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Original Article

Developmental expression of the POU transcription factor qBrn-2 during somitic myogenesis in quail

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ABSTRACT We prepared a specific antiserum to the qBrn-2 protein and examined the developmental distribution of this protein during quail somitic myogenesis. In contrast to its mammalian homolog N-Oct-3, qBrn-2 exhibited an impressive spatio-temporal profile in somitic myogenesis, in addition to the orthodox expression observed in the developing neural tube. In somites, qBrn-2 was expressed in the outer epithelial cells, but not in the core cells. During the somite differentiation, qBrn-2 expression was enhanced and restricted to myotome. The location of qBrn-2 expression seemed to overlap with that of myf5 and myoD in myotome. However, in cells that just began to express myf5 or myoD, qBrn-2 expression was not obvious. As embryonic development proceeded, qBrn-2 positive cells in myotome migrated dorsally and ventrally, and qBrn-2 expression was still observed at dorsal and ventral muscle masses in the forelimb. On the basis of our observations, it seems that qBrn-2 may play important roles in the determination, differentiation and migration of muscle precursor cells, in addition to its known roles in neurogenesis.

KEY WORDS: *myogenesis, somite, POU, developmental expression, quail*

Introduction

POU domain is a bipartite DNA binding domain, consisting of POU specific domain and POU homeodomain tethered by a variable linker. Ever since its discovery, POU proteins from diverse range of species have been identified and grouped into six classes based on their amino acid sequences of the POU domain and the conservation of the linker. The spatiotemporal expression patterns and function analyses of POU proteins have demonstrated that POU proteins play critical roles in embryogenesis (reviewed in Ryan and Rosenfeld, 1997; Veenstra *et al.*, 1997; Schonemann *et al.*, 1998; Latchman, 1999). For example, mouse N-Oct-3 is required for the development and survival of the endocrine hypothalamus and posterior pituitary gland in mouse (Nakai *et al.*, 1995; Schonemann *et al.*, 1995).

Previously we cloned a novel POU box gene *qBrn-2* from quail. Analysis of the predicted amino acid sequence showed that the most related genes were *N-Oct-3* in mammal and *XLPOU 3* in Xenopus (Liu *et al.*, 2000). Despite the similarity in primary structure, qBrn-2 exhibited distinct features in its developmental expression profile compared with that of N-Oct-3 (Liu *et al.*, 2001). In this manuscript we examined the developmental expression of qBrn-2 during somitic myogenesis in quail. Upon the data available, qBrn-2 was the first reported POU factor that exhibited restricted expression pattern in somitic myogenesis. The impressive profile highly suggested that qBrn-2 played some roles in the determination, differentiation and migration of muscle precursor cells (MPC) besides its roles in neurogenesis.

Results

Preparation of a specific antiserum against the qBrn-2 protein

To raise a specific antiserum against qBrn-2 protein, we constructed an vector to express the N-terminal peptide of qBrn-2 (residues 1-207 excluding POU domain, P207). The structure of the plasmid was shown (Fig. 1A). In pHis 207, P207 was expressed as a fusion peptide with a His-tag and a short T7-tag (11 aa) at its N-terminus under the control of T7Φ10 promoter and T7Φ transcription terminator.

Expression of pHis 207 proved to be satisfactory (Fig. 1B). After IPTG induction, a dark extra protein band of the predicted

Abbreviations used in this paper: DM, dorsal muscle mass; EMSA, Electrophoresis mobility shift assay; H, condensing humerus; MPC, muscle precursor cells; Myo, myotome; N, notochord; NT, neural tube; R, condensing radius; U, condensing ulna; VM, ventral muscle mass.

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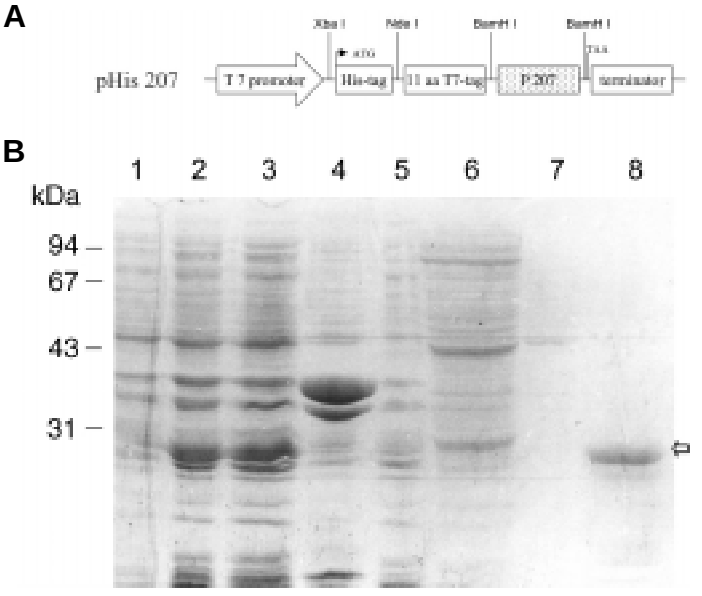


Fig. 1. Expression and purification of recombinant peptide His-P207. (A) *Schematic diagram of expression plasmid pHis 207. ATG, initial codon of translation; TAA, stop codon of translation; P207, the N-terminal segment of qBrn-2 (residues 1-207).* **(B)** *Purification profile of the recombinant peptide His-P207. The position corresponding to His-P207 is indicated by an arrow. Lanes 1-2, total bacterial extracts of BL21(DE3)/pHis 207 before and after IPTG induction, respectively; lanes 3-8, samples at different steps of His-P207 purification: 3, supernatant of sonicated lysate, 4, precipitate of sonicated lysate, 5, flow through, 6, 20 mM imidazole wash, 7, 50 mM imidazole wash, 8, 200 mM imidazole elution.*

size was observed, indicating that intact P207 fusion was overexpressed. The bands corresponding to His-P207 in the whole cell extracts and the supernatant of sonicated lysate showed almost the same density, indicating that His-P207 was highly expressed in soluble form. By passing a Ni^{++}-chelating Sepharose 4B column, most contaminant proteins were washed off by 50 mM imidazole. Then His-P207 was eluted by 200 mM imidazole. The eluted fraction migrated as a single band on SDS-PAGE with an apparent molecular mass as 26 kDa, the same as the predicted one.

Using the purified His-P207 as an antigen, we immunized New Zealand white rabbits and obtained an antiserum designated as anti-qBrn-2. The specificity of anti-qBrn-2 in binding proteins was demonstrated by Western blot analysis. The antiserum recognized a single band in the SDS-buffer boiled tissues of whole E4 quail embryos, while the preimmune serum detected no band (Fig. 2, lanes 1 and 2), indicating anti-qBrn-2 bound protein specifically. The protein anti-qBrn-2 bound was located in the nucleus, which was supported by immuno-detection of a single band in the nuclear extracts from E3.5 quail embryos (Fig. 2, lane 3). Interestingly, the apparent molecular mass of the detected protein (53 kDa) was lager than that predicted from its cDNA (43.7 kDa). Nevertheless, the protein detected in Western blot 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 (Fig. 2, lanes 4 and 5). More convincingly, immunocytochemistry data with the antiserum were highly consistent with those of *in situ* hybridization (Liu *et al.*, 2001). Further,

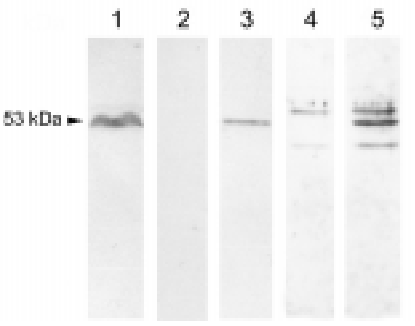


Fig. 2. Specificity of anti-qBrn-2. *anti-qBrn-2 recognized a single protein (53 kDa) in Western blot. SDS-buffer boiled tissues of whole E4 embryos (70 μg, lanes 1 and 2), nuclear proteins of E3.5 quail embryos (15 μg, lane 3), rabbit reticulocyte lysate without qBrn-2 cDNA (8 μl of the reaction system, lane 4), in vitro translated qBrn-2 protein by rabbit reticulocyte lysate (8 μl of the reaction system, lane 5) were subjected to 12% SDS-PAGE, and then transferred to nitrocellulose membrane. Proteins in lane 1 and lanes 3-5 were detected by anti-qBrn-2 (1:500). Proteins in lane 2 were detected by the preimmune serum (1:500) as a negative control. The bound primary antibody was visualized by HRP-goat anti-rabbit IgG.*

the antiserum recognized the developing paraventricular nuclei which were the orthodox expression of mammalian N-Oct-3, and the immunolabeling was predominantly located in the cell nucleus (Fig. 4). Lastly, EMSA also supported that anti-qBrn-2 specifically recognized qBrn-2 protein *in vivo*. Since qBrn-2 and mouse N-Oct-3 contain the same amino acid sequence within their POU domain (Liu *et al.*, 2000), it is conceivable that they could bind the same DNA motif. We synthesized an oligo DNA according to the promoter of corticotropin-releasing hormone (CRH) II site, the highest affinity site of mouse N-Oct-3 (Li *et al.*, 1993). In EMSA addition of anti-qBrn-2 to the mixture of E3.5 nuclear extracts and

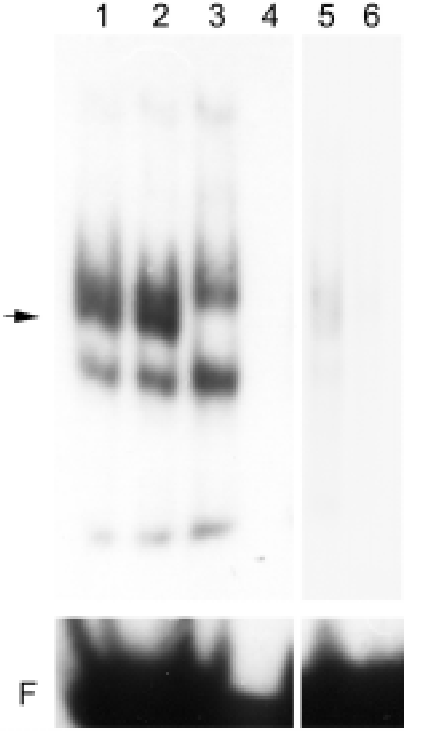
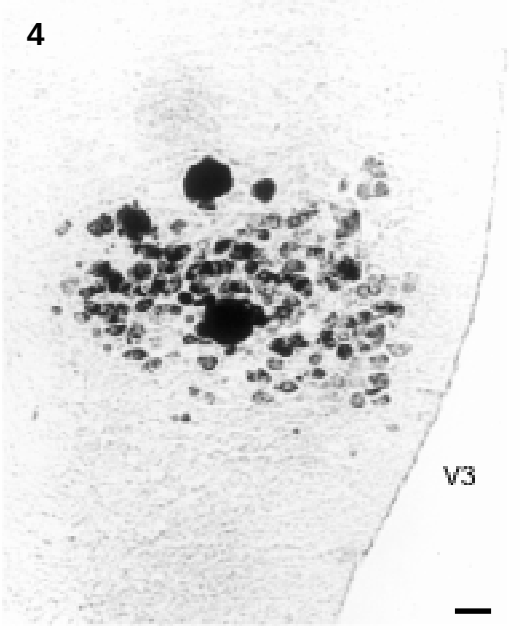
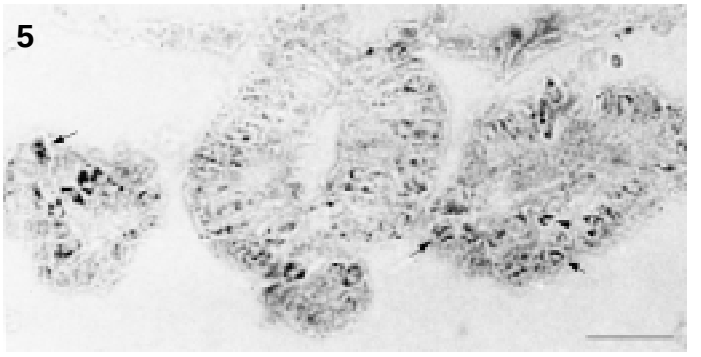


Fig. 3. anti-qBrn-2 recognized qBrn-2 protein *in vivo* revealed by EMSA. *Nuclear proteins of E3.5 quail embryos were used to bind the ^{32}-P probe and the change in the band pattern by addition of anti-qBrn-2 to the binding system is indicated by an arrow. Lane 1, nuclear proteins with the probe; lane 2, nuclear proteins with the probe plus preimmune serum; lane 3, nuclear proteins with probe plus anti-qBrn-2; lane 4, probe plus anti-qBrn-2; lanes 5-6, nuclear proteins with the probe plus the cold probe as a competitor, ten or a hundred times of the hot probe (molar rate), respectively. In each of the reactions 2 μg of poly(dIdC) was added to block non-specific binding. F, free probe.*





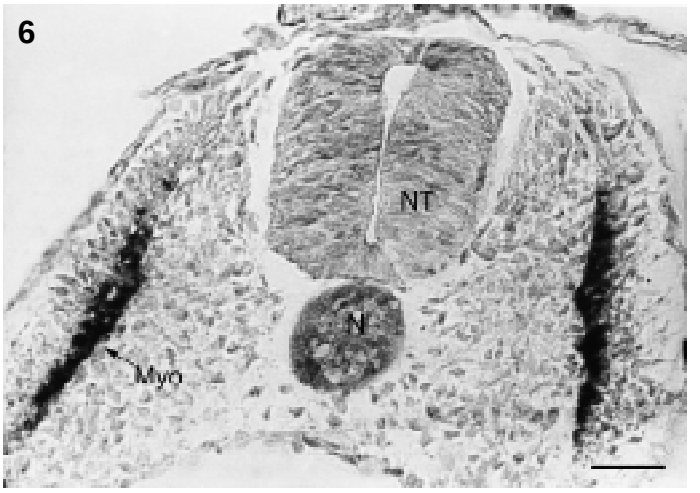


Fig. 4. qBrn-2 expression in developing paraventricular nuclei. *A coronal section of an E6 quail brain is illustrated. V3, ventricle 3. Scale bar, 13.3 μm.*

Fig. 5. qBrn-2 was expressed in outer epithelial cells, but not in the core cells of somites. *Immunolabeling is indicated by arrows. Transverse sections of an 10-somite quail embryo were subjected to immunocytochemistry with the anti-qBrn-2 antiserum. Scale bar, 35 μm.*

Fig. 6. Restriction of qBrn-2 expression to myotome. *Transverse sections of an E2 quail embryo were subjected to immunocytochemistry with the anti-qBrn-2 antiserum. Myo, Myotome; NT, neural tube; N, notochord. Scale bar, 27 μm.*

the ^{32}-P labeled probe caused displacement of a specific band, while addition of the preimmune serum did not affect the original band pattern at all (Fig. 3, arrow). It seemed that the interaction between qBrn-2 protein and anti-qBrn-2 interrupted the DNA-protein binding. When cold probe was added to the binding mixture as a competitor, density of the binding bands were dramatically reduced (Fig. 3, lanes 5 and 6). Those EMSA results again confirmed the specificity of anti-qBrn-2.

Developmental expression of qBrn-2 during somitic myogenesis in the quail

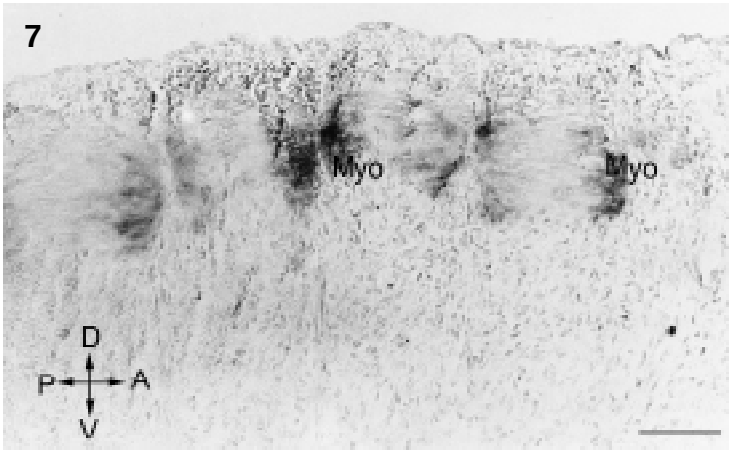
We examined temporal-spatial profile of qBrn-2 expression during somitic myogenesis in quail. qBrn-2 was clearly expressed in outer epithelial cells of somite, and the protein displayed restricted distribution within a somite cell. Interestingly, in a mature somite, no obvious expression was observed in the core cells (Fig. 5). During the differentiation of somite, qBrn-2 expression became progressively restricted into myotome, and the expression level in myotome was obviously higher than that at the previous stage (Fig. 6). By immunocytochemistry, we observed that myotome was formed only at the anterior and posterior part of a somite (Fig. 7). At later stages, some qBrn-2 positive MPC migrated dorsally and ventrally, and the expression level became lower than that in the nascent myotome (Fig. 6; Fig. 8A). After long-range migration, some of the qBrn-2 positive MPC arrived at limb buds and formed dorsal muscle mass (DM) and ventral muscle mass (VM) (Fig. 8 B,C,D), where MPC underwent further differentiation and formed specific muscles.

Discussion

Restricted developmental expression and putative roles of qBrn-2 in quail somitic myogenesis

In this manuscript we report preparation of a specific antiserum against qBrn-2 protein and the developmental distribution of *qBrn-2* product during quail somitic myogenesis. The antiserum was proved to specifically recognize qBrn-2 protein by Western blot, EMSA, consistency of immunocytochemistry and *in situ* hybridization and negative controls of immunocytochemistry with either solid-phase absorbed antiserum or the preimmune serum. The antiserum adds a new tool in exploration of the mechanisms of development.

The developmental expression of qBrn-2 during somitic myogenesis in quail was impressive. In somite qBrn-2 was obviously expressed in the cells of outer epithelial layer, but not the core (Fig. 5), suggesting that molecular identities of these cells can be distinguished at least by the presence of qBrn-2 protein. During the differentiation of somite, qBrn-2 expression was only restricted to myotome, which was derived from dorsal lateral part of somite. Further, the expression level of qBrn-2 in myotome was obviously enhanced compared with that at the previous stages (Fig. 6). According to their locations, the qBrn-2 positive cells in myotome



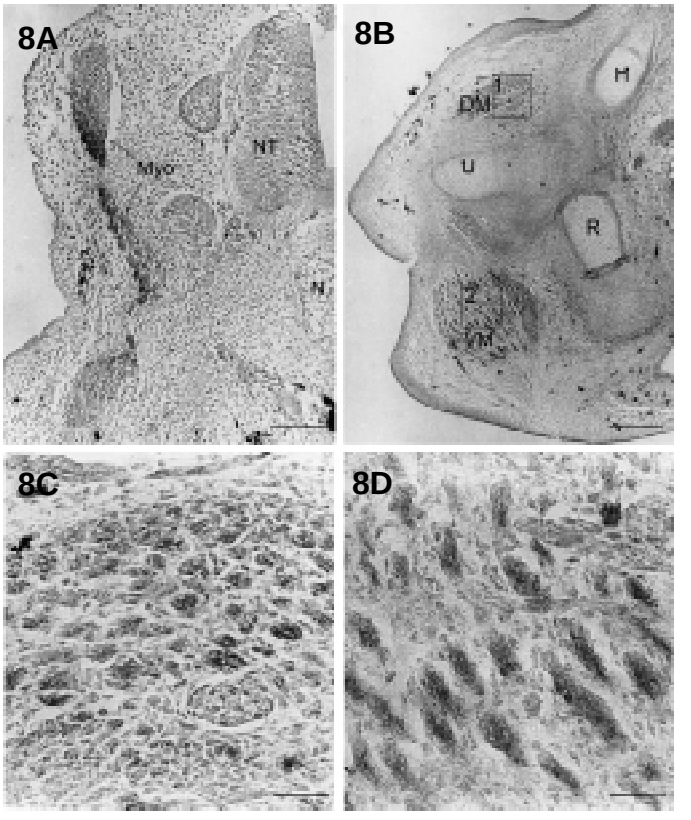


Fig. 7. Myotome was formed only at the anterior and posterior parts of a somite. *Parasagittal sections of an E3 quail embryo at the thoracic level were subjected to immunocytochemistry with the anti-qBrn-2 antiserum. A, anterior; D, dorsal; Myo, Myotome; P, posterior; V, ventral. Scale bar, 53 μm.*

Fig. 8. Migration of qBrn-2 positive muscle precursor cells. (A) *qBrn-2 positive precursor muscle cells in myotome migrated dorsally and ventrally. Transverse sections of an E4 quail embryo at the thoracic level were subjected to immunocytochemistry using the anti-qBrn-2 antiserum.* **(B)** *In dorsal and ventral muscle masses of the forelimb, obvious qBrn-2 expression was observed. Transverse sections of an E6 quail embryo at forelimb level were subjected to immunocytochemistry by anti-qBrn-2. Micrographs* **(C)** *and* **(D)** *are the framed areas 1 and 2 in* **(B)**, *respectively. DM, dorsal muscle mass; H, condensing humerus; Myo, myotome; N, notochord; NT, neural tube; R, condensing radius; U, condensing ulna; VM, ventral muscle mass. Scale bar, 85 μm* **(A)**, *178 μm* **(B)**, *and 27 μm* **(C,D)**.

were epaxial myocytes and hypaxial myocytes and should also be myf5 and myoD positive. However, qBrn-2 expression was not obvious in the cells either at dorsomedial lip of the dermomyotome, where myf 5 began to express, or at dermomyotome adjacent to the ventrolateral myotome, where myoD began to express (Fig. 6; Yun and Wold, 1996; Tajbakhsh *et al.*, 1997). Thus, the restriction of qBrn-2 expression to myotome seemed to be a downstream event to the onset of myf5 and myoD expression. qBrn-2 might also be involved migration and further differentiation of MPC, since qBrn-2 positive MPC were observed in DM and VM in forelimb. From the developmental expression profile, qBrn-2 seemed to be highly involved in somitic myogenesis. Integration of qBrn-2 with the myogenic network including muscle regulatory factors and myocyte enhancer factors is a future challenge.

The impressive qBrn-2 expression in myogenesis was intriguing in the light of evolution. The mammalian homolog of *qBrn-2* was a gene specifically expressed in central nervous system (He *et al.*, 1989; Alvares-Bolado *et al.*, 1995). Indeed, qBrn-2 expression in developing neural tube showed a similar pattern compared with its mammalian homolog although obvious differences were observed (Figs. 4-6; Liu *et al.*, 2001). However, qBrn-2 was also expressed during myogenesis. Upon the data available, qBrn-2 was the first reported POU factor that exhibited restricted expression during myogenesis. The distinct qBrn-2 expression suggests that the interpretation context of POU box genes might vary to some degree during the evolution of the two classes. Further, qBrn-2 expression during both quail neurogenesis and myogenesis might provide one molecular basis for ultimately establishing correct connections between neurons and their target muscles.

Materials and Methods

Embryos

Embryos of the quail (*Coturnix coturnix japonica*) species were used. Eggs were incubated 38±1°C and staged as described before (Hamburger and Hamilton, 1951; Zacchei, 1961; Xue *et al.*, 1993).

Construction of plasmids

The *Hinf* I-*Sma*I fragment of *qBrn-2* cDNA (from -4 to +621; Genbank accession number: AF091043) was first filled by Klenow DNA polymerase and then ligated with *Bam*HI linker (12mer, Promega). After *Bam*HI digestion, the fragment was in frame cloned into the *Bam*HI site of pET 3b (Novagen) with correct orientation, resulting pET 3b 207. Sequence at the junction was confirmed by DNA sequencing. pHis 207 was constructed by replacing the *Xba*I-*Nde*I fragment of pET 3b 207 with the *Xba*I-*Nde*I fragment of pET 28a (Novagen) which encodes a His-tag. Bacterial strain BL21(DE3) was used as the host of either pET 3b 207 or pHis 207 (Studier and Moffatt, 1986).

Expression and purification of recombinant peptide His-P207

An overnight bacterial culture was diluted 1 in 50 LB medium and cultured at 37°C to an OD600=0.6~0.8. Overexpression was induced by addition of IPTG at a proper concentration. After further 3-hour growth at 37°C, the bacteria were harvested and suspended in PBS buffer containing 1 μg/ml of aprotinin and 100 μg/ml of PMSF, and sonicated in ice by several bursts. Then Triton X-100 was added (1% as a final concentra-

tion) with gentle shaking for 30 min. Supernatant was obtained by centrifugation at 4°C. Peptide His-P207 was purified by passing the supernatant through a Ni^{++}-chelating Sepharose 4B column (Pharmacia) according to the instructions of manufactures.

Production of antiserum

Two of 8-week-old female New Zealand white rabbits were each immunized subcutaneously with 1.0 mg of purified His-P207 emulsified with Freund's complete adjuvant (1:1 v/v). One month later, the rabbits were first boosted with 0.5 mg of the peptide emulsified with Freund's incomplete adjuvant. After that, the rabbits were boosted in every the other week until a good immune response resulted. Rabbits were bled via the marginal ear vein 10 days after each inoculation, beginning after the second boost.

Preparation of nuclear protein extracts

Several E3.5 quail embryos were collected and washed with phosphate-buffered saline (PBS, 140 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3), then homogenized in 3 volumes of buffer A (10 mM Hepes (pH7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.5 mM phenylmethylsulphony fluoride (PMSF)) containing 0.05 % Nonidet P-40 for the release of nuclei. Then the nuclei were collected by centrifugation at 250g for 10 minutes, and suspended in buffer C (5 mM Hepes (pH 7.9), 26% glycerol (v/v), 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF). NaCl was added to a final concentration of 300 mM. After incubation on ice for 30 minutes, supernatant was collected by centrifugation at 24000g for 20 minutes at 4°C, then snap-frozen in dry ice/ethanol and aliquoted at -70°C.

Western blot analysis

Nuclear proteins of E3.5 quail embryos, SDS-buffer boiled tissues of whole E4 quail embryos, *in vitro* translated qBrn-2 protein (TNT^R coupled reticulocyte lysate, Promega) were subjected to 12% SDS-PAGE, and then transferred to nitrocellulose membrane. Proteins on the membrane were detected by anti-qBrn-2 or the preimmune (1:500) and visualized by HRP-goat anti-rabbit IgG.

Electrophoresis mobility shift assay (EMSA)

Oligo DNA was synthesized according to the DNA motif of mouse N-Oct-3 (Li *et al.*, 1993). For the ease of labeling, *Hin*dIII and *Xho*I ends were incorporated at the two ends. Oligo a: 5' AGCT TGCAT AAATA ATAGG C 3'; oligo b: 5' TCGA GCCTA TTATT TATGC A 3'. The two strands were annealed and labeled by ^{32}-P with filling-in reaction. Binding reaction was made by mixing 20 mM Hepes (pH7.9), 1 mM $MgCl_2$, 4% Ficoll, 0.5 mM DTT, KCl to a final salt concentration of 50 mM, 2 μg of poly(dIdC) and 15 μg of nuclear proteins. For reactions to test the function of antiserum, 1μl of additional anti-qBrn-2 (1:10) or preimmune serum (1:10) was put into the binding system. For competition test of EMSA ten or a hundred times of cold probe was added to the binding systems. The mixtures stood on ice for 30 min. Then 1 μl (10 fmol) ^{32}-P labeled probe was added to each of the mixtures and kept in ice for another 40 min. Finally the samples were subjected to 4% PAGE with 0.25 times of TBE, and exposed to X-film.

Solid-phase absorption

Purified His-P207 was coupled to CNBr-activated Sepharose 4B (8 mg protein/ml bed volume approximately) according to the instruction of manufacture (Pharmacia). Proper amount of anti-qBrn-2 was incubated with excess of His-P207-Sepharose 4B overnight at 4°C on rotary mixer. Then the supernatant was applied to immunocytochemistry.

Immunocytochemistry in sections

Fresh quail embryos were collected and fixed in 4% cold paraformaldehyde-PBS for 3 h, and then embedded in paraffin and sectioned (6-8 μm). Sections were dewaxed and re-hydrated before immunocytochemistry. Nonspecific binding sites in the sections were saturated by incubation with PBS containing 0.15% Tween-20, 3% skim milk powder and 5% goat serum (PBS-B) for 1 h. Sections were incubated overnight with anti-qBrn-2 or solid-phase absorbed antiserum or preimmune serum (1:100) in PBS-B at 4°C, and then washed 4 times in PBS-B, 15 min each time. HRP-second antibody (goat anti-rabbit IgG, 1:2000 in PBS-B, Vector) was applied to the sections for 2 h at room temperature. Excess of the second antibody was washed away by PBS containing 0.15% Tween 20 for 4 times, 15 min each. The bound antibody was visualized by DAB method. For localization of the immunolabeling in sections, cresyletcht violet stain of the connective sections was performed.

In situ *hybridization*

The conditions for *in situ* hybridization were as described before (Liu *et al.*, 2000).

Acknowledgements

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References

ALVAREZ-BOLADO, A., ROSENFELD, M.G. and SWANSON, L.W. (1995). Model of forebrain regionalization based on spatiotemporal patterns of POU-III homeobox gene expression, birthdates, and morphological features. *J. Comp. Neurol.* 355: 237-295.

HAMBURGER, V. and HAMILTON, H.L. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol.* 88: 49-92.

HE, X., TREACY, M.N., SIMMONS, D.M., INGRAHAM, H.A., SWANSON, L.W. and ROSENFELD, M.G. (1989). Expression of a large family of POU-domain regulatory genes in mammalian brain development. *Nature* 340: 35-42.

LATCHMAN, D.S. (1999). POU family transcription factors in the nervous system. *J. Cell Physiol.* 179: 126-33.

LI, P., HE, X., GERRERO, M.R., MOK, M., AGGARWAL, A. and ROSENFELD, M.G. (1993). Spacing and orientation of bipartite DNA-binding motifs as potential functional determinants for POU domain factors. *Gene Dev.* 7: 2483-2496.

LIU, W., XUE, J.X., HE, R.Q. and XUE, Z.G. (2001). qBrn-2, a POU III gene in quail: distinct developmental expression revealed by a specific antibody. *Mech. of Dev.*, 100: 349-352.

LIU, Y., XUE, J.X., ZHANG, W., FU, D.C., HE, R.Q. and XUE, Z.G. (2000). qBrn-2, a POU domain gene expressed in quail embryo. *Biochim. Biophys. Acta.* 1491: 27-36.

NAKAI, S., KAWANO, H., YUDATE, T., NISHI, M., KUNO, J., NAGATA, A., JISHAGE, K., HAMADA, H., FUJII, H., KAWAMURA, K., SHIBA, K. and NODA, T. (1995). The POU domain transcription factor Brn-2 is required for the determination of specific neuronal lineages in the hypothalamus of the mouse. *Genes Dev.* 9, 3109-3121.

RYAN, A.K. and ROSENFELD, M.G. (1997). POU domain family value: flexibility, partnership, and developmental codes. *Genes Dev.* 11: 1207-25.

SCHONEMANN, M.D., RYAN, A.K., ERKMAN, L., MCEVILLY, R.J., BERMINGHAM, J. and ROSENFELD, M.G. (1998). POU domain factors in neural development. *Adv. Exp. Med. Biol.* 449: 39-53.

SCHONEMANN, M.D., RYAN, A.K., MCEVILLY, R.J., O'CONNELL, S.M., ARIAS, C.A., KALLA, K.A., LI, P., SAWCHENKO, P. and ROSENFELD, M.G. (1995). Development and survival of the endocrine hypothalamus and posterior pituitary gland requires the neuronal POU domain factor Brn-2. *Genes Dev.* 9: 3122-3135.

STUDIER, D.B. and MOFFATT, B.A. (1986). Use of Bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* 189: 113-130.

TAJBAKHSH, S. and COSSU, G. (1997). Establishing myogenic identity during

somitogenesis. *Curr. Opin. Genet. Dev.* 7: 634-641.

VEENSTRA, G.J., VAN DER VLIET, P.C. and DESTREE, O.H. (1997). POU domain transcription factors in embryonic development. *Mol. Biol. Rep.* 24: 139-55.

XUE, Z. XUE, X. and LE DOUARIN, N.M. (1993). Quox-1, an Antp-like homeobox gene of the avian embryo: a developmental study using a Quox-1 specific antiserum. *Mech. Dev.* 43: 149-158.

YUN, K. and WOLD, B. (1996). Skeletal muscle determination and differentiation: story of core regulatory network and its context. *Curr. Opin. Cell Biol.* 8: 877-889.

ZACCHEI, A.M. (1961). Lo sviluppo embryonale della quaglia giapponese (Coturnix coturnix japonica, T. e S.). *Arch. Ital. Anat. Embryol.* 66: 36-62.

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