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RESEARCH REPORT

Patterning of brain precursors in ascidian embryos

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ABSTRACT

In terms of their embryonic origins, the anterior and posterior parts of the ascidian central nervous system (CNS) are associated with distinct germ layers. The anterior part of the sensory vesicle, or brain, originates from ectoderm lineages following a neuro-epidermal binary fate decision. In contrast, a large part of the remaining posterior CNS is generated following neuro-mesodermal binary fate decisions. Here, we address the mechanisms that pattern the anterior brain precursors along the medial-lateral axis (future ventral-dorsal) at neural plate stages. Our functional studies show that Nodal signals are required for induction of lateral genes, including *Delta-like*, *Snail*, *Msxb* and *Trp*. Delta-like/Notch signalling induces intermediate (*Gsx*) over medial (*Meis*) gene expression in intermediate cells, whereas the combinatorial action of Snail and Msxb prevents the expression of *Gsx* in lateral cells. We conclude that despite the distinct embryonic lineage origins within the larval CNS, the mechanisms that pattern neural precursors are remarkably similar.

KEY WORDS: Ascidian, *Ciona*, Brain, Sensory vesicle, Neural patterning

INTRODUCTION

The chordate super-phylum is characterised by a well patterned dorsal tubular central nervous system (CNS) (Satoh et al., 2014). Ascidians belong to the urochordates, or tunicates, a phylum of invertebrate chordates closely related to vertebrates (Delsuc et al., 2006; Satoh et al., 2014). Ascidian embryos develop with very few numbers of cells and a fixed cell lineage, features enabling the step-by-step analysis of developmental cell fate choices with a single-cell level of precision (Hudson, 2016).

Founder cell lineages of the ascidian embryo are established at the 8-cell stage, when the embryo divides along the animal-vegetal axis to produce two pairs of animal cells (the a- and b-lineages) and two pairs of vegetal cells (the A- and B-lineages). The CNS arises from the a-, b- and A-lineages (Nicol and Meinertzhagen, 1988a,b; Nishida, 1987). The anterior-most part of the sensory vesicle, including the pigmented cells, has an a-lineage origin and thus shares a common origin with anterior epidermis. The dorsal-most cells of the remaining CNS arise from the b-lineage, with the rest of the CNS arising from the A-lineage cells, which share a common lineage origin with mesoderm (notochord). At mid-gastrula stages, A- and a-lineage CNS precursors are arranged in a neural plate that consists of six rows of cells along the anterior-posterior (A-P) axis, such that row I is the most posterior and row VI the most anterior (Fig. 1A). The posterior-most two rows (I-II) of cells are A-lineage, and the anterior four rows (III-VI) of cells are a-lineage. Cells are aligned in columns along the medial-lateral axis, with column 1 the medial-most pair of columns and column 3 the lateral-most, although the A-lineage has an additional fourth column. The b-lineage cells are positioned lateral to this grid-like array. Of the four rows of a-lineage cells, only rows III and IV will actually contribute to the CNS, generating the anterior part of the sensory vesicle, the ascidian 'brain', and contributing to the oral siphon primordium (Christiaen et al., 2007; Cole and Meinertzhagen, 2004; Nishida, 1987; Taniguchi and Nishida, 2004; Veeman et al., 2010). Rows V and VI will form a specialised region of anterior epidermis, including a placode-like territory and the palps (Abitua et al., 2015; Nishida, 1987).

Patterning of the A-lineage-derived neural plate involves combinatorial inputs of FGF/ERK, Nodal and two temporally separable Delta/Notch signals (Hudson and Yasuo, 2005; Hudson et al., 2007; Imai et al., 2006; Mita and Fujiwara, 2007). Each cell, present on both sides of the bilaterally symmetrical embryo, receives a unique combination of these three signalling pathways, which determine the eight distinct cell types (Hudson et al., 2007). Like the A-lineage-derived neural plate, differential FGF/ERK signalling also patterns the a-lineage-derived neural plate along its anterior-posterior axis. Specifically, FGF/ERK signalling is required to promote row III over row IV cell identities (Haupaix et al., 2014; Racioppi et al., 2014). Similarly, as in the A-lineage neural plate, Nodal signalling is implicated in specification of the lateral part of the a-lineage neural plate, as lateral gene expression is lost in the a-lineage cells when Nodal signalling is inhibited (Hudson and Yasuo, 2005; Imai et al., 2006; Ohtsuka et al., 2014). In this study, we investigate in detail the mechanisms responsible for patterning of the a-lineage row III brain precursors of *Ciona* embryos.

RESULTS AND DISCUSSION

Nodal is required for medial-lateral patterning of the a-lineage-derived neural plate

In order to investigate patterning of the ascidian brain precursors, we used a set of three genes, *Trp*, *Gsx* and *Meis*, which label row III cells in columns 3 (lateral), 2 (intermediate) and 1 (medial), respectively, at neurula stages. The expression of *Trp* and *Meis* was analysed at the neurula stage (~8.25 h of development at 18°C), when all of the 6-row neural plate cells have divided along the A-P axis (Fig. 1A). *Trp* is expressed in column 3, with stronger expression in the posterior cell, a10.97, whereas *Meis* is expressed in column 1, with stronger expression in the posterior cell a10.73 (Fig. 1A, Fig. 2A). *Gsx* expression was analysed in slightly earlier neurula stage embryos (7.5 h of development at 18°C), when it is expressed in both row IIIa and row IIIp (a10.66 and a10.65

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