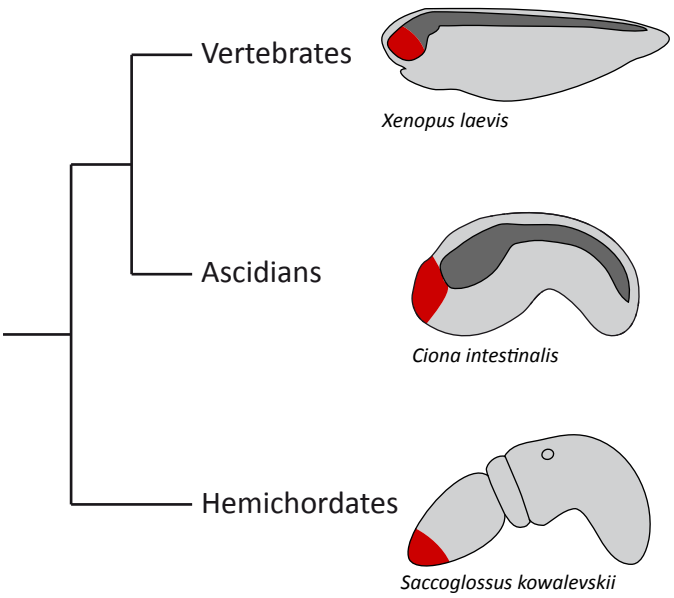
Comparative development of anterior neurectoderm in deuterostomes.

Anterior forebrain or telencephalon is a highly developed structure in vertebrates that contains the olfactory bulb and regions of higher-order brain functions. Understanding its evolutionary origins has thus been a long standing question. Developmental genetic has identifyed a specific combination of which defines a conserved molecular identity of the telencephalon. Decades of molecular embryology have proposed the following two-step model for the early definition of the central nervous system (CNS): signals from the organizer (BMP antagonists being key players) induce ectodermal cells to become neural; these cells have an anterior identity and posteriorizing signals (Fgf, Wnt, Nodal, BMP and Retinoic acid) act to define more posterior CNS territories. One of the fascinating outcome of neural induction in vertebrates is that ANE is the 'default' fate of ectodermal cells: without any influence from neighboring cells – in the silence – cells become anterior and neural. During normal embryonic development, anterior CNS has thus to be actively protected from mesoderm-inducing or posteriorizing molecules by secreted antagonists for example. In particular it has been proposed that the triple inhibition of Wnt, Nodal and BMP signals is necessary and sufficient for head formation.



Comparative studies in deuterostomes, the group of animals that include the vertebrates, the invertebrate chordates (ascidians and amphioxus) and the ambulacrarians (echinoderms and hemichordates), have shown that orthologs of telencephalon markers are expressed at the anterior end of embryos, a region that we will refer to Anterior NeuroEctoderm (ANE). In addition, we have produced published and preliminary data that support a role for Wnt, Nodal and BMP pathways in ANE specification and patterning in invertebrate deuterostomes (the ascidian *Ciona intestinalis* and the hemichordate *Saccoglossus kowalevskii*) [1–4]. Such comparative approaches are also challenging the vertebrate two-step model of CNS formation by suggesting that nervous system specification and AP patterning could be separate processes. First, hemichordates do not have an embryonic CNS but a diffuse nervous system that is radially patterned by the same genes that pattern the chordate CNS, and that is specified autonomously. Second, in ascidians, we have shown that, not only the CNS, but also the peripheral nervous system and the epidermis are patterned along the AP axis under the control of Wnt. These observations are unlikely to be solely linked to species divergence since recent studies from vertebrates point to similar conclusions [5,6].

The PhD project thus aims, through a comparative approach using *Ciona* and *Saccoglossus*, at identifying the mechanisms that specify and pattern the ANE, and at comparing these findings with vertebrate data. We will combine molecular embryology and transcriptomics to address the following questions:

- is the ANE the 'default' fate of the ectoderm? In both species, ANE precursors can be isolated from the embryo at the time they are born (8-cell stage). In *Saccoglossus*, these cells differentiate into ANE and this is suppressed by activating Wnt, Nodal or BMP. We aim at determining the temporal window of such activity by progressively delaying the onset of treatment; and this will provide a first hint at epistatic relationships between pathways. In *Ciona*, similar explants develop into epidermis and they can be induced into neural fate by activating Fgf signaling. For both types of explants, we will determine their AP and ANE identity and test the effect of modulating Wnt, BMP and Nodal. We will also determine the fate of the cells derived from ANE precursors that develop without cell-cell communication (through sister cell separation after every division).

- what are the roles of BMP, Wnt and Nodal signaling pathways in ANE specification? We aim at determining the precise function of the three signaling pathways and their epistatic relationships. We will determine the spatio-temporal activation of these pathways by immunofluorescence and/or reporterassays. In parallel, we will modulate positively and negatively each pathway using whole embryo treatment in a time-controlled manner. Finally, we will combine modulation of pathways to determine their interactions and test whether their triple inhibition is sufficient for ANE fate. While the focus will be on ANE, we will also examine additional markers to determine the precise function of each pathway (posteriorization, ventralization, cell fate specification…).

- are the ANE developmental programs conserved, and do they lead to specification of similar neurons? Using results from the above two sections, we will generate key biological samples (explants and/or whole embryos with pathway modulations at specific developmental times) that will be processed for RNA-seq to identify ANE genes. The analysis of our datasets will be complemented by scRNA-seq available for both species and should produce a global atlas/network of ANE specification in each species, and shed light on the possible cellular homology at the level of ANE neurons. Comparison will be extended to vertebrates using finely resolved expression data and methods developed for vertebrate ectoderm patterning.

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