

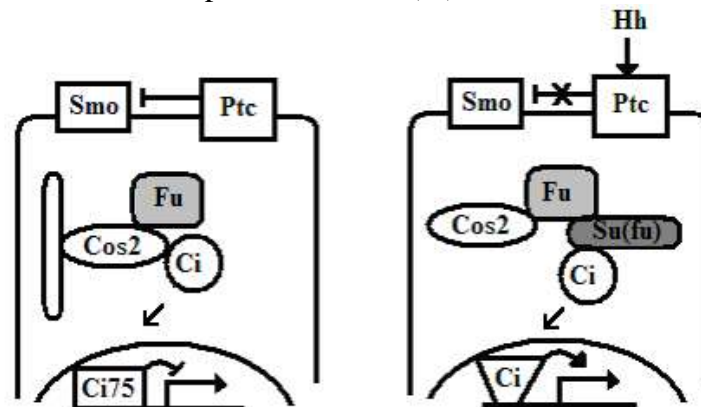
## Hedgehog Cell Signaling: Fused and Suppressor of Fused

### INTRODUCTION

The hedgehog or Hh signal transduction pathway is important in embryogenesis, cell fate determination, and tissue differentiation. The pathway mechanism is very complex and parts of it are still very poorly understood. Since the pathway is highly conserved in the animal kingdom, it is not surprising that disruptions in the pathway can cause severe fetal developmental effects such as cyclopia and polydactyly, and cancer such as basal cell carcinoma (the most prevalent type of cancer in humans) and prostate cancer. As a result, the Hh pathway has been identified as a potential therapeutic target for many cancers. My research will focus on the activity and structure of Fused (Fu), a protein kinase in the Hh signal transduction cascade. The proposed research will be conducted during the summer of 2007 in the Holmgren lab.

### BACKGROUND

General scheme of Hh signaling is as follows: without the Hh signal, Patched (Ptc) a transmembrane protein inhibits Smoothed (Smo), another transmembrane protein, from activating the pathway. The transcriptional factor Cubitus Interruptus (Ci) is partially degraded into a repressor form, which enters the nucleus, binds to DNA and prevents transcription of target genes. In the presence of the Hh signal, Ptc no longer inhibits Smo, allowing Smo to activate the pathway. Ci is no longer partially degraded, and the full-length active form is released from the cytoplasm, enters the nucleus, and activates transcription. The cytoplasmic complex from which Ci is released is known to be composed of three other proteins: Fu, Su(fu), and Cos2.



*Figure:* The Hh pathway is turned off in the left cell, and on in the right cell. Proteins of interest are highlighted in gray. In the left cell a truncated form of Ci acts as a repressor; in the right cell the full-length form of Ci acts as an activator.

### LITERATURE REVIEW

The kinase activity of Fu regulates nuclear import of Ci. However, the process by which this regulation occurs is unknown. Several observations suggest that Fu may regulate Ci via phosphorylation. First, Ci has a highly conserved domain located in the N-terminus called NR. The NR domain is 49 amino acids long and is known to regulate Ci nuclear import and to be phosphorylated by an unknown kinase. Thus, it is plausible that Fu is the kinase that phosphorylates NR.

A second player in Ci nuclear import is Su(fu), a novel 468 amino acid cytoplasmic protein that functions as a negative regulator of the Hh pathway. In the absence of Hh signaling, Su(fu) binds and inhibits Ci, while in the presence of Hh, Su(fu) function is counteracted by Fu kinase activity. It has also been demonstrated that Fu binds to Su(fu), and this binding is likely to be important for the regulation of Ci nuclear import. However, the location of the binding sites is unknown. In the case of Su(fu), the binding sites could be located either on the globular N-terminal domain or on the C-terminal trail of Su(fu) or involve both domains.

## RESEARCH QUESTIONS

The purpose of the proposed project is two-fold. The first aim is to assay the potential phosphorylation of the NR domain of Ci by the kinase domain of Fu. The second aim is to map the Fu binding site on Su(fu).

## METHODOLOGY

The following procedure will be used to determine whether or not Fu phosphorylates NR. The GAL4/UAS system will be used to express the Fu kinase and NR domains in *Drosophila melanogaster* S2 cells. The GAL4 transcription factor is under the regulation of a metallothionein promoter so its production can be induced by the addition of copper sulfate. I have generated constructs in pUAST in which the Fu kinase and NR-GFP are under the regulation of a GAL4 UAS. Once the GAL4 transcription factor is produced, it binds to the target sequence on the pUAST vector called the Upstream Activating Sequence and drives expression of genes downstream of the target sequence. The proteins will be expressed in cells either treated or not treated with Hh and extracts prepared. Then, radiolabeled - ATP will be added to the extract. NR-GFP will be immunoprecipitated using anti-GFP antibodies and fractionated by gel electrophoresis. The presence of radioactive phosphates on the NR will indicate that it has been phosphorylated by Fu kinase. The presence of radioactivity in +/- Hh cell lines will also be compared to determine how the presence of Hh affects kinase activity. Control experiments will include assaying the phosphorylation of GFP on its own and performing the phosphorylation experiments in the absence of the Fu kinase domain. It is also possible that parts of Fu outside the kinase domain are involved in kinase function, so in anticipation of this possibility, I have made a full-length Fu pUAST DNA construct.

To determine the Fu binding site on Su(fu), I will use a newly developed assay. Fu tagged with GFP (a fluorescent protein) will be combined with several deletion variants of Su(fu) tagged with GST. Su(fu)-GST will be bound to sepharose beads covered with glutathione. Then, the beads are washed to remove unbound proteins and incubated with an S2 extract containing Fu-GFP. If Fu-GFP binds to the Su(fu) deletion variant, the fluorescent GFP tag will accumulate around the surface of the bead. This can be directly visualized using a fluorescent microscope. The first modified version of Su(fu) that will be used will only include the C-terminal tail. If Fu still binds, then the C-terminal tail will be systematically shortened to identify the exact binding site. If Fu does not bind, then a systematic modification of the surface residues of the N-terminal globular domain will be used to identify the binding site.

All of necessary DNA constructs for conducting the experiments outlined above have been constructed, and transfected into S2 *Drosophila* cells. The protein products expressed have been verified by Western blots as well. Thus, very little time this summer will be spent building DNA constructs and the majority of the time will be spent conducting experiments and obtaining data.

## PREPARATION

I have been conducting research in Professor Holmgren's lab since the summer of 2006. Throughout the academic year, I have been enrolled in 398 and subsequently 399 Independent Research courses. I am familiar with the lab and many basic laboratory techniques. In addition, I have completed the full year sequences of general chemistry, organic chemistry and biology. I am hoping that the research conducted this summer will contribute to my senior honors thesis.

### **General comments:**

The project is well developed and it shows ownership in the part of the student.

The student has clearly described the protocol to carry out this experiment. If her model turns out to be true, this will be an important contribution to our understanding of Hh signal transduction. However, it is possible that the Fu kinase has other targets. To account for that, she has a second part in the project that will provide important information regardless of the result. Having a contingency plan is a great plus.

The project has publication potential.

The student shows preparation to carry out the work.

### **Improvements:**

No references to prior work in the area are presented  
Should have added how this research will help in accomplishing long term goals for the student.