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Abortive BmNPV Replication in Two Non-Permissive Cell Lines, Sf9 and HighFive, is Caused by Defective Nuclear Import of the Virus

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Bombyx mori nucleopolyhedrovirus (BmNPV) and *Autographa californica* multicapsid NPV (AcMNPV) are insect-specific, large, enveloped, double-stranded DNA viruses that belong to the family Baculoviridae. These two viruses have been demonstrated to have a distinct host specificity property, exhibiting narrow host range in BmNPV and relatively broad host range in AcMNPV, yet comparative analysis of viral genomes has demonstrated that these two viruses are genetically closely related. Specifically, BmNPV that grows to a high titer in *Bombyx* BmN-4 cells is considered to be non-permissive in *Spodoptera frugiperda* Sf9 cells, whereas AcMNPV that productively infects Sf9 cells is considered to be unable to multiply successfully in BmN-4 cells. In this study, BmNPV replication was examined in Sf9 and *Trichoplusia ni* HighFive cells that were non-productive for BmNPV infection but support a high titer of AcMNPV replication.

To determine if BmNPV genomic DNA was delivered into the nucleus of Sf9 and HighFive cells in expressible form, a recombinant BmNPV, vBmgfp/lac, that contained *bm-ie1* promoter-driven *egfp* was generated, and EGFP expression was examined in the cells infected with vBmgfp/lac and transfected with vBmgfp/lac DNA. The results showed that few, if any, proportion of Sf9 and HighFive cells infected with vBmgfp/lac expressed EGFP, while large proportion of EGFP-expressing Sf9 and HighFive cells were observed in the culture transfected with vBmgfp/lac genomic DNA. By immunocytochemical analysis with the antibody against major capsid protein, VP39, it was shown that BmNPV virions were internalized into Sf9 and HighFive cells and transported to the vicinity of the nucleus, without being imported into the nucleus. On the other hand, recombinant BmNPV, vBmΔ64/ac-gp64, that possessed *bm-gp64* promoter-driven *ac-gp64* in place of its own *bm-gp64* was imported into the nucleus in both Sf9 and HighFive cells, and yielded substantial amounts of budded virions and polyhedra in HighFive cells. In syncytium formation assay, pH threshold for syncytium formation was

lower in BmNPV GP64-expressing Sf9 cells than in AcMNPV GP64-expressing Sf9 cells and at certain low pHs, AcMNPV GP64-expressing Sf9 cells fused more extensively than did BmNPV GP64 expressing Sf9 cells.

These results indicate that BmNPV replication in Sf9 and HighFive cells is restricted at a step of viral genome import into the nucleus in expressible form and suggest that observed restriction of the viral genome import is attributed to lower fusogenic activity of BmNPV GP64 as compared to that of AcMNPV GP64 in Sf9 and HighFive cells.