Formulaire de stage (sur une page maximum)

Parcours M2 GGBS 2019-20

Laboratoire : CRTI/UMR1064 Intitulé/N° d’équipe : 2

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Titre du stage : **Targeted proteomic analysis of epigenetically programmed genes in the sperm chromatin**

Résumé du projet proposé :

**Background**

We recently demonstrated that the sperm deliver to the eggs epigenetic informations necessary for the expression of genes in the developing embryos. We now want to understand how the epigenetic informations associated with such programmed genes exert its effect on the developing embryos. In particular we would like to understand the mechanism of recognition of such sperm derived epigenetic cues by egg factors after fertilization. We hope that uncovering this mechanism could help elucidate the basis of some case of infertility.

**Aims**

The goal of this project is to identify the maternal factors that specifically bind to genes that are epigenetically programmed in sperm. We expect to find factors that are required for the recognition of sperm-derived epigenetic informations, for the propagation of these instructions in the cells of the developing embryos, and to ultimately regulate expression of programmed genes.

**Methods**

We have already identified genes that are epigenetically programmed in the sperm of frogs and human. Following incubation of sperm chromatin in Xenopus egg extract, we will apply genomic locus proteomics (*i.e* GLoPro, Myers *et al*., Nature methods, 2018) to these programmed genes. For that purpose, genes will be targeted with CRISPR-based targeting using a nuclease dead Cas9 fused to an enzyme that can be induced to carry out proximity biotin labelling (dcas9-Apex2 or dcas9-turboIDs). Egg factors associated with these genes will be purified by streptavidin precipitation and subsequently identified through quantitative proteomic.

**Prospect**

Following the identification of egg factors that bind to epigenetically programmed genes on sperm chromatin we will address the following questions:

(i) What are the sperm epigenetics mark required for egg factor recruitment to programmed genes? To that end we will interfere with the epigenetic status of the sperm prior to exposure to the egg environment and assess the effect on maternal factor recruitment.

(ii) What are the consequences of preventing egg factor recruitment on sperm chromatin? For that purpose we will deploy Trim away (Clift *et al*., 2017) as a maternal factor depletion strategy and evaluate the effect on programmed gene expression.