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Temporal coupling between specifications of neuronal and macular fates of the inner ear

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ABSTRACT

The inner ear is a complex organ comprised of various specialized sensory organs for detecting sound and head movements. The timing of specification for these sensory organs, however, is not clear. Previous fate mapping results of the inner ear indicate that vestibular and auditory ganglia and two of the vestibular sensory organs, the utricular macula (UM) and saccular macula (SM), are lineage related. Based on the medial-lateral relationship where respective auditory and vestibular neuroblasts exit from the otic epithelium and the subsequent formation of the medial SM and lateral UM in these regions, we hypothesized that specification of the two lateral structures, the vestibular ganglion and the UM are coupled and likewise for the two medial structures, the auditory ganglion and the SM. We tested this hypothesis by surgically inverting the primary axes of the otic cup in ovo and investigating the fate of the vestibular neurogenic region, which had been spotted with a lipophilic dye. Our results showed that the laterally-positioned, dye-associated, vestibular ganglion and UM were largely normal in transplanted ears, whereas both auditory ganglion and SM showed abnormalities suggesting the lateral but not the medial-derived structures were mostly specified at the time of transplantation. Both of these results are consistent with a temporal coupling between neuronal and macular fate specifications.

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1. Introduction

During early stages of inner ear development in both chicken and mice, cells within the otic epithelium can be categorized into neuronal, sensory and non-sensory fates. Based largely on gene expression patterns, neuronal and sensory precursor domains overlap in the anterior otic cup region and are collectively named as the neural-sensory competent domain (NSC) (Fekete and Wu, 2002). Neuronal-fated cells delaminate from this NSC to form the neurons of the cochleovestibular ganglion (CVG), which later divides into the vestibular and auditory ganglion. Cells that remained in the NSC after neuroblast delamination are thought to split into various sensory organs over time (Fekete and Wu, 2002).

In the chicken otic placode, cell-tracing experiments using lipophilic dye suggest that the sensory cells of an individual sensory organ and its innervating neurons are derived from the same region of the placode (Bell et al., 2008). More specifically, cell lineage studies using replication incompetent retrovirus in chicken indicate that neurons of the CVG and cells within the utricle share a common lineage (Satoh and Fekete, 2005). Furthermore, genetic fate mapping of the neuronal lineage in the mouse inner ear using the *Neurogenin1-cre* (*Neurog1-cre*) strain indicates that the vestibular and auditory neurons share a common lineage with the two maculae, utricular macula (UM) and saccular macula (SM) (Raft et al., 2007). However, the relationships between the neuronal and macular fate specifications are not known.

Among the three primary fates of the inner ear, the neuronal fate is likely to be the first to be specified, since the earliest morphological change evident in the shallow otic cup is the delamination of neuroblasts from the anterior-ventral region (Alvarez and Navascues, 1990; Carney and Couve, 1989). Within the NSC, there are also spatial and temporal differences in the generation of neuronal subtypes: the lateral NSC gives rise to vestibular neuroblasts (Fig. 1A, red colors) at an earlier time than the medial NSC gives rise to auditory neuroblasts (Fig. 1A, purple colors) (Bell et al., 2008; Koundakjian et al., 2007). While these spatial and temporal differences suggest that neuroblasts may have already acquired some of their identities prior to exiting from the otic epithelium, the timing of specification for each neuronal type is not clear. Based on the evidence of shared lineage between neurons and the two maculae in both chicken and mice (Raft et al., 2007; Satoh and Fekete, 2005), we hypothesized that location within the NSC not only dictates neuronal subtype fate but also specifies the type of macula that develops subsequently in the

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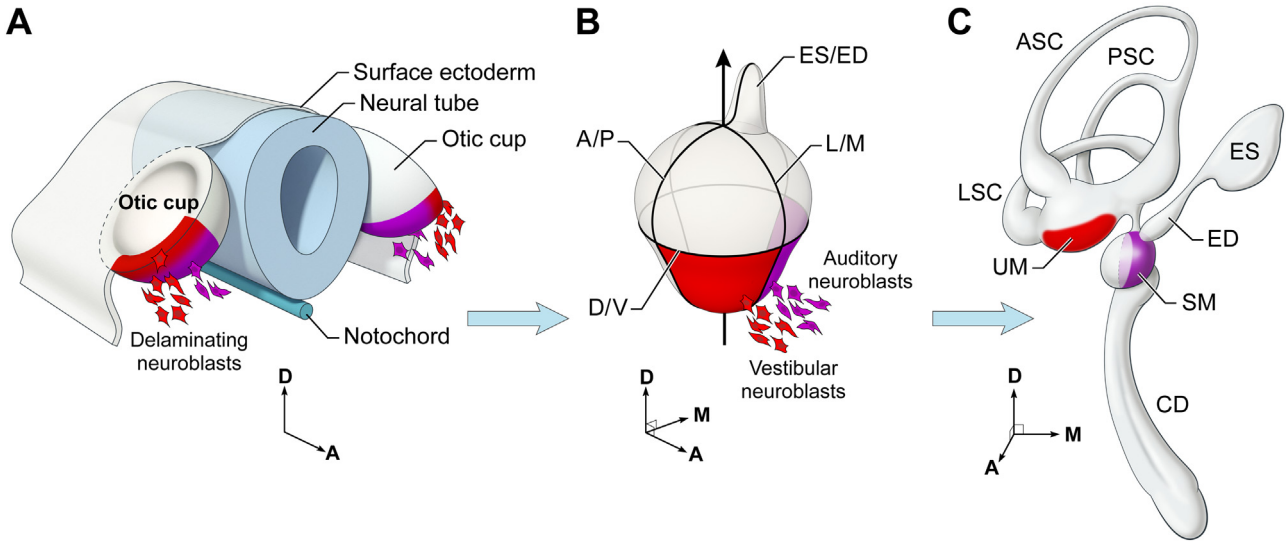

Fig. 1. Schematic diagram of an otic cup (A), otocyst (B), and a mature inner ear (C). (A) and (B) illustrate the consensus that the vestibular neurogenic region (red color) is located lateral to the auditory neurogenic region (purple color), where respective neuroblasts delaminate. Based on the spatial locations of the sensory organs in the mature inner ear, we propose that after neuroblasts delamination, the lateral neurogenic region develops into the utricular macula (red color, C) and the medial region develops into the saccular macula (purple color, C). For simplification, the vestibular and auditory ganglia are not shown in (C). Abbreviations: ASC, anterior semicircular canal; CD, cochlear duct; ED, endolymphatic duct; ES, endolymphatic sac; LSC, lateral semicircular canal; PSC, posterior semicircular canal; SM, saccular macula; UM, utricular macula. Orientations: A, anterior; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral.

same region. This hypothesis predicts that the lateral NSC specifies the fate of both vestibular neurons and the laterally-positioned UM, whereas the medial NSC specifies the formation of auditory neurons and the medially-positioned sensory organs such as the SM (Fig. 1). More importantly, this model predicts that specification of both neuronal and macula fates are coupled and this

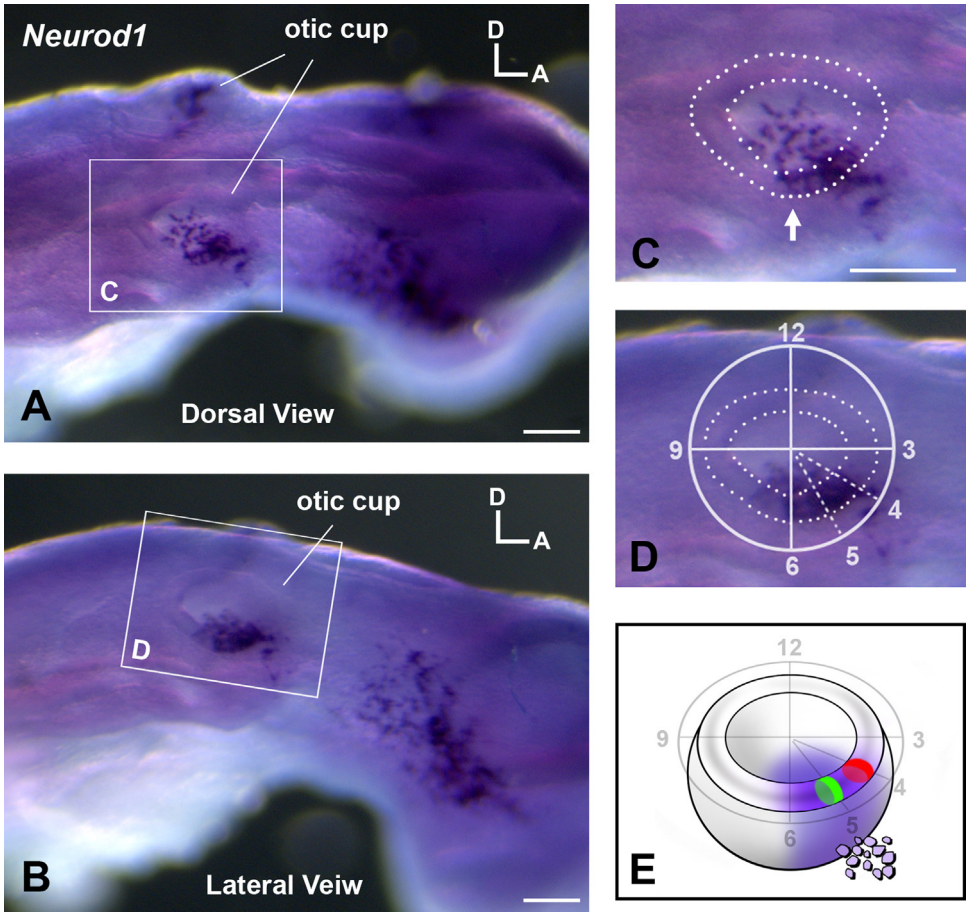

Fig. 2. *Neurod1* expression in the chicken otic cup. (A-D) Dorsal and lateral views of an otic cup at 19ss. (C) and (D) are higher magnification of the otic cup shown in (A, B). By aligning the ventral tip of the otic cup (C, arrow) as the 6 o'clock position of a clock face, the *Neurod1* domain at the rim of the otic cup always falls between 4 and 6 o'clock positions (D). (E) Schematic diagram of the neurogenic domain, its delaminating neuroblasts and locations of dye injections. Scale bars: 100 μm.