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Identification and Functional Analysis of *iap* Genes of *Hyphantria cunea* Nucleopolyhedrovirus

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The inhibitor of apoptosis genes (*iap* genes) have been shown to prevent the apoptosis of the cells triggered not only by baculovirus infection but also by variety of apoptosis inducers. In the present study, we identified and functionally analyzed anti-apoptotic genes from *Hyphantria cunea* nucleopolyhedrovirus (HycuNPV). HycuNPV possessed three members of *iaps*, *hycu-iap1*, *hycu-iap2* and *hycu-iap3*, while homologues of *p35* that is an anti-apoptotic gene from *Autographa californica* MNPV (AcMNPV) and *iap4* were not identified on HycuNPV genome by hybridization. To examine whether the Hycu-IAPs have ability to block actinomycin D (AmD)-induced apoptosis in Sf9 cells, cells were transfected with *hycu-iap*-expression plasmids, and at 24 h posttransfection, cells were treated with AmD for 6 h. Significantly greater survivals in *hycu-iap3* transfected cells were observed (77.5%) as compared with cells mock- transfected or transfected with *hycu-iap1*- and *hycu-iap2*-expression plasmids. To examine whether the Hycu-IAPs have ability to rescue the replication of *p35*-deficient AcMNPV (vΔp35) in Sf9 cells, cells were co-transfected with vΔp35 DNA and *hycu-iap*-expression plasmids. Severe apoptosis was induced in cells transfected with vΔp35 DNA alone or co-transfected with vΔp35 DNA and either *hycu-iap1*- or *hycu-iap2*-expression plasmid. In contrast, by co-transfection with vΔp35 DNA and *hycu-iap3*-expression plasmid, most of cells were resisted to apoptosis and formed a number of polyhedra. These results indicate that Hycu-IAP3 is a functional inhibitor of apoptosis in HycuNPV. To examine whether anti-apoptotic activity of Hycu-IAP3 is essential for HycuNPV infection, knockdown of Hycu-IAP3 expression was performed by double-stranded RNA (dsRNA)-mediated RNA interference. HycuNPV genomic DNA and *hycu-iap3* dsRNA were co-transfected into SpIm cells that are permissive for HycuNPV infection. As the control, *hycu-iap1* or *hycu-iap2* dsRNA was used instead of *hycu-iap3* dsRNA. By 5 days posttransfection, apoptosis was not induced and polyhedra formation was observed in control cells transfected with HycuNPV DNA alone or co-transfected with HycuNPV DNA and either *hycu-iap1* or *hycu-iap2* dsRNA. On the other hand, apoptosis was induced in cells co-transfected with virus DNA and *hycu-iap3* dsRNA, indicating that knockdown of Hycu-IAP3 during HycuNPV transfection induced apoptosis in SpIm cells. These results demonstrate that HycuNPV infection induces apoptosis in SpIm cells and this apoptosis is suppressed by Hycu-IAP3.