Microarray analysis of gene expression during anoxia in *C. elegans*

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Background

- In humans, periods of hypoxia (low oxygen) or anoxia (no oxygen) are associated with many diseases, including cancer, stroke, and cardiac failure.
- A better understanding of the cellular changes occurring during oxygen deprivation will be important in increasing the survival rate for patients suffering from a stroke.
- The nematode *C. elegans* is an excellent model organism for these studies, because their neurons go through cell death pathways similar to humans and their genome has been completely sequenced.

Goal

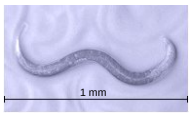
- Use DNA microarrays to analyze the entire *C. elegans* genome and determine changes in gene expression that occur during exposure to anoxic conditions.

Specific Aims

- Conduct viability assays at 24, 36, 72, and 96 hours of exposure to anoxia to confirm the ability of *C. elegans* to survive extended periods in anoxic conditions.
- To determine which neuronal genes are changing expression (upregulated/ downregulated) in response to anoxia.

Experimental advantages of *C. elegans*

- Free-living soil nematode
- Genome is fully sequenced
- Easy and economical to manipulate gene function
- Easy to grow and culture
- Neuronal death pathways similar to humans



Methods

Viability Assay

Synchronize worms

↓

Expose worms to anoxia for 24, 36, 48, 72 or 96 hours

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Remove worms from anoxia

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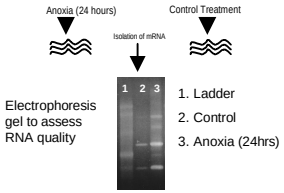
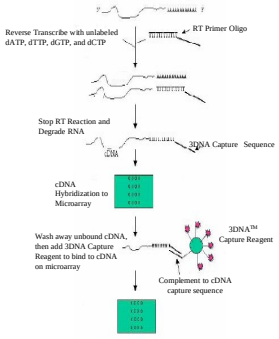
After 24 hours in normal oxygen levels (normoxia), count dead and alive

Microarray Procedure

I. Expose worms to anoxia / Isolate / Assess RNA



II. cDNA Synthesis / Labeling / Hybridization

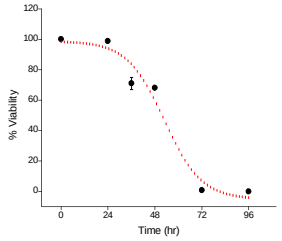


III. Send slides to GCAT for scanning

IV. Eagerly await results

Results

C. elegans can survive extended periods without oxygen

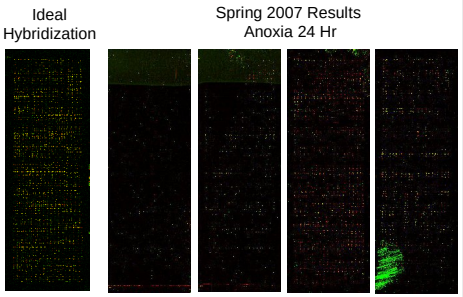


Interpreting Microarray Results

- All control DNA was labeled with Alexa 546 (green)
- All anoxia DNA was labeled with Alexa 647 (red)
- Relative gene expression in anoxia vs. control conditions is indicated by the color intensity of each spot representing a single gene

Anoxia > Control
Anoxia < Control
Anoxia = Control

Microarray results



Summary of Microarray Results

- An ideally hybridized microarray chip (slide) from the Washington University *C. elegans* Center is shown for comparison
- As demonstrated on the 24 hr anoxia chips, hybridization quality improved as we increased starting RNA concentration
- However, hybridizations are still not optimal since dye-swapping results are not consistent

Conclusions

- Our studies confirm previous data demonstrating that *C. elegans* can survive over 48 hours in anoxic conditions without profound effects on viability
- Hybridization procedure needs optimization
- Our microarray data need further analysis to confirm hybridization quality and validate results

Future Studies

- Modify microarray hybridization to improve cDNA binding
  - Test different hybridization temperatures
  - Confirm RNA quality (denaturing gels)
  - Increase initial RNA concentration
- Thoroughly analyze microarray results

Acknowledgements

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  - GCAT (Genome Consortium for Active Teaching) for microarray slides and scanning services.