Researching the Hedgehog Pathway

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I - INTRODUCTION

The **Hedgehog pathway (Hh)** is a cell signaling pathway that functions in tissue development and is associated with many varieties of cancer (Briscoe and Therond, 2013, Pak and Segal, 2016). The work in this project involves ascertaining what role cholesterol bonding has on the Ptch protein in the Hh pathway, and which sequences in Ptch are important for successful cholesterol binding.

II - BACKGROUND AND SIGNIFICANCE

Fundamentally, the end goal of this project is to learn more about a cell pathway connected to cancer, in the hopes of someday contributing to the effort against it.

Cancer is caused primarily by malfunctioning cell processes and pathways, one such being Hh (see above). The section of the pathway my project focuses on involves regulation of Hh in cells. In order to have Hh activated, cells receive a protein called **Sonic Hedgehog**[1]**(Shh)**; this protein serves to inhibit **Patched (Ptch)**, a membrane protein very important in Hh. Ptch in turn inhibits **Smoothened (Smo)**, another protein that's connected to the rest of Hh downstream. When uninhibited, Smo activates the rest of the pathway (like a double negative - see Fig. 2). Mutations in Ptch and Smo are the cause of most Hh-dependent cancers (Briscoe and Therond, 2013, Pak and Segal, 2016). Regulated activation of Hh is important to correct development (Fig. 1).

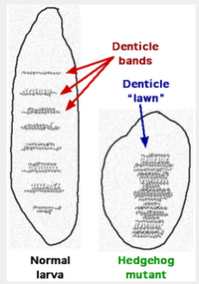

Fig. 1: *Lack of a Hh gene in* Drosophila *larvae results in shortening/denticles (hairs) uniformly distributed on the larva; hence the name "Hedgehog".*

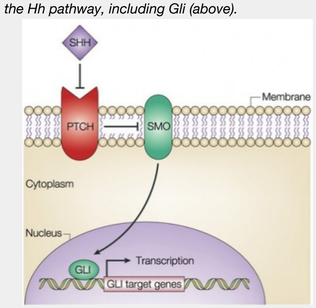

Fig 2: *The Shh protein inhibits Ptch (indicated by the line with the bar at the end), which inhibits Smo. Introduction of Shh will activate Smo and the rest of the Hh pathway, including Gli (above).*

1: Sonic Hedgehog (Shh) is Indeed named after the Sega character. Other related proteins include Indian Hedgehog (Ihh) and Desert Hedgehog (Dhh).

III - RESEARCH METHODOLOGIES

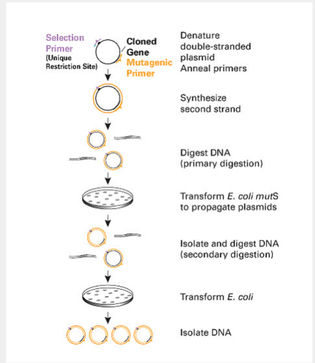

Fig. 3: *The general procedure for creating mutant DNA. This mutant DNA is used by cells to make mutant Ptch.*

It has been proposed that cholesterol has significance in regulating Ptch (Myers, Sever, et. al. 2013). The idea came from the unexpected discovery of a hydrophobic pocket (that might bind to a lipid like cholesterol) in the crystal structure of NPC1, a Ptch-like protein involved in the transport of cholesterol in lysosomes (Li, Saha, et. al. 2016). Since these residues were conserved between Ptch and NPC1, Ptch was also likely to have a similar hydrophobic pocket. The central focus of this project has thus been to mutate the residues within Ptch's hydrophobic pocket using molecular biology techniques, and then to assess the consequences of these mutations on Ptch function in living cells. In these mutants of wild-type (normal) Ptch, nine individual amino acids that are thought to contact cholesterol in cholesterol binding are converted to tryptophan, a large fatty amino acid that generally neutralizes whatever function the original amino acid had. One amino acid is substituted per mutant.

After the mutant Ptch gene is isolated (Fig. 3), it is inserted into cells that then synthesize mutated Ptch. The assay used to study these Ptch mutants is a **Gli-transcriptional reporter assay**. This assay provides a quick and easy quantitative measure of Hh pathway activity, and thus of the Ptch protein's functionality.

Generally, any changes in Hh activity would be considered worthy of further investigation, the expected result being a marked decrease in Hh activity with the mutations present.

IV - DATA: MOVING FORWARD

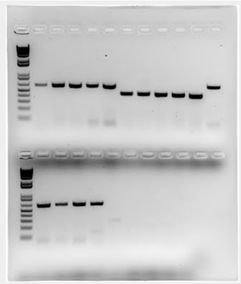

Fig. 4: *A photo of a gel (an electric current is used to pull DNA fragments along a thick gel, sorting them by size) taken using UV light to make the bands visible. This gel is from when I was incorporating the mutations into plasmids with PCR.*

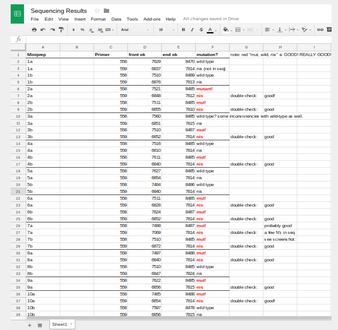

Fig. 5: *analysis of data on the mutant gene's sequence and whether or not the mutations were successful.*

In the course of this project, abundant preliminary data has been collected[2]. Fig. 4 shows a PCR assay, used to determine the success of initial DNA mutations. More recently, gene sequencing data (Fig. 5) has shown the transformations (mutations of DNA) were successful; however, the final results of the G-protein assay have yet to be realized.

Although this project has not produced concrete data yet, this work will continue as a means of exploring the impact of cholesterol binding on Ptch. I've learned a tremendous amount in the lab and have gained valuable experience. I plan on staying at this lab over the summer and reaching new, meaningful conclusions in my work.

Thanks so much for your time!

2: Other preliminary data that has accrued has been omitted for space reasons.

V - ACKNOWLEDGEMENTS / REFERENCES

Special thanks to my mentor Ben Myers, my other mentor Randall Mann, Aaron Kershner, and many others at Phil Beachy's lab for helping me make this project possible.

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