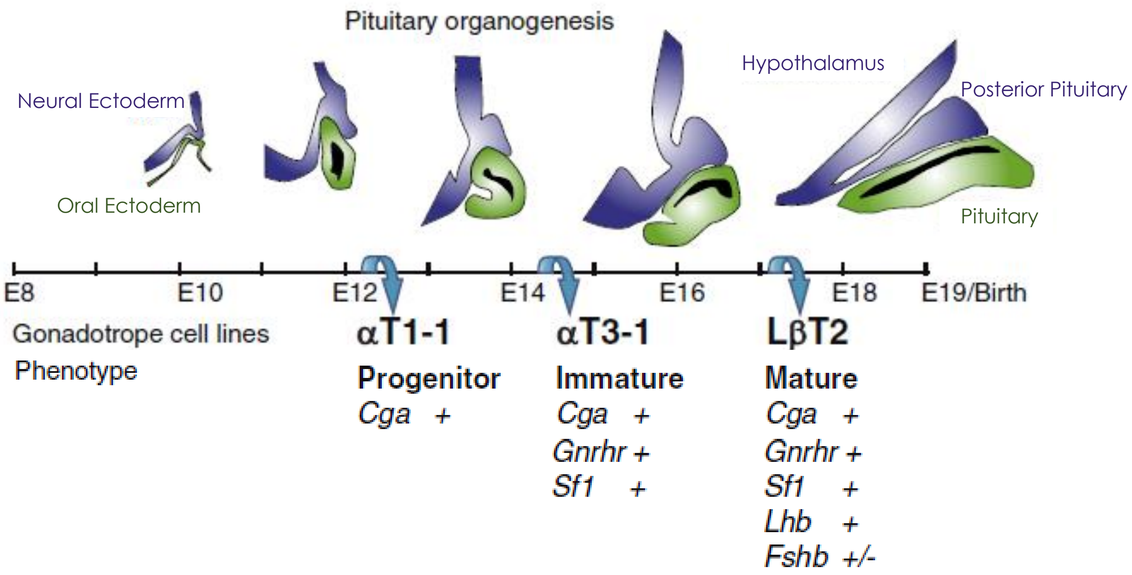
Involvement of oestrogenic pathway in *Sf-1* epigenetic regulation during the differentiation of pituitary gonadotrope lineage

V. Pacini (M.), JN. Laverriere (Dr), F. Petit (Mlle), J. Cohen Tannoudji (Pr), D. L'Hôte (Dr)
Team Physiologie de l'Axe Gonadotrope, INSERM U1133 - University Paris Diderot, Sorbonne Paris Cité, Unity of Biologie Fonctionnelle et Adaptative (BFA), CNRS UMR 8251, F-75013, Paris, France
E-mail : vincent.pacini@hotmail.fr

Background

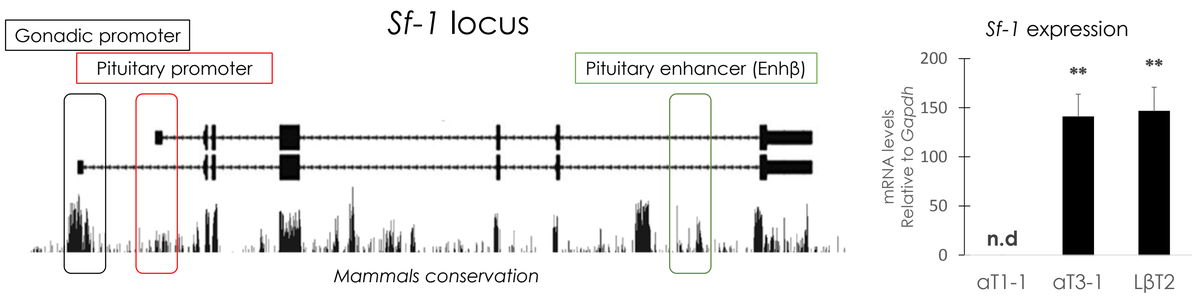


Sf-1 (also known as *Nr5a1*) is a trancription factor belonging to the nuclear receptor family early expressed during pituitary gonadotrope differentiation, and is essential for the reproductive function. Pituitary inactivation of this gene leads to a hypogonadotropic hypogonadism and sterility. *Sf-1* regulates indeed the expression of key genes such as the GnRH receptor and gonadrotopins sub-units genes.

Sf-1 expression is regulated by a pituitary promoter (Kimura R. and al, 2000) and an enhancer which we called enhβ (Shima Y. and al, 2008). Using three cell lines recapitulating three stages of gonadotrope differentiation (αT1-1 : progenitor, αT3-1 : immature and LβT2 : mature), we conducted an epigenetic study of *Sf-1* regulatory sequences. This study suggests that enhβ is inactive in immature gonadotropes cells αT3-1 although *Sf-1* is expressed (Laverrière J-N. and al, 2016).

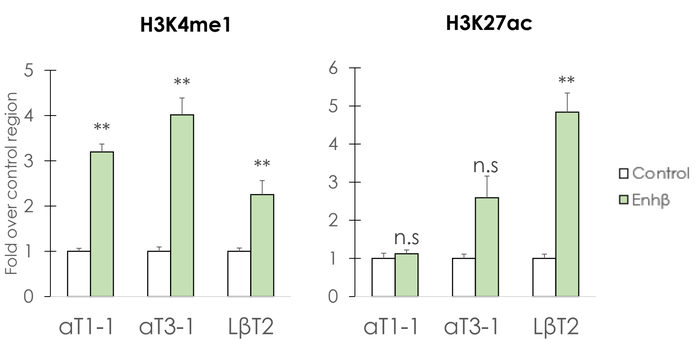
In order to understand molecular mechanisms that control *Sf-1* expression during the differentiation of gonadotrope lineage, we thus performed a high-throughtput genome-wide chromatin accessibility (ATAC-Seq) in these three gonadotropes cells lines.

Pituitary enhancer (Enhβ) functionnality in gonadotropes cells :

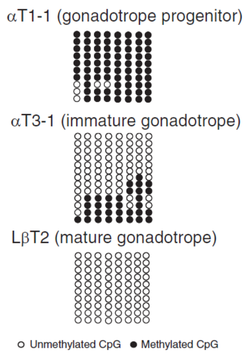


_Sf-1_ is expressed at the same level in immature and mature gondotropes cells.

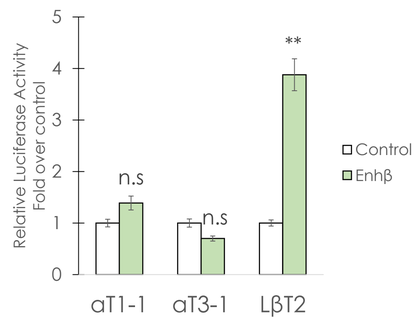
Epigenetic marks enrichment of Enhβ (ChIP-PCR) :



Méthylation states of Enhβ CpG islets (Bisulfitage-PCR) :



Enhβ luciférase activity :



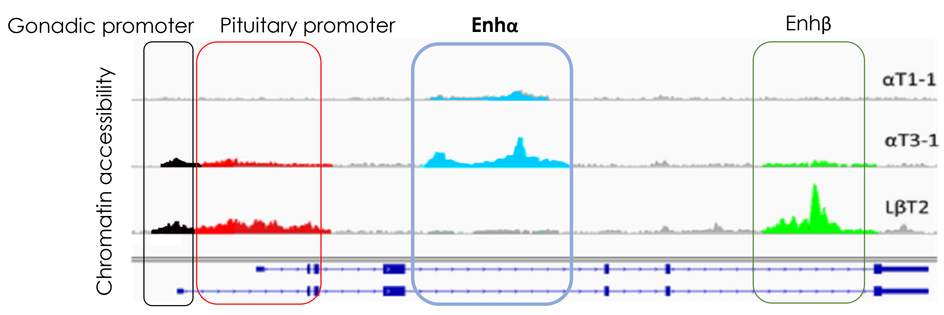
Enhβ, while displaying active enhancer epigenetic marks and transcriptional activity in mature gonadotrope cells, is inactive in immature gonadotrope cells.

So, which are the epigenetic mechanisms regulating *Sf-1* expression in immature gonadotrope cells ?

All data were expressed as the mean ± SEM. Statistical comparisons were performed using ANOVA Dunnett's test (* : p< 0;05; **: p<0.01 vs its respective controls).

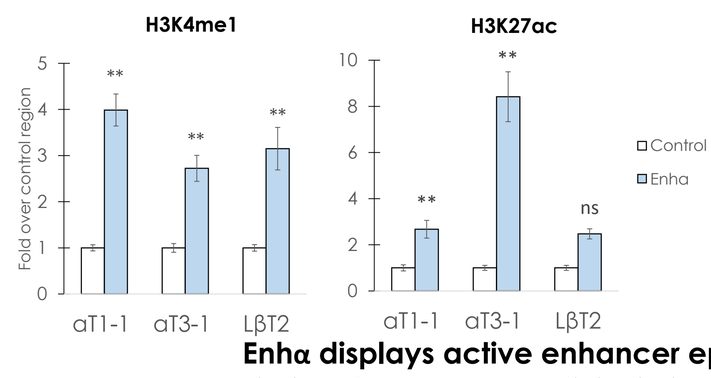
Discovery of an immature gonadotrope specific enhancer designated as Enhα :

Chromatin accessibility of *Sf-1* locus in gonadotrope cells lines accessed by ATAC-Seq

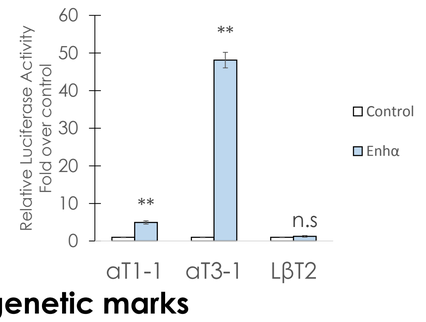


Besides the Enhβ chromatin accessibility in LβT2, we observed chromatin accessibility in an undescribed region in the 4th intron, specific of the immature stage (Enhα).

Epigenetic marks enrichment of Enhα (ChIP-PCR) :

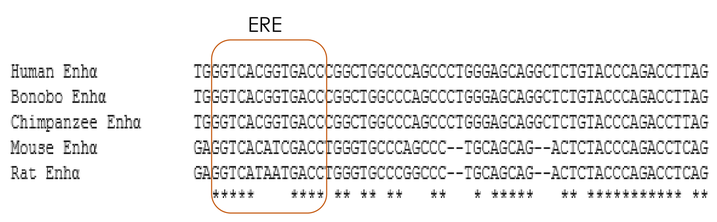


Enha luciférase activity :

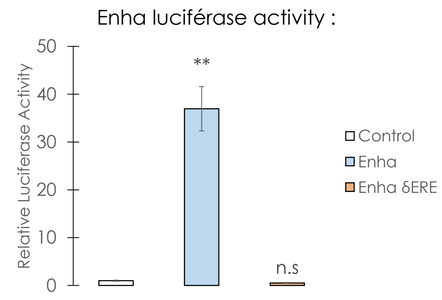


Enhα displays active enhancer epigenetic marks and transcriptional regulatory activity in immature gonadotrope cells.

A highly conserved sequence with potential Estrogenic Receptor Element - ERE (ClustalW/Cis-BP) :



Enha luciférase activity :



We identified a conserved Estrogenic Response Element, essential for Enha activity.

Enhα is an enhancer specific of immature stage whose activity is strictly dependent of an ERE motif.

All data were expressed as the mean ± SEM. Statistical comparisons were performed using ANOVA Dunnett's test (* : p< 0;05; **: p<0.01 vs its respective controls).

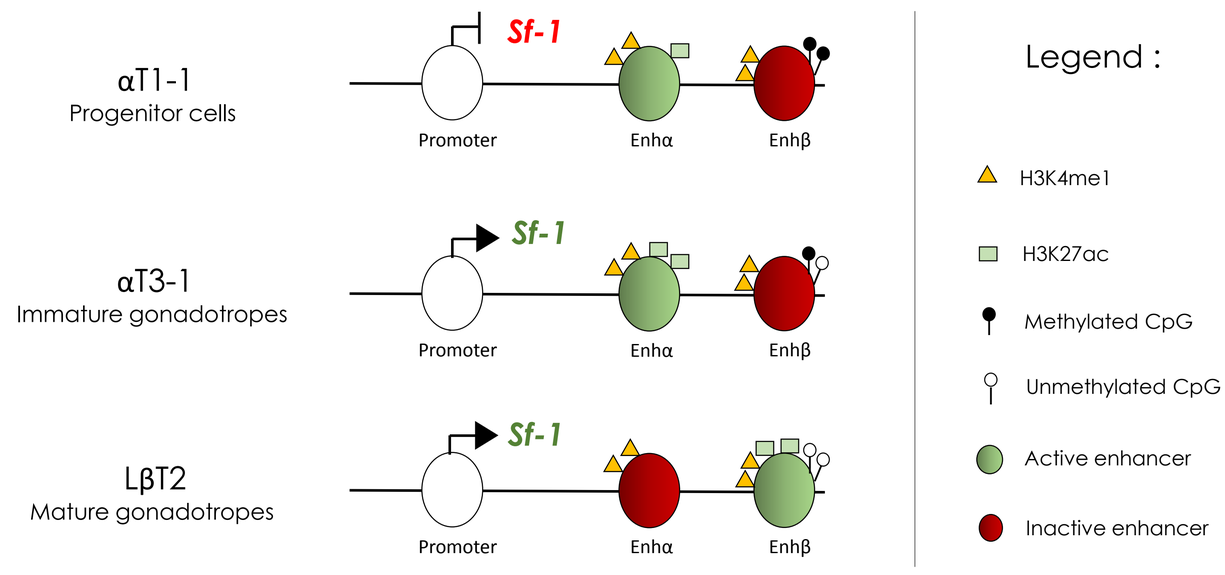
SFE project :

- Evaluation of the **Enhα implication in *Sf-1* expression** in immature gonadotrope cells by using Crispr/Cas9 and identification of the **molecular mechanisms that control Enha activity** during the specification of pituitary gonadotrope cells.

- Investigation of the **involvement of estrogenic receptor / estrogenic pathway for Enha activity** in immature gonadotrope cells by small interfering RNA and pharmacological approaches.

- Studies of the **ontogenesis of Enha activity** and its **implication for *in vivo* *Sf-1* expression** during development ; **implication of estrogenic pathway in *in vivo* Enha activity** using ER-a or ER-β knockout mice models.

Model of *Sf-1* expression regulation :



These results suggest that *Sf-1* expression involves an early undescribed enhancer (Enha) which recruitment precedes the mature gonadotrope enhancer (Enhβ). Enha displays active epigenetic marks (H3K4me1 and H3K27ac) and transcriptional activity specifically in immature gonadotrope cells contrary to Enhβ. Enha activity is dependent of Estrogenic Response Element. These results suggest an undescribed involvement of the estrogenic pathway during pituitary development.

Establishing a direct link between estrogenic pathway and regulation of *Sf-1* expression could be essential to explain idiopathic endocrine disorders, whether endogenous (genetic) or environmental origins (such as xenobiotics).