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Mechanisms of Development 100 (2001) 349–352

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Gene expression pattern

qBrn-2, a POU III gene in quail: distinct developmental expression revealed by a specific antibody

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Received 13 September 2000; received in revised form 27 October 2000; accepted 2 November 2000

Abstract

We examined qBrn-2 protein expression in quail from its onset to final profile with a specific antibody we prepared. qBrn-2 expression employed onset-widespread-restriction pattern, and precisely concurred with formation and differentiation of neural tube. qBrn-2 protein was also located outside neural tube. Obvious differences in expression were observed compared with that of Brn-2. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Homeodomain; POU; Developmental expression; Immunocytochemistry; Neural tube; Somite; Notochord; Dorsal root ganglion; Anlagen of eye and ear; Visual; Quail

1. Results and discussion

Expression pattern and functional analysis demonstrate POU proteins play critical roles in neural development (reviewed in Ryan and Rosenfeld, 1997; Veenstra et al., 1997; Schonemann et al., 1998; Latchman, 1999). By screening a quail cDNA library we isolated qBrn-2, which was closely related to Brn-2 and XLPOU 3 (Liu et al., 2000). Although their expression profiles (He et al., 1989; Baltzinger et al., 1996; Alvarez-Bolado et al., 1995; Hagino-Yamagishi et al., 1997) and knock-out of Brn-2 (Nakai et al., 1995; Schonemann et al., 1995) were reported, localization of their proteins has been rarely described. Furthermore, gene expression from their onset to final patterns was still incomplete. Hence we prepared a specific antibody against qBrn-2 protein to address that subject.

We obtained the antibody anti-qBrn-2 by immunizing rabbits with a purified recombinant peptide of qBrn-2 (aa 1–207). In Western blot the antibody recognized a single protein (53 kDa, larger than the predicted one 43.7 kDa from qBrn-2 cDNA) in SDS-buffer boiled tissues, while the pre-immunized serum gave no detectable band (Fig. 1A). The revealed protein as qBrn-2 protein in vivo was strongly supported by the result that in vitro translated qBrn-2 protein in reticulocyte lysate also migrated as 53 kDa in SDS-PAGE (not shown). More convincingly, immunocytochemistry data with the antibody were highly consistent with those of in situ hybridization (Fig. 1B,C), and the pre-immunized serum gave no signals.

qBrn-2 protein in vivo bound to the promoter site of corticotropin-releasing hormone (Li et al., 1993), which was demonstrated by displacement of a specific band in EMSA after addition of anti-qBrn-2 to the mixture of E3.5 whole cell extracts and the probe (Fig. 1D, arrow). Interestingly, three other embryonic proteins in vivo also interacted with the motif.

Developmental qBrn-2 expression precisely concurred with formation and differentiation of neural tube. The first visible immunolabeling appeared at H–H stage 6 when neural plate had just formed, and the labeling was only located at the anterior margin and median shallow groove of incipient neural plate and primitive pit (Fig. 2A). At two-somite stage, when the neural plate began to fold at the presumptive midbrain, robust immunolabeling was observed precisely at that region. At the more caudal position where neural folds were still rather wide apart, however, immunolabeling was much weak (Fig. 2B). At three-somite stage, with the folding of neural tube rostrally and caudally, immunolabeling spread along the tube (Fig. 2C). qBrn-2 expression reached optic vesicles when they began to form (Fig. 2D). At 14-somite stage the labeling clearly delineated the brain vesicles, spinal cord and eye

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PII: S0925-4773(00)00531-1

cups (Fig. 2E). In early embryos we did not observe any expression gap at the junction of midbrain and hindbrain as that of Brn-2 (Schonemann et al., 1995).

qBrn-2 was widely expressed in proliferating neuroepithelium of developing neural tube (Fig. 3A) with various spatio-temporal strengths. With advancement of embryogenesis qBrn-2 expression progressively became restricted, and some features was obvious different from that of Brn-2. At E4 stage expression gaps in the floor plate at cervical level and the roof plate at sacral level became visible (Fig. 3B,C). At E6 stage restriction became more obvious: typical sites were the differentiating ventral and lateral neurons in spinal cord (Fig. 3F) and the paraventricular nuclei (Fig. 3G) and supraoptic nuclei in brain. According to their positions, the qBrn-2 positive neurons in spinal cord overlapped with LIM positive neurons. However, LIM protein assumed such pattern by a different strategy (Ericson et al., 1992; Tsuchida et al., 1994). In the brain of neonatal quail, typical subsets included olfactory bulb, hyperstriatum ventrale, paraventricular (Fig. 3H) and supraoptic nuclei, isthmi

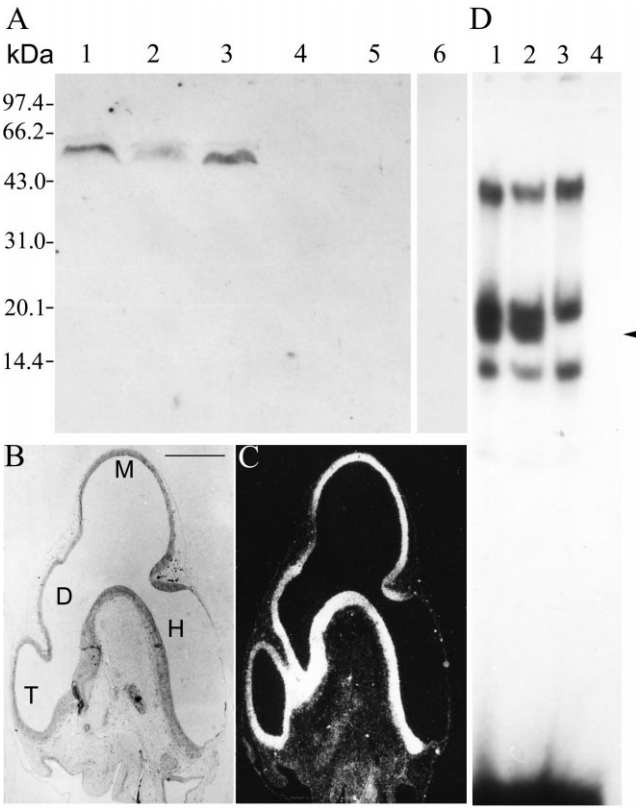

Fig. 1. Specificity of anti-qBrn-2. (A) Detection of a single protein (53 kDa) in quail embryos by anti-qBrn-2. About 70 μg protein of SDS-buffer boiled tissues was used. Lane 1–5: detected by anti-qBrn-2 (1:800), head of E3.5, trunk of E3.5, head of E5.5, trunk of E5.5, adult brain, respectively; lane 6, detected by the pre-immunized serum, head of E3.5. (B,C) qBrn-2 was expressed in the ventricular zones of brain revealed by both immunocytochemistry (anti-qBrn-2 1:100) and in situ hybridization (Liu et al., 2000). Connective parasagittal paraffin sections of E4 quail embryo fixed in 4% paraformaldehyde were used. T, telencephalon; D, diencephalon; M, mesencephalon; R, rhombencephalon. Scale bar, 400 μm. (D) qBrn-2 protein in vivo bound to the promoter site of corticotropin-releasing hormone revealed by displacement of a specific band in EMSA after addition of anti-qBrn-2 to the mixture of E3.5 whole cell extracts and a ^{32}P-labeled probe that was synthesized according to the sequence of CRH II sites (Li et al., 1993). In each of the reactions 2 μg of poly(dIdC) was added to block non-specific binding. Lane 1–4: whole cell extracts with the probe, whole cell extracts with the probe plus pre-immunized serum, whole cell extracts with the probe plus anti-qBrn-2, the probe plus anti-qBrn-2, respectively.

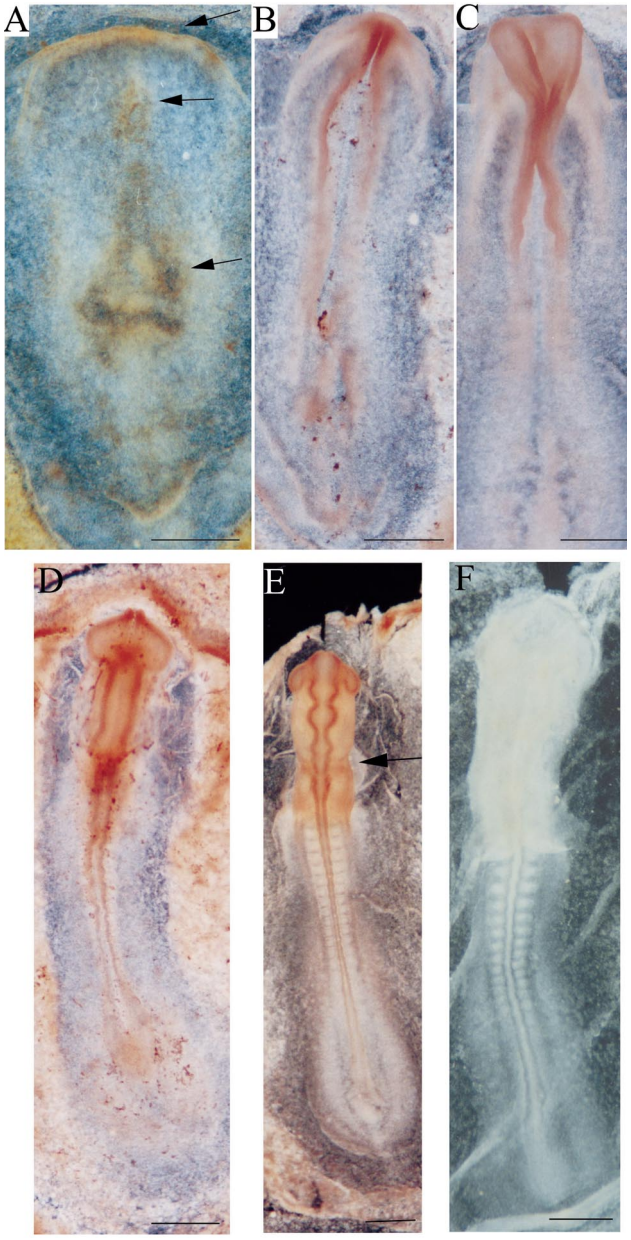

Fig. 2. Onset of qBrn-2 protein expression. Embryos of the quail (Coturnix coturnix japonica) were incubated and staged as described (Hamburger and Hamiltom, 1951; Zacchei, 1961). Fresh embryos were fixed in 4% paraformaldehyde, soaked in 1.5% H_2O_2, then subjected to whole-mount immunocytochemistry (anti-qBrn-2 1:100). (A) H–H 6 stage. Localization of immunolabeling was indicated by arrows. (B) Two-somite stage. (C) Three-somite stage. (D) Eight-somite stage. (E) Fourteen-somite stage. qBrn-2 expression in auditory pit was indicated by an arrow. (F) Negative control with pre-immunized serum, 16-somite. Scale bars, 500 μm.

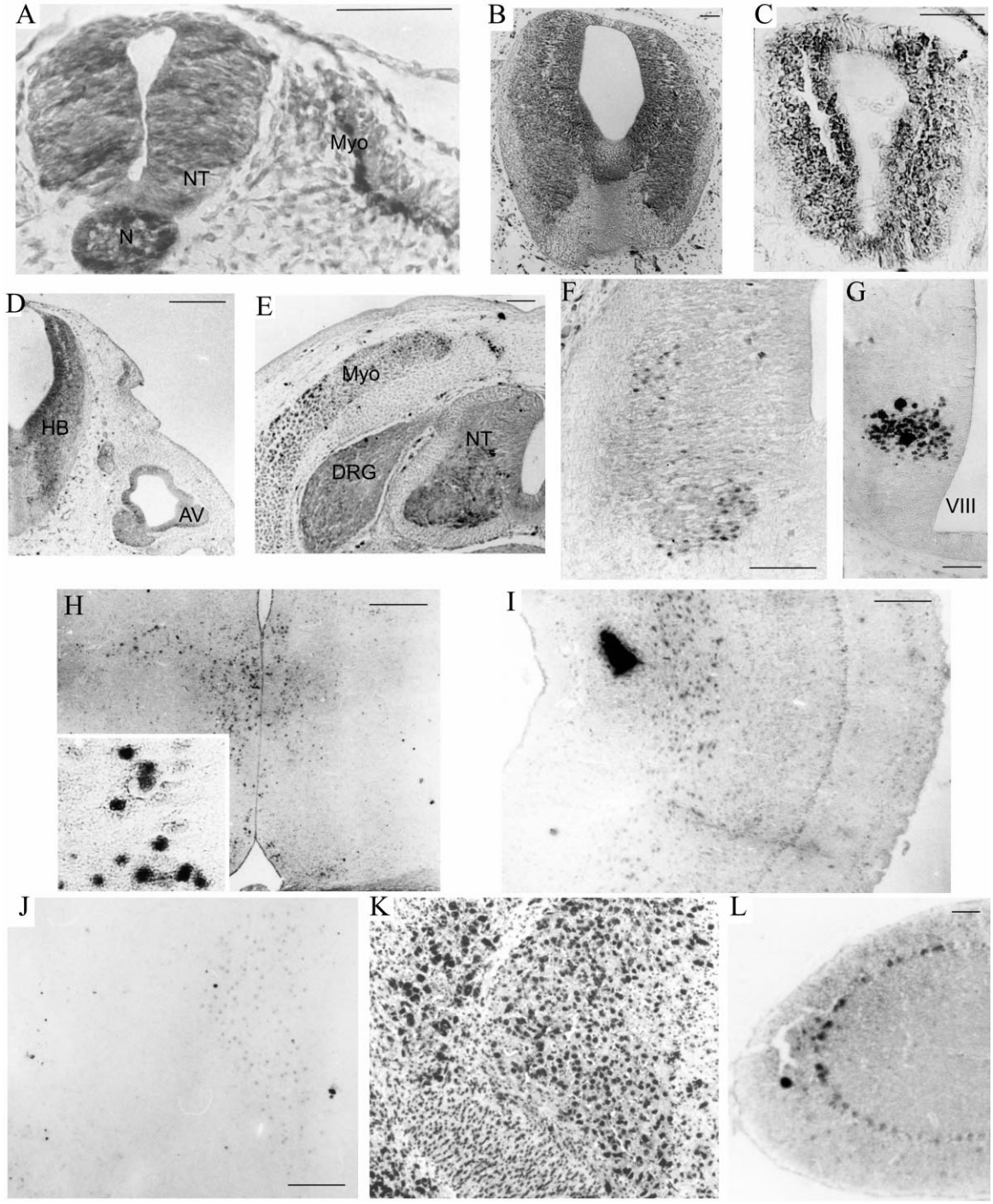

Fig. 3. Progressive restriction of qBrn-2 expression. Conditions for immunocytochemistry were the same as in Fig. 1. Localization of the immunolabeled nuclei in postnatal brain was according to the reference (Kuenzel and Masson, 1988). (A–F) Transverse sections of E2, E4 at cervical level, E4 at sacral level, E4 through hindbrain, E6 at branchial level, E6 at cervical level, respectively; (G) E6 coronal section through paraventricular nuclei; (H–L) neonatal coronal sections through paraventricular nuclei, tectum, isthmi nuclei pars parvocellularis, isthmi nuclei pars parvocellularis stained by cresylecht violet, cerebellum, respectively. The insert in (H) was the qBrn-2 positive cell at high magnification. AV, auditory vesicle; DRG, dorsal root ganglion; HB, hindbrain; Myo, myotome; N, notochord; NT, neural tube; VIII, ventricular III. Scale bar, 36 μm (B,C), 43 μm (L), 53 μm (A,E–G), 107 μm (I), 133 μm (J), 222 μm (D), 267 μm (H).

nuclei pars parvocellularis (Fig. 3J; Gruberg, 1983; Wang, 1988), optic tectum (Fig. 3I; (Grüsser-Cornehls, 1984; Székely and Lázár, 1976), Purkinje neurons (Fig. 3L), nuclei pontis medialis and lateralis.

In contrast to Brn-2, qBrn-2 protein was also expressed outside neural tube in early embryogenesis. Obvious immunolabeling in neural crest and dorsal root ganglion (DRG) was observed (Fig. 3E). The qBrn-2 positive cells in DRG

were postmitotic neurons based on the birth date of DRG neurons (Xue et al., 1998) and double labeling of the cultured E10 DRG cells by antibodies anti-qBrn-2 and anti-neurofilament (not shown). qBrn-2 was also expressed in eye and ear anlagen (Figs. 2D,E and 3D). Furthermore, qBrn-2 protein was localized in mesoderm including notochord, somite, myotome (Fig. 3A,E). Interestingly, the onset of qBrn-2 expression in notochord and somite was later than that in neural tube (Fig. 2B,C).

Acknowledgements

We are grateful to Dr Shu-Rong Wang for his constant support and Dr Ying Liu for communicating data before publication. This project was supported by the National Natural Sciences Foundation (No. 39770254) and Foundation of Chinese Academy of Sciences.

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