Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Fiche à nommer selon le format Nom_Prénom_ProjetED2021, à enregistrer en format PDF et à renvoyer à l’adresse : edcdv@sorbonne-universite.fr

Nom et prénom du directeur de thèse (et si besoin du co-directeur) : FRE Silvia
Le directeur de thèse et le co-directeur doivent impérativement avoir l’HDR ou équivalent
Coordonnées Tel : 01 56246936
e-mail : silvia.fre@curie.fr

Nom et prénom du co-encadrant (non HdR) (s’il y a lieu) :
Coordonnées Tel :
e-mail :

Y-a-t-il un candidat déjà identifié pour le projet: OUI ✓

Nom et prénom du responsable de l’équipe : FRE Silvia
Intitulé de l’équipe : Notch signaling in stem cells and tumors
Nombre de chercheurs et enseignants-chercheurs statutaires de l’équipe titulaires d’une HDR (ou équivalent) : 1

Nom et prénom du responsable d’UMR ou de département: LEOPOLD Pierre
Intitulé et N° d’UMR ou de département : Unité de Génétique et Biologie du Développement UMR 3215 - U934 - Institut Curie - Section Recherche
Titre du projet de thèse : Defining cell fate specification of mouse mammary stem cells

Signature du directeur d’UMR ou de département (vaut avis favorable pour le dépôt du projet) :

Spécialité : Biologie du développement, Cancérologie

Résumé du projet de thèse (1 page maximum, en anglais)
Pour les thèses avec 2 co-directeurs, ou en partenariat entre 2 laboratoires ou structures, indiquer la participation de chaque co-directeur et structure dans la gestion du projet.
Coordination of cell fate acquisition and morphogenesis is necessary to generate functional organs. During development, specialised cell types are generated from stem cells in a spatiotemporal manner tightly regulated to fulfil the tissue functions. In the case of complex tissue shape such as the one of branched organs, the patterns followed are generated by individual and collective cell movements. Yet, how stem cell differentiation and cell dynamics are orchestrated to generate tissues of complex shapes harbouring defined cell types remains a major question in the field of development and cell biology.

Mammary gland development requires extensive epithelial remodeling and proliferation to generate the highly branched epithelial ductal network that allows milk production and flow toward the nipple to feed the offspring. The mammary tree is composed of an inner layer of polarised luminal cells (LCs) and a surrounding layer of basal cells (BCs) adjacent to the basement membrane. It is a highly dynamic epithelium, with an extraordinary capacity for rapid growth and tissue remodelling throughout reproductive life. This remarkable regeneration potential is attributed to the presence of long-lived mammary stem cells (MaSCs). Multipotent MaSCs, capable of generating all mammary lineages, become fate-restricted during embryonic development and the epithelium is maintained in homeostatic conditions exclusively by uninotent progenitors $^{1-7}$. The embryonic mammary gland represents therefore a powerful tissue paradigm to study spatial and temporal integration of stem cell potential and lineage specification. We have recently shown that this key lineage potential restriction occurs progressively within a narrow developmental window that coincides with the initial branching events that generate the mammary tree$^{8}$. Strikingly, this lineage restriction coincides temporally with remarkable epithelial remodelling associated with the first morphogenetic events in the embryonic bud.

Interestingly, cell movements and proximity to local cues, such as attachment to the basement membrane$^9$, local tissue deformations, cell division and even the shape of individual cells, can contribute to cell fate determination$^2$. Recently, endocrine fate allocation and timing in the developing pancreas have been reported to act in concert to coordinate morphogenesis with lineage diversification$^{10,11}$. We therefore hypothesize that the loss of multipotency in the mammary gland is linked to cell rearrangements and tissue morphogenesis during the initial steps of mammogenesis, leading to the branching of embryonic placodes. Testing this hypothesis will be key to understand how the switch to unipotency is dynamically orchestrated with the morphogenetic events and more specifically with cell rearrangements, position or proliferation. The primary aim of this project is therefore to decipher the molecular and cellular mechanisms underlying cell fate acquisition and investigate how cell differentiation is coordinated with tissue morphogenesis in the murine embryonic mammary gland.

We will combine original reporter mice and lineage tracing tools with single cell analyses, time-lapse of branching morphogenesis, modelling of clonal dynamics and functional studies in mouse mutants, to address the following fundamental questions:

1) Which signals determine cell identity and potency during mammary development?
2) What are the cellular dynamics of embryonic mammary stem cells?
3) How branching morphogenesis is linked to lineage specification during embryonic patterning?

Most of the scientific questions we will tackle will be addressed in the context of mouse mammary embryonic development. This stage is studied by only a few laboratories, mostly due to intrinsic technical difficulties, which we have now largely overcome. The embryonic phases of mammary gland development have become a topic of growing interest, given that the switch from multipotency to unipotency occurs at embryonic stages$^{5,6,12}$. We thus believe the time is right for us to address these important questions and that by now we have acquired the relevant expertise as well as the necessary reagents and animal models to do so. We consider this proposal both original and potentially ground breaking. The single cell analyses (scRNAseq and scATACseq) will be performed in vivo in mammary embryonic buds. However, as this stage of mammogenesis cannot be investigated dynamically in vivo, the student will study 3D embryonic mammary explant cultures at defined developmental stages and image the branching morphogenesis and lineage specification events that occur during embryogenesis in real time. Furthermore, mouse mammary organoids will also be established from both embryonic and pubertal mammary tissues. This system will entail a compromise in terms of in vivo physiology that will allow to assess a wide variety of candidates. On one hand, CRISPR/Cas9-mediated gene knock-out will be used to probe the function of candidate genes identified in Aim 1. On the other hand, signalling molecules will be added to the culture medium allowing to investigate both intra and extracellular regulators. The ability to analyse the process occurring using time-lapse microscopy and in controlled culture conditions, is a significant benefit. These studies will uncover the molecular circuitries driving cell fate specification in mammogenesis and should reveal the underlying mechanisms linking specific stem cell states to cell dynamics during tissue morphogenesis, fundamentally advancing our understanding of how organs are formed during development.

**Faisabilité du projet de thèse (1/2 page maximum, en anglais)**

Expliciter la faisabilité du projet en terme d'expertise de l'équipe d'accueil, des collaborations potentielles qui pourront être mises en place pour certains aspects du projet, de la disponibilité des appareils nécessaires au bon déroulement du projet...
In vivo studies addressing how cells coordinate morphogenetic cues and fate specification during development have been hindered by the lack of 4D resolution at the cell and tissue scale in complex organs. Here, we will address this fundamental question, leveraging on our recent findings and expertise in the understudied embryonic mouse mammary gland and in quantitative analysis of tissue dynamics. By correlating quantitative tracing data, single cell resolution transcriptomics and dynamic information acquired by time-lapse imaging of morphogenesis, we should be able to assign transcriptional signatures to specific cell fates and cellular dynamics. Overall, our findings will provide unique and original insights into how stem cell lineage restriction is achieved and integrated with mammary morphogenesis.

In recent years, our team has generated a rich body of data on mammary hierarchies and cell fate specification, as well as developed and optimized new tools and protocols. We have contributed to the understanding of the heterogeneity of MaSCs\textsuperscript{13,14} and established the time of their commitment during mammary development\textsuperscript{2}. In addition, some of the datasets and tools necessary for the successful completion of the present PhD project have been generated by another student in the lab, Claudia Carabana Garcia, currently performing her 3\textsuperscript{rd} year of PhD, ensuring project feasibility and safe passage of competences between the two students. This combination of expertise and approaches allow us to undertake the proposed project in a straightforward manner. We have also established collaboration with the team of Y. Bellaïche (DRCE, CNRS, Curie Institute), who has extensive expertise in epithelial cell division and tissue morphogenesis. In particular, in close collaboration with mouse and organoids experts, this group has already demonstrated that the image analysis methods they implemented can be used to achieve a broader understanding of epithelial morphogenesis across model systems\textsuperscript{15,16}. This close interaction, along with our already established collaboration with the labs of B. Simons (Cambridge University, UK) and S. Rulands (Max Planck Dresden, DE) will be instrumental to productively accomplish this project, representing an original line of research, fostering strong exchanges of know-how and expertise to optimize feasibility and facilitate rapid and smooth achievements. Finally, Institut Curie has outstanding facilities, including advanced imaging platforms for time-lapse imaging and complex image analysis, which, combined with our expertise in studying stem cells in vivo and the fact that the other techniques are established, proves that we are in an advantageous position to pursue these studies and to overcome potential obstacles.

Thèses actuellement en cours dans l’équipe

Tous les encadrés doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d’une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu’IPV, FDV...)

<table>
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<th>Directeur(s) de thèse</th>
<th>Année de 1ère inscription</th>
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<td>CARABANA Claudia</td>
<td>FRE Silvia</td>
<td>2018</td>
<td>CdV ED515</td>
<td>EU COFUND IC-3i</td>
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Trois publications récentes du directeur de thèse (du co-directeur ou du co-encadrant s’il y a lieu). Mettre en gras le nom du directeur de thèse.


Docteurs encadrés par le directeur de thèse ayant soutenu entre la date de dépôt de ce dossier et il y a 5 ans et publications relatives à leur sujet de thèse. Mettre en gras le nom du directeur de thèse et celui du docteur.

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Publications :


Nom Prénom : JACQUEMIN Guillaume Date de soutenance : 26/09/2019
Durée de thèse (en mois) : 47
Ecole Doctorale : CdV ED515 - PSL

Publications :


References