the nuclear location of the protein (1). HA-tagged YY1 deletion mutants were expressed in BSC40 cells from the nuclear CMV promoter and infected with vaccinia virus. The mutant with amino acids 2 to 273 deleted, preserving only the zinc finger domain, colocalized with viral replication complexes in the cytoplasm, similar to what was seen with the full-length protein (Fig. 4). Mutants with either zinc finger 1 (A296-331) or finger 4 (A399-414) deleted, by contrast, remained in the nucleus during the course of infection. Impairment of DNA binding to a YY1 consensus site by the mutants with zinc fingers 1 and 4 deleted has been confirmed by electrophoretic DNA band shift analysis (data not shown). Based on these results, it was concluded that only the DNA binding domain of YY1 was required to target it to the virus replication complex in the cytoplasm.

**Localization of other nuclear proteins during vaccinia virus infection.** The identification of nuclear transcription factors in the cytoplasm of vaccinia virus-infected cells raised the issue of whether the phenomenon was specific for transcription factors or if it was affecting nuclear proteins in general. Other nuclear non-transcription factor proteins were monitored for their behavior following virus infection. As representative nuclear proteins, PolIII, TFIID component TAFIIP32, and HDAC8 were monitored for their intracellular location during the course of vaccinia virus infection. All three proteins were confirmed as being located in the nucleus in uninfected BSC40 cells (Fig. 5). The distribution of these three proteins in vaccinia virus-infected cells, however, differed from what was observed for transcription factors. PolIII, TAFIIP32, and HDAC8 were found to be dispersed throughout the cytoplasm 5 h after infection by vaccinia virus and not localized to virus replication complexes or any other specific structure in the cell.

The localization of nuclear proteins after vaccinia virus infection was characterized in greater detail by confocal immunofluorescence microscopy. SP1 and PolIII were chosen as proteins representative of those that colocalized with vaccinia virus replication complexes and those that appeared dispersed by viral infection as determined by conventional fluorescence microscopy, respectively. Lamin B was chosen as a control because it is a structural protein associated with the inner nuclear membrane. Staining of SP1 showed colocalization with viral replication complexes as observed before (Fig. 6). Staining of PolIII and observation by confocal microscopy resulted in a different pattern, however. Rather than a uniformly di-

![Subcellular fractionation and immunoblotting of nuclear proteins from uninfected and vaccinia virus-infected cells.](http://jvi.asm.org/)

To address the mechanism of relocation of nuclear transcription factors to cytoplasmic viral replication complexes, the stage to which the virus infectious process must progress in order to observe the relocation was determined. The RFP-YY1 fusion protein was expressed prior to infection with vaccinia virus, and inhibitors of viral DNA and protein synthesis were used as blocks at various stages of virus development. Infection in the presence of the DNA synthesis inhibitor hydroxyurea (HU) blocked the appearance of DAPI staining in the cytoplasm (Fig. 3), as expected. No red fluorescence in the cytoplasm was observed either. Inhibition of DNA synthesis was confirmed by slot blot hybridization with a viral DNA probe (data not shown). This result indicates that DNA synthesis or postreplicative protein synthesis is a requirement for the cytoplasmic localization of the protein. To discriminate between these two alternatives, the following experiment was performed. Cells were infected for 3 h in the presence of HU to block DNA replication and postreplicative protein synthesis. The medium containing HU subsequently was removed and replaced with medium containing cycloheximide to allow DNA synthesis to begin in the absence of postreplicative protein synthesis. Following this regimen, DNA was observed to accumulate in the cytoplasm, and red fluorescence indicative of the location of the YY1-RFP fusion protein was observed to colocalize with replicated DNA in the cytoplasm. Identical results were observed with native YY1 in indirect immunofluorescence experiments (data not shown). These results indicate
persed pattern of staining, the fluorescent stain appeared to colocalize with DNA staining in the cytoplasm in addition to nuclear staining. Thus, PolII appears also to localize with viral replication complexes in the cytoplasm. The difference in the pattern observed here for PolII staining in infected cells compared to conventional microscopy shown in Fig. 5 is attributed to the superior optics and resolution of the confocal instrument. Lamin B staining was primarily a perinuclear pattern in both infected and uninfected cells, consistent with its location on the nuclear membrane.

The intracellular localization of the proteins described above was confirmed by subcellular fractionation and analysis by immunoblotting with the same antibody used for immunofluorescence. Infected and uninfected cells were lysed with the detergent Nonidet P-40, and nuclei were pelleted by centrifugation (5). Lamin B was used as a nuclear protein marker, and heat shock protein Hsp27 served as a cytoplasmic marker protein. After subcellular fractionation, the nuclear fraction was highly enriched for lamin B, and the cytoplasmic fraction contained the majority of Hsp27 (Fig. 7). TBP, YY1, and HDAC8 were tested for their subcellular localization before and after vaccinia virus infection. TBP appeared to be slightly enhanced in the cytoplasm of virus-infected cells relative to that of uninfected cells; however, no difference in subcellular distribution was noted for YY1. The level of HDAC8 in the nuclei of infected cells appeared lower relative to nuclei from uninfected cells, but no corresponding increases in the cytoplasmic levels of the protein were observed. It appeared that HDAC8 levels declined, suggesting that this protein may have a short half-life and would decay after virus-induced host protein shut-off. Thus, for all three nuclear proteins, most of the protein was found in the nuclear fraction in both uninfected and infected cells, and their distributions did not appear to shift significantly as a result of vaccinia virus infection.

**Nucleocytoplasmic transport in vaccinia virus-infected cells.** The redistribution of nuclear proteins in vaccinia virus-infected cells suggested that nuclear import or export pathways may be altered. A synthetic fusion protein consisting of the human immunodeficiency virus type 1 Rev protein and the ligand binding domain of the glucocorticoid receptor linked to green fluorescent protein (GFP) was used to monitor nucleocytoplasmic transport (10). This protein, referred to as XRGG, has nuclear import signals in both the Rev and glucocorticoid receptor domains. The Rev domain also has a classical leucine-
rich nuclear export signal peptide that mediates nuclear export through the Crm1-dependent pathway (6). The localization of the protein is monitored by green fluorescence microscopy. The fusion protein normally resides in the cytoplasm of the cell. Upon treatment of cells with Dex, the protein translocates to the nucleolus, where the Rev protein normally resides in human immunodeficiency virus type 1-infected cells. Removal of Dex returns the protein to the cytoplasm. The nucleocytoplasmic shuttling of the XRGG protein in vaccinia virus-infected cells was compared to that of uninfected cells. Cytoplasmic fluorescence was observed initially in both uninfected and infected cells (Fig. 8). Treatment of cells with 5 μM Dex for 2 h resulted in translocation of the XRGG protein to the nucleoli within the nuclei in infected cells as well as uninfected cells. Thus, nuclear import appeared not to be significantly impaired in vaccinia virus-infected cells. Removal of the Dex-containing medium followed by washing and replacement with fresh medium for 2 h resulted in a shift of fluorescence from the nucleoli to the cytoplasm in both uninfected and vaccinia virus-infected cells. It was concluded that nuclear export mediated by Crm1 was also functional in vaccinia virus-infected cells. Nuclear export of XRGG was blocked by 5 nM leptomycin B, an inhibitor of Crm1 (17), in vaccinia virus-infected cells, demonstrating that the leptomycin B was active. Based on these results, it was concluded that vaccinia virus infection does not impede nuclear import or export.

Effect of nuclear transport inhibitors on localization of nuclear proteins in vaccinia virus-infected cells. The localization of nuclear proteins in the cytoplasm of vaccinia virus-infected cells could be the result of effects on nucleocytoplasmic transport. The contribution of nuclear export to this phenomenon was tested by inhibition of export with leptomycin B. This antibiotic targets the Crm1 component of the nuclear export pathway (17). It was observed that 5 nM leptomycin B had no effect on the colocalization of YY1 with cytoplasmic vaccinia virus replication complexes (Fig. 9). The drug thapsigargin induces release of endoplasmic calcium stores, resulting in inhibition of nuclear import (7). Thapsigargin had no discernible effect on localization of YY1 in vaccinia virus-infected cells.

Fate of nucleoporins in vaccinia virus-infected cells. The properties of vaccinia virus-infected cells suggest that the barriers at the nuclear pore may somehow be compromised as a result of virus infection. The mechanisms and proteins that are responsible for protein gating at the nuclear pore have not been well characterized; however, nucleoporin proteins are believed to have a role in the gating mechanism. Immunoblot analysis of vaccinia virus-infected cells did not reveal any significant differences in the levels of nucleoporins Nup98 or Nup62 (data not shown).

DISCUSSION

The results presented here indicate that a wide range of nuclear proteins can colocalize with vaccinia virus replication complexes in the cytoplasm of the infected cell. While a limited number of nuclear proteins was tested here, only lamin B was identified as resistant to colocalization with virus replication complexes. Lamin B is a structural protein that is anchored on the inner surface of the nuclear membrane by prenyl modification. The proteins examined here were not detected in the cytoplasm when viral DNA replication was blocked, and the colocalization of YY1 with replication complexes required only its DNA binding domain. The ability to bind to viral DNA in the cytoplasm may be a requirement for nuclear transcription factors to move to those sites. High-affinity DNA binding may not be a requirement, however, because SP1 relocated to virus replication complexes, and only two SP1 binding sites exist in the A-T rich vaccinia virus genome. All proteins that target specific sequences in DNA also have low affinity for DNA of any sequence. It is noted that much of each of the nuclear transcription factors examined here remained in the nucleus following infection with vaccinia virus. Perhaps these proteins are anchored on DNA in chromatin such that high levels of DNA in the cell cytoplasm are required to colocalize the proteins on viral replication complexes in the cytoplasm.

Results were presented here showing a lack of effect of the antibiotic leptomycin B on cytoplasmic location of YY1 in vaccinia virus-infected cells. This result contradicts those of Slezak et al. who reported that leptomycin B blocked the cytoplasmic location of YY1 in vaccinia virus-infected cells (14). The antibiotic, however, was shown here to block the nucleolar-to-cytoplasmic transport of the XRGG protein.
known to require Crm1-mediated export from the nucleus. The reasons for the discrepancy are not clear.

The results presented here indicate that nuclear import and export are not dramatically affected by vaccinia virus infection. The colocalization of nuclear proteins with viral replication complexes in the cytoplasm of the cell therefore suggests that some of the proteins may leak out of the nucleus to associate with viral DNA or proteins in the complexes. Alternatively, the nuclear proteins may be located in the cytoplasm in levels too low to observe until they become concentrated through association with viral replication complexes. Some of the results presented in this study suggest that leakage from the nucleus better explains the origin of the nuclear proteins in the replication complexes. When tagged versions of YY1 were overexpressed from the CMV promoter, the proteins were almost exclusively located in the nucleus of uninfected cells (Fig. 2 and 4). After infection with vaccinia virus, the tagged YY1 proteins were found at high levels in the cytoplasm, sometimes apparently at much higher levels than in the nucleus. It would seem that the overexpressed proteins were drawn into the nucleus by active import but were more easily drawn out of the nucleus by virus infection because their natural sites of association with nuclear DNA might have been saturated.

The results presented here indicate that vaccinia virus has open access to a variety of nuclear proteins provided that they are not anchored to a nuclear structure such as the membrane. Despite having a very large DNA genome, it is likely that the vaccinia virus genome is incapable of encoding all the proteins required for virus replication. Access to the large number of nuclear proteins that potentially could function in viral DNA or proteins in the complexes. Alternatively, the nuclear proteins may be located in the cytoplasm in levels too low to observe until they become concentrated through association with viral replication complexes. Some of the results presented in this study suggest that leakage from the nucleus better explains the origin of the nuclear proteins in the replication complexes. When tagged versions of YY1 were overexpressed from the CMV promoter, the proteins were almost exclusively located in the nucleus of uninfected cells (Fig. 2 and 4). After infection with vaccinia virus, the tagged YY1 proteins were found at high levels in the cytoplasm, sometimes apparently at much higher levels than in the nucleus. It would seem that the overexpressed proteins were drawn into the nucleus by active import but were more easily drawn out of the nucleus by virus infection because their natural sites of association with nuclear DNA might have been saturated.

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ACKNOWLEDGMENTS

We are grateful to Bernhard Lüsher for the YY1 cDNA and deletion constructs and to John Hanover for the XRGG expression vector. We thank Jennifer Sturgis of the Purdue Cytometry Center for confocal microscopy.

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