Using Fluorescence Assays to Explore the Regulation of the Kynurenine Pathway in *Neurospora crassa*

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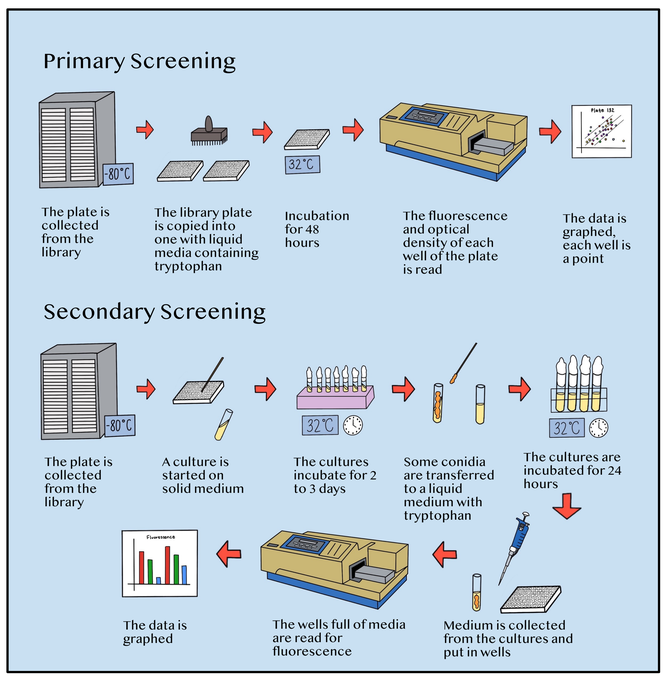
Introduction

In *Neurospora crassa* (a filamentous fungus), there are several enzymes that cause the breakdown of tryptophan into fluorescent anthranilic acid, several of whose genes have the chromatin marker that our lab studies, specifically methylation of lysine 27 of histone H3. If we give a *N. crassa* culture tryptophan and it fluoresces under UV light, this indicates that the genes are "turned on" normally; if it doesn't, they could be abnormally "turned off." Understanding which genes contribute to the regulation of this pathway could illuminate general chromatin control processes. Conveniently, our lab already has a collection of mutants, the FGSC knockout library, that include mutants with knockouts of almost every gene in the *N. crassa* genome.

Research Question

We wanted to know if there are any additional unknown genes that affect the regulation of the kynurenine pathway. If we could identify genes that are essential in the functioning of the pathway, these could potentially be affecting the H3K27 methylation. Using the FGSC knockout library, I conducted a screen of the collection that used relative fluorescence data after the strains had been given liquid medium containing tryptophan.

Methods

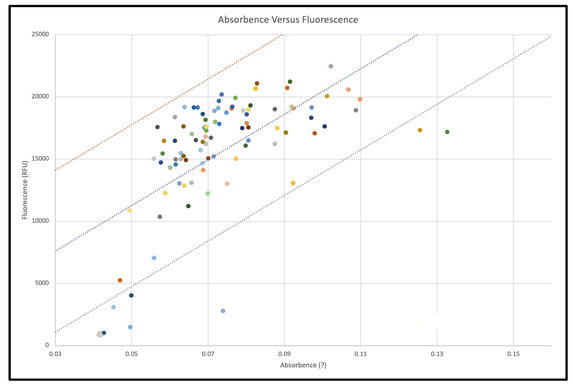


This figure pictorially represents the methods used in the primary and secondary screenings of the FGSC knockout library.

Primary Screening

For the primary screen, the strains were analyzed using fluorescence data and optical density data (i.e. how much tissue grew). The strains that showed tissue growth coupled with low or high fluorescence were advanced to the next screening round. There were around 100 strains identified from this screening that moved on.

To the right is an example graph from plate 16 . There were 135 others similar to this. The three lines on the graph are the linear line of best fit, and plus and minus one tenth of a standard deviation away.



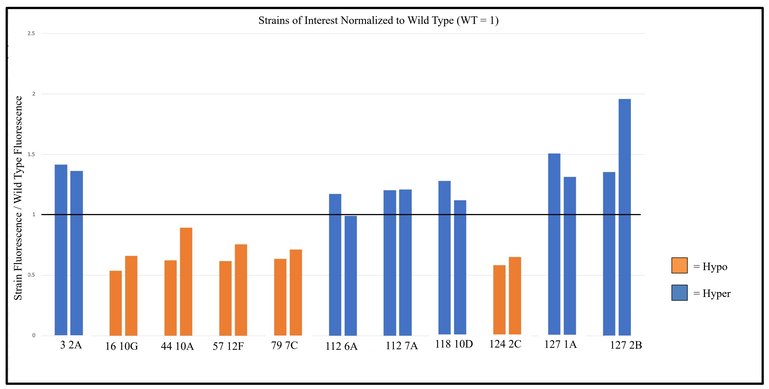
Secondary Screening

To the right is a table of the strains that passed the secondary screening, their various identifying numbers, and their phenotype.

NCU Number (gene database number)	Library Plate	Library Well	Hypo/Hyper Fluorescence Phenotype
NCU09252	3	2 A	Hyper
NCU06801	16	10 G	Hypo
NCU04105	44	10 A	Hypo
NCU05559	57	12 F	Hypo
NCU05950	79	7 C	Hypo
NCU10762	112	6 A 7 A	Hyper
NCU08046	118	10 D	Hyper
NCU16316	124	2 C	Hypo
NCU16805	127	1 A	Hyper
NCU16823	127	2 B	Hyper


A culture of *N. crassa*

The bar chart shows the fluorescence of two cultures per strain in relation to wild type (WT = 1). The blue bars are hyperfluorescent and the orange are hypofluorescent.



Additional Characterization

- PCR confirmation of the mutants by using a forward primer in the upstream region of the gene and a reverse primer in the hygromycin resistance cassette that replaced the gene
- Searching the BLAST database for homology of the proteins made by the genes
- A time course that allowed the mutants I identified and previously known mutants that disrupt the regulation of this system to be compared over three hours, and another at two days. Some of this time course was inconclusive, but mainly the strains I identified were on par with the strains previously known to affect the chromatin markers of the kynurenine pathway.

Future Directions

Actions Already Underway:

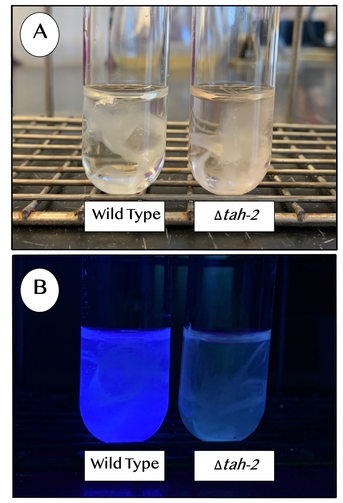
- Complementation by crossing the strains of interest with a *his3-* strain to see if the genes that are knocked out cause the phenotype

Future Actions:

- Re-attempting to tag *tah-2* (a previously known gene that is required for the function of the kynurenine pathway)
- Western blots on enzymes in the kynurenine pathway whose genes are marked by H3K27me. This would see if the enzyme transcription is being affected or if something else in the pathway is
- More generally: answering the question of why these mutants have these phenotypes.

Figures A and B to the right show two cultures, wild type and Δ*tah-2* (a gene known to disrupt the kynurenine pathway). The cultures are shown in daylight and in UV light. The wild type culture is noticeably more fluorescent than the Δ*tah-2* culture, meaning that it is producing more anthranilic acid, as anticipated.

Note: While *Neurospora crassa* is typically orange, in liquid cultures it is white. See the figure in the Secondary Screening section for a picture of orange *Neurospora crassa*.



References

Storck, William. Characterization of LSD Complex Function, Histone Exchange, and Regulation of a Tryptophan Catabolism Gene Pair in *Neurospora crassa*. 2020. University of Oregon, PhD dissertation.

Acknowledgments

I would like to acknowledge Eric Selker, Colleen Mumford, Jeannie Selker, Will Storck, and Kevin McNaught in this project so far. Without any of them this project could not be where it is. I would also like to acknowledge the FYRE fellowship for allowing me to work all of last summer on this project and really get started in a way that made everything else possible.