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) : Sylvie MAZAN
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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 : Sylvie Mazan
Intitulé de l'équipe : Développement et évolution des vertébrés
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: Hector Escriva
Intitulé et N° d'UMR ou de département : UMR7232 Biologie Intégrative des Organismes Marins

Titre du projet de thèse :
Diversification of habenular asymmetries in vertebrates: a mechanistic approach in the lamprey and catshark

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

Spécialité : Biologie du développement, Evolution

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.
Since their first description in humans by De Broca in 1865, cerebral asymmetries have been found in a wide range of animals [1]. In vertebrates, habenulae, a bilateral epithalamic structure involved in the integration of environmental signals and organismal responses, constitute the reference model for their study. Asymmetries are indeed frequently encountered across the taxon, albeit with considerable variation in degree and nature [2-3]. The mechanistic basis underlying these variations remains poorly known. To date, mechanisms of habenular asymmetry formation have only been analysed in three species: the zebrafish, the only vertebrate genetic model organism to exhibit marked habenular asymmetries, and two non-conventional models, the catshark (chondrichthyan) and the lamprey (cyclostome). All three species share an early, left restricted, window of Nodal activity in the dorsal diencephalon shortly after neural tube closure [4-7]. This activity is essential for habenular asymmetry formation in the catshark and the lamprey, but it is dispensable in the zebrafish, the only effect of its inhibition in the latter being to randomise asymmetry laterality [5-7]. In the catshark, the inhibition of Nodal signalling has multiple effects. In this species, habenular asymmetries include size differences between the left and right, with a larger left habenula, and a highly asymmetric organisation in three main subdomains, each characterised by distinct neuronal identity signatures (Fig. 1A). Nodal signalling has been shown to control neurogenesis, including the timing of neuronal differentiation (which starts on the left as in the zebrafish [8]) and the maintenance of neural progenitor pools (submitted to a differential regulation between early and late progenitors: [9]). It also represses on the left a Wnt signalling activity, which promotes right neuronal identities (Lanoizelet et al, ms in prep). In the lamprey, the molecular and cellular roles of Nodal remain largely unknown, but several intriguing differences are observed with the catshark. The laterality of size asymmetry, since its earliest appearance, is inverted between the two species (with a larger left habenula in the catshark, and a larger right one in the lamprey, Fig. 1B). Despite the conservation of an early, left restricted Nodal activity, neuronal differentiation also starts on the right in the lamprey rather than on the left as in the catshark (Jordan and Lagadec, unpublished). To explain these discrepancies, we propose that in the vertebrate ancestral state, asymmetry formation may have involved several distinct Nodal dependent mechanisms, asymmetrically regulating neuronal identity choices and different aspects of neurogenesis, and differentially conserved depending on species (Fig 1C). The general objective of the project is to test this hypothesis, focussing on systematic comparisons between the lamprey and the catshark.

Two main questions will be addressed:

1. How do Nodal dependent molecular and cellular mechanisms controlling asymmetry elaboration in the lamprey compare to those already identified in the catshark (Fig. 1C)? These mechanisms will be analysed in the lamprey Lampetra fluviatilis during pro-larval stages. The work will focus on a characterisation of proliferation-differentiation patterns and on the search of early habenular neuronal identity markers, using IHC, ISH and RNA tomography ([10], see Feasibility below), an innovative 3D RNA profiling technique adapted to the catshark and lamprey in the laboratory. The Nodal or Wnt dependence of detected asymmetries will be analysed by pharmacological treatments using antagonists of these signaling activities, in conditions already established in the laboratory [7,9].

2. Are early targets of Nodal signaling conserved between the lamprey and catshark? Candidate Nodal target genes will first be identified, taking advantage of 3D RNA profiles of catshark and lamprey embryonic heads during the window of Nodal activity (3D profiles currently available, bioinformatic analysis in progress; see feasibility and Fig. 2 below). Their dependence on Nodal activity will be assessed using pharmacological treatments, as described in [7,9]. Functional analyses will be conducted for selected genes using in ovo pharmacological approaches in the catshark, and a CRISPR-Cas9 approach in the lamprey. This part of the work will be conducted in collaboration with P. Blader, CBI, Toulouse.

Beyond specific results on the driving forces and constraints shaping habenular asymmetries across vertebrates, this project should generate reference transcriptomic and functional data, using innovative techniques (RNA tomography,CRISPR-Cas9 gene inactivation) and exploiting the characteristics of two original model organisms.

References


Figure 1. Habenular asymmetries in the catshark and lamprey. (A) Subdomain organisation of habenulae in the catshark, as inferred from fluorescent ISH of transverse sections. (B) Transverse section of prolarval lamprey habenae showing a major size asymmetry. (C) Nodal roles identified in vertebrates and unanswered questions addressed by the project (blue/green arrowheads resp.: gnathostomes/cyclostomes). Photographs: Lanoizelet, Pain and Lagadec, unpublished.
All standard techniques needed for this project (ISH, IHC, in ovo injections, pharmacological treatments) are routinely used by the DEV group and his collaborator P. Blader (CBI, Toulouse). The project will also benefit from the technical platforms of the Banyuls Oceanological Observatory and UMR7232: confocal imaging including a workstation equipped with the Imaris software; histology, molecular biology; aquariology service providing all facilities for lamprey and catshark housing and manipulation.

Concerning the most innovative approaches to be conducted, i.e. RNA tomography and CRISPR-Cas9 gene inactivations: the DEV group has recently adapted the former to the lamprey and catshark and 3D RNA profiles of the head are already available in both species (see Fig. 2). The bioinformatic expertise needed to extract information from these data will be provided by the support of an UMR7232 engineer with an advanced expertise in statistical analyses of transcriptomic data, including 3D RNA profiles (H. Mayeur). CRISPR-Cas9 analyses, which remain an original approach in the lamprey, will be conducted in collaboration with the group of P. Blader (CBI, Toulouse), who routinely uses them in the zebrafish.

Figure 2. 3D RNA profile of a catshark embryonic head (stage 17).
(A) Digital sagittal section extracted from a 3D genome-wide RNA profile obtained by RNA tomography, with in green, blue, pink and red voxels expressing Fgf17, Emx3, Lhx5 and Fgf8 respectively. The profile was generated at stage 17, corresponding to the window of left restricted Nodal diencephalic activity. (B) Left views of stage 17 catshark embryonic heads following in situ hybridisation (ISH) with probes for Fgf17, Lhx5, Emx3 and Fgf8 as indicated. Digital profiles are consistent with those obtained by ISH. Expected strongly asymmetric, left restricted digital profiles are also obtained for known targets of Nodal signalling, such as Lefty2 (not shown). Data: Mayeur et Lagadec, ms in preparation.

Thèses actuellement en cours dans l’équipe

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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.


**Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS**

**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 (sur le travail de thèse uniquement)** :


