## *ABSTRACT*

**TITLE : STUDY OF PROTEINS PATCHED AND SUFU INVOLVED IN THE HEDGEHOG SIGNALLING PATHWAY**

The Hedgehog signaling pathway is responsible for cell differentiation during embryogenesis. It is also part of the stem cells maintenance during adulthood. This pathway was discovered during the 1980's by Nüsslein-Volhard and Wieschaus. The HH pathway is very conserved and its disruption causes many human diseases such as holoprosencephaly and some mutations of proteins in this pathway are associated with cancers. The HH pathway activation occurs when the morphogen hedgehog binds its receptor Patched. This activation leads to the expression of some genes *via* the activation of its transcription factor belonging to the Zn finger transcription factor family Gli in human and Cubitus interruptus in drosophila (CI/Gli). On the other hand, when the pathway is switched off, CI/Gli is phophorylated, partly degraded into a shorter, repressing form and inhibited by another protein, Suppressor of fused (SUFU).

The Hedgehog signaling pathway includes the 152 kDa transmembrane protein patched. This protein has 12 transmembrane segments and two large extracellular domains. Biochemistry studies realized by Marigo et al showed that the two large extracellular domains are sufficient for the binding of its ligand Hedgehog. Moreover, Isabelle Mus-Veteau showed that Patched is involved in anticancer drugs efflux. However, few structural studies were conducted on this protein. In order to structurally study Patched, I have designed 2 constructions of the human Patched (hPTC) protein. The first construction is the two extracellular domains attached to the lysozyme T4 (hPTCD1D2), the second one is the protein truncated of its N and C terminal domains (hPTCΔNΔC). hPTCD1D2 was expressed in bacteria as inclusion bodies which might be refolded but, this long procedure wasn't done during this Ph.D. However, I have cloned the gene expressing hPTCD1D2 in a yeast vector. Expressing experiments are planned in the laboratory. I have also cloned the gene coding for hPTCΔNΔC in a yeast-expressing vector. Experiments conducted by Annick Pacquelin and Chloé Galbert show that this protein is expressed at the plasma membrane of the yeast. Its solubilisation conditions are being optimised.

This work was done in collaboration with Isabelle Mus-Veteau who provides gene and vector used to obtain these constructions.

The Hedgehog signalling pathway also includes SUFU, a 52 kDa soluble protein organized in α helices and β sheets and whose 3D structure has been published in 2013. Atomic emission spectroscopy allowed us to determine that drosophila SUFU (dSUFU) binds Zn and that this binding increases with the pH. Due to a colorimetric compound, we were able to determine that dSUFU has a nanomolar affinity for the Zn. In addition, circular dichroism spectra showed that the secondary structure content of SUFU in solution was similar to that in crystals. These results were submitted for publication to a peer-reviewed journal.

**BIOPHYSICAL CHARACTERISATION OF THE NOVEL ZINC BINDING PROPERTY IN SUPPRESSOR OF FUSED.**

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Furthermore, SAXS data obtained with dSUFU, human SUFU (hSUFU) and zebrafish SUFU (zSUFU) show that hSUFU and zSUFU present different oligomerization states than dSUFU in solution. The N-terminal residues of hSUFU are likely to be involved in dimer formation. In addition, the mutation of some residues in the N-terminal of hSUFU is involved in some cancers. We designed a truncated mutant of hSUFU to obtain hSUFUΔ30. This mutant is being tested in human cells for its ability to inhibit the HH pathway.