Developmental Biology 323 (2008) 230–247

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Genomes & Developmental Control

Hox gene colinear expression in the avian medulla oblongata is correlated with pseudorhombomeric domains

Faustino Marín *, Pilar Aroca, Luis Puelles

Department of Human Anatomy and Psychobiology, School of Medicine, University of Murcia, CIBER de Enfermedades Raras (CIBERER), Instituto Universitario de Envejecimiento, E-30100 Murcia, Spain

ARTICLE INFO

Article history:
Received for publication 30 April 2008
Revised 29 July 2008
Accepted 15 August 2008
Available online 27 August 2008

Keywords:
Hindbrain
Rhombomeres
Transcription factor
Pattern formation
Chick embryo
Chick brain
Myelencephalon
Segmentation

ABSTRACT

The medulla oblongata (or caudal hindbrain) is not overtly segmented, since it lacks observable interrhombomeric boundaries. However, quail–chick fate maps showed that it is formed by 5 pseudorhombomeres (r7–r11) which were empirically found to be delimited consistently at planes crossing through adjacent somites (Cambronero and Puelles, 2000). We aimed to reexamine the possible segmentation or rostrocaudal regionalisation of this brain region attending to molecular criteria. To this end, we studied the expression of *Hox* genes from groups 3 to 7 correlative to the differentiating nuclei of the medulla oblongata. Our results show that these genes are differentially expressed in the mature medulla oblongata, displaying instances of typical antero-posterior (3′ to 5′) Hox colinearity. The different sensory and motor columns, as well as the reticular formation, appear rostrocaudally regionalised according to spaced steps in their Hox expression pattern. The anterior limits of the respective expression domains largely fit boundaries defined between the experimental pseudorhombomeres. Therefore the medulla oblongata shows a Hox-related rostrocaudal molecular regionalisation comparable to that found among rhombomeres, and numerically consistent with the pseudorhombomere list. This suggests that medullary pseudorhombomeres share some AP patterning mechanisms with the rhombomeres present in the rostral, overtly-segmented hindbrain, irrespective of variant boundary properties.

Introduction

The developing vertebrate hindbrain displays segments (rhombomeres) along its rostrocaudal axis that show a fundamental metameric cellular organisation modified by specific local identity. At early embryonic stages, overt rhombomeres are limited by boundaries that display typical characteristics. These include clonal restriction (Fraser et al., 1990), aggregation of early axons (Lumsden and Keynes, 1989), a differential pattern of interkinetic nuclear dynamic (Guthrie et al., 1991), reduced gap-junctional permeability (Martínez et al., 1992) and expression of distinct molecular markers (Heyman et al., 1993, 1995). However, all these characteristics are only observable in boundaries r1/r2 to r6/r7. Rostrally there appears a brain region formed by r1, isthmus and caudal midbrain, whose patterning is governed not by intersegmental boundaries but by gradiental signaling from the isthmic organiser (reviews by Puelles et al., 1996; Martínez, 2001; Wrust and Bally-Cuif, 2001). The hindbrain portion lying caudal to the r6/r7 boundary was subdivided by some authors into rhombomeres r7 and r8, using the rostral end of the hypoglossal nucleus to define the r7/8 boundary (Vaage, 1969; Lumsden and Keynes, 1989; Lumsden, 1990). However, this putative boundary lacks the cellular and molecular features described above and r8 is considerably larger than r7. The caudal hindbrain appears therefore as a non-segmented region, according to standard criteria as well as gross morphology (i.e. scanning images of the ventricular surface; Tanaka et al., 1987). Indeed, even the medullo-spinal boundary is not recognisable without referring to experimental fate maps (Cambronero and Puelles, 2000).

Another well-known characteristic of typical rhombomeres (r2–r6) is that they form segmentally iterated cell groups with a heterochronic pattern of neurogenesis (Amat, 1986; Clarke and Lumsden, 1993; the even-numbered rhombomeres associated to

Abbreviations: 6, abducens nucleus; 6n, abducens nerve; 8cn, cochlear nerve; 9, glossopharyngeal dorsal motor nucleus; 9n, glossopharyngeal nerve; 10, dorsal motor nucleus of the vagus; 10n, vagus nerve; 11, accessory nerve nucleus; 12, hypoglossal nucleus; Amb, ambiguus nucleus; Ang, angular cochlear nucleus; dcn, dorsal column nuclei; Gi, gigantocellular nucleus; Gu, gustatory nucleus; IOD, inferior olivary nucleus, dorsal lamina; IOV, inferior olivary nucleus, ventral lamina; IRt, intermediate reticular nucleus; IZ, intermediate zone; LVeD, lateral vestibular nucleus, dorsal part; LVeV, lateral vestibular nucleus, ventral part; MCC, magnocellular cochlear nucleus; mlf, medial longitudinal fasciculus; MSO, medial superior olivary nucleus; MVe, medial vesticbular nucleus; my, myelomere; PCRt, parvicellar reticular nucleus; Pe, periventricllar stratum; PMn, paramedian reticular nucleus; Pn, pontine nuclei, Pr, prepositus nucleus; r, rhombomere; RF, retrofacial nucleus; RO, raphe obscurus; RPa, raphe pallidus; Rt, reticular formation; RVL, rostroventrolateral reticular nucleus; Sol, nucleus of the solitary tract; sol, solitary tract; Sp5C, spinal trigeminal nucleus, caudal part; SP5I, spinal trigeminal nucleus, interpolar part; SpVe, spinal vestibular nucleus; vc, ventral column of the spinal cord; VH, ventral horn of spinal cord; xc, cochlear decussation; xsol, decussation of the solitary tract; xv, vestibular commissure.

* Corresponding author. Fax: +34 968363955.
E-mail address: marin@um.es (F. Marín).

0012-1606/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.08.017

nerve roots develop in advance relative to the odd ones). These discrete neuronal groups are encompassed within motor or sensory nuclei (Lumsden, 1990; Simon et al., 1995). In contrast, the caudal hindbrain shows a neurogenetic pattern that is more homogenous along the rostrocaudal axis. There appears instead a graded pattern of neurogenesis with several longitudinal columns extending caudalwards into the spinal cord (Amat, 1986). Clarke and Lumsden (1993) noted that the caudal hindbrain lacks some neuronal types typical of rhombomeres (r2–r6), and shows other types characteristic of the spinal cord. The caudal hindbrain also resembles the spinal cord in that both are flanked by somites, while typical rhombomeres are flanked by the otic placode and head mesoderm. The caudal hindbrain thus seems to lack the typical differentiated segmented structure of the rostral hindbrain or other brain regions (i.e. prosomeres) and shares instead cellular and morphological features with the non-overtly segmented spinal cord.

The ontogenesis of this brain region has been analysed descriptively and by fate maps that show its correspondance with the classic myelencephalon or medulla oblongata (His, 1893; Vaage, 1969; Tan and Le Douarin, 1991; Cambronero and Puelles 2000; review by Puelles et al., 2007). Its alar plate forms the caudal part of the cochlear, vestibular, trigeminal and viscerosensory columns, each of them internally regionalised along the rostrocaudal axis. For example, the viscerosensory column, that includes the gustatory and solitary nuclei, has a very complex structure in terms of morphology, function and neurochemistry (Dubbeldam et al., 1979). In the basal plate of the medulla there are highly regionalised motor complexes such as those of vagal and hypoglossal nerves, as well as diverse specialised areas of the reticular formation (Katz and Karten, 1983a; Wild, 1981; review by Puelles et al., 2007). On the other hand, the rhombic lip at medullary levels gives rise to distant tangentially migrated derivatives, such as the pontine and inferior olivary nuclei, which display their own complex internal regionalisation (review by Sotelo, 2004).

Cambronero and Puelles (2000) first analysed the possible existence of a hidden segmental Bauplan within the medulla oblongata. Reasoning with Vaage (1969) that hidden intersegmental boundaries would be expected to occur antimeric to intersomitic limits (as occurs in the spinal cord), they fate-mapped in the 2-day-old chick embryo empirically defined segments (pseudorhombomeres) limited by planes bisecting the adjacent somites. They found that each pseudorhombomere gives rise to a well-defined transverse portion of the mature medulla oblongata, as occurs with the typical rhombomeres r2–r6 (Marín and Puelles, 1995). Significantly, the limits between pseudorhombomeres precisely fitted the morphological transverse limits of a number of medullary neuronal groups, bespeaking thus of a hidden metameric process in their causal background.

In this report we addressed the question whether there is an underlying molecular basis for this hypothetical hidden metameric organisation of the caudal hindbrain. We decided to analyse in detail the expression pattern of *Hox* genes within this structure.

Hox genes provide positional information for the metazoan body plan (reviews by Krumlauf, 1994; Kmita and Duboule, 2003; Deschamps and van Nes, 2005; Iimura and Pourquié, 2007). In vertebrates these genes are organised into four clusters (A–D), whose respective linearly arranged genes (paralogue groups 1–13) show a temporal and spatial expression gradient according to their 3′ to 5′ positions within the cluster (colinearity). In the early neural tube they are expressed continuously from the spinal cord to the hindbrain, each differing in their respective anterior limits arranged according to the 3′–5′ order. It is well known that the anterior boundaries of 3′ *Hox* genes forming groups 1 to 3 fit interrhombomeric boundaries within the overtly segmented part of the hindbrain — review by Nolte and Krumlauf, (2005). On the other hand, 5′ *Hox* genes from groups 8 to 10 are distributed within the spinal cord, where they correlate functionally with the differentiation of brachial, thoracic or lumbar motoneuron phenotypes (Dasen et al., 2003; Shah et al., 2004) or dorsal horn neurons (Holstege et al., 2008).

The medulla oblongata is positioned between these two regions and contains the anterior expression boundary of *Hox* genes from groups 4 to 7. This pattern was initially described in mouse embryos (i.e. Deschamps et al., 1987; Krumlauf et al., 1987; Toth et al., 1987; Gaunt, 1988; Graham et al., 1988; Gaunt et al., 1989, 1990; Mahon et al., 1988; Schughart et al., 1988). Comparative analysis in chicken (i.e. Grapin-Botton et al., 1995; Gaunt and Strachan, 1996; Gaunt et al., 1999) uncovered similar expression patterns. When compared at early stages, these group 4–7 *Hox* genes display their typical spatial colinearity in the caudal hindbrain neuroepithelium (i.e. mouse Hoxc4 and c5 — Geada et al., 1992; Hoxa4, a5 and a6 — Gaunt, 2000). Unfortunately, all these studies, as well as those ulterior ones involving mouse transgenic lines, involved analysis of early stages, so that we still lack an assessment of the relation of these Hox expression patterns with mature medullary neuromorphological features.

We approached this issue using as a model system the medulla oblongata of midgestational chick embryos (10 to 15 days in ovo), and analysed therein the expression pattern of different *Hox* genes from the 4 to 7 groups relative to observable nuclear boundaries. We included also the analysis of Hoxb3 in order to compare the other results with a 3′ *Hox* gene pattern whose expression is characteristic of the overtly segmented hindbrain. Our findings are largely supportive of the hypothesis of a hidden segmentation of the avian medulla oblongata.

Materials and methods

All experiments were done in accordance with the European Communities Council Directive (86/609/EEC) and the ethical guidelines on animal experiments of our institutions.

Brains from chick embryos ranging from 10 to 12 days of incubation (stages HH36 to HH38 of Hamburger and Hamilton, 1951) were dissected out and fixed by immersion in 4% paraformaldehyde in PBS 0.1 M, pH 7.4. In the case of older embryos (13–15 days of incubation, stages HH39 to HH41) they were previously perfused intracardially with 4% paraformaldehyde in PBS, followed by brain dissection and immersion overnight in the same fixative solution.

Fixed brains were embedded in a gelatine–albumine–sucrose mixture and sectioned in a vibratome to obtain 50–75 μm thick slices.

Table 1

HH	Plane	Riboprobes
35	S	a4, a5, a6
36	S	b4, d4
37	S	a4, a5, a6
		a4, b4, a5, b5
		d4, c5, c6
		b6, b7
		b3, a7, b7
		a4, d4
		b4, a5, b5, c5
		a4, b6, a6
37	T	a4, a5, a6
		d4, c5
		b3, b5, b6, b7
38	S	a4, d4
		a5, c5
		b6, b7
39	S	a4, a5, a6
41	S	a4, a5, a6
		b4, b5
		d4, c5, c6
		b3, b4, d4
41	T	b4, b5
		a4, a5
		a7, b7