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Focal Distribution of Baculovirus IE1 Triggered by Its Binding to the *hr* DNA Elements

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In BmN cells infected with the baculovirus *Bombyx mori* nucleopolyhedrovirus (BmNPV), IE1, a principal transcriptional activator, localizes to sites of viral DNA replication. IE1 initially displays focal distribution in BmNPV-infected cells prior to DNA synthesis, whereas the protein expressed by transfection with the *ie1* gene is distributed throughout the nucleoplasm instead of localized to discrete subnuclear structures. To identify the inducer of focus formation for IE1, we conducted transfection experiments with an *IE1-GFP* construct and found that cotransfection with genomic DNA fragments bearing the homologous region (*hr*) sequences caused the formation of IE1-green fluorescent protein (GFP) foci. The transfection of insect cells with a single plasmid containing exclusively the *hr*3 sequence and the *IE1-GFP* gene was sufficient to form IE1-GFP foci. These results suggest that *hr* elements are a primary determinant of the focal distribution of IE1. An analysis of a series of *hr*3 deletion mutants showed that a single copy of the direct repeat could induce the formation of IE1 foci. Targeted mutagenesis within the *hr*-binding domain of IE1-GFP caused impairment of the *hr*-dependent IE1 localization, suggesting that binding of IE1 to the *hr* elements is essential for the onset of IE1 focus formation. The observation of BmNPV IE1 foci in non-BmNPV-susceptible cells suggests that no species-specific factors are required for *hr*-dependent IE1 focus formation.

The immediate early gene 1 (*ie1*) of *Bombyx mori* nucleopolyhedrovirus (BmNPV) encodes a 67-kDa nuclear protein (IE1) that plays crucial roles in viral replication (reviewed in reference 6). In BmNPV-infected BmN cells, IE1 localizes to discrete subnuclear structures in which viral DNA synthesis occurs (26). While IE1 was originally characterized as a transcriptional activator of baculovirus early gene expression (2, 3, 10, 11, 13, 17, 18, 19, 25, 30, 31, 34), it has also been demonstrated to be essential for the replication of plasmids carrying putative origins of virus DNA replication, known as homologous region (*hr*) sequences (1, 15, 21).

hr sequence elements have been identified in the genomes of many baculoviruses (see reference 20). The BmNPV genome possesses five *hr* elements, which are dispersed at map units (m.u.) ~18 (*hr*2-left), ~19 (*hr*2-right), ~51 (*hr*3), ~67.5 (*hr*4-left), ~70 (*hr*4-right), ~83.5 (*hr*5), and ~96.5 (*hr*1) on the ~128-kbp genome (23). The BmNPV *hr*s are characterized by a series of ~75-bp DNA repeats, with each containing a 30-bp imperfect palindrome with an EcoRI site at its core (23). When viral early genes are *cis*-linked to the *hr* sequences, IE1-mediated activation of their transcription is greatly enhanced (9, 12, 25). Gel shift assays and mutational analyses have shown that IE1 binds directly to the *hr* sequences, which serve both as enhancers and as putative replication origins (3, 4, 8, 12, 16, 25, 28, 32, 33). It has also been reported that the palindrome in the *hr* sequences is essential for the direct interaction between the *hr* sequences and the IE1 protein (5, 7, 18, 32). While the binding of IE1 to *hr* sequences is clearly evident, the functional details of its binding for DNA replication and RNA transcription are still unknown.

By employing green fluorescent protein-tagged IE1 (IE1-GFP) as a reporter protein, we recently demonstrated temporal changes in BmNPV IE1 localization in living cells (13a). IE1 initially appears as small foci in the nucleus prior to the onset of DNA replication. As viral DNA synthesis proceeds (<8 to 24 h postinfection [hpi]), these IE1-associated structures gradually expand (26). At the end of the DNA replication period (24 to ~36 hpi), the expanded structures containing IE1 accumulate large amounts of DNA and seem to establish the site for nucleocapsid assembly, the virogenic stroma (13a). Although IE1 shows focal distribution at early stages of viral infection, the transfection of insect cells with a DNA fragment containing the *ie1* gene led to a relatively diffuse distribution of IE1 throughout the nucleoplasm rather than subnuclear localization (29; also see below). The establishment of IE1 localization during infection therefore must require a viral factor(s) other than IE1. What is the factor(s) that determines IE1 distribution? A previous report by Okano et al. showed that the number of IE1 foci in a nucleus is affected by the multiplicity of infection (MOI) (with a range of 0.4 to ~10), suggesting that the viral DNA genome itself is important for establishing IE1 localization (26).

In this study, we show that the *hr* DNA elements of the viral genome are needed for the focal distribution of IE1 and that no viral proteins other than IE1 are required for IE1 localization. In addition, by using a mutant IE1-GFP that lacks *hr*-binding ability, we demonstrate that direct binding of IE1 to the *hr* elements is essential for the subnuclear localization of IE1. We also report that BmNPV IE1, in conjunction with BmNPV *hr*, forms discrete foci in non-BmNPV-susceptible cells. This result suggests that no species-specific factors are required for *hr*-dependent IE1 focus formation.

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MATERIALS AND METHODS

Cells and viruses. BmN cells were maintained in TC100 medium (Funakoshi Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (26). High Five cells (Invitrogen) and S2 cells were maintained in SF900-II (Invitrogen) (24), and Sf21 cells were maintained in EX-CELL 420 (JRH Biosciences). The BmNPV wild-type isolate T3 (22) was propagated on BmN cells.

Plasmid construction. A plasmid expressing GFP-tagged IE1 (pKm-IE1-GFP) was constructed as described elsewhere (13a). For the cloning of BmNPV *hr*3, the termination codon of the *p91* coding sequence and the next 3 bp (TAAACA) in the PstI-K fragment (m.u. 48.4 to 52.3) of the BmNPV genome were replaced with an XhoI site (CTCGAG) by site-directed mutagenesis. The XhoI-NdeI subfragment of the mutagenized fragment (a 630-bp fragment of the *hr*3 region) was inserted into pBluescript (Stratagene). The SmaI-XhoI fragment from the resulting plasmid (pBS-hr3) was subcloned into the Eco47III and XhoI sites of pKm-IE1-GFP to create pKm-IE1-GFP-hr3, which carries both the *IE1-GFP* gene and *hr*3. Deletion mutants of *hr*3 were generated from pBS-hr3 by use of a Kilo-Sequence deletion kit (Takara Bio, Shiga, Japan). A plasmid containing the *hr* palindrome, pBS-palind, was constructed by ligating the annealed oligonucleotides 5′-TCGACGTTTTACACGTAGAATTCTACTCGTAAAGCA-3′ and 5′-AGCTTGCTTTACGAGTAGAATTCTACGTGTAAAACG-3′ into the XhoI and HindIII sites of pBluescript (Stratagene). For the construction of a plasmid expressing a mutant IE1-GFP that lacks *hr*-binding ability, the *IE1-GFP* gene (BglII-XbaI fragment) from pKm-IE1-GFP was inserted into the BamHI and XbaI sites of pUC19 to facilitate site-directed mutagenesis. The resulting plasmid (pUC-IE1-GFP) was mutagenized to make a plasmid (pUC-BIB-GFP) that has the sequence GGATCC (G162/S163 in IE1) instead of the sequence AAGAAA (K162/K163). The *hr*3 sequence (XhoI-SmaI fragment) in pBS-hr3 was inserted into the XhoI and SmaI sites of the plasmids pUC-IE1-GFP and pUC-BIB-GFP to produce pUC-IE1-GFP-hr3 and pUC-BIB-GFP-hr3, respectively. Transfections of BmN cells with pUC-IE1-GFP-hr3 showed similar efficiencies of IE1 focus formation to those with pKm-IE1-GFP-hr3 (data not shown).

Plasmid transfection and viral infection. For live-cell microscopy, all cell lines were seeded onto 27-mm-diameter glass-bottomed dishes (Matsunami, Tokyo, Japan) and allowed to stand for several hours or overnight for cell attachment. For the introduction of plasmids, the cells were transfected with 0.5 μg of each plasmid DNA sample by the use of Lipofectin reagent (Invitrogen). The transfected cells were incubated at 28°C for 24 h and analyzed directly with a confocal microscope or were infected with BmNPV at an MOI of 10. For immunocytochemistry, BmN cells were plated onto 22- by 22-mm coverslips (no. 1; Matsunami). The cells were allowed to attach for several hours and then transfected by the use of Lipofectin reagent (Invitrogen). Transfected BmN cells on coverslips were fixed for 10 min with 3.7% paraformaldehyde in phosphate-buffered saline (PBS) and then were washed three times with PBS. The fixed cells were treated with 0.1% Triton X-100 and 10% fetal bovine serum in PBS (TF-PBS) for 1 h and then incubated with a 1:100 dilution of guinea pig anti-IE1 antiserum (a gift from H. Bando, Hokkaido University) in TF-PBS for 1 h at room temperature. After four wash steps with PBS, incubation with fluorescein isothiocyanate-conjugated goat anti-guinea pig immunoglobulin G (1:200 dilution in TF-PBS; Cappel) was performed at room temperature for 1 h. The stained cells were washed four times with PBS and mounted by use of a Slow Fade light antifade kit (Molecular Probes).

Confocal microscopy. Confocal images were obtained with a Leica TCS NT microscope. For evaluations of the numbers of IE1-GFP foci within the entire nuclei, randomly selected fields of view which contained 5 to 10 cells expressing IE1-GFP were analyzed by use of the confocal microscope system. Eight consecutive optical sections of cell nuclei for each field were collected and projected onto a single image by the use of Leica software (26). The numbers of foci were counted in the cells presenting GFP fluorescence, which formed ~50% of all cells under our transfection conditions.

RESULTS

Formation of nuclear IE1 foci is dependent on *hr* DNA elements. In the initial stage of viral infection, IE1 accumulates in several small foci within the nucleus (26) (Fig. 1D). However, when it is expressed by plasmid transfections, this protein shows a relatively diffuse distribution throughout the nucleoplasm rather than a focal distribution (29) (Fig. 1B). This difference in IE1 distribution suggests that a viral factor(s) other than IE1 is required for the formation of nuclear IE1 foci. To identify the viral factor(s), we transfected BmN cells with a plasmid(s) containing a subfragment of the BmNPV genome simultaneously with the gene for IE1-GFP. We recently demonstrated that the intracellular distribution of the IE1-GFP used in this study is quite similar to that of untagged IE1 in both infected and uninfected cells (13a). When almost all of the subfragments in the genomic library were applied together for cotransfection, a focal distribution of IE1-GFP was observed (data not shown). By dividing the genomic library into two sublibraries, we could see that both halves of the sublibrary possessed the ability to form IE1-GFP foci (data not shown). This result suggested that the viral factors needed to make IE1 foci are dispersed throughout the viral genome. Previous studies have shown that IE1 can bind *hr* DNA sequences, which are likewise dispersed throughout the viral genome (reviewed in references 6 and 20). Hence, we reasoned that *hr*s were good candidates for the viral factors that are necessary to form IE1 foci. To investigate this possibility, we cotransfected BmN cells with the *IE1-GFP* construct together with subfragments of the viral genome containing *hr* sequences. As shown in Fig. 1E, G, I, K, and L, all *hr* sequences possessed the ability to produce IE1-GFP foci. The sizes of these foci (0.5 to 2 μm in diameter) were similar to those of the IE1-GFP foci observed in infected cells at 2 hpi (Fig. 1D). In contrast, infected cells that were transfected with subfragments that lacked *hr* sequences showed a diffuse distribution pattern of IE1-GFP (Fig. 1F, H, J, and M). This result suggests that the *hr* DNA elements are the viral factors that are needed for IE1 focus formation. However, each subfragment had regions other than the *hr* sequence that may have had an effect on IE1 focus formation. We therefore transfected BmN cells with a single plasmid bearing the *IE1-GFP* gene (containing the *ie1* promoter region [760 bp]) and an ~600-bp DNA fragment specific to the *hr*3 region. As shown in Fig. 2, IE1-GFP foci within the nucleus were seen in >80% of the cells that were transfected with the plasmid carrying *IE1-GFP* and *hr*3, while ~80% of the cells that were transfected with the *IE1-GFP* gene alone failed to form IE1-GFP foci. This result demonstrates that IE1-GFP foci can be induced by the *hr* element alone. On the other hand, since the BmNPV genome was sparsely examined (Fig. 1A), we could not exclude the possibility that viral factors other than *hr* function as inducers of focus formation for IE1. Next, to rule out the suspicion that the GFP tag had an effect on the formation of the IE1-GFP foci, we conducted an immunocytochemical experiment with an anti-IE1 antibody instead of employing the GFP fusion construct. BmN cells were cotransfected with two plasmids, one carrying the *hr*3 region and the other carrying the EcoRI G fragment (m.u. 90.5 to 96.4) of the BmNPV genome, which carries the *ie1* gene. When stained with an anti-IE1 antibody, the transfected cells exhibited IE1 foci (Fig. 2D). Together, these results suggest that the presence of the *hr* DNA elements is sufficient to generate IE1 foci in *ie1*-expressing insect cells.

A single direct repeat of an *hr* sequence or even its 30-bp imperfect palindrome can produce IE1 foci. Each BmNPV *hr* element contains two to eight direct repeats of a homologous nucleotide sequence that are, on average, about 75 bp long (23). To determine how many repeats are required for the formation of IE1 foci, we prepared a series of *hr*3 deletion

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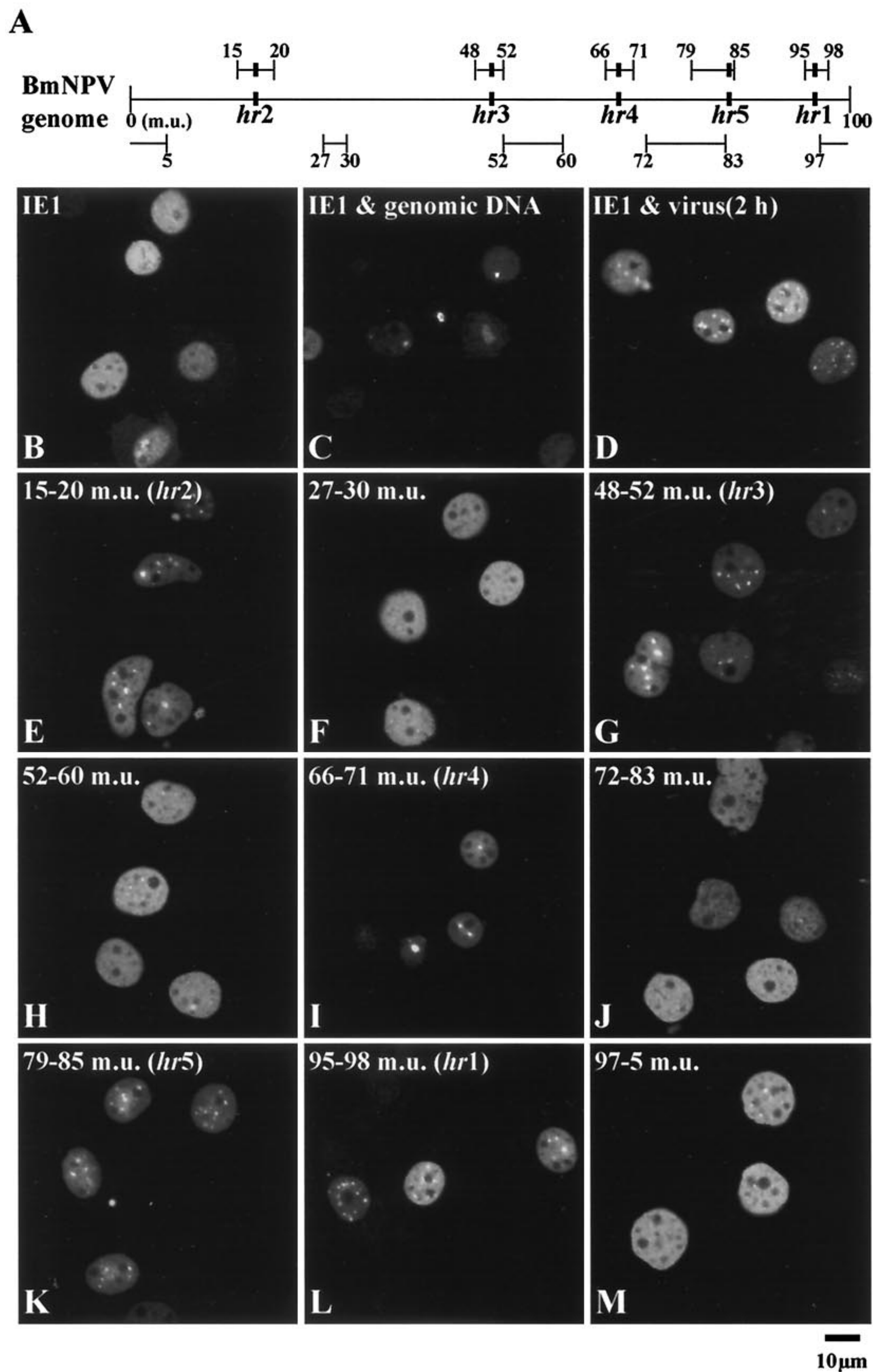

FIG. 1. Effects of BmNPV genomic fragments on IE1-GFP distribution. (A) Diagram showing the BmNPV genomic fragments used for transfection of BmN cells. Solid bars indicate the regions of the *hr* sequences. (B to M) Confocal images of BmN cells. Cells were transfected with a plasmid expressing IE1-GFP (B), infected with BmNPV (D), and cotransfected with a plasmid expressing IE1-GFP and either the entire BmNPV genomic DNA (C) or the indicated fragments of the genome (E to M). The transfected and infected cells were analyzed by confocal microscopy at 24 h posttransfection and at 2 h postinfection, respectively.

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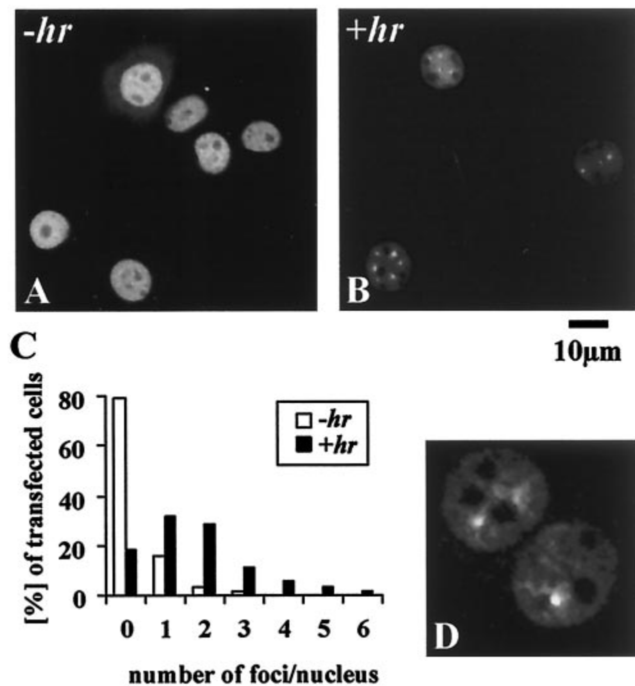

FIG. 2. Effect of *hr*3 on IE1-GFP distribution. BmN cells were transfected with a plasmid containing either the *IE1-GFP* gene alone (A) or the *IE1-GFP* gene and *hr*3 (B) and were analyzed by confocal microscopy at 24 h posttransfection. (C) Proportion of transfected cells containing IE1-GFP foci, with the number of foci per nucleus being estimated for a total of 119 cells without *hr* (open columns) and 108 cells with *hr* (closed columns). (D) Immunochemical detection of IE1 foci. Cells were transfected together with *hr*3 and the *ie1* gene and then stained with an anti-IE1 antibody.

constructs (Fig. 3A). As seen in Fig. 3E and F, a single repeat was enough to lead to IE1 focus formation. On the other hand, cells that were transfected with a deletion mutant that lacked most of the 75-bp repeats showed a diffuse distribution of IE1-GFP (Fig. 3G), demonstrating the importance of this repetitive sequence for IE1 focus formation.

Previous studies have shown that IE1 binds to the 30-bp imperfect palindrome that is at the core of the repeat sequence in *hr* (5, 7, 18, 32). Therefore, to determine if the 30-bp imperfect palindrome was enough to produce IE1 foci, we cotransfected BmN cells with two plasmids, one carrying the *IE1-GFP* gene and the other containing one copy of the 30-bp imperfect palindrome. As shown in Fig. 3H, IE1 foci were observed in the cotransfected cells, although the difference in fluorescence intensity between the foci and the surrounding nucleoplasm was quite small. This result suggests that a single copy of the 30-bp imperfect palindrome is sufficient for IE1 focus formation but that its ability to produce IE1 foci is less effective than that of a single copy of the 75-bp repeat sequence.

IE1-GFP with a mutation in the *hr*-binding domain results in a loss of *hr*-dependent IE1 focus formation. As described above, we have shown that *hr* is required for the formation of IE1 foci. Several lines of evidence have already demonstrated the direct binding of IE1 to *hr* (4, 7, 16, 18, 32). Consequently, we next examined whether direct binding of IE1 to the *hr* elements is necessary for IE1 focus formation. To this end, we used an *hr*-binding-domain mutant of IE1-GFP in which two basic residues (K162/K163) that are essential for *hr* binding were replaced with noncharged residues (G162/S163) (Fig. 4A). This type of mutation in IE1 results in defective *hr* binding and a loss of *hr*-dependent transcriptional enhancement but has no effect on IE1 oligomerization or nuclear localization (27). In the absence of *hr* elements, BmN cells transfected with a plasmid containing a gene for a mutant IE1-GFP exhibited GFP fluorescence that was evenly distributed throughout the nucleoplasm, similar to unmodified IE1-GFP (Fig. 4B and D). As expected, this mutation had no effect on nuclear localization. In the presence of *hr* elements (i.e., transfected with a single plasmid carrying both the mutated *IE1-GFP* gene and *hr*3), while the unmodified IE1-GFP localized to small foci, the mutated IE1-GFP failed to form nuclear foci (Fig. 4C and D). This result, therefore, indicates that the *hr*-binding ability of IE1 is essential for the induction of IE1 focus formation.

We next examined the effect of viral infection on the intracellular distribution of mutant IE1-GFP. A viral infection can supply wild-type IE1 to cells that produce mutant IE1-GFP. As seen in Fig. 4E, viral infection led to the recruitment of mutant IE1-GFP to nuclear foci at 4 hpi. At 8 hpi, nuclear foci containing the mutant IE1-GFP became enlarged, and at 24 hpi, they occupied a large proportion of the infected cell nuclei (Fig. 4F and G). This distribution pattern of the mutant IE1-GFP clearly indicated a colocalization pattern with wild-type IE1 in the IE1-associated structures (i.e., the virogenic stroma) (13a). Together, these results demonstrate that the interaction between IE1 and the *hr* elements is essential for the formation of the IE1 foci, whereas an IE1 protein that is deficient in *hr* binding can be recruited into IE1-associated structures in virally infected cells.

***B. mori* cell-specific factors are not required for *hr*-dependent focus formation of BmNPV IE1.** BmNPV replicates in *B. mori* (BmN) cells, but not in *Trichoplusia ni* (High Five) cells or *Spodoptera frugiperda* (Sf21) cells (14). To examine if BmNPV IE1 focus formation is also species specific, we used these non-BmNPV-permissive cells for transfections with BmNPV genes. In High Five cells and Sf21 cells that were transfected with the *IE1-GFP* gene in the absence of *hr* elements, diffuse distributions of nuclear GFP fluorescence were observed, revealing that the BmNPV *ie1* gene promoter and its protein nuclear localization signal function in both cell lines (Fig. 5A, C, and G). When High Five cells and Sf21 cells were transfected with the BmNPV *IE1-GFP* gene in association with BmNPV *hr*, IE1-GFP foci were clearly detected in both species of the non-BmNPV-permissive cells as well as in BmN cells (Fig. 5B, D, and G). This suggests that no species-specific factors are required for *hr*-dependent IE1 focus formation.

BmNPV is closely related to *Autographa californica* nucleopolyhedrovirus (AcNPV), which replicates in both *T. ni* cells and *S. frugiperda* cells (14). These cells, therefore, may synthesize a specific factor that functions in the focus formation of BmNPV IE1 as well as AcNPV IE1. To address this issue, we examined the *hr*-dependent focus formation of BmNPV IE1 in *Drosophila melanogaster* (S2) cells, which belong to a different order (Diptera) than *B. mori*, *T. ni*, or *S. frugiperda* (Lepidoptera). To our knowledge, no NPV infections in *Drosophila* have been recorded thus far. When expressed without *hr* elements,

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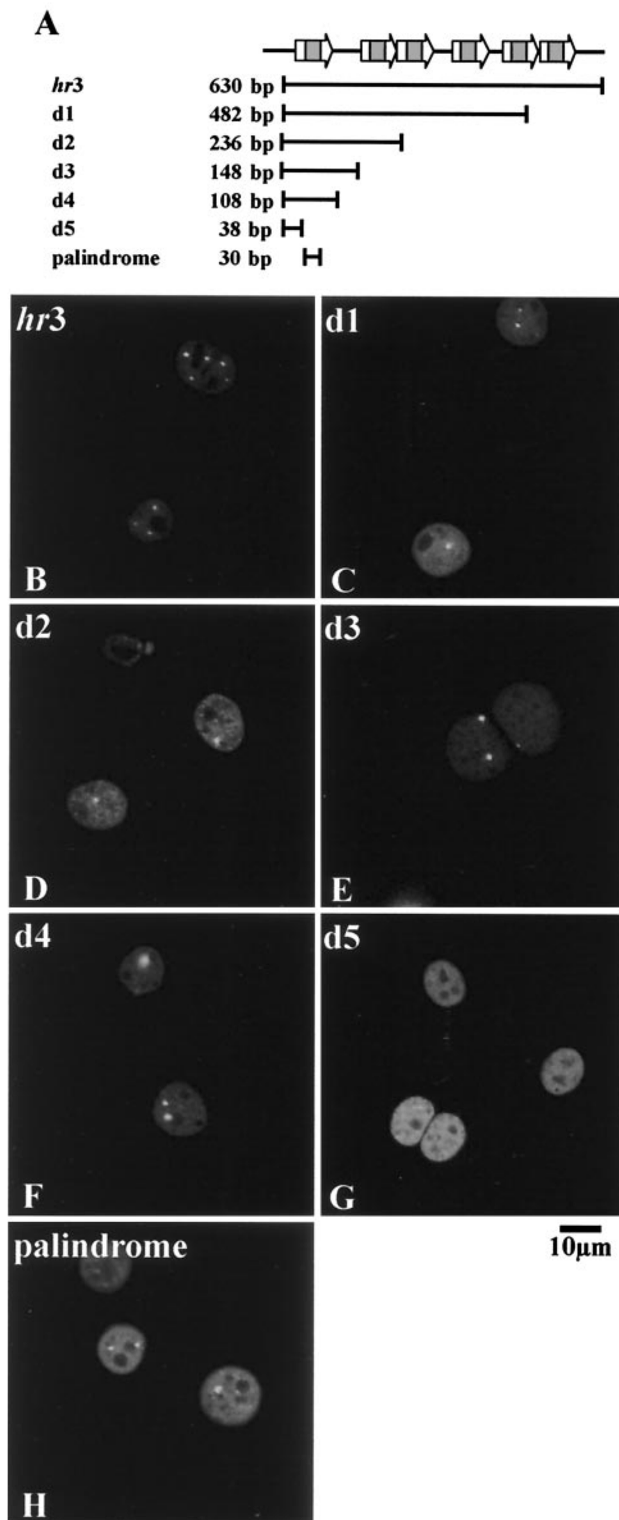

FIG. 3. Effects of *hr*3 deletion mutants on IE1-GFP distribution. (A) Diagram showing the construction of deletion mutants. Arrows and their hatched regions indicate the direct repeat sequences of *hr*3 and their imperfect palindrome, respectively. (B to H) BmN cells were cotransfected with a plasmid expressing IE1-GFP and the indicated deletion construct and were analyzed at 24 h posttransfection.

BmNPV IE1-GFP was distributed throughout the nucleoplasm in *Drosophila* cell nuclei, whereas IE1-GFP foci were clearly observed within the nuclei of cells that were cotransfected with the *IE1-GFP* gene and *hr* (Fig. 5E to G). Collectively, these results suggest that the *hr*-dependent focus formation of BmNPV IE1 is independent of the insect species and can occur in non-baculovirus-susceptible cells.

DISCUSSION

While IE1 initially localizes to small foci within the nuclei of BmNPV-infected cells (26) (Fig. 1D), the protein expressed by transfection is diffusely distributed throughout the nucleoplasm (29) (Fig. 1B). In the present study, therefore, we screened viral genes with IE1-GFP via confocal microscopy to identify the determinant of IE1 focal distribution in infected cells. Our results show that the introduction of *hr* DNA elements, specific regions of the BmNPV genome, is sufficient for the focus formation of IE1-GFP. In addition, an analysis of mutant IE1-GFP lacking the *hr*-binding ability revealed that direct binding of IE1-GFP to *hr* is essential for focal distribution. For the convenience of IE1 observation, we used GFP-tagged IE1 (IE1-GFP) instead of untagged IE1 in this study. Recently, we demonstrated that the intracellular distribution of IE1-GFP used in this study is quite similar to that of untagged IE1 in both infected and uninfected cells, suggesting that the GFP tag does not interfere with IE1 localization (13a). Furthermore, by performing an immunocytochemical analysis, we confirmed that the *hr* DNA elements induce the focus formation of untagged IE1 (Fig. 2D). Hence, we concluded that the GFP tag has no effect on *hr*-dependent IE1 localization and that the binding of IE1 to *hr* is required for the focal distribution of untagged IE1 as well as IE1-GFP.

IE1 foci (0.5 to 2 μm in diameter) induced by *hr* transfection are similar to those generated at the early stages of viral infection (~4 hpi) in size and shape (26) (Fig. 1). At present, however, we cannot definitively determine the identities of these two types of IE1 foci, i.e., we cannot provide direct evidence to prove that in infected cells the initial step in the formation of IE1 foci and IE1-associated structures (virogenic stroma) that subsequently develop is the assembly of IE1-*hr* complexes on parental viral genomes. In the baculovirus infection cycle, after the entrance of parental genomes into the nucleus, immediate-early genes on the genomes are transcribed more rapidly than other genes (6). After the translation of its mRNA in the cytoplasm, the immediate-early protein IE1 is transported into the nucleus. Some of the IE1 molecules in the nucleus must encounter and interact with *hr*s on the parental genomes to activate IE1-dependent genes prior to viral DNA replication (6). These IE1-*hr* complexes can induce IE1 focus formation, as they do in *hr*-transfected cells. Therefore, it is most likely that the IE1 foci that appear early during infection develop from IE1-*hr* complexes assembling on parental genomes. Moreover, Okano et al. previously demonstrated that the average number of IE1 foci per infected cell nucleus is well correlated with the MOI (with a range of 0.4 to 10), suggesting that the number of viral genomes that enter the nucleus exerts a critical influence on the number of IE1 foci (26). This observation also supports the parental genome-dependent mechanism of IE1 focus formation. We thus propose

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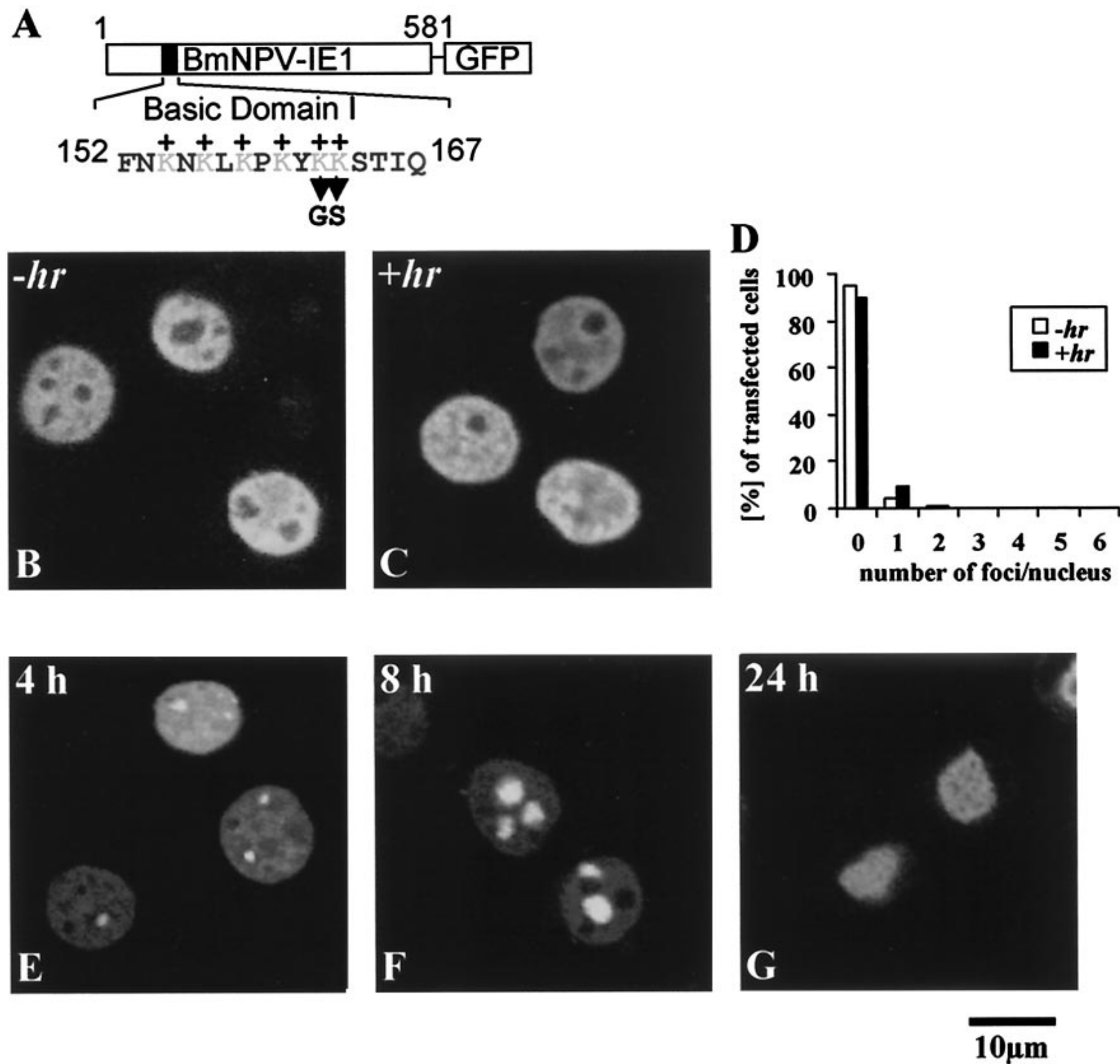

FIG. 4. Intracellular distribution of mutant IE1-GFP lacking *hr*-binding ability. (A) Diagram showing the mutagenized site of IE1-GFP. (B and C) BmN cells were transfected with a plasmid containing either the mutant *IE1-GFP* gene alone (−*hr*) or the mutant *IE1-GFP* gene and *hr*3 (+*hr*) and were analyzed by confocal microscopy at 24 h posttransfection. (D) Proportion of transfected cells containing mutant IE1-GFP foci, with the number of foci per nucleus being estimated for a total of 110 cells without *hr* (open columns) and 121 cells with *hr* (closed columns). (E to G) Cells were transfected with a plasmid expressing mutant IE1-GFP and then infected with BmNPV. At the indicated times postinfection, the infected cells were analyzed by confocal microscopy.

that the IE1 foci that appear during the early stages of infection are generated from IE1-*hr* complexes assembling on parental viral genomes. This mechanism of *hr*-dependent IE1 focus formation may serve to exclude the formation of prereplication compartments lacking viral genomes, as the IE1 foci later develop into sites for viral DNA replication (26).

Several studies of IE1 function have reported by the use of plasmid transfection assays that IE1 can dramatically augment the expression of genes that are *cis*-linked to *hr* elements (9, 12, 25). In similar assays, IE1 has also been shown to be essential for the replication of *hr*-containing plasmid DNAs (1, 15, 21). In these plasmid transfection assays, in which insect cells were cotransfected with plasmids containing both the *ie1* gene and *hr* elements, *hr*-containing plasmids should have induced IE1 focus formation. Hence, the formation of IE1 foci in these assays may have significant effects on IE1-mediated transactivation or DNA replication. It is possible that IE1 and *hr* contribute to IE1 focus formation in plasmid transfection assays such that the IE1 foci serve as nuclear compartments that promote DNA replication or RNA transcription.

This study sheds light on the initial step of IE1 focus formation. At the early stages of infection, the IE1-*hr* complex associated with the parental genome appears to induce the formation of IE1 foci in which DNA replication and the transcription of early genes occurs. IE1 foci therefore appear to be functionally important for baculovirus replication, although their structural details are not clear. Our finding that only two viral factors, IE1 and *hr*, are sufficient for IE1 focus formation should serve to facilitate future work to explore the molecular architecture of IE1 foci, and possibly the virogenic

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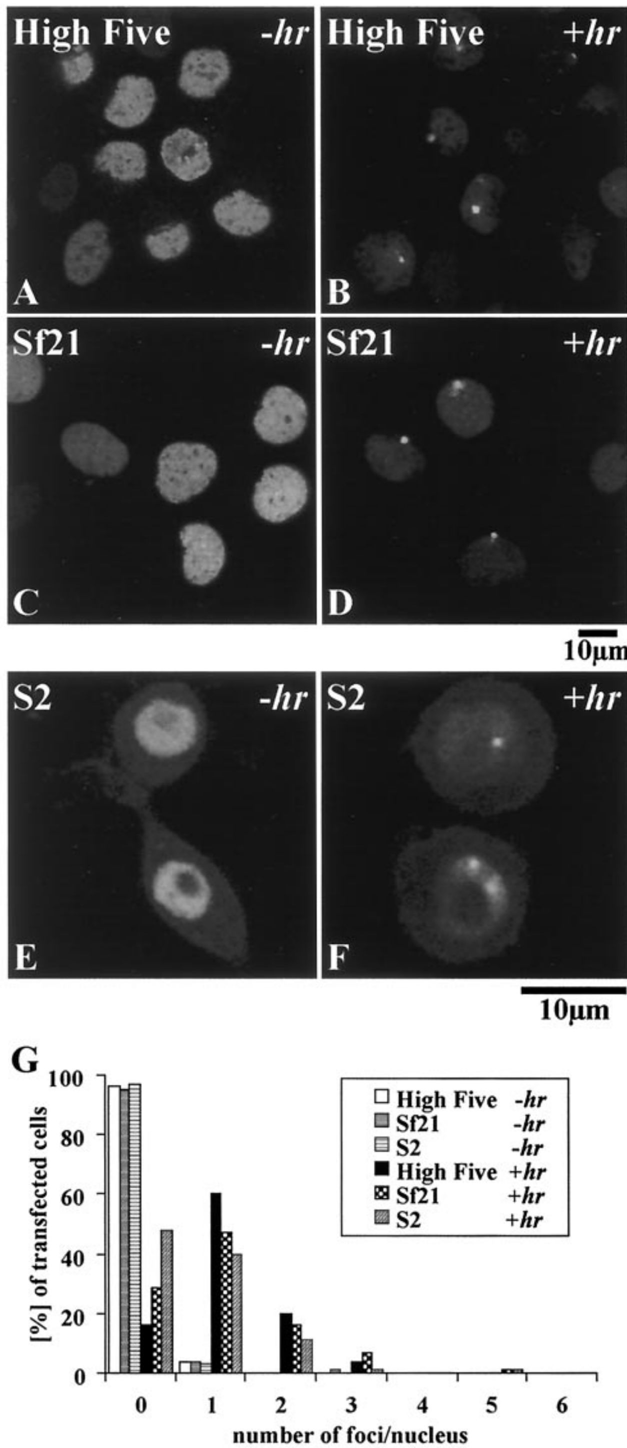

FIG. 5. IE1-GFP focus formation in different cell lines. High Five cells (A and B), Sf21 cells (C and D), and S2 cells (E and F) were transfected with a plasmid containing either the *IE1-GFP* gene alone (A, C, and E) or the *IE1-GFP* gene and *hr*3 (B, D, and F) and were analyzed by confocal microscopy at 24 h posttransfection. (G) Proportion of transfected cells containing IE1-GFP foci, with the number of foci per nucleus being estimated for a total of 124 (−*hr*) and 126 (+*hr*) High Five cells, 104 (−*hr*) and 104 (+*hr*) Sf21 cells, and 107 (−*hr*) and 101 (+*hr*) S2 cells.

stroma. The structural information gleaned from further studies should help us to more precisely define the functions of these nuclear structures.

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