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Origin and plasticity of the subdivisions of the inferior olivary complex

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ABSTRACT

The precerebellar nuclei (PCN) originate from the rhombic lip, a germinal neuroepithelium adjacent to the roof plate of the fourth ventricle. We first report here that, in chicken, the *Brn3a*-expressing postmitotic medullary cells that produce the inferior olive (ION, the source of cerebellar climbing fibres) originate from a dorso-ventral domain roughly coinciding with the hindbrain vestibular column. Whereas *Foxd3* expression labels the whole mature ION but is only detected in a subpopulation of ION neuroblasts initiating their migration, we report that *Brn3a* allows the visualization of the whole population of ION neurons from the very beginning of their migration. We show that *Brn3a*-positive neurons migrate tangentially ventralwards through a characteristic dorso-ventral double submarginal stream. *Cath1* expressing progenitors lying just dorsal to the ION origin correlated dorso-ventral topography with the prospective cochlear column (caudal to it) and generate precerebellar nuclei emitting mossy-fiber cerebellar afferents. We used the chick–quail chimaera technique with homotopic grafts at HH10 to determine the precise fate map of ION precursors across the caudal cryptorhombomeric subdivisions of the medullary hindbrain (r8–r11). We demonstrate that each crypto-rhombomere contributes to two lamellae of the ION, while each ION sub-nucleus originates from at least two contiguous crypto-rhombomeres. We then questioned how rhombomere identity is related to the plasticity of cell type specification in the dorsal hindbrain. The potential plasticity of ectopically HH10 grafted ION progenitors to change their original fate in alternative rostrocaudal environments was examined. Heterotopic grafts from the presumptive ION territory to the pontine region (r4–r5) caused a change of fate, since the migrated derivatives adopted a pontine phenotype. The reverse experiment caused pontine progenitors to produce derivatives appropriately integrated into the ION complex. Grafts of ION progenitor domains to myelomeres (my) 2–3 also showed complete fate regulation, reproducing spinal cord-like structures, whereas the reverse experiment revealed the inability of my2–3 to generate ION cell types. This was not the case with more caudal, relatively less specified myelomeres (my5–6). Interestingly, when heterotopically grafted cells are integrated dorsally, they do not change their phenotype. Our results support the hypothesis that positional information present in the hindbrain and spinal cord at early neural tube stages controls the specific fates of ventrally migrating PCN precursors.

Introduction

The precerebellar nuclei (PCN) originate from the embryonic rhombic lip, which constitutes a singular germinative epithelium in the dorsalmost portion of the medullary hindbrain. The pontine nuclei (PN), the external cuneate nucleus (ECN), the lateral reticular nucleus (LRN), and the inferior olivary nucleus (ION) are distinct rhombic lip derivatives identified in mammals (Altman and Bayer, 1987a,b; Rakic, 1990). The axons of mature precerebellar neurons end in the cerebellum as mossy or climbing fibres, the latter originating exclusively from the ION (see Sotelo, 2008). During embryonic development, two ventrally directed streams of tangentially migrating rhombic lip neurons have been reported: a marginal (superficial) stream contains exclusively neurons fated to integrate the LRN, ECN, and PN, whereas cells targeting the ION follow exclusively a submarginal (deep) stream towards their final destination (His, 1890; Essick, 1912; Ellenberger et al., 1969; Altman and Bayer, 1987a,b; Bourrat and Sotelo, 1988; reviewed by Sotelo, 2004).

The combination of anatomical data together with genetic fate mapping and conditional mutagenesis has allowed the identification of sub-domains in the rhombic lip, that give rise to the distinct PCN progenitors. The precerebellar primordium would be divided along the dorso-ventral axis into discrete molecularly defined pools of progenitor cells (Lmx1a, Math1, Ngn1, Mash1 and Ptf1a pools) jointly with a delimitation with Wnt1 and Olig3 (Ray and Dymecki, 2009). In mice, *Math1* has been implicated, among others, in the generation of mossy-fibre neurons that enter the medullary marginal stream (Ben-Arie et al., 2000; Landsberg et al., 2005;

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Machold and Fishell, 2005; Wang et al., 2005; Farago et al., 2006). Its expression defines the precerebellar primordia (r7–r11) but *Math1* is not necessary for ION development (Wang et al., 2005). Conversely, the co-expression of Ptf1a and Olig3 has been shown to be required for the specification of climbing fiber neurons and ION neurons have been proposed to rise from the area with combined expression of Ptf1, Olig3 and a weak Wnt1 level. Interestingly, the *Brn3* family of genes, members of the POU-IV class of transcription factors (Turner et al., 1994), is involved in CNS patterning and specification of some mature neuronal phenotypes (Fedtsova and Turner, 1995; McEvilly et al., 1996; Xiang et al., 1996; Eng et al., 2004). In mammals, *Brn3b* expression, first present ventral to the cochlear primordium in the rhombic lip territory, allows visualizing the migrating ION cells along the submarginal stream and their mature cytoarchitectonic arrangement (Fedtsova and Turner, 1995; McEvilly et al., 1996; Xiang et al., 1996; Bloch-Gallego et al., 1999; Trieu et al., 1999; de Diego et al., 2002; Marillat et al., 2004; Di Meglio et al., 2008).

Once genetically determined to their ION fate, young postmitotic neurons will migrate and will be integrated in the various sub-domains of the ION nucleus. Classic studies in birds showed that the incipient ION has a bi-lamellar structure constituted by dorsal and ventral lamellae (Kooy, 1917; Harkmark, 1954; Vogt-Nilsen, 1954). As development proceeds, these two lamellae transform into the mature ION, which is subdivided into smaller interconnected cells groups. According to Kooy's homology studies, the dorsal lamella will develop medial and lateral olivary parts (MAO, the homologue of the mammalian medial accessory olive, versus DAO, the homologue of the mammalian dorsal accessory olive; see also Vogt-Nilsen, 1954). The MAO, found adjacent to the midline raphe, is constituted by dorsal, middle, and ventral subdivisions (dMAO, mMAO, vMAO). The DAO, located between the MAO and the ventral lamella, consists of lateral and medial portions (lDAO and mDAO). The ventral ION lamella also forms lateral and medial cell aggregates (lVL and mVL). This subdivision is held to be homologous as a whole with the mammalian principal ION subnucleus (Kooy, 1917; Vogt-Nilsen, 1954; Watson and Herron, 1977; Furber, 1983; Whitworth and Haines, 1986).

In birds, the origin of the ION complex in the cryptically segmented caudal hindbrain (medulla oblongata; Marin et al., 2008) has been fate mapped using the chick/quail chimaera technique. Extending the reports of Vogt-Nilsen, 1954 and Tan and Le Douarin (1991), whose focus was the origin of the dorsal and ventral lamellae, Cambronero and Puelles (2000) showed that the complete chick ION originates within crypto-rhombomeres (r) r8 to r11. However, a detailed correlation of the origin of each ION subnucleus with one or several neuromeric subdivisions of the caudal hindbrain was first attempted in the present report.

The plasticity of rhombomeric identity has been extensively studied, both in avian and mouse embryos (Kuratani and Eichele, 1993; Grapin-Botton et al., 1995, 1997, 1998, 1999; Martinez et al., 1995; Simon and Gordon, 1995; Itasaki et al., 1996; Saldivar et al., 1996; Gould et al., 1998; Marin and Charnay, 2000). The specific molecular identity of each segmental unit is acquired by a unique combination of genetic marker expressions, best exemplified by the well-known 3′ to 5′ spatial colinearity of *Hox* genes (Marin et al., 2008). Differential specification entails restrictions in cell movements between adjacent hindbrain units, and causes the emergence of the interposed interrhombomeric limits (Fraser et al., 1990; Martinez et al., 1992). Planar and vertical signaling from the neural tube and the paraxial mesoderm, respectively, seems responsible for these patterning inductions (Grapin-Botton et al., 1995, 1997, 1998; Itasaki et al., 1996; Gould et al., 1998).

We aimed at studying the plasticity of cell type specification in the various rhombomeres – for PCN neuroepithelial precursors – in the dorsal hindbrain. Thus, in the present work, we first characterized the medullary expression in the chick of various relevant markers, focusing in particular on *Brn3a*-positive cells, attending both to their dorsal cell source and migration in the hindbrain alar plate and their postmigratory incorporation within the ventrally placed mature olivary complex. We compared *Brn3a* expression with *Foxd3* expression that had been already proposed to label ION (Storm et al., 2009). The area containing the ION precursors colocalizes with the presumptive vestibular column in the alar plate of the developing chick hindbrain, always located ventrally to the dorsal rhombic lip subarea characterized by *Cath1* expression. In the rostralmost ION source domain (r8), located at a sizeable distance from the choroidal insertion line, *Brn3a*-positive migrating cells invade the submarginal stream via an initial radial course. Caudalwards, the source domain occupies progressively a more dorsal position, but this lies always ventral to the *Cath1*-positive rhombic lip area. The caudalmost *Brn3a*-expressing presumptive ION elements tend to follow a nearly tangential course into the submarginal stream.

We next performed an exhaustive neuromeric fate map of the ION subregions, using the quail–chick chimaera procedure. Altogether, the results show that each sub-nucleus has a well-defined origin, from at least two contiguous crypto-rhombomeres. We also analyzed the plasticity of the rhombic lip progenitor territory via heterotopic grafts of r4–r5 (normal cochlear fate) into r9–r10 (ION source site) or vice versa, as well as grafts of r9–r10 rhombic lip into myelomeres 2–3 or 5–6 (spinal cord) and vice versa. Our experimental results are consistent with the hypothesis that positional signals along the antero-posterior and dorso-ventral axes of the hindbrain and spinal cord alar plates control the specific fate of ventrally migrating PCN precursors.

Material and methods

Tissue processing

Fertilized White Leghorn chick and Japanese quail eggs were incubated in a humidified atmosphere at 38 ± 1 °C. Normal and chimaeric embryos were fixed by immersion or intracardiac perfusion with 4% paraformaldehyde solution and processed for cryostat sections as described by Sanchez-Guardado et al. (2011). The recommendations of the European Union and the Spanish government were followed.

Grafting experiments

Chick and quail embryos were used to obtain chimaeras by transferring excised portions of the hindbrain and spinal cord (Vaage, 1969) at stage HH10 (Hamburger and Hamilton, 1951) in either homotopic or heterotopic transplants. For grafts of the cryptically segmented caudal hindbrain, the center of adjacent somites was taken as a landmark to identify the crypto-rhombomeric limits (Cambronero and Puelles, 2000). In all cases, the chick embryo was the host and the quail embryo the donor. The surgical procedure has been described in detail (Alvarado-Mallart and Sotelo, 1984).

Chick cDNA and in situ hybridization

Chicken *Brn3a* plasmid was kindly provided by Antonio Simeone and *Foxd3* by Thomas Müller. *Brn3* is a cDNA fragment generated by RT-PCR, spanning the region between codon 6 and 117 (335 bp). *Brn3a* probe were obtained using HindIII (Invitrogen) and T7 (Roche) to generate an antisense probe. *Cath1* and *Hoxb7* riboprobes were synthesised from cDNA ESTs no. ChEST686k4 and ChEST611c8, respectively. *Foxd3* probe was obtained using HindIII (Invitrogen) and T3 (Roche) to generate an antisense probe. *Delta1* probes (NM_204973.1) were obtained