2023-12-19

International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Coordinating lumen formation and cell fate specification in embryonic epithelial morphogenesis

General information

Call	2024
Reference	2024-05-FRE_MARTIN-FARALDO
Keyword(s)	Lumen formation; cell fate specification; embryonic tissue explants; time-lapse microscopy; stem cells

Director(s) and team

Thesis director(s)	Silvia Fre & Maria Luisa Martin-Faraldo
Research team	Notch Signaling in Stem Cells and Tumors
Research department	UMR3215 / U934 - Genetics and Developmental Biology

Description of the PhD thesis project

Abstract

A major challenge in biology is to explain how complex tissues arise from the collective action of individual cells. Lumen formation is a fundamental step in the development of the structural and functional units of glandular organs, such as alveoli and ducts. A well-orchestrated and coordinated action between epithelial cells and surrounding mesenchymal cells within 3- dimensional space, impacting cell differentiation and polarization dynamics, is necessary to form a lumen during embryonic morphogenesis. How an apical lumen is formed *in vivo* is a major question in the field of developmental cell biology, and it is central as defective lumen formation is responsible for a variety of human diseases. We propose to elucidate the molecular machinery mediating the coordination between polarization of epithelial cells and apical lumen formation. We will unravel the conserved **mechanistic rules driving cell fate specification and lumen formation** in four mouse embryonic glandular epithelia: **mammary gland (MG), prostate (PR), salivary gland (SG) and lacrimal gland (LG)**, which share critical features in terms of tissue organization and stem cell hierarchy. Our findings will provide unique and original insights into how stem cell lineage decisions are integrated with mammary branching and lumen formation. As perturbation of normal morphogenesis is a key driver of tumorigenesis, these studies are also important for an improved understanding of cancer initiation and growth.

Background

Different mechanisms of lumen formation have been proposed: epithelial lumens can form from pre-existing polarized epithelial structures by either folding of the epithelial sheet or budding/sprouting from epithelial tubes (Andrew & Ewald 2010). In addition, *de novo* lumen formation from nonpolarized cells has also been documented, in which polarization of epithelial cells happens simultaneously with the establishment of a new lumen. This can happen by cavitation (also called lumenization), the creation of a luminal space through apoptosis of inner cells within the developing lumen. Cavitation has been initially observed as a result of apoptosis in spheres of mammary epithelial cells grown *in vitro* (Debnath & Brugge 2005 PubMed:

16148884). However, *in vivo*, there is no conspicuous apoptosis during mammary gland morphogenesis in the embryo or at puberty. Indeed, although the cavitation model explains lumen formation in some tissues, in many cases apoptosis is not required to form an apical lumen. Lumens can form de novo as the result of coordinated cell division and polarization (Bryant et al. 2010 PubMed: 20890297).
Our understanding of apical lumen formation has been derived primarily through *in vitro* 3D organoid models exclusively composed of epithelial cells, lacking the inherent complexity of *in vivo* tissue morphogenesis, which draw on dynamic polarity cues and positional information that cannot be fully replicated in cell culture. As a consequence, our understanding of how lumens form in vivo remains very limited and incomplete.

Objectives
Apical lumen formation is a complex process that involves dynamic restructuring of the cytoskeleton and the formation of specialized polarity complexes allowing selective directional transport of specific molecules. This project is focused on studying the process of lumenogenesis in four glandular epithelia that develop an apical lumen during mouse branching morphogenesis. We will address the following questions:
How do cells establish a single apical lumen site forming a continuous space along the ducts? How do cells coordinate differentiation and lumen formation? Does commitment to a luminal fate precede lumen opening? And, if so, is luminal differentiation an obligatory step in lumenogenesis? Finally, are stromal signals also involved in lumen formation or is this a cell autonomous phenomenon intrinsically regulated by the epithelium?
In Aim 1 we will create a comprehensive "fate-behaviour-morphogenic map" of branching embryonic explants before and after lumen formation. This map, albeit extensively descriptive, will provide a detailed and quantitative characterization of the localization of actin-rich foci to the site of nascent lumens and of the specific proteins that selectively accumulate at the apical surface of polarized cells. In Aim 2 we will use mutant mice for signalling molecules involved in cell fate specification and/or morphogenesis (Notch, Wnt, YAP and retinoic acid) and assess lumen formation in epithelial or stromal-specific mutants.

Experimental approaches
Improvement in live imaging and establishment of 3D complex tissue culture systems permit us to study the dynamics of stem cell differentiation and lumen formation during branching morphogenesis. We will use embryonic tissue explants composed of the epithelium and its surrounding native stroma, that we have optimised to make them amenable to genetic and pharmacological manipulation coupled to live tracking of individual cells by time-lapse microscopy (Carabana et al. Biorxiv 2022). To establish a ground-truth on the behaviour of epithelial cells during lumenogenesis, we will perform continuous time-lapse imaging of growing tissue explants until they form a lumen and track sparsely labelled GFP cells by genetic lineage tracing to assign them to a specific differentiated cell type. We will then functionally test how perturbations to cell dynamics regulate cell fate specification and lumen formation, by virally transducing embryonic explants with mutants targeting cell polarity and cell adhesion genes. We will pharmacologically inhibit cell division or Na/K ATPases, potentially required for the paracellular ion transport necessary for lumen fluid accumulation and initial lumen expansion and test the effects of these drugs on lumen formation. Finally, mutations in pathways involved in cell fate choices (Notch, Wnt) or in morphogenesis (Hippo, Hh, retinoic acid) will be targeted to epithelial or stromal cells (fibroblasts) in our explant cultures, to define how lineage segregation is orchestrated with morphogenetic cues to establish the proper final tissue and lumen shape.

International, interdisciplinary & intersectoral aspects of the project

This project will be performed in tight collaboration with Prof. Benjamin Simons (University of Cambridge, UK), Dr. Steffen Rulands (Max Planck Dresden and University of Munich, DE) and Dr. Edouard Hannezo (ISTA Vienna, Austria). Prof. Simons has a world-wide recognized expertise in studying the clonal dynamics of stem cells in several tissues and already collaborates with us to develop mathematical models of cell fate acquisition during mammary gland development and branching morphogenesis. Dr. Rulands will implement statistical models of lumen formation that will be tested in our experimental settings in the four branching

tissues under study. Dr. Hannezo, a theoretical physicist who has been collaborating with us for the past 15 years, will be instrumental in developing statistical frameworks to explain if lumen expansion and coalescence can be mediated through fluid accumulation and the generation of a turgor force. Turgor is generated by hydrostatic pressure, which builds through the accumulation of ion channels and pumps at the apical membrane. At the national level, we engaged collaboration with Dr. Leo Guignard (IBDM, Marseille), Dr. Yohanns Bellaiche (Institut Curie) and Dr. Jean-Ives Tinevez (Image Analysis Hub, Institut Pasteur, Paris, France), mainly for data mining and image analysis. These ongoing collaborations will ensure broadening of the international networks of the PhD student and will be crucial for teaching him/her to develop a leading role in managing collaborative projects.

This project involves 4 disciplines: Biology, Mathematics (modelling of lumen formation in collaboration with Prof. Simons, Dr. Rulands and Dr. Hannezo), Imaging (collaboration with Dr. Guignard, Dr. Bellaiche and Dr. Tinevez) and Biophysics (turgor forces with Prof. Simons, Dr. Rulands and Dr. Hannezo). Of note, the PhD student will be strongly encouraged to follow bioinformatics and statistics training courses as well as imaging workshops to acquire autonomy in processing and analysing their data.

Recent publications

1. Sumbal J, **Fre S**, Sumbalova Koledova Z. (2023). *Fibroblast-induced mammary epithelial branching depends on fibroblast contractility.* Plos Biology, in press.

2. Jacquemin G, Wurmser A, Huyghe M, Sun W, Homayed Z, Merle C, Qasrawi F, Perkins M Richon S, Dingli F, Arras G, Loew D, Vignjevic D, Pannequin J and **Fre S.** (2022). *Paracrine signalling between intestinal epithelial and tumour cells induces a regenerative programme.* eLife 11:e76541. doi: 10.7554/eLife.76541. PMID: 35543624.

3. Lloyd-Lewis B, Gobbo F, Perkins M, Jacquemin G, Huyghe M, **Faraldo M** and **Fre S.** (2022). *In vivo imaging of mammary epithelial cell dynamics in response to lineage-biased Wnt/β-catenin activation.* Cell Reports 38 (10) 110461, ISSN 2211-1247, doi: 10.1016/j.celrep.2022.110461. PMID: 35263603.

4. Jacquemin* G, Benavente-Diaz M, Djaber S, Bore A, Dangles-Marie V, Surdez D, Tajbakhsh S, **Fre* S** and Lloyd-Lewis* B. (2021). *Longitudinal high-resolution imaging through a flexible intravital imaging window.* Science Advances 7 (25), eabg7663
doi: 10.1126/sciadv.abg7663. * co-corresponding authors.

5. Lilja*, A., Rodilla*, V., Huyghe, M., Hannezo, E., Landragin, C., Renaud, O., Leroy, O., Ruland, S., Simons, B.D and **Fre, S.** (2018). *Clonal analysis of Notch1-expressing cells reveals the existence of unipotent stem cells that retain long-term plasticity in the embryonic mammary gland*. Nature Cell Biology 20(6), p. 677–687. DOI:10.1038/s41556-018-0108-1. PMID: 29784917

Expected profile of the candidate

Candidate's requirements:

- Master's degree or equivalent
- Enthusiastic and highly motivated researcher with strong interest in stem cell and developmental biology, morphogenesis
- Ability to work independently, good team spirit, social skills are a plus
- Good communication skills, proficiency in oral and written English
- Candidates with competence in image analysis as well as previous experience in mouse handling, developmental biology, molecular biology or 3D cultures will be prioritised.