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Identifying Novel Triggers of the Intracellular Pathogen Response (IPR) in C. elegans

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Abstract

The intracellular pathogen response (IPR) is a cell signaling pathway found in *C. elegans* that is triggered when pathogenic microorganisms invade and immune responses attempt to eliminate the threat. Due to the relative simplicity of *C. elegans*, they are an excellent model organism to analyze the cell signaling pathways triggered by various pathogens. They lack the complex immune systems of larger organisms, making it easier to study the involved cell signaling pathways. Past studies have shown that the IPR can also be triggered via intestinal wounding. Heat stress, viral infection, and proteasome stress are all known triggers of the IPR. The long-term goal of this project is to generate potential new triggers, the response of which will be compared to the response generated by known triggers. The IPR will be quantified by measuring nanoluciferase expression, which is driven by an IPR gene. The immediate goal of this project is to develop this nanoluciferase assay system to generate IPR activity data in a high-throughput fashion. We hope to eventually be able to relate our findings to similar pathways in humans and other vertebrates. While humans do not have the IPR that is found in *C. elegans*, they have similar pathways that perform equivalent functions. In particular, we are interested in how intestinal damage could be related to activation of innate immunity. This information could be used to gain a better understanding of gastrointestinal diseases, specifically inflammatory bowel diseases.

Methods

Creating a Dual Luciferase Reporter Strain

We currently have possession of a *pals-5p::nanoluc* strain of *C. elegans* containing the *pals-5* promoter driving nanoluciferase (Nanoluc) expression. We want to add to this strain a Firefly luciferase expressed from a housekeeping gene promoter. Using a dual reporter assay system kit will allow for the study of Nanoluc activity and Firefly activity at the same time (Nano-Glo® Dual-Luciferase® Reporter Assay System) . This will allow us to normalize for differences in worm size or number using Firefly as an internal control, greatly increasing the precision of our IPR activity measurements.

First, we will selectively breed a strain of *C. elegans* that are homozygous for both Nanoluc and Firefly expression. We will do this by crossing a strain homozygous for the *pals-5p::Nanoluc* to a strain homozygous for *sur-5p::Firefly luc*. The F1 generation will contain only heterozygotes for both traits, but upon examination of the F2 generation, 1/16th of the progeny should be homozygous for both luciferase-encoding genes. To determine if an individual is homozygous for both traits, its progeny will be examined using both PCR and the dual reporter assay system kit. If the individual is truly homozygous for the expression of both traits, all of the progeny should also be homozygous for the expression of both traits.

Testing IPR Triggers

Once a viable homozygous strain is produced, the organisms will be used to test the effect of various substances and environmental pressures on IPR activation in *C. elegans*. We will do this by measuring the activity of the *pals-5p::Nanoluc* gene in comparison to the activity of the *sur-5p::Firefly luc* gene. Different stimuli turn on the IPR to different degrees, which can be measured with the sensitivity of the dual reporter assay system. Bortezomib is a chemical that is known to strongly activate the IPR by creating proteasome stress (Reddy et al., 2017). Heat stress and viral infection are also known triggers of the IPR, but the IPR is less sensitive to these triggers (Reddy et al., 2017; Sowa et al., 2020). We will use these known triggers as a comparison to identify additional triggers of the IPR. Unpublished preliminary evidence shows that intestinal damage also activates the IPR. Therefore, we plan to test multiple forms of intestinal wounding.

Nanoluciferase Dual Reporter Assay

To perform the experiment, we will first treat the worms with their respective stressor (or a control condition), grind the worms using silicon beads, and add NanoGlo Reagent, which will allow for the quantification of the activity of both Nanoluc and Firefly luciferases. The samples will be placed in 24-well plate, and luminescence will be measured. The results will be expressed as a ratio of Nanoluc to Firefly expression.

Background

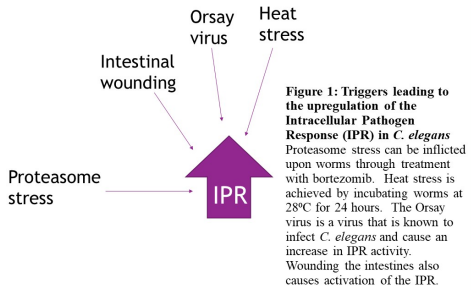
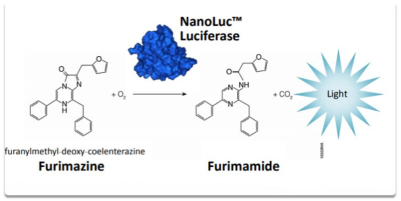
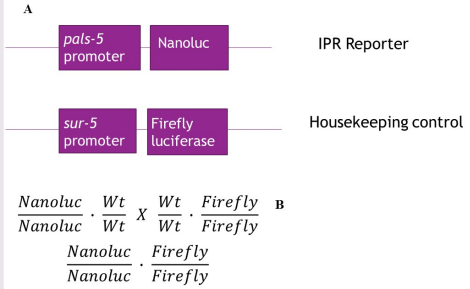


Figure 1: Triggers leading to the upregulation of the Intracellular Pathogen Response (IPR) in *C. elegans*
Proteasome stress can be inflicted upon worms through treatment with bortezomib. Heat stress is achieved by incubating worms at 28ºC for 24 hours. The Orsay virus is a virus that is known to infect *C. elegans* and cause an increase in IPR activity. Wounding the intestines also causes activation of the IPR.



Kopish et al., 2012

Figure 2: Diagram of substrate oxidation in nanoluciferase assay
Unlike florescence, as seen in GFPs, bioluminescence is caused by oxidation of a substrate, which, in this case, is furimazine. The oxidation of furimazine produces furimamide, carbon dioxide and light.

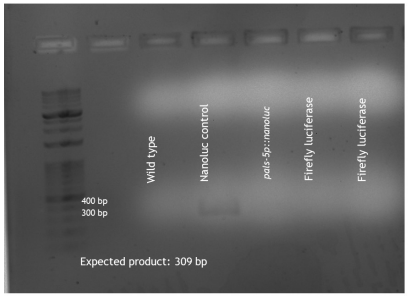


$$\frac{Nanoluc}{Nanoluc} \cdot \frac{Wt}{Wt} \; X \; \frac{Wt}{Wt} \cdot \frac{Firefly}{Firefly}$$

$$\frac{Nanoluc}{Nanoluc} \cdot \frac{Firefly}{Firefly}$$

Figure 3: Nanoluc and Firefly luciferase crossing strategy
A. The *pals-5* promoter drives the expression of nanoluciferase, the IPR reporter, and the *sur-5* promoter drives the expression of firefly luciferase, the housekeeping gene. **B.** When a strain homozygous for Nanoluc expression is crossed with a strain homozygous for Firely expression, Mendelian genetics predicts that 1/16th of the progeny will be homozygous for both Nanoluc and Firely expression. This strain will be used for experimentation to allow for the dual reporting of both luciferase genes in a single strain.

Results



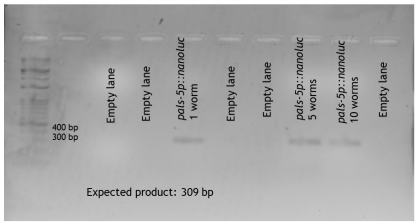


Figure 4: Verification of genotyping strategy via PCR
PCR was performed to amplify the segment of the genome containing the *nanoluc* coding sequence, and the resulting PCR products were analyzed using gel electrophoresis. The expected product was 309 bp, and the bands present aligned with that region of the ladder. Bands were present in strains containing the *nanoluc* coding region, including the *nanoluc* control and the *pals-5p::nanoluc* strain. Bands were not the Firely strains or wildtype control, which do not contain this region.

Experimental Plan

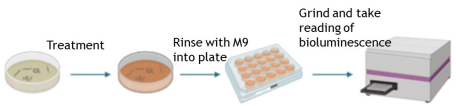


Figure 5: Using bioluminescence to quantify levels of IPR activation
To test levels of various factors, the worms will first be treated with the substance being tested. Afterwards, they will be rinsed into a plate using M9 (Sfarcic et al. 2019). Then, lysis buffer will be added to the worms and they will be ground using silicon carbide beads (Sfarcic et al. 2019). The NanoGlo reagent will then be added to the ground worm lysate (Sfarcic et al. 2019) and a reading will be taken on the plate reader and can be expressed as a ratio of Nanoluc to Firefly luciferase.

Conclusions

The proposed genotyping strategy using PCR and gel electrophoresis is viable for the purposes of this experiment

Future Directions

- Perform genetic cross to create a worm strain homozygous for both Nanoluciferase and Firefly luciferase expression
- Trial run of the Nanoluc Dual Reporter Assay System using known triggers of the IPR such as viral infection, heat shock, and proteasome stress via the chemical Bortezomib
- Identification of additional triggers of the IPR through various intestinal wounding methods such as feeding worms diamond dust, treatment with chemicals such as TNBS and oxalazone, and electroporation
- Development of a high-throughput method of inflicting intestinal wounding on worms

Acknowledgements

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