Finding *dex-1* Phenotype Suppressing Components

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PRECS Phenotypic Plasticity Research Experience for Community College Students

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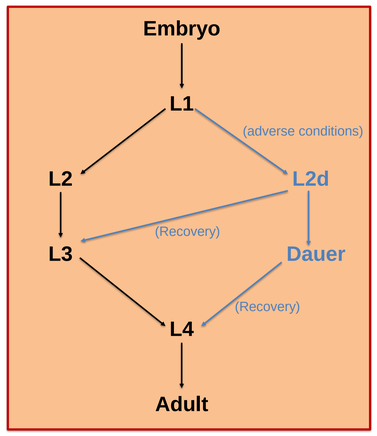
Background

***C. elegans*:**

- *Caenorhabditis elegans* is a species of microscopic round worm that has been used as a genetic model for over forty years.

- When in an adverse environment, *C. elegans* larvae cease reproductive development and enter the stress-resistant dauer stage[1] (*Fig.2*).



- The focus of our lab is characterizing the genetic pathways that facilitate morphological changes that occur during the dauer stage.

dex-1 mutants

***dex-1* mutants of *C. elegans*:**

- DEX-1 is an extracellular matrix protein in *C. elegans* responsible for proper dendrite morphology in the early stages of the worms growth.[2]

- *dex-1* mutants are deficient in this protein, resulting in shortened dendrites and a sensitivity to sodium dodecyl sulfate (SDS).

- SDS will kill any non-dauer *C. elegans*, but wild type dauers will survive well past the standard concentration of 1% SDS. Thus, treatment with SDS is commonly how labs isolate dauers.

- In contrast, *dex-1* dauers (*Fig.3*) will die when exposed to 1% SDS, but can potentially survive in less (*Fig.1*).

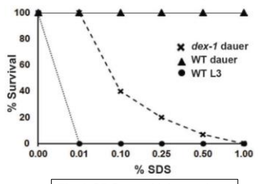


Fig.1 SDS sensitivity scale.

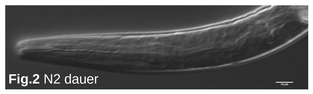


Fig.2 N2 dauer

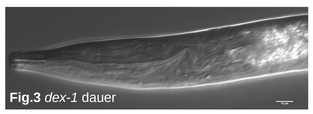


Fig.3 *dex-1* dauer

Project Outline

Our project set out to find potential interactors of *dex-1* during dauer by conducting a suppressor screen

Suppressor Screening

- We started by finding *dex-1* mutants that've suppressed their SDS sensitivity, and we did this through **mutagenesis** and **SDS selection**.

- This involved mutating *dex-1* mutants with EMS, and exposing their F2 progeny to SDS while in dauer. We then moved on to **mapping the survivors mutation**.

Gene Mapping

- Once we isolate suppressor mutants, we will perform several **crosses between these mutants and mapping strains**.

- Based on the crosses **progeny's phenotypic ratios**, these crosses will tell us what chromosome the mutated gene lies on, and further crosses will narrow down and eventually determine the map position.

- After finding that position, we will track the new gene's resulting protein and its effect.

Mutagenesis

Mutating the worms:

- We synced worms up at the L4 stage (*Fig.4*)

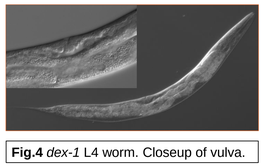


Fig.4 *dex-1* L4 worm. Closeup of vulva.

- Mutated synced L4's with Ethyl Methanosulfonate
  - We were sure to use proper safety equipment, as EMS is toxic

- Checking every day and separating individuals when needed, we allowed the mutated P0 generation to lay the F1 generation, then we let those F1 lay the F2 (which are able to have recessive phenotypes).[3]

- For this reason, these F2 are the worms we screened with SDS.

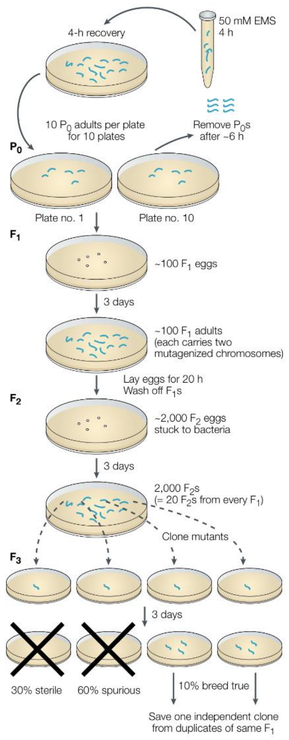


***Fig.5** Protocol for EMS mutagenesis[3]*

SDS selection

Screening the worms:

- Got F2 gen to become dauers
  - We did this by placing adults older than L1 at 25°C. This causes their offspring to reach dauer thanks to a secondary mutation in our worms known as daf-7

- Exposed the dauer F2 to SDS

- Picked survivors onto their own plates

- From here we can move onto gene mapping

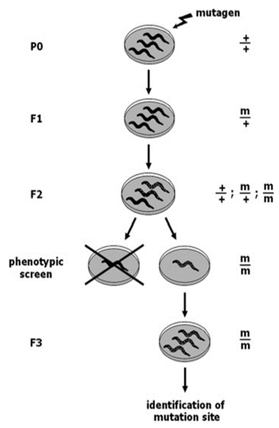


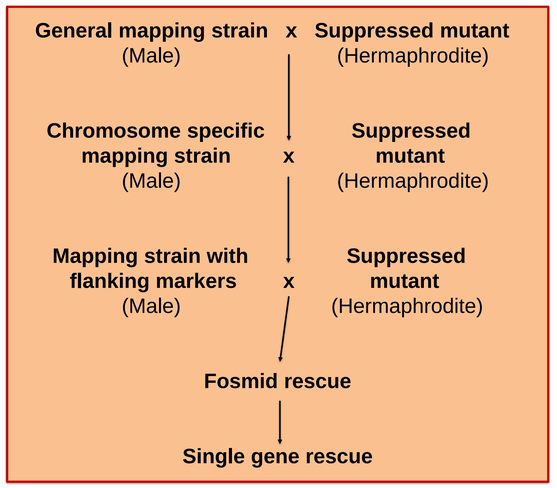
Fig.6 Protocol for a suppressor screen.[4]

Findings

- After washing off a bit over 100 plates, and exposing the worms to SDS, we found **7 independent mutants** that suppressed their *dex-1* phenotype.

Further Research

Mapping a Gene:



- With the exact mutation site found, we can track the gene's resulting protein's pathway and determine what kind of suppression it is.

Potential types of suppression mutation

Intragenic mutation

- Same site replacement**:** a form of suppression wherein the originally mutated gene is simply mutated back to N2.

AGA CCC CGA CCA	(N2 protein)
AGA CCC TGA CCA	(*dex-1* protein)[2]
AGA CCC CGA CCA	(Suppressor's protein)

- Compensatory second site mutation**:** a mutation elsewhere on the protein that affects it in such a way that the original mutation's phenotype is reduced. Rare.

Extragenic

- Bypass suppression**:** a mutation that activates an alternate pathway, "bypassing" the defective components.
  - For example, a mutation in a non-coding region that results in a change in the level of *dex-1* expression.

- Suppression by interacting components: a mutation that restores an abnormal protein to proper functionality by changing an interacting protein.[5]
  - For example, the CUT protein is an interactor with the DEX-1 protein, so a change in it could potentially cause a suppression by interacting components.

References

1) Cassada, R. C., & Russell, R. L. (1975)

2) Heiman, M. G., & Shaham, S. (2009).

3) Jorgensen, E. M., & Mango, S. E. (2002)

4) Sin, O., Michels, H., & Nollen, E. (2014)

5) Hodgkin, J. Genetic suppression (2005)

Acknowledgments

Financial support was provided by the National Science Foundation under grant #NSF REU 1559908/1559929, as part of the Phenotypic Plasticity Research Experience for Community College Students, through the University of Illinois at Urbana-Champaign Institute for Genomic Biology and Parkland College. http://precs.igb.illinois.edu/

I'd like to thank the project directors, Dr. Nathan Schroeder and Dr. C. Britt Carlson. Thank you as well to my mentor Kristen Flatt and the whole Schroeder lab for all the encouragement.

NSF