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Generating a novel fly line to quantify cellular oxidative stress on developing Drosophila melanogaster

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Abstract & Background

The redox condition of the cell, the ratio of reactive oxygen species to reducing agents present, can indicate the likelihood of oncogenesis. There are several genetic pathways present, such as the Nrf2 pathway in humans, which moderate the level of reactive oxygen species (ROS) within the cell. The Nrf2 pathway is an orthologue to the cncC pathway in *Drosophila melanogaster*, making *Drosophila* an ideal model organism to study redox pathways in the cell.

Previous work has shown that cncC alterations can alter oncogene levels in *Drosophila*, however it hasn't been shown that this manipulation truly alters ROS levels within the cell. To solve this, a fly line was created which could indicate and quantify ROS levels via fluorescence microscopy. This was done through use of the UAS-GAL4 system; a system which allows for the introduction and expression of desired genes within the *Drosophila melanogaster* genome. For this desired purpose, both the UAS-GAL4 indicator and ROS GFP reporter need to be on the same chromosome, so recombination was necessary for the success of this introduction. Through several rounds of genetic crosses, a fly line was established with a UAS-GAL4 indicator and a ROS GFP reporter on the same chromosome within the selected flies. Recombination occurred in 26% of 250 offspring.

This recombination will allow quantification of ROS in developing wings, guts, and testes via GFP fluorescence.

Methods

Stock fly lines were crossed to create heterozygotic offspring with both desired genes present. First, this cross was conducted with UAS-GAL4 indicator flies and cytoplasmic ROS GFP reporter flies. Both UAS-GAL4 and ROS GFP are necessary for oxidative stress indication in the developing larvae.

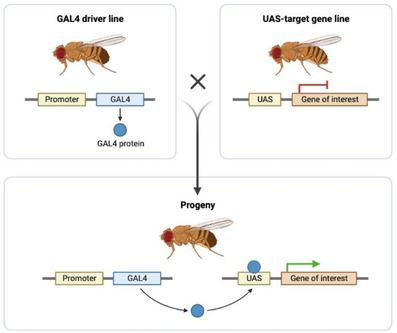

Figure 1. **GFP fluoresces in the presence of reactive oxygen species (ROS).** Image from BioRender.

Flies with both genes of interest were selected and crossed with their-eyed, wild-type flies to easily identify recombination. To perform future experiments on whether cncC alters cellular ROS levels, both the UAS-GAL4 sequence and ROS GFP need to be on the same chromosome. Thus, we selected for offspring in which recombination has occurred, as seen through eye color and fluorescence.

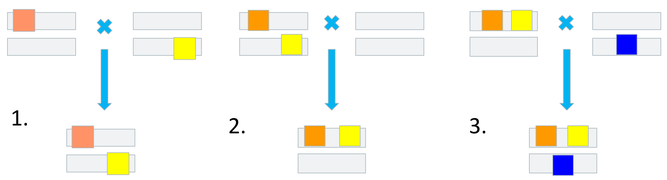

Figure 2. **Sample illustration of genetic scheme.** In order for subsequent experiments, recombination is necessary for both genes of interest to be on the same chromosome.

Results

26% of the 250 offspring from heterozygous parents showed recombination of both indicator and reporter on the same chromosome. 24% of the offspring collected had white eyes, indicative of neither indicator nor reporter. The flies with either indicator or reporter were also found in near 1:1 ratio to each other, at approx. 25% of the offspring each.

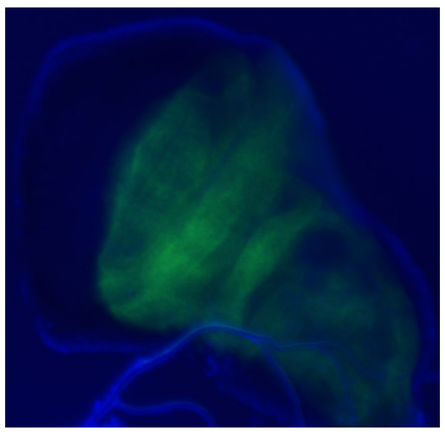

Figure 3. **Composite image of wing disc from *Drosophila melanogaster* larvae.** The wing discs can be easily dissected from *Drosophila* larvae. The apterous GAL4 UAS sequence causes the GFP to fluoresce in the dorsal compartment of the wing disc (green). The blue DAPI stain shows the nuclei within the disc of the wing disc. This image confirms the expression of UAS-Gal4/ROS GFP within the selected offspring.

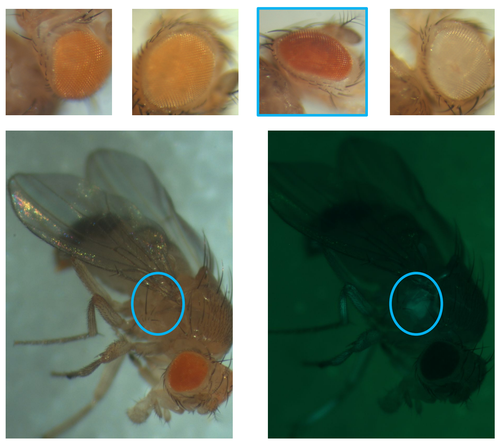

Figure 4. **Recombinant fly eye color and fluorescence under UV light.** The recombinant flies were identified first by eye color, then by fluorescence microscopy. Figure 4A-D show the four distinct eye colors present in the offspring: Both the indicator and reporter are linked with orange-yellow eye color, thus, offspring with both genes will have dark red eyes due to the cumulative effect of the two eye color genes. Figure 4E shows the dark red eye color associated with recombinant flies. Figure 4F shows the same fly under UV light. Recombinant flies were confirmed via fluorescence at the junction between the wing and abdomen, seen circled above.

Conclusions & Future Directions

Several conclusions can be drawn from the results of this experiment.

1. The UAS-Gal4/ROS GFP system is effective at measuring ROS levels in developing wing discs.
2. The UAS-GAL4 indicator and ROS GFP reporter are both on chromosome II, but they are not near each other. This allowed for recombination to occur readily.

Based on these conclusions, further research can be conducted to quantify whether cncC actually alters cellular ROS levels. This will also enable us to investigate the effect of overexpressing cncC in these flies to determine the role cncC plays in managing oxidative stress. This will provide insight in to the ability of oncogenes to promote cancer.

This model system can be used to better understand how antioxidants relate to cancer development in the human system. By understanding the role of ROS in oncogene expression, treatments and targets for cancer prevention can be better understood.

Acknowledgements

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