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Functional Sites within the IHNV NonVirion Protein that Regulate Host Cellular Responses

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Functional Sites within the IHNV NonVirion Protein that Regulate Host Cellular Responses

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Introduction

Fish *Rhabdoviruses* are responsible for causing fatal epizootics within commercial and wild populations of various fish species around the world. Infectious hematopoietic necrosis virus (IHNV), also known as the *Salmonid novirhabdovirus,* is enzootic along the Pacific Coast of North America and is comprised of five genogroups, each of which is endemic to a specific geographical location. Once the virus enters the host through the fin epithelia, IHNV infection causes infectious hematopoietic necrosis in salmonid species. The disease is highly fatal and presents with signs such as abdominal distension, bulging of the eyes, anemia, and necrosis of vital organs such as the liver and kidneys, all caused by systemic hemorrhaging within the host.

The 11-kb negative-sense, ssRNA viral genome within IHNV consists of six genes that encode the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), nonvirion protein (NV), and RNA-dependent RNA polymerase (L), in order from 3' to 5', respectively (Fig. 1). While most of the protein products from the IHNV genome have been studied and elucidated, the precise function of the NV protein remains unknown. While multiple studies have reported various roles for NV, such as suppression of apoptosis, interferon (IFN) induction, and NF-κB activation, data from our lab suggest that NV augments transcriptional or translational responses in the host. Using transient transfections and luciferase reporter assays, we have observed upregulation of host cell transcription/translation and innate immune responses. Regardless of the proposed functions of NV, functional sites within the viral protein are poorly defined. With the introduction of C- and N-terminal deletion mutations (ΔNV), we were able to characterize the effects of mutated NV on rainbow trout gill epithelial cell (RT-Gill) constitutive and induced transcriptional responses using specific luciferase reporter plasmids, pCAG*luc* and RT-IFN*luc*. Our results suggest that while all ΔNV mutants showed a decrease in the augmented luciferase expression obtained with WT-NV, mutations within the N-terminal region of the protein led to an inhibitory effect on constitutive or induced luciferase expression. These data suggest that the N-terminal region of NV plays a critical role in the upregulation of host cell expression.

Materials and Methods

PCR Site-Directed Mutagenesis

- Deletion mutations were introduced within the NV gene of the IHNV genogroup "M" viral backbone, by site-directed mutagenesis PCR. Sequence-verified mutants were then cloned into the pcDNA3.1 expression vector.

Transfection Protocol

- RT-Gill epithelial cells were maintained at 20°C in L-15 medium (HyClone), with 10% FBS (Corning) and 1% Penicillin/Streptomycin (Corning) with cellular transfection being carried out in L15-10%, without PS. Viafect transfection reagent (Promega) was used at a 3:1 volume ratio of the total pDNA in OptiMEM (Gibco). At 48hpt, cells are quickly washed with 1x PBS, lysed with 1.5x Luciferase Cell Culture Lysis Reagent (Promega), and processed after 10-minute shaking incubation at room temp to ensure total cell lysis.

Luciferase Assay Protocol

- Luminescence data, obtained using a Synergy H1 microplate reader (BioTek), were normalized to the total protein load in each cell lysate sample using a Bradford assay (BioRad). Expression levels were normalized to the constitutive expression in the internal transfection control. The significance of the average fold change between the control and each NV plasmid was analyzed by one-way ANOVA and LSD post hoc test for comparison of the group means. Data are presented as group means (+ SEM) indicating the p-value, when applicable, as: **p <0.01; ***p <0.001.

Figure 1. The Infectious hematopoietic necrosis virus genome (negative-sense, ssRNA).

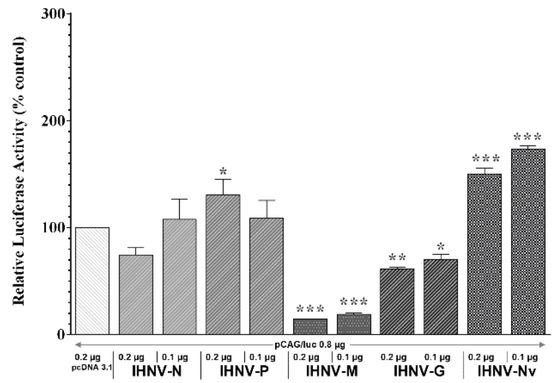

Figure 2. Comparative modulation of host constitutive transcription by single IHNV genes. Luciferase reporter assay comparatively assessing the effect of IHNV genes on the host general transcription upon co-transfection with pCAGluc.

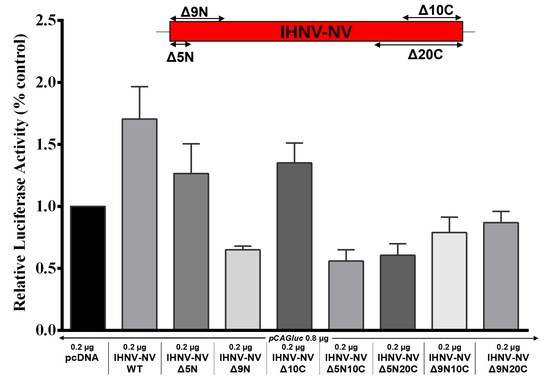

Figure 3. Comparative modulation of host constitutive transcription by IHNV-NV WT and ΔNV Mutants. Luciferase reporter assays comparatively assessing the effect of the NV gene from IHNV-WT and ΔNV mutants on the host general transcription upon co -transfection with pCAGluc. Deletion mutations are shown within the NV gene diagram.

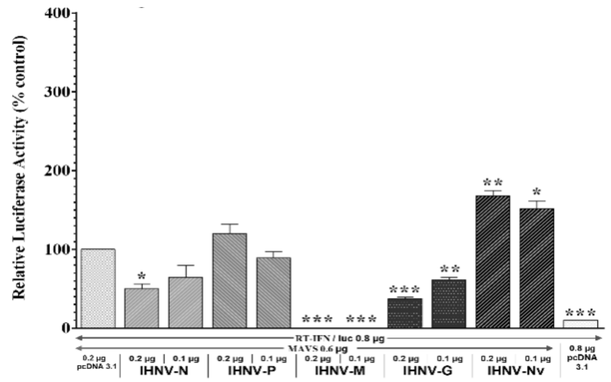

Figure 4. Comparative modulation of host innate antiviral response by single IHNV genes. Luciferase reporter assay comparatively assessing the effect of IHNV genes on the host innate antiviral response upon co-transfection with RT-IFN*luc* induced by Mitochondrial Antiviral Signaling Protein (MAVS).

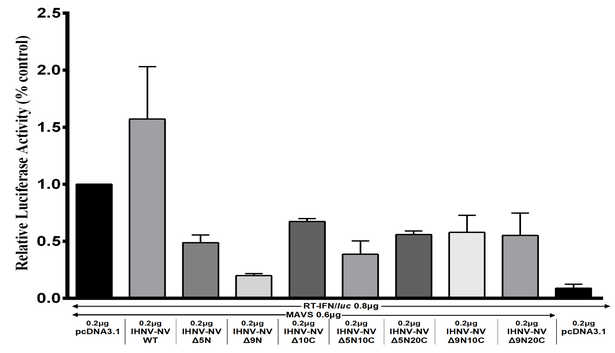

Figure 5. Comparative modulation of host innate antiviral response by IHNV-NV WT and ΔNV Mutants. Luciferase reporter assays comparatively assessing the effect of the NV gene from IHNV-WT and ΔNV mutants on the host innate antiviral response upon co-transfection with RT-IFN*luc* induced by MAVS. Deletion mutations are shown within the NV gene diagram (Fig. 3).

Conclusions and Future Directions

- Unlike other IHNV genes that had either no effect or suppressed host transcriptional/translational responses, NV elicited the highest increase in expression of co-transfected constitutive or inducible reporter plasmids
- All N- and C-terminal deletion mutations of IHNV-NV showed a decrease in effect as compared to WT NV
- IHNV-NV Δ9N, Δ5N10C, and Δ5N20C were the mutants with the most perturbed activities, in some cases suppressing luciferase expression as compared to the empty vector control
- Perform western blots to confirm ΔNV protein expression
- Create NV Δ5C single and double mutants to further narrow down functional regions within the C-terminus
- Determine mechanism of NV expression modulation based on newly discovered functional sites

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