Signaling Dynamics of SMAD Proteins

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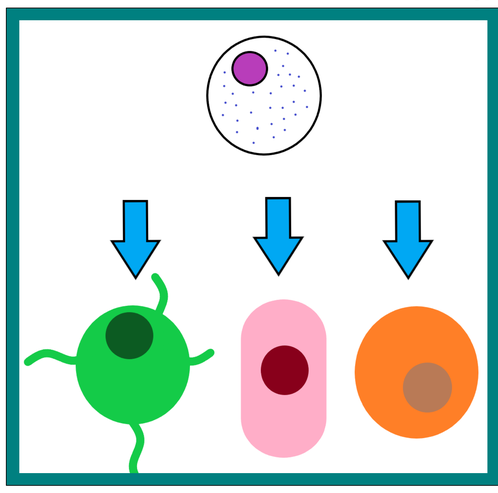
1. Introduction

Background: SMAD proteins are found in every mammalian cell and play important roles in stem cell differentiation and embryonic development.



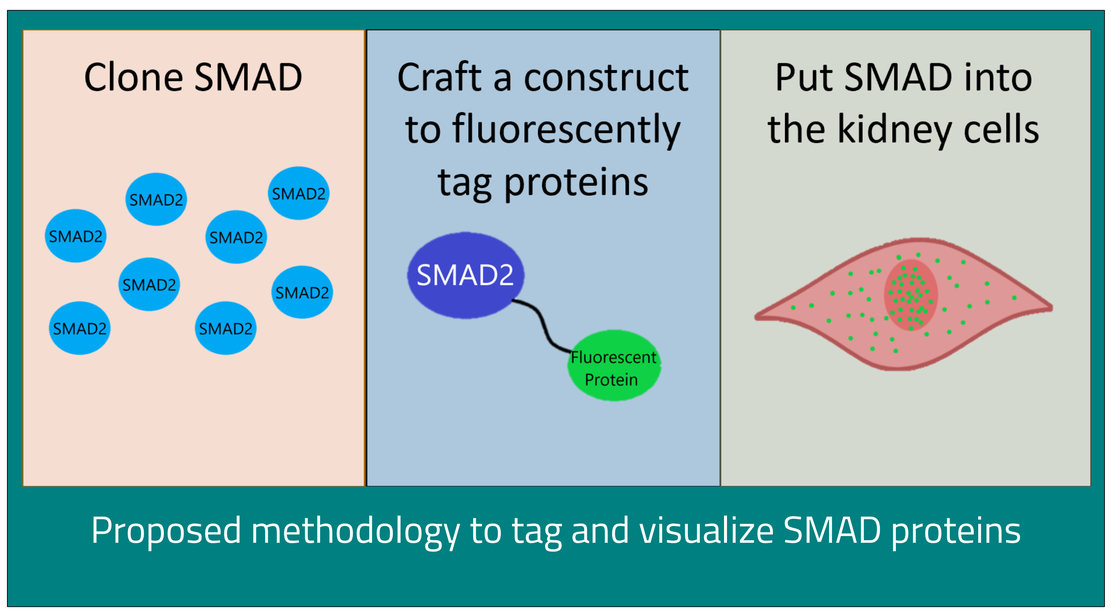
Stem cells have the ability to transform into more than 100 types of cells in the human body!

Motivation: In learning more about this process, we hope to understand its intricacies and be able to have control over some of these processes and decide what types of cells we want. Little research has been done in the time it takes for these proteins to interact with the cell.

Claim: We expect the SMAD proteins shuttle in and out of the nucleus due to the fact that these proteins are needed as transcriptional cofactors.

2. Methodology

To visualize the dynamics of SMAD proteins, a construct was built to fluorescently tag SMAD proteins. With the use of a special microscope we will be able to analyze in real time their journey to the cell's nucleus.

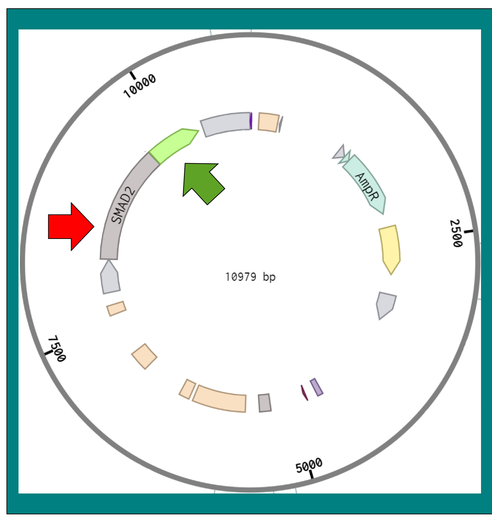


Proposed methodology to tag and visualize SMAD proteins

In this model, we used kidney cells instead of Stem cells. This is because of the conserved function and pathway of the SMAD proteins.

3. Results

Once we successfully cloned SMAD2 and the backbone containing the fluorescent tag, Gibson assembly is used to complete the construction of the plasmid.



The construct contains the SMAD protein and the fluorescent tag. In this case, we started with a green fluorescent color. The red arrow points to the SMAD2 insert and the green points to the fluorescent tag

The construct contains Ampicicilin resistance that allows for selection in bacteria culture. Other constructs with different fluorescent tags are needed to simultaneously observe the dynamics of different SMAD proteins.

4. Future Plans

Once we understand the time SMAD proteins take to reach the nucleus, we can start thinking about modifying their behavior using Optogenetics.

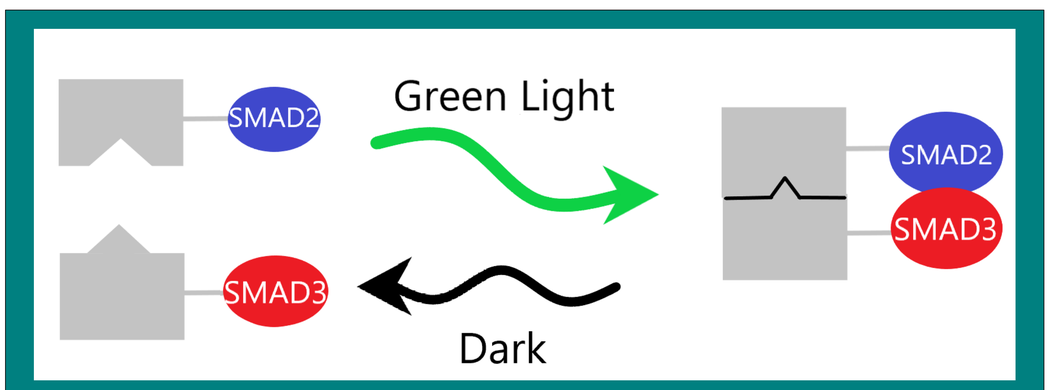


Diagram for the function of an Optogenetic tool which reacts to green light and combines SMAD proteins. In the absence of light, the optogenetic tool will separate SMAD proteins

In using an optogenetic tool, we will be able to make different combinations of SMAD proteins and analyze the cell's response to these changes. Moreover, we will be able to explore the significance of each protein as transcriptional cofactors.

Acknowledgements

I thank the Wilson's lab team for instructing and helping me structure this project. Furthermore, the CSEP team for guiding me through the basics of finding a scientific community and help me take part on this project.