Sorbonne Université/China Scholarship Council program 2021

Thesis proposal

Title of the research project: ***Hnf1b* regulation during kidney development and regeneration**

Joint supervision: no

Joint PhD (cotutelle): no

Thesis supervisor: … Muriel Umbhauer.

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Institution: …… Sorbonne Université ….

Doctoral school (N°+name): ED …. …… ED 515 Complexité du Vivant

Research laboratory: UMR7622 CNRS Laboratory of developmental biology

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Subject description (2 pages max):

1) Study context

The POU homeodomain transcription factor hepatocyte nuclear factor 1β (Hnf1b) plays an essential role in vertebrate kidney development. Heterozygous mutations in human HNF1B cause the complex multisystem syndrome known as Renal Cysts And Diabetes (RCAD). The most prominent clinical features of this autosomal dominant disorder are non-diabetic renal disease resulting from abnormal renal development and diabetes mellitus (reviewed by Clissold RL et al., 2015). During early mouse kidney development, Hnf1b has been shown to be required for ureteric bud branching and initiation of nephrogenesis (Lokmane et al., 2010). *Hnf1b* conditional inactivation in murine nephron progenitors has

revealed an additional role in segment fate Xenopus is a well established and attractive model to study kidney development (Krneta-Stankic V. et al, 2017). Renal function at larval stages relies on two pronephroi located on both sides of the body, each consisting on one giant nephron displaying the same structural and functional organisation than the mammalian nephron. Moreover, pronephric and metanephric differentiation and morphogenesis share most of the signalling cascades and gene regulatory networks. Development of the pronephros is completed within 2 days and can be analysed in hundreds of synchronous embryos generated by in vitro fertilization. Microinjection of morpholino oligonucleotides or synthetic mRNA into specific blastomeres allows to target gain and loss of function experiments in the developing pronephric territory. Routine methods for generating transgenic animals are also available (Ogino et al., 2006), while gene knock-out using genome editing tools (Crispr/Cas9, TALENs) has already proven to be efficient in generating Xenopus models of human diseases (Szenker-Ravi et al., 2018). Resources including genomes browsers, gene expression data, epigenome reference maps (www.xenbase.org), and single cell transcriptomics data (http://www.xenbase.org/entry/doNewsRead.do?id=595) are accessible.

In Xenopus, *hnf1b* expression is first observed in the pronephric field at neurula stage and is maintained throughout the entire pronephros development. Expression of HNF1B carrying human mutations in the DNA binding domain leads to pronephros abnormal development (Bohn S et al., 2003). Overexpression of an HNF1B dominant negative construct caused an inhibition of proximal and intermediate tubule marker genes, whereas distal expression remained normal (Heliot et al., 2013). Interestingly, *hnf1b* expression is activated in regenerating nephrons within 24 hr after nephrectomy in the Xenopus tadpole (Suzuki et al., 2019).

How *hnf1b* expression is specifically activated and maintained in the developing Xenopus pronephros, and how it is activated in regenerating nephron is still poorly understood. Our data indicate that *hnf1b* is a potential target gene of the transcription factor pax8 (Buisson et al., 2015). However, other inputs and transcriptional regulators need to be discovered. We propose to elucidate the mechanisms regulating *hnf1b* expression through the identification and characterization of *hnf1b* regulatory sequences responsible for *hnf1b* expression in the developing pronephros and/or in the context of nephron regeneration.

2) Details of the proposal

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1/ Identification of *hnf1b* nephric enhancers during kidney development

Comparative genomics-based approaches have markedly improved our efficiency in identifying enhancers that are often located far from promoters or inside introns (Ogino et al., 2012). Alignment of human *HNF1B* gene sequence with different vertebrate species including *Xenopus tropicalis* has allowed us to identify 8 non-coding sequences (CNSs) conserved in tetrapods that are likely candidates for *hnf1b* cis-regulatory sequences. CNSs' examination of epigenetic marks that characterize active enhancers such as H3K4me1 and P300 will be performed using epigenome reference maps (http://veenstra.science.ru.nl/trackhubx.htm). Possible enhancer activity in the developing kidney of the identified sequences will be assessed in vivo using a transgenic reporter assay in *Xenopus laevis*. Transactivation of the identified CNS will also be tested in kidney-derived cell lines. Using this approach, we have already identified a 300 bp CNS able to drive reporter gene expression in the Xenopus pronephros, as well as in MDCK and IMCD3 cells. In order to analyse the in vivo importance of the idenfied CNSs, Crispr/cas9 technology will be used to analyse the consequences of CNSs deletion on endogenous *hnf1b* expression in *Xenopus tropicalis*. Stable *Xenopus tropicalis* transgenic lines will be established if relevant.

2/ Dissecting the transcriptional inputs for the identified *hnf1b* nephric enhancers

In order to examine the transcriptional mechanisms for the identified *hnf1b* nephric enhancers, we will search for putative transcription factors binding motifs (TFBM) using publicly available tools (JASPAR - http://jaspar.genereg.net). Candidate transcription factors will be narrowed on the basis of their nephric expressions (Xenbase, GUDMAP and Expression Atlas) and by phylogenetic footprinting. Then, the role of the resulting putative candidates on enhancer activity and *hnf1b* expression will be tested by gain and loss of function experiments in Xenopus embryos. Physical interaction between candidate transcription factors and enhancer sequences will be investigated by EMSA and ChIP experiments in vivo. The effects resulting from TFBM mutation on enhancer activity will be assessed using EMSA, transactivation assays in cell lines, and Xenopus F0 transgenesis.

3/ Identification and characterisation of *hnf1b* nephric enhancers during kidney regeneration

Numerous vertebrates have the ability to repair damage to nephric structures, although with distinct mechanisms and extent. In mammals, the regenerative capacity is restricted to the reconstruction of nephric epithelial cells in

damaged regions. In contrast, *Xenopus laevis* tadpole regenerate fully functional nephric tubule architecture after severe damage of the proximal tubules. (Caine and Mclaughlin, 2013, Suzuki et al, 2019). Regeneration appears to involve regrowth of existing tubules and the use of the remaining tubule cells to produce the functional pronephros. We will use the transgenic reporter assay (as in 1/) combined with the surgical removal of nephric tubules to analyse the ability of the identified CNSs to function as regeneration signal-response enhancers. Nephrectomy will be performed on transgenic embryos at stage 37 and reporter gene expression examined 48 hrs after. Characterisation of the CNSs that show enhancer activity will be performed as in 2/ with a particular focus on CNSs able to activate reporter gene expression in regenerating nephrons but not in the developing nephrons, if any.

This project will provide interesting novel insights concerning transcriptional regulation of *hnf1b* during pronephros nephrogenesis and nephron regeneration in Xenopus. Because the role of hnf1b is evolutionary conserved from Xenopus to mammals, we expect that characterization of conserved enhancers will help understanding pathologies caused by HNF1b mutations. The HNF1B-related syndrome is considered to result from *HNF1B* haploinsufficiency. Further understanding transcriptional regulation of HNF1B is therefore essential to decipher how we can modulate its expression and could lead to the identification of new disease-causing mutations of the *HNF1B* locus

3) References

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Szenker-Ravi E et al. , Nature. 2018 Sep;561(7722)

4°) Profile of the Applicant (skills/diploma…)

Applicants should have a Master's level degree in biology. She/he should be highly motivated and be able to work productively in a team as well as independently. The successful applicant has affinity with the fields of molecular biology and cellular biology, well-developed general molecular biology laboratory skills and is keen to embrace both experimental and computational/bioinformatics approaches. Basic background in developmental biology is welcome.

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