

Molecular characterization of IPNV RNA replication intermediates during the viral infective cycle

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Infectious Pancreatic Necrosis Virus (IPNV) is a bisegmented, double-stranded RNA virus, which belongs to the Birnaviridae family. In the current study, we have analyzed the RNA replication intermediates (RI) purified throughout the viral replication cycle in cultured cells. Equilibrium ultracentrifugation of infected cellular lysates resulted in two major peaks of viral components. The first peak, at a buoyant density of 1.33 g/cm³, contained assembled IPNV viral particles A and B, whereas the second peak, located at buoyant densities >1.4 g/cm³, contained a higher molecular weight viral ribonucleoprotein complex composed of, at least, VPg/VP1 and a heterogeneous population of single- and double-stranded viral RNA species. Interestingly, analyses of these dsRNA RI indicated that they contain single-stranded segments of incompletely synthesized positive-strands of RNA. Northern blot experiments of total RNA isolated from infected cells confirmed our proposed configuration of the RNA RI