Function of the Putative Thioredoxin Reductase Gene *trxr-2* in *Drosophila melanogaster*

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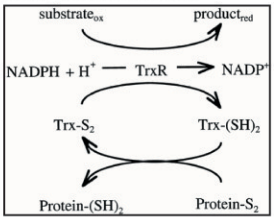
Abstract

The *trxr-2* gene is currently an uncharacterized gene that on the basis of sequence homology probably encodes an enzyme which plays a role in cellular defenses against oxidative damage and aging. No one has described any mutants in the *trxr-2* gene. We are developing RNAi plasmid constructs to create flies which do not express the product of the *trxr-2* gene in order to determine what role it plays in *Drosophila*. RNAi (post-transcriptional gene silencing) is a novel gene silencing technique that uses double stranded RNA homologous to portions of the target gene sequence to destroy the mRNA of the target gene product. The molecular construct necessary for this gene silencing will be prepared in the form of a stable transgene that produces a dsRNA hairpin loop that will silence the expression of the *trxr-2* gene. We will examine these *trxr-2* mutants for abnormalities in development, morphology, behavior, and longevity in order to deduce the general function and importance of the *trxr-2* gene.

Introduction

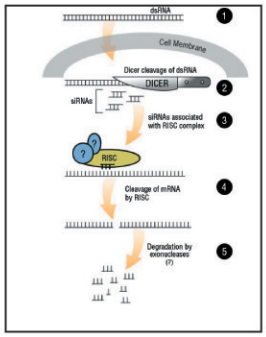
The *trxr-2* gene has not yet been characterized but on the basis of sequence homology may encode an enzyme which plays a role in cellular defenses against oxidative damage and aging. All aerobic organisms produce reactive oxygen species (ROS) as byproducts of oxygen metabolism. ROS molecules such as hydroxide radicals and hydrogen peroxide are neutralized by the actions of flavoenzymes such as glutathione reductase and thioredoxin reductase.



Glutathione (GSH) is a tripeptide antioxidant that is ubiquitously present in mammals, including humans. In the presence of ROS molecules, GSH is oxidized to GSSG, saving other cell components, and is then reduced back to GSH by the enzyme glutathione reductase (GR). *Drosophila melanogaster* does not have any GR; instead its cellular defense system depends on thioredoxin reductase (TR). Thioredoxin reductase is a disulfide reducing enzyme that reduces thioredoxin which then reduces molecules such as glutathione.

There are two closely related genes for thioredoxin reductase in the *Drosophila* genome, *trxr-1* and *trxr-2*. Severe mutations of the *trxr-1* gene result in larval lethality. No one has yet characterized any mutants in *trxr-2* gene. It is not known whether the *trxr-2* gene is functional or if it plays any role in antioxidant defense. I plan to silence the *trxr-2* gene through RNA interference (RNAi) in order to study the general function and importance of the *trxr-2* gene product in *Drosophila*.

RNAi is the process in which small fragments of double-stranded RNA (dsRNA) induce the silencing of homologous endogenous genes. Transcription of the target gene is unaffected, but gene expression is silenced as the mRNA molecules are degraded. RNAi is a phenomenon which occurs naturally in organisms in which an enzyme called dicer encounters double-stranded RNA and chops it into small pieces called small interfering RNAs (siRNAs). These siRNAs are collected by a complex of proteins called the RNA-induced silencing complex (RISC), which uses the coding sequence of these siRNAs to search out and destroy all mRNAs in the cell with a matching sequence.



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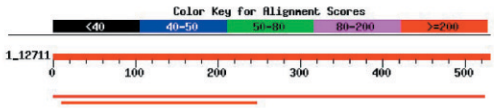
Research Methods

Polymerase chain reaction (PCR):
Polymerase chain reaction (PCR) is a common method for creating copies of specific DNA fragments. We amplified our trxr-2 gene sequence using the two primer sequences shown in bold.

(**GACTAGTTCAAGCCCACTCC**AGTGGGCACCAAGTGGGGCATCGGCGGCACCTGCGTGAATGTGGGCTGCATC
CCCAAGAAGCTAATGCACCAGGCCTCGCTGCTGGGCGAGGCTGTTCACGAGGCGGTGGCCTACGGCTGGAATG
TAGACGACACGAACATACGGCCCGATTGGCGCAAGTTGGTGCGCTCCGTCCAGAACCACATCAAGTCCGTCAAC
TGGGTGACCCGCGTGGACCTGCGCGACAAAAAGGTGGAGTACGTAAATTCGATGGCCACCTTTCGCGACAGCCA
CACCATCGAGTATGTGGCGATGCCAGGTGCCGAGCACCGTCAAGTGACCTCAGAGTACGTGGTGGTAGCCGTCG
GCGGGAGGCCACGCTACCCGGACATTCCCGGAGCCGTTGAACTGGGCATCACCAGCGACGATATATTCAGCTAC
GAGCGAGAGCCGGGTCGTACCCTTGTGGTGGGCGCCGGATACGTGGGTCTCGAGTGCGCCT**GTTTCCTTAAGG
GTCTCGGCTAGCAGC**)

Blast Search Results:
Distribution of 2 Blast Hits on the Query Sequence



Sequences producing significant alignments:

Drosophila melanogaster chromosome 3L, *trxr-2* gene sequence
Drosophila melanogaster chromosome X, *trxr-1* gene sequence

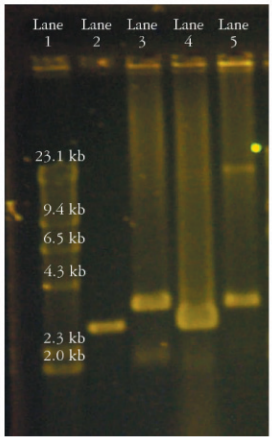
The section of the *trxr-2* gene that I amplified is specific for the *trxr-2* gene located on the 3rd chromosome in *Drosophila*.

Restriction enzyme digest:
Restriction enzymes are enzymes that recognize specific sequences in DNA and cleave the DNA at those recognition sequences to produce the restriction fragments.

Gel electrophoresis:
A technique that uses an electrical current to separate the fragments of DNA molecules based on size. Smaller DNA fragments move through the gel faster than larger DNA fragments. Gel electrophoresis is used to check the size of DNA molecules or separate DNA fragments after the DNA has been cut with restriction enzymes.

Results and Future Directions

We have successfully cloned the *trxr-2* gene sequence into the LITMUS28i plasmid, from which we cut and inserted the *trxr-2* gene sequence into the pHIBS plasmid. We are currently working on the insertion of *trxr-2* gene sequence into the pUdsGFP plasmid. This last step of our project will result in the final molecular plasmid ready for injection into *Drosophila* eggs. After the injection of white-eyed *Drosophila* eggs with pUdsGFP plasmid containing the inverted repeat *trxr-2* gene sequence, we will identify the "right" fruit flies (those that incorporated the pUdsGFP plasmid into their genome) by their red eye color. We will characterize these red-eyed mutants phenotypically and investigate the role of *trxr-2* gene in cellular defenses against oxidative damage and aging.

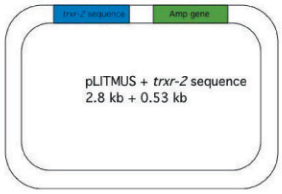


Lane 1 - HindIII lambda marker
Lane 2 - LITMUS28i plasmid cut with XbaI restriction enzyme
Lane 3 - LITMUS28i plasmid containing *trxr-2* gene sequence insert cut with EcoRI restriction enzyme
Lane 4 - pHIBS plasmid cut with EcorI restriction enzyme
Lane 5 - pHIBS plasmid containing *trxr-2* gene sequence insert cut with XhoI restriction enzyme

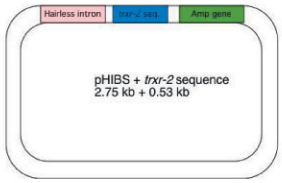
Plasmid Maps

Individual plasmids used in the cloning strategy to develop the final pUdsGFP plasmid forming a dsRNA hairpin loop

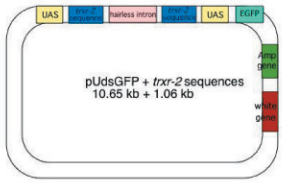
LITMUS28i plasmid containing the *trxr-2* gene fragment



pHIBS plasmid containing the *trxr-2* gene fragment



Final pUdsGFP plasmid containing two *trxr-2* gene fragments



Conclusion

We are in the process of preparing the molecular construct which will act as a stable transgene that forms a dsRNA hairpin loop that will silence the expression of the *trxr-2* gene. We hope to have this construct ready in the next few months. Moravian students who decide to continue this research project in the future will have an opportunity to examine these *trxr-2* mutants for any abnormalities in development, morphology, behavior, and longevity in order to deduce the general function and importance of the *trxr-2* gene in *Drosophila* biology.

Works Cited

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RNAi Figure accessed at:
http://www.ambion.com/techlib/tn/101/7.html

Acknowledgements

Moravian College Honors Program

Student Opportunities for Academic Research (SOAR) Program, Moravian College

Kristine Kotsch, StudioSouth designer, Moravian College

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