The gene regulatory network in the anterior neural plate border of ascidian embryos

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Background

It has been proposed that cranial placodes and neural crest are vertebrate innovations that give rise to structures including sensory organs, skeleton and muscles in the head, and thereby that these structures had contributed to the emergence of a ‘‘new head” in vertebrates. Ascidians, closest relatives of vertebrates, possess cells similar to cranial placode and neural crest cells. These ascidian cells are differentiated in the anterior neural plate border (ANB) and the lateral neural plate border, as in vertebrate embryos.

In ascidian embryos, *Foxg* is expressed in palps, which are ANB-derived adhesive organs with sensory neurons. Its vertebrate ortholog, *Foxg1*, is expressed in the telencephalon and cranial placodes. In the present study, to understand the evolutionary origin of the vertebrate placodes, I examined how *Foxg* is activated and functions in the ascidian embryo.

Methods

The spatio-temporal gene expression patterns were examined by fluorescence *in situ* hybridization. For gene knockdown experiments, morpholino antisense oligonucleotides were injected. Mis/overexpression DNA constructs were introduced by electroporation. Activities of the MAPK (mitogen activated protein kinase) and Bmp (bone morphogenetic protein) signaling pathways were examined by immunostaining.

Results

First, I precisely determined the spatio-temporal expression patterns of *Foxg*, *Emx*, *Zf220* and *Isl* in the ANB. *Foxg* was initially expressed at the most anterior and posterior rows in the ANB at the early neurula stage. *Emx* was expressed between the two rows with *Foxg* expression. *Zf220* was expressed in a subset of cells with *Foxg* or *Emx* expression at the initial tailbud stage. At the middle tailbud stage, three distinct regions expressed *Foxg*. These regions also expressed *Isl*, which is a marker for cells that contribute to protrusions formed in the palp. *Emx* and *Zf220* were expressed in the intervening regions of the presumptive palp protrusion cells at this stage.

By knockdown and mis/overexpression of *Foxg*, I found that *Foxg* was required for proper palp formation, and that *Foxg* activated *Isl* and repressed *Emx*. While *Foxg* was also expressed in the oral siphon primordium, which is also derived from the ANB, no defects were found in this region of *Foxg* morphants. Similarly, no defects were observed in the anterior part of the brain or atrial siphon primordia (counterparts of the otic placodes in vertebrate embryos), although *Foxg* plays an important role in these tissues of vertebrate embryos.

Foxc is required for the initiation of *Foxg* expression. I found several additional factors that regulated *Foxg*; *Tfap2-r.b* regulated *Foxg* in the anterior row; the MAPK signaling pathway is used to specifically induce *Foxg* in the most anterior and posterior rows of the four rows in the ANB; *Zf220* repressed *Foxg*.

The Bmp signaling pathway was activated differentially in the ANB. A gene expression profile assay suggested that this differential activation pattern was established by two Bmp ligands expressed in ANB cells (*Admp* and *Bmp5/6/7/8*) and two Bmp-antagonist genes expressed in cells laterally and posteriorly adjacent to the ANB (*Chordin* and *Noggin*). I also present evidence suggesting that a low level of Bmp signaling activity was required for *Six1/2* expression, and that a high level of Bmp signaling activity was required for palp formation.

Discussion

The present study uncovered the gene regulatory circuit in the ANB. This circuit strongly indicates that ANB in ascidians shares the evolutionary origin with the cranial placodes in vertebrates with the following reasons. First, *Foxg* is important for differentiation of the cranial placodes in vertebrates and for differentiation of the palps in ascidians. The vertebrate placodes and ascidian palps are both derived from the anterior border of the neural plate. Second, in both of vertebrates and ascidians, *Foxg* is positively regulated through activation of the MAPK pathway. Third, different levels of Bmp signaling activity induce different cell types within the anterior border of the neural plate in embryos of vertebrates and ascidians.

In vertebrate embryos, *Foxg* is expressed in the telencephalon (the most anterior brain region), and plays a role in specifying this region. In vertebrate embryos, *Foxg* expression is induced by *Fgf8* (fibroblast growth factor 8) in the anterior border of the neural plate and in the telencephalon, and *Foxg* maintains *Fgf8* expression. However, in ascidian embryos, *Foxg* is expressed only in the ANB but not in the brain. Although this *Foxg* expression is under the control of the MAPK signaling pathway, the sole ortholog for *Fgf8* is not expressed in the anterior part of the brain. Ascidians do not have a structure morphologically similar to the telencephalon, which is consistent with the *Foxg* expression patterns. On the basis of these observations, I propose a model of evolution of the telencephalon. Specifically, recruitment of *Fgf8* as a downstream gene of *Foxg* would constitute the *Fgf8*-*Foxg* regulator loop, which is seen in extant vertebrates, and this loop could expand *Foxg* expression to the adjacent brain region, because Fgf8 is a secreted molecule.