Identification of a nucleocapsid protein (VP35) gene of shrimp white spot syndrome virus and characterization of the motif important for targeting VP35 to the nuclei of transfected insect cells.

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摘要.

To identify the protein encoded by a 687-bp open reading frame (ORF) of a salI genomic DNA fragment of shrimp white spot syndrome virus (WSSV), we expressed the ORF in a baculovirus/insect cell expression system. The apparent molecular mass of the recombinant protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was 35 kDa in insect cells. Antibody raised against bacterially synthesized protein of the ORF identified a nucleocapsid protein (VP35) in the extracts of both the purified WSSV virions and the nucleocapsids which comigrated with the 35-kDa baculovirus-expressed recombinant protein on SDS-PAGE. We also show by transient expression in insect cells (Sf9) that VP35 targets the nucleus. Two potential nuclear localization signals (NLSs) were characterized, but only one of them was important for targeting VP35 to the nuclei of transfected insect cells. Replacement of a cluster of four positively charged residues ((24)KRKR(27)) at the N terminus of the protein with AAAA resulted in mutant proteins that were distributed only in the cytoplasm, thus confirming that this sequence is a critical part of the functionally active NLS of VP35.

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