4-27-2020

Effects of individual Orsay virus proteins on the Intracellular Pathogen Response of C. elegans

Barbara Chen

Jessica N. Sowa

Effects of individual Orsay virus proteins on the Intracellular Pathogen Response of *C. elegans*

Chen, B. C & Sowa, J. N.

WCU WEST CHESTER UNIVERSITY

Abstract

In order to cause infection, pathogens must avoid destruction by the host immune system. However, how pathogens evolved to evade host immunity is not fully understood. Mammals have two systems of immunity, innate and adaptive, which are regulated by complex interactions between the two systems. In order to study innate immunity exclusively, the roundworm *C. elegans* is a useful invertebrate model because it lacks adaptive immunity and the complexities that arise from interactions between the two types of immunity. The goal of this project is to investigate whether the individual expression of the Orsay virus capsid and delta-fusion proteins can suppress the Intracellular Pathogen Response (IPR) in *C. elegans*. The IPR is an innate immune response activated by the Orsay virus. To investigate the effects of the viral proteins on the IPR, molecular cloning will be used to construct plasmids that will be microinjected into *C. elegans*, creating transgenic animals that overexpress each of the two Orsay proteins. We will then test the animals to observe the individual effects of the capsid protein and delta-fusion protein on IPR suppression. From this we will better understand how viruses evolved to evade the host immune system.

Methods

Plasmid Construction

To begin characterizing Orsay virus capsid and delta-fusion proteins' effect on the IPR, we first needed to create transgenic strains of *C. elegans* that overexpress each of the two Orsay proteins. Each protein is expressed on an open reading frame (ORF), ORFI and ORFII. To create the transgenic strains, plasmids containing the ORFs for each of these proteins with a *C. elegans promoter* to drive expression were constructed using Gibson cloning. Premade plasmids from a collaborator's lab containing a *C. elegans* promoter and the entire Orsay RNA2 segment were used as a starting point. Various fragments were amplified using polymerase chain reaction (PCR) (see Figure 5). The PCRs were done using the NEB Q5® High-Fidelity 2X Master Mix PCR kit.

Gibson Cloning

Gibson cloning is a reaction in which multiple overlapping DNA fragments are joined to form a new plasmid. The first step of Gibson cloning was choosing primers with overlapping ends to the ORF and the annealing ends of the plasmid vector. Using the chosen primers, we have amplified the ORF inserts for the capsid and delta-fusion proteins by PCR and the plasmid vector has been digested via restriction digest reaction. The Gibson cloning reaction will then join the ORF insert and the vector together to form the desired plasmid product.

Background

In the evolutionary host-pathogens arms race, selective pressure between a pathogen and its host lead to the necessity for pathogens to evolve to evade detection by the host immune system, thus weakening the host's ability to effectively resist the pathogen. The goal of this project is to better understand the interactions between viruses and the immune systems of their hosts. In order to study innate immunity exclusively, the roundworm *C. elegans* is a useful invertebrate model because it lacks adaptive immunity and the complexities that arise from interactions between the two types of immunity.

Currently, the Orsay virus is the only known virus that naturally infects *C. elegans*. The Orsay virus is an RNA virus that activates an immune response in *C. elegans* called the Intracellular Pathogen Response (IPR). The Orsay virus has a very small genome containing only four proteins. Preliminary evidence suggests that either one or a combination of these Orsay virus proteins can suppress the IPR (unpublished data) In this project, the effects of the Orsay viral capsid protein and a novel capsid-delta fusion protein located on the RNA2 genome segment of the Orsay virus on the IPR will be investigated.

Background

Figure 1: ***C. elegans* as a model organism**
The roundworm *C. elegans* is a useful invertebrate model for studying innate immunity because it lacks an adaptive immune system. Additionally, the short 3-day life cycle of *C. elegans* and its high progeny production make it amenable to genetic crosses and modification. *C. elegans* is also a transparent, allowing for easy visualization of fluorescence.

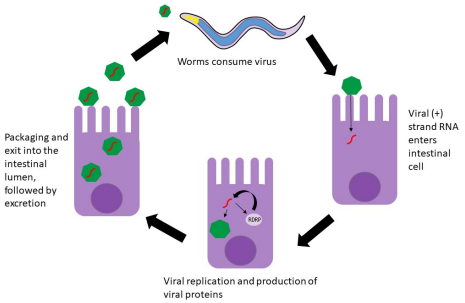

Figure 2: The Orsay virus is a positive-sense single-stranded RNA virus (or (+)ssRNA). Its genome consists of only four proteins, and it is a natural pathogen that infects *C. elegans*.

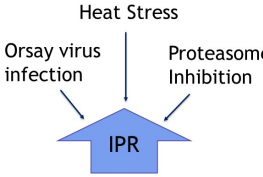

Figure 3: Stresses that upregulate the Intracellular Pathogen Response (IPR) Pathway in *C. elegans*
The IPR is a set of 80 genes that are upregulated when *C. elegans* is infected with an intracellular pathogen. Infection by the Orsay virus is known to activate the IPR. Heat stress can be accomplished by incubating the worms at 28°C for 24 hours. Proteasome inhibition can be achieved through treatment with Bortezomib.

Figure 4: Reporter for IPR gene expression
pals-5p is one of the genes of the IPR. IPR expression can be tracked using the *pals-5p::GFP* reporter, which drives expression of green fluorescent protein (GFP). Green fluorescence can be seen through the transparent bodies of *C. elegans* using a microscope.

Results

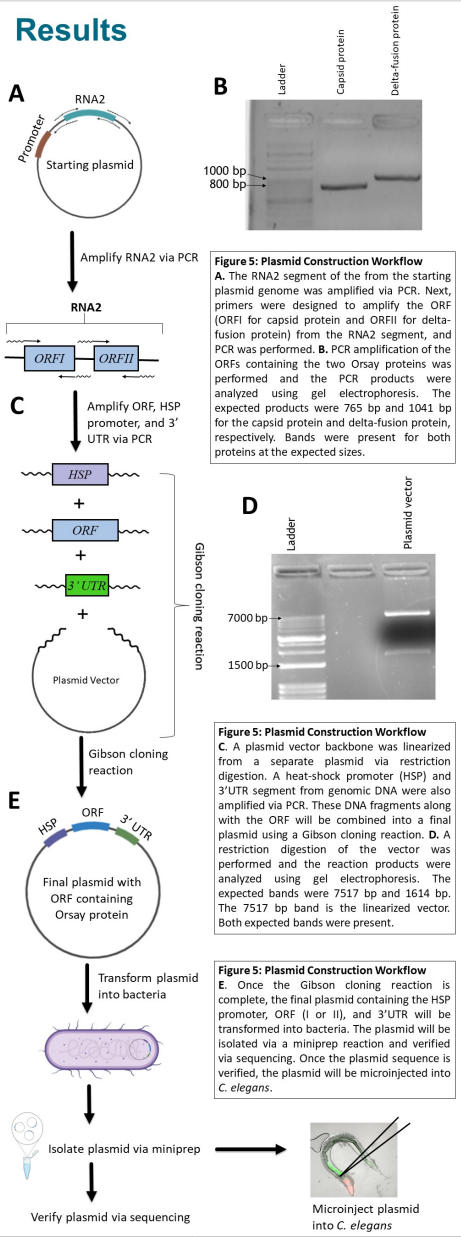

Figure 5: Plasmid Construction Workflow
A. The RNA2 segment of the from the starting plasmid genome was amplified via PCR. Next, primers were designed to amplify the ORF (ORFI for capsid protein and ORFII for delta-fusion protein) from the RNA2 segment, and PCR was performed. **B.** PCR amplification of the ORFs containing the two Orsay proteins was performed and the PCR products were analyzed using gel electrophoresis. The expected products were 765 bp and 1041 bp for the capsid protein and delta-fusion protein, respectively. Bands were present for both proteins at the expected sizes.

Figure 5: Plasmid Construction Workflow
C. A plasmid vector backbone was linearized from a separate plasmid via restriction digestion. A heat-shock promoter (HSP) and 3'UTR segment from genomic DNA were also amplified via PCR. These DNA fragments along with the ORF will be combined into a final plasmid using a Gibson cloning reaction. **D.** A restriction digestion of the vector was performed and the reaction products were analyzed using gel electrophoresis. The expected bands were 7517 bp and 1614 bp. The 7517 bp band is the linearized vector. Both expected bands were present.

Figure 5: Plasmid Construction Workflow
E. Once the Gibson cloning reaction is complete, the final plasmid containing the HSP promoter, ORF (I or II), and 3'UTR will be transformed into bacteria. The plasmid will be isolated via a miniprep reaction and verified via sequencing. Once the plasmid sequence is verified, the plasmid will be microinjected into *C. elegans*.

Conclusions

The ORFs, HSP promoter, and 3'UTR have all been successfully amplified via PCR. The backbone vector has also been successfully linearized via restriction digestion. All these DNA fragments are ready to be combined into a Gibson cloning reaction to form the final plasmid constructs that will express the Orsay virus capsid and delta-fusion proteins.

Future Directions

Gibson cloning and microinjection

The DNA fragments will be combined via Gibson cloning to form the final plasmid constructs containing the Orsay virus genes. The constructs will be verified and microinjected into the distal gonads of *C. elegans*, creating extrachromosomal arrays. Plasmids microinjected into the distal gonads can be transmitted to many progeny. Progeny carrying the viral protein expression array will be used to establish transgenic lines.

Stress Comparison

Once the constructs are microinjected, various stresses will be applied to *C. elegans* containing the Orsay virus genes (array) and a control group of worms without the Orsay genes (non-array). Through this comparison, we hope to determine if the worms with the Orsay genes activate the IPR to the same degree as those without the array. The stresses we will use are heat stress and Bortezomib. To determine levels of IPR activation, levels of the *pals-5p::GFP* reporter will be compared in worms with and without the viral protein expression array after the stresses are applied. Each array will also have a treatment control group. The heat stressed worms will be incubated at 28ºC for 24 hours, while the treatment controls will be incubated at 20ºC. Bortezomib is a proteasome inhibitor, and to induce the IPR using this method, 25mM Bortezomib dissolved in DMSO will be added to the plates the worms are on. The treatment controls for this method will have only DMSO added to their plates. We will be comparing eight groups of worms for each of the arrays: array and non-array at 20ºC, array and non-array under 28ºC heat stress, array and non-array with bortezomib, and array and non-array with DMSO.

Acknowledgements

Plasmids:

Dr. David Wang & Dr. Hongbing Jiang, Washington University in St. Louis School of Medicine

Dr. Emily Troemel, University of California San Diego

Funding:

West Chester University

Literature Cited

Félix, M.-A. et al. Natural and experimental infection of Caenorhabditis nematodes by novel viruses related to nodaviruses. PLoS Biol. 9, e1000586 (2011).

Bakowski, M. A. et al. Ubiquitin-mediated response to microsporidia and virus infection in C. elegans. PLoS Pathog. 10, e1004200 (2014).

Pratheek, B. M., Saha, S., Matti, P. K., Chattopadhyay, S. & Chattopadhyay, S. Immune Regulation and Evasion of Mammalian Host Cell Immunity During Viral Infection. Indian J. Virol. 24, 1–15 (2013).

Jiang, H. et al. Orsay virus utilizes ribosomal frameshifting to express a novel protein that is incorporated into virions. Virology 450–451, 213–221 (2014).

Yuan, W., Zhou, Y., Fan, Y., Tao, Y. J. & Zhong, W. Orsay δ Protein Is Required for Nonlytic Viral Egress. J. Virol. 92, (2018).

Jiang, H., Franz, C. J. & Wang, D. Engineering recombinant Orsay virus directly in the metazoan host Caenorhabditis elegans. J. Virol. 88, 11774–11781 (2014).