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Novel sites of expression of the bHLH gene *NSCL1* in the developing nervous system

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Abstract

We report on novel sites of expression of the bHLH transcription factor *NSCL1* in the developing forebrain, hindbrain and spinal cord in chick and mouse. In the hindbrain in particular, *NSCL1* is the first bHLH transcription factor detected so far in rhombomere boundaries and its expression is coincident with boundary formation and maintenance. Novel sites of expression of this gene include the hippocampus, septum, tectum and hypothalamic nuclei. *NSCL1* is thus expressed in various neuronal populations that are either not actively proliferating or postmitotic.

Keywords: bHLH; Transcription factors; Chick/avian; Rhombomere boundaries; Cranial ganglia; Spinal cord; Dorsal root ganglia; *NSCL1*; *TAG-1/axonin-1*

1. Results and discussion

1.1. Expression pattern of avian and mouse NSCL1 in rhombomere boundaries

We have isolated *cNSCL1*, the avian homologue of mouse and human *NSCL1* (neurologic stem cell leukemia, Begley et al., 1992; Li et al., 1999a). *NSCL1* is expressed in various neuronal populations (Begley et al., 1992; Lipkowitz et al., 1992; Li et al., 1999a). Some details of the expression pattern of chick *NSCL1* in the central nervous system (CNS) have been described (Li et al., 1999a,b; Uittenbogaard et al., 1999) with particular attention to the retina and cerebellum. We have noticed novel sites of expression of the gene that have not been described previously, in the developing brain. In the hindbrain, a strong *NSCL1* signal is detected in rhombomere boundaries in both chick and mouse (Fig. 1, B1, B2, C, D, H). In addition, a stronger *NSCL1* signal is observed in r4, corresponding to facial branchiomotor (fbm) neurons (Fig. 1, B1, G) (Lumsden and Keynes, 1989; Varela-Echevarria et al., 1996). *NSCL1* signal is detected in stages HH15–HH20 (Fig. 1A–D) and is evident through HH24 (not shown), when rhombomere boundaries are not morphologically evident any more. *NSCL1* is one of the few transcription factors associated with rhombomere boundaries, together with the zinc-finger protein PLZF (Cook et al., 1995) and Pax-6 (Heyman et al., 1995). These structures consist of specialized cells that form at the boundaries of rhombomeres and help in maintaining their segregation (Lumsden and Keynes, 1989; Fraser et al., 1990; Guthrie et al., 1991a; Guthrie and Lumsden, 1991b; Nittenberg et al., 1997; Lumsden, 1999). They are characterized by large intercellular spaces, early differentiation of radial glia, and precocious development of an axon between rich marginal zone. It will be of interest to further investigate the particular role of *NSCL1* in this region. The hindbrain expression of *NSCL1* described above for the chick is also conserved in the mouse embryo (Fig. 1G–J). In both chick and mouse tissues, the adhesion molecule *TAG-1*(rodent)/*axonin-1*(chick) is used as a marker of the basal plate (Fig. 1E, F, K–M), fbm (Fig. 1E, F, K–M) and the lateral part of the neural tube (Fig. 1F, K–N).

1.2. Expression of NSCL1 in other CNS structures

NSCL1 is detected in the cerebellar rhombic lip (Fig. 2A, B) but not in the posterior myelencephalic rhombic lip and in postmitotic granule cells (Fig. 2C) (Li et al., 1999a; Uittenbogaard et al., 1999). The expression of *NSCL1* is similar to the described pattern of *TAG-1/axonin-1* in postmitotic granule cells (Fig. 2D) (Yamamoto et. al., 1986; Furley et.al., 1990). We also noticed other sites of expres-

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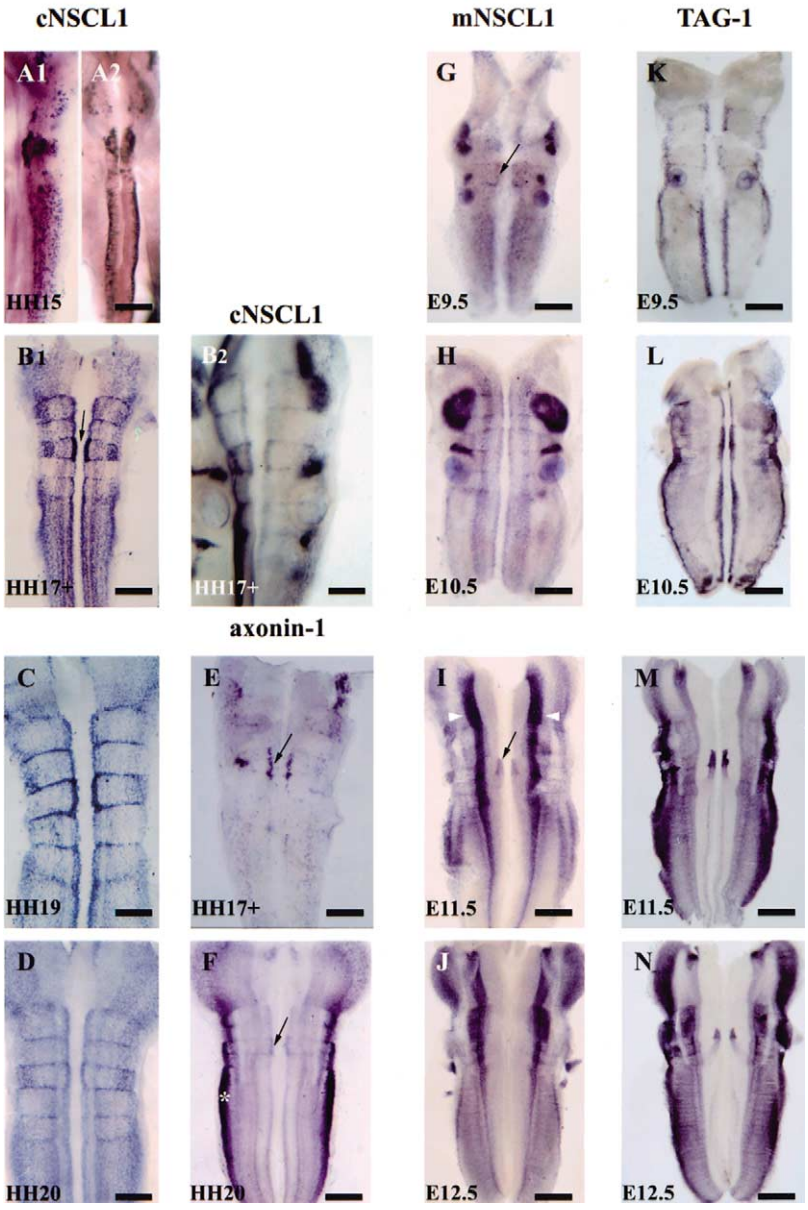

Fig. 1. Chick and mouse *NSCL1* expression pattern in the developing hindbrain in comparison to *TAG-1*/*axonin-1*. The developmental stages are indicated in each panel. Whole mount in situ hybridization with *cNSCL1* (A–D), *axonin-1* (E–F), mNSCL1 (G–J), *TAG-1* (K–N) antisense probes. In stage HH15 (A1 – lateral view, A2 – dorsal view), punctate signal is detected in the lateral part of the neural tube. A strong *cNSCL1* signal is detected in rhombomere 4 (r4) while there is no remarkable signal in r3 and r5. In B1, *cNSCL1* transcripts are present in rhombomere boundaries as well as in the basal plate, and are particularly intense in r4 (arrow). In (B2), a strong *cNSCL1* signal can be observed in the cranial ganglia (whole mount chick embryo HH17+). In (C), the expression in the rhombomere boundaries and basal plate is the highest and appears more uniform in all rhombomeres. The stronger signal in r4 is maintained. In (D), the expression in the boundaries is reduced and in the basal plate has almost disappeared. This pattern of *cNSCL1* expression is compared to *axonin-1*, expressed at the basal plate of r4 in stage HH17+ (arrow in E) and HH20 (arrow in F). In (F), a strong signal is detected in the lateral part of the neural tube (rhombic lip, asterisk) while low level staining is observed at r3/r4 and r4/r5 boundaries and is barely visible at the r2/r3 boundary. In comparison to the chick, mouse *NSCL1* at E9.5 (G) is detected in cranial ganglia and at r4 (arrow). At E10.5 (H), mouse *NSCL1* is expressed in cranial ganglia and all rhombomere boundaries except the r7/r8, albeit, less intensely than in chick. At E11.5, the signal is mainly detected in migrating fbm neurons located in r4 (arrow in I) and in two longitudinal streams at the middle of the dorsoventral axis (between arrowheads). Faint signal is observed in some rhombomere boundaries. In E12.5 (J), the signal in fbm neurons and boundaries is eliminated. In comparison, *TAG-1* is expressed in the basal plate of r2 and r4 and posteriorly to r6 in E9.5. In E10.5 (L), *TAG-1* is expressed in the basal plate all along the AP axis, as well as in the lateral part of the neural tube. In E11.5 (M), the signal in the basal plate is restricted in r4 to r6 where the migration of fbm neurons is taking place. In E12.5 (N), the *TAG-1* signal in fbm neurons persists contrary to the *NSCL1* signal at the equivalent stage (J). Scale bars in A: 200 μm; in B1,C,D: 300 μm; in E: 170 μm, in F,G: 450 μm, in H,I,L: 600 μm, in B2,J,M,N: 770 μm, in K: 370 μm.

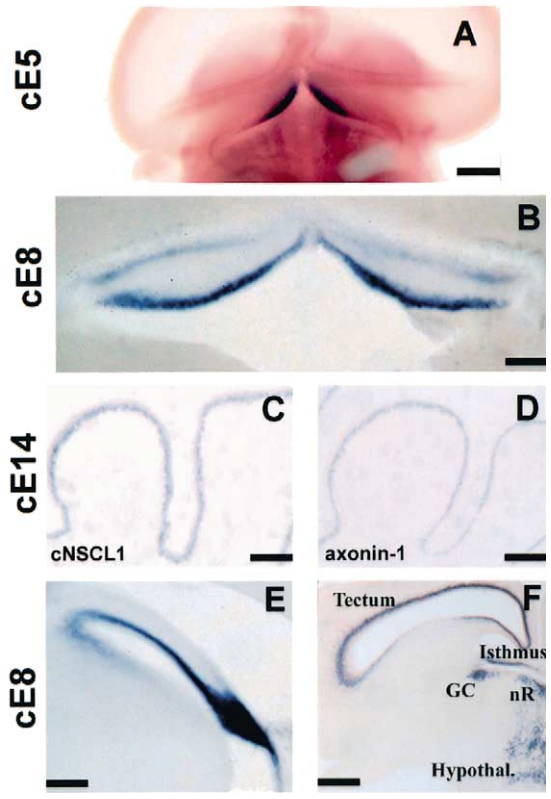

Fig. 2. Expression pattern of *cNSCL1* and *axonin-1* in the developing brain. (A) Whole-mount in situ hybridization for *cNSCL1* in E5 embryo brain, dorsal side up. *cNSCL1* is expressed in the cerebellar primordia. (B) Transverse vibratome section of E8 chick embryo showing in situ hybridization signal for *cNSCL1* in the developing cerebellum. (C,D) In situ hybridization for the detection of *cNSCL1* (C) and *axonin-1* (D) on sagittal cryostat sections of E14 chick embryo. *cNSCL1*and *axonin-1* are both expressed by the postmitotic granule cells of the external granule layer. (E,F) In situ hybridization for *cNSCL1* on coronal vibratome sections of E8 chick embryo brain. (E) *cNSCL1* is expressed in the medial and ventral hippocampus and septum. (F) Signal is also detected in the tectum, the isthmic region, the central gray (GC, griseum centrale), the raphe nuclei (nR) and the hypothalamus. Scale bars in A, E: 300 μm; in B,D: 240 μm; in C: 160 μm, in F: 360 μm.

sion of *NSCL1* such as the hippocampus and septum (Fig. 2E) as well as the tectum and hypothalamic nuclei (Fig. 2F).

1.3. Expression of avian and murine NSCL1 in the developing spinal cord and dorsal root ganglia and comparison with TAG-1/axonin-1

In order to determine the expression of *NSCL1* in the spinal cord and dorsal root ganglia, we have compared it with that of *TAG-1/axonin-1*, a well studied marker for this structure (Dodd et al., 1988; Ruegg et. al, 1989). The spatial and temporal pattern of expression of *NSCL1* (Fig. 3A, D, G) shows limited similarities with that of *TAG-1/axonin-1* (Fig. 3B, E, H). Namely, while *NSCL1* and *TAG-1/axonin-1* are expressed in commissural neurons at HH19 (Fig. 3A, B), *NSCL1* is still detected in postmitotic neurons later (Fig. 3D, G) but *TAG-1/axonin-1* is not detected in a likewise manner (Fig. 3E, H). Another difference includes the stronger *TAG-1/axonin-1* signal in the dorsal root ganglia (Fig. 3D, E, G, H).

2. Experimental procedures

2.1. Cloning of avian NSCL1

A 271 bp fragment was first isolated by polymerase chain reaction (PCR) using DNA from a Uni-ZAP cDNA library from 5-day chick embryos (Stratagene #937405) and primers: 5′-GCCCACGCCACGAGGGAACGCATCC-3′ and 5′-AATTAACCCTCACTAAAGGG-3′. This fragment, corresponding to 165 bp of the bHLH region and 106 bp of the 3′UTR, was used to screen the above cDNA library. Three clones were obtained covering the entire coding region of chick *NSCL1* gene (Accession number AF 393850).

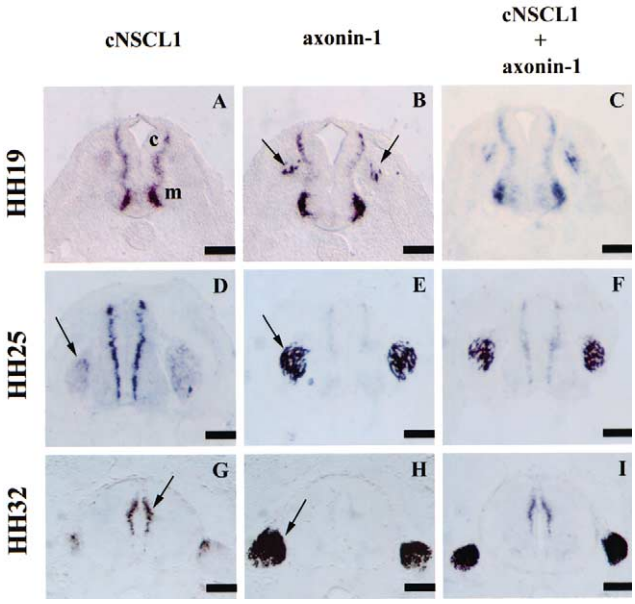

Fig. 3. Expression pattern of *cNSCL1* in comparison to *axonin-1* in the developing spinal cord at HH 19, HH 25 and HH32. All panels show transverse cryostat sections hybridized with *cNSCL1* (A,D,G), *axonin-1* (B,E,H) and both *cNSCL1* and *axonin-1* (C,F,I). At stage HH19 (A), the expression of *cNSCL1* is widespread at the neural tube in commissural (c) and motor neuron progenitors (m). In comparison, *axonin-1* is expressed by a different, more laterally localized population of motor neurons at stages HH19 (B), commissural neurons and dorsal root ganglia (arrows in B). The gap in the double in situ image (C) observed in the ventral spinal cord indicates that there is no overlap in the *NSCL1* and *axonin-1*- positive motor neuron populations. The *cNSCL1* signal is observed in postmitotic neurons in the subventricular zone at stage HH25 and low level staining is observed in the dorsal root ganglia (arrow in D). At this stage, *axonin-1* expression is very faint in the spinal cord (E), contrary to its very strong expression in the dorsal root ganglia (arrow in E). At stage HH32 (G), *cNSCL1* expression is restricted in a subpopulation at the ventricular zone of the dorsal spinal cord (arrow in G). *Axonin-1* expression is much more intense in the dorsal root ganglia at this stage (arrow in H). Scale bars in A: 170 μm; in B,C: 160 μm; D,E,F: 240 μm, in G,H: 300 μm, in I: 360 μm.

2.2. Eggs and embryos

Fertilized eggs were incubated in a humidified atmosphere at 38°C. The embryos were staged according to Hamburger and Hamilton (1951). CBAxC57Bl/10 mice were used and the day of vaginal plug detection was considered as embryonic day 0.5 (E0.5).

2.3. In situ hybridization

Full length chick *NSCL1* cDNA clones were obtained in pBluescript SK(+/−) by in vivo excision and recircularization (Stratagene, in vivo excision protocol). A 1790 bp fragment, obtained by digestion with SacII, was used as a probe for in situ hybridization using T7 RNA polymerase (Scharen-Wiemers and Gefrin-Moser, 1993). To test the specificity of the probe, we also used a fragment encompassing most of the 3′ UTR (without any coding sequence) for in situ hybridization experiments, giving identical results. No staining was obtained with sense probes.

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