

Confirmation of Interaction of a Novel HIV NS2 Protein with Host Proteins in Living Mammalian Cells

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Abstract

NS2 is a novel HIV protein confirmed to be expressed from an open reading frame alongside the Pol open reading frame. Furthermore, NS2 has been shown to be critical to viral replication and to localize in the nucleolus of the nucleus. The mechanism of NS2 function is currently unknown. A yeast-2-hybrid assay (Y2H) yielded a number of possible mammalian protein interactions with NS2. Bimolecular fluorescence complementation (BiFC) is utilized to confirm these positive interactions. The first step to performing a BiFC experiment is inserting the coding sequence for the protein of interest into a vector plasmid. From the list of Y2H positive interactions, we chose to analyze programmed cell death 5 (PDCD5), heat shock protein-40 (DNAJB1), and lantibiotic synthetase C-like protein-2 (LANCL2). Our results presented here are the successful plasmid construction of pBiFC-PDCD5-YN155 and pBiFC-DNAJB1-YN155. Both of these proteins are now ready for BiFC experiments, however, LANCL2 will require a site-directed mutagenesis to remove a restriction site within the coding sequence. The next step in this project is constructing the NS2 plasmid, pBiFC-NS2-YC155. Afterwards, BiFC experiments can be conducted and finally, the mechanism of NS2 determined, which may potentially lead to HIV-1 drug therapy.

Introduction

HIV-1 has become a pandemic, infecting millions across the world. The HIV-1 genome has 9 genes coding for 19 essential proteins. The Pastey laboratory has discovered a novel HIV protein coined NS2, which may be a target for drug therapy in the future. Our laboratory has shown that NS2 is expressed, is critical to viral replication, and localizes in the nucleus. The NS2 open reading frame is along the Pol open reading frame and consists of 192 base pairs. Western blot analysis of a strain with a mutated start codon, delta-NS2GFP, confirmed that the NS2 gene is expressed from our predicted start codon. Various cell lines were then transfected with both the NS2 wild type strain and the mutated delta-NS2GFP strain. The samples were assayed for viral capsid protein concentrations across a 10-day time period and the results concluded that NS2 is critical to viral replication since the delta-NS2GFP viral capsid protein concentrations were significantly less than the wild type. Finally, the laboratory determined localization of NS2 by transfecting cells with the NS2GFP construct and observing under fluorescent microscopy. Dr. Manoj Pastey determined localization to be in the nucleolus of the nucleus. The hypothesized function of NS2 is that NS2 interacts with mammalian proteins in the nucleus to facilitate viral infection.

A yeast-2-hybrid screening (Y2H) of NS2 with human cDNA yielded 19 positive protein-protein interactions. Bimolecular Fluorescence Complementation (BiFC) is a method used to confirm these positive interactions since Y2H yields a number of false positives. BiFC tags the two proteins of interest with half of a yellow fluorescent protein (YFP), and then both proteins are co-transfected into mammalian cells. If the two proteins interact, YFP simultaneously recombines and fluoresces under fluorescent microscopy. Our laboratory chose three proteins to analyze as the first group: programmed cell death 5 (PDCD5), heat shock protein-40 (DNAJB1), and lantibiotic synthetase c-like protein-2 (LANCL2).

PDCD5 plays a critical role in apoptosis. After apoptosis is induced, PDCD5 relocates to the nucleus from the cytoplasm and programs for cell death (NCBI, 2011). NS2 may inhibit PDCD5 and its ability to carry out cell death by a protein-protein interaction in the nucleus. Based on the localization and function of PDCD5, it was given top priority.

DNAJB1 has been shown to interact with HIV-1 infected cells via the Nef protein (Kumar and Mitra, 2005). This interaction leads to increased DNAJB1 expression and translocation to the nucleus, facilitating viral gene expression (Kumar and Mitra, 2005). Although Nef signals for DNAJB1 upregulation and translocation, NS2 binding via a protein-protein interaction in the nucleus may be what initiates viral gene expression. Likewise, this protein was given priority due to its localization and its previously determined association with HIV-1 infected cells.

LANCL2 is required for abscisic acid binding and signaling pathways in mammalian granulocyte cells (Allegretti, 2009). This interaction with granulocyte cells, which play a key role in the immune system, is hint at a possible role in HIV infections. Furthermore, LANCL2 localizes in the nucleus, which is a hint at a possible NS2 interaction.

Our laboratory chose to give these three Y2H positive protein interactions priority over the others for BiFC analysis. The first step to conducting a BiFC experiment is constructing the plasmids that house NS2 tagged by a half of YFP, and our protein of interest tagged by the other half of YFP. Positive BiFC results will lead to further research to determine the mechanism of interaction.

Materials & Methods

cDNA Extraction – HeLa mammalian cells were cultured and seeded to a total cell count of approximately 5×10^6 . An RNA Extraction kit was used to extract mammalian RNA from the HeLa cells. Afterwards, Invitrogen SuperScript III First-Strand Synthesis SuperMix was utilized for an RT-PCR reaction to convert the RNA to DNA.

Plasmid Construction – Plasmid, pBiFC-bJunYN155, was utilized as the vector for the Y2H proteins. The insert site has restriction site, HindIII, upstream and restriction site, Xba1, downstream, thus both were used for our plasmid construction. Primers were designed that had HindIII and Xba1 restriction sites on the ends. The primers were ordered from Eurofins MWG Operon. PCR amplification was done on the cDNA library using the designed primers. Gel electrophoresis was used to isolate the amplified sequence from the original cDNA compound. The amplified sequence and the pBiFC-bJunYN155 vector were subject to a double digest reaction, creating sticky ends at the restriction sites. Both the vector and protein coding sequence were then ligated together constructing a complete plasmid with ampicillin resistance. This plasmid was then placed into competent DH5-alpha *Escherichia coli* cells, which were cultured on an ampicillin plate for 24-48 hours. Colonies from this plate were picked and the plasmid constructs were isolated and sequenced to confirm correct alignment. Oregon State's CGRB Laboratory analyzed these sequences.

Results and Discussion

Plasmid Construction – The plasmids, pBiFC-PDCD5-YN155 and pBiFC-DNAJB1-YN155, were both constructed. To ensure correct insert site and ensure site is in frame with YFP, both plasmids were sequenced (refer to **Figure 1**). Plasmid, pBiFC-LANCL2-YN155, has not yet been constructed because the presence of an Xba1 restriction site in the LANCL2 coding sequence caused some issues. pBiFC-LANCL2-YN155 plasmid construction will proceed as described in the methods section after the LANCL2 coding sequence has been subjected to a site-directed mutagenesis eliminating the Xba1 site. The PDCD5 and DNAJB1 plasmids are ready to be tested via BiFC, however, the pBiFC-NS2-YN155 plasmid has not yet been constructed. This plasmid will be constructed using pBiFC-bFoS-YN155 as the vector and EcoRI/KpnI as the restriction sites on either side. Once the NS2 plasmid has been constructed, BiFC experiments can be conducted and the interactions tested.

References

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Kumar M., Mitra D., 2005, Heat Shock Protein 40 is Necessary for Human Immunodeficiency Virus-1 Nef-mediated Enhancement of Viral Gene Expression and Replication, *The Journal of Biological Chemistry*, Vol. 280, p. 40041-40050

NCBI, Dec 2011, PDCD5 programmed cell death 5 (Homo Sapiens), *NCBI Gene Result*, <http://www.ncbi.nlm.nih.gov/gene/9141>

Figure Legends

Figure 1. pBiFC-PDCD5-YN155/pBiFC-DNAJB1-YN155 sequencing results – Sequencing data from Oregon State's CGRB laboratory to confirm both inserts, PDCD5 (top) and DNAJB1 (bottom), are correct and in frame with the YFP component. The highlighted portion is the start of the respective protein sequences.