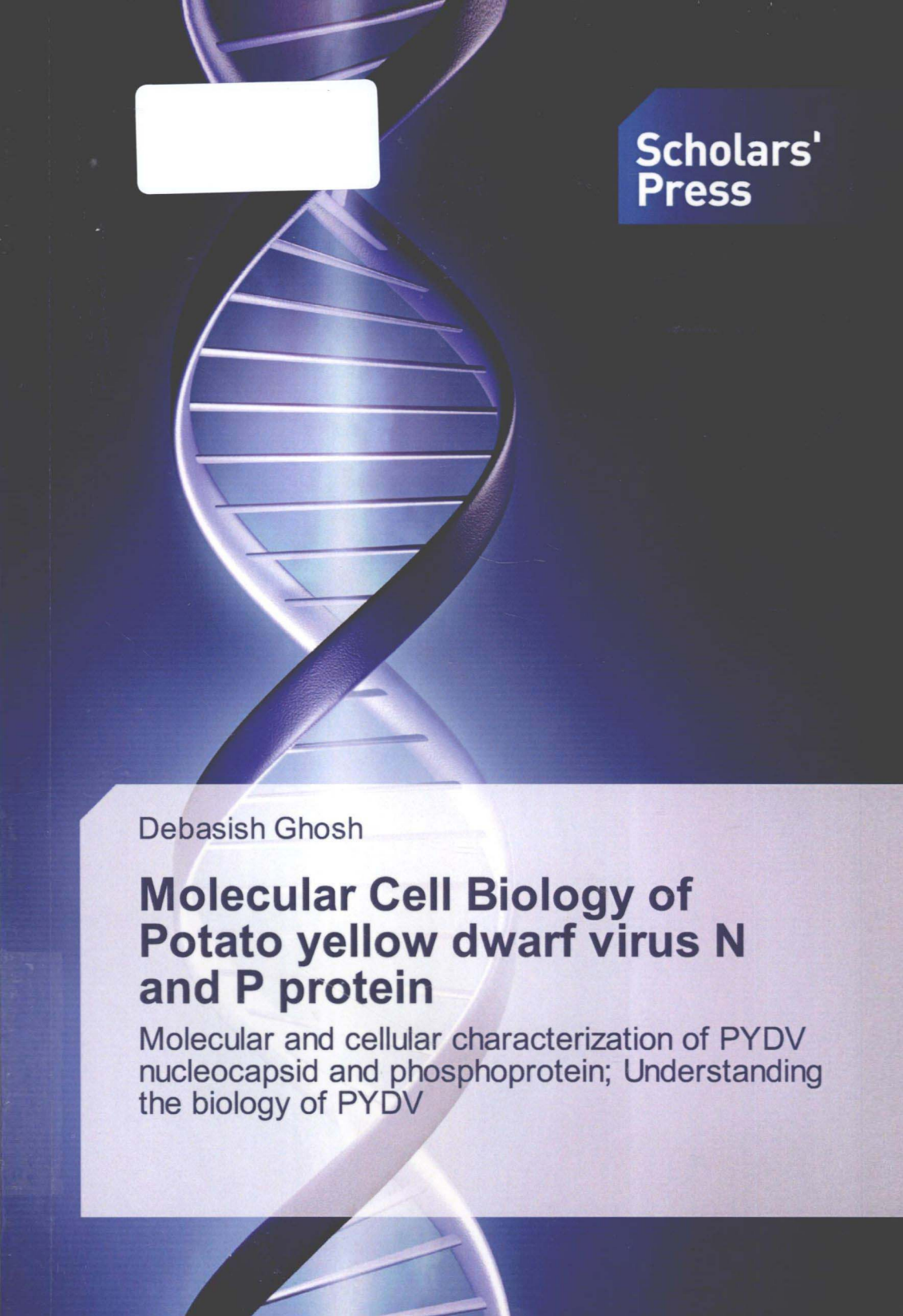

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Debasish Ghosh

Molecular/ Cell Biology of Potato yellow dwarf virus N and P protein

Molecular Cell Biology of Potato yellow dwarf virus N and P protein

Dr. Debasish Ghosh M.Sc., Ph.D.

Dedicated to the loving memory of my grandfather, Late Mr. Chitta Ranjan Ghose
and my mother, Late Mrs. Dipti Ghose

MOLECULAR CELL BIOLOGY OF THE N (NUCLEOCAPSID) AND P (PHOSPHOPROTEIN) PROTEIN OF THE SYDV (*POTATO YELLOW DWARF VIRUS – SANGUINOLENTA* STRAIN)

Potato yellow dwarf virus (PYDV) is the type member of the genus *Nucleorhabdovirus*. The virus replicates in the nuclei of infected cells and mature virions accumulate in the perinuclear space after viral cores bud through the inner nuclear membrane. The virus was first described as an extremely destructive pathogen of potato (*Solanum tuberosum*) and other members of family *Solanaceae*. There are two different strains of PYDV based on their insect-vector specificity, namely SYDV (*sanguinolenta* strain) and CYDV (*constricta* strain). PYDV is considered a model system to study virus-vector relationship, particularly for agriculturally harmful rhabdoviruses. However, very little is known about the molecular aspects and cell biology of PYDV. Preliminary studies showed that infection of transgenic *Nicotiana benthamiana* plants that constitutively express GFP targeted to endomembranes with SYDV and SYNV (*Sonchus yellow net virus*, another member of genus *Nucleorhabdovirus*) results in increased accumulation of GFP and membrane within the infected nuclei, though the pattern of GFP accumulation is completely different for the two viruses. GFP accumulation was found mainly in the external and internal loci of the nucleus in SYDV-infected cells, where as, in the case of SYNV infection, the GFP accumulation was scattered throughout the nucleus of the infected cell. Molecular characterization of SYDV was undertaken to better understand the cellular difference between these two members of *Nucleorhabdoviruses*. This dissertation describes the determination of the complete nucleotide and ORF (open reading frame) sequences of N (nucleocapsid) and P (Phosphoprotein) gene of SYDV from cDNA clones of both viral genomic and messenger RNAs. Analyses of sequence showed that SYDV-N mRNA contains an 11 nucleotide (nt) untranslated region followed by a 1416 nt ORF encoding a 472 amino acid (aa) protein and P-mRNA contains an 18 nt 5' untranslated region followed by 840 nt ORF encoding a 280 aa protein. Characterization of SYDV-N and P protein using bioinformatic algorithms predict basic hydrophilic and coiled coil regions that may posses the putative nuclear localization signal and protein-protein interaction domain, respectively. Comparison of the SYDV-N ORF with orthologous regions from other plant and animal rhabdoviruses showed

statistically significant identity. Phylogenetic analysis based on consensus N-ORFs placed SYDV into the same group with other *Nucleorhabdoviruses*. Localization studies of SYDV-N and P protein as autofluorescent protein fusions revealed that both proteins are exclusively nuclear localized. Taken together, this dissertation reports a detailed analysis of the biology of SYDV-N and P protein at the molecular and cellular level for the first time towards the long term goal to characterize the entire SYDV genome and to better understand SYDV-host interaction.

Keywords: rhabdovirus, SYDV, SYNV, nucleus, transgenic "16c" plant,

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Table of Contents

List of Tables

List of Figures