

Identification of a developmentally regulated striatum-enriched zinc-finger gene, *Nolz-1*, in the mammalian brain

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Neural information processed through the striatum of the basal ganglia is crucial for sensorimotor and psychomotor functions. Genes that are highly expressed in the striatum during development may be involved in neural development and plasticity in the striatum. We report in the present study the identification of a previously uncharacterized mammalian member of the *noca/elB/itp-1* family, *Nolz-1*, that is preferentially expressed at high levels in the developing striatum. *Nolz-1* mRNA was expressed as soon as striatal anlage began to form at embryonic day 13 in the rat. *Nolz-1* mRNA was predominantly expressed in the lateral ganglionic eminence (striatal primordium) and was nearly absent in the adjacent structures of the medial ganglionic eminence and the cerebral cortex. Moreover, *Nolz-1* was highly expressed in the subventricular zone of the lateral ganglionic eminence and was colocalized with the early neuronal differentiation markers of *TuJ1* and *Isl1* and the projection neuron marker of *DARPP-32*, suggesting that *Nolz-1* was expressed in differentiating progenitors of striatal projection neurons. A time course study showed that *Nolz-1* mRNA was developmentally regulated, as its expression was down-regulated postnatally with low levels remaining in the ventral striatum at adulthood. As the tagged *Nolz-1* protein was localized in the nucleus, *Nolz-1* may function as transcriptional regulator. In a model system for neural differentiation, *Nolz-1* mRNA was dramatically induced on neural induction of P19 embryonal carcinoma cells by retinoic acid, suggesting that *Nolz-1* activation may be involved in neural differentiation. Our study suggests that *Nolz-1* is preferentially expressed in differentiating striatal progenitors and may be engaged in the genetic program for controlling striatal development.

The striatum is the major input information processing unit in the basal ganglia circuits of the mammalian forebrain. What makes the striatum an attractive and important system for neurobiological study is its involvement in multiple neurological functions ranging from motor control, cognition, emotion, and reinforcement to plasticity underlying learning and memory (1). The importance of the striatum is also reflected in a number of neurological disorders, including Parkinson's disease, Huntington's disease, and obsessive-compulsive disorders in which malfunction of neurotransmission or neurodegeneration are associated with the striatal system (2, 3). The study of development and function of the striatum is thus fundamentally important not only to the understanding of integrative brain function, but also to the development of therapeutic approaches to neurological diseases.

An experimental approach to study the development and function of the striatum is to identify genes that are preferentially expressed by striatal neurons. Based on the study of cDNA subtractive hybridization cloning, it has been estimated that

there are about 100 genes that are preferentially expressed at high levels in the striatum (4). The striatum-enriched genes comprise at least two classes of genes with distinct function. One class of genes are transcriptional regulators that control neuronal development, including the *Dlx* family, *Mash1*, *Gsh2*, *Ebf1/Olf1*, *Six3*, *Isl1*, *Foxp1*, and *Foxp2* (5–11). The other class of genes are signaling transduction molecules related to neurotransmission, including dopamine D1 and D2 receptors; the G protein, G_{OIR} ; the adenylyl cyclase type V; the serine/threonine protein phosphatase-1 inhibitor, *DARPP-32*; the striatal-enriched tyrosine phosphatase, *STEP*; and the Rap guanine nucleotide exchange factor, *CalDAG-GEF I* (12–16). The enrichment of these signaling molecules in the striatum constitutes parts of the specificity of neurotransmission in the striatal system.

These two classes of striatum-enriched genes are important for setting up the cellular and molecular infrastructure of the striatal signaling system. We report in the present study the identification of a striatum-enriched gene, *Nolz-1*, that is a previously uncharacterized mammalian member of the *noca/elB/itp-1* family. The spatiotemporal expression pattern of *Nolz-1* mRNA suggests that it may regulate development and function of striatal neurons.

Materials and Methods

Subtractive Hybridization of cDNA Cloning. The lateral ganglionic eminence (LGE) and the overlying cerebral cortex were dissected from the embryonic day (E) 15 rat forebrain (Sprague-Dawley, National Yang-Ming University). Total RNA was extracted by using the guanidinium thiocyanate extraction method and was then reverse transcribed into cDNAs. The PCR-select cDNA subtraction was performed by following the manufacturer's instruction (Clontech). The subtracted cDNAs were subcloned into the pCRII vector (Invitrogen) and were sequenced. The protocol of animal use conformed to National Institutes of Health guidelines.

Northern Blotting. Total RNA was isolated from rat tissues and P19 embryonal carcinoma cells. The random hexamer method was used to synthesize [³²P]dCTP-labeled probes from pCRII-

Abbreviations: C form, constitutive form; En, embryonic day *n*; E form, embryonic form; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; RA, retinoic acid; RARE, RA response element; SVZ, subventricular zone.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AY281290 and AY281291).

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LGEC-18 plasmid that contained 3' UTR of rat *Nolz-1* cDNA (506 bp). The procedures of hybridization and autoradiography were as described (17).

In Situ Hybridization. The isotope and nonisotope methods of *in situ* hybridization were performed as described (11, 18). The [³⁵S]UTP-labeled and digoxigenin-UTP-labeled antisense and sense *Nolz-1* riboprobes were synthesized by *in vitro* transcription with *Hind*III or *Xho*I linearized pCRII-LGEC-18 plasmids as templates. The rat *Raldh-3* probe (461 bp) was derived from PCR cloning. After *in situ* hybridization, some of the sections were further processed for immunostaining for Mash1 (1:100, kindly provided by D. J. Anderson, California Institute of Technology, Pasadena), Isl1 (1:1,000, 3A4, Developmental Studies Hybridoma Bank), DARPP-32 (1:20,000, kindly provided by H. C. Hemmings, Weill Medical College, Cornell University, Ithaca, NY), and Tuj1 (1:5,000, Promega) with the tyramide amplification method, except Tuj1 (19, 20).

Plasmids. The full-length *mNolz-1*-FLAG cDNA was subcloned into pcDNA3.1 and pEF1/Myc-His (Invitrogen) vectors to generate the expression plasmids of pCMV-*Nolz-1*-FLAG and pEF1-*Nolz-1*-FLAG, respectively. The pGL3-*mNolz-1*-P1.4 and pGL3-*mNolz-1*-dRARE [RARE, retinoic acid (RA) response element] vectors were constructed by subcloning ≈1.4-kb (nucleotides -235 to -1648) and ≈1.27-kb (nucleotides -235 to -1509) 5' flanking regions, respectively, of *mNolz-1* into pGL3 vectors (Promega). The plasmids were verified by sequencing.

Cell Culture and Transfection. HEK293T cells were cultured in DMEM with 10% FBS. P19 cells were cultured with α-MEM containing 7.5% newborn calf serum and 2.5% FBS. The pCMV-*Nolz-1*-FLAG and pEF1-*Nolz-1*-FLAG expression plasmids were cotransfected with the reporter plasmid pGFP-C1 (5:1; Clontech) into HEK293T cells and P19 cells, respectively, by calcium phosphate-mediated transfection. Neural induction of P19 cells was performed by treating aggregated P19 cells with *all-trans* RA (1 μM) in α-MEM with 5% FBS. Four days after the RA induction, the cell aggregates were trypsinized and cultured on poly(D)-lysine-coated dishes with SF21 medium (19) to promote neural differentiation.

Electroporation. The pCMV-*Nolz-1*-FLAG plasmid was transfected into E13.5 mouse forebrains by using the method of *in vivo* electroporation (21). The brains were harvested for immunostaining 1 day after electroporation. The pGL3-*mNolz-1*-P1.4 or pGL3-*mNolz-1*-dRARE plasmids were coelectroporated with pCMV-β-gal plasmid into the LGE of E16 rats, and the transfected tissues were cultured as explants for 1 day *in vitro*. The luciferase activity was normalized with the β-gal activity.

Immunocytochemistry. The transfected HEK293T, P19 cells and the brain tissue were processed for immunocytochemistry with FLAG antibodies (monoclonal antibody, 1:8,000, Sigma; polyclonal antiserum, 1:10,000, kindly provided by F.-J. Lee, National Taiwan University) and Nkx2.1 antibody (1:100, NeoMarkers, Fremont, CA) as described (19). The immunostained cells and tissue were counterstained with the DNA dye Hoechst 33258.

Results

Identification of *Nolz-1* Gene. To search for novel striatum-enriched genes, we used the method of cDNA subtractive hybridization cloning. We subtracted the cDNAs of the LGE (striatal primordium) from the cDNAs of the cerebral cortex of E15 rats. Of 86 subtracted clones, there were 60 independent cDNAs in which 24 were known genes, 16 were uncharacterized genes, 17 were unknown EST clones, and 3 were novel clones.

The RT-PCR analysis of 53 subtracted clones showed that three clones including *Dlx2* (5), *Six3* (8), and a previously uncharacterized gene (clone LGEC-18) were preferentially expressed at high levels in the LGE. We identified and sequenced two clones containing the full-length cDNA of LGEC-18 from the RIKEN mouse full-length cDNA library (RIKEN 41-22401-M18, B830032-M18). The ORFs of these two clones were identical, but the clones differed at the 5' and the 3' UTR, where several nucleotide variations occurred (data not shown).

The mouse gene encoded a putative protein of 652 aa that contained a serine-rich and two glycine-rich domains in its amino terminus, and five stretches of alanine-rich domains and a C₂H₂ zinc-finger motif (amino acids 519–550) in its carboxyl terminus (Fig. 1 *A* and *C*). The zinc-finger motif of this protein shared 48%, 61%, 55%, 87%, and 94% identity with the proteins encoded by *Caenorhabditis elegans tlp-1* (22), *Drosophila* 1 (2)35*Ba/no ocelli (nocA)* and *elB* (23, 24), *Zebrafish Noz1/Nlz* (25, 26), and *Xenopus xNolz* (GenBank accession no. BC047263), respectively (Fig. 1*C*). The alignments showed that the amino terminus (amino acids 66–144) and the carboxyl terminus (519–652) of this protein had high homology with *nocA*, *elB*, *Noz1/Nlz*, and *xNolz* proteins (Fig. 1 *B* and *C*). Thus, the murine homologue of *nocA*-like zinc-finger protein (*Nolz-1*) represented a previously uncharacterized murine member of the *nocA/elB/tlp-1* family. The search of GenBank further identified its human homologue, *hNolz-1* (BC011625) and its rat homologue, *rNolz-1* (a composite cDNA-derived from EST clones AW915098, A1600276, and AI599428 and the genomic clone AC128846) (Fig. 1 *B* and *C*). The entire amino acid sequence of mouse *Nolz-1* (*mNolz-1*) was 99% and 96% identical to *rNolz-1* and *hNolz-1* proteins, respectively.

Expression of *Nolz-1* Transcripts in Developing Rat Embryos. The *Nolz-1* transcript appeared as a major band of ≈3.5 kb in different tissues of E20 rat embryos, including the brain, lung, muscle, heart, thymus, kidney, stomach, and spleen (Fig. 2*A*). Within the E20 rat brain, *Nolz-1* was highly expressed in the striatum, confirming *Nolz-1* as a striatum-enriched gene (Fig. 2*B*). Notably, the striatal *Nolz-1* transcripts comprised a doublet of a major ≈3.5-kb band and a minor ≈4-kb band. More interestingly, the doublet transcripts were present only in the striatum. A single ≈3.5-kb transcript was detected in other examined brain regions (Fig. 2*B*).

Preferential Expression of *Nolz-1* in the Developing Striatum. The detailed expression pattern of *Nolz-1* mRNA in the developing rat brains was examined by *in situ* hybridization with the same 3' UTR probe of rat *Nolz-1* for Northern blotting. *Nolz-1* mRNA was detected in the lateral parts of the ventral forebrain at E13 (Fig. 3*A*). *Nolz-1* mRNA was expressed in the Mash1-positive and Nkx2.1-negative LGE, except that the dorsal part of LGE lacked *Nolz-1* (Fig. 3 *B–D*). *Nolz-1* was not expressed in the adjacent Nkx2.1-positive medial ganglionic eminence (MGE), except for a strip of *Nolz-1*-positive cells across the borders between the MGE and the LGE (Fig. 3 *C* and *D*).

At E15, high levels of *Nolz-1* mRNA were present in the LGE (striatal anlage) (Fig. 4 *A–E*). There were two interesting features of *Nolz-1* expression in the ventral telencephalon at this developmental stage. First, *Nolz-1* mRNA was not detectable in the Mash1-positive ventricular zone (VZ) (Fig. 4*D*), whereas it was dramatically up-regulated in the adjacent subventricular zone (SVZ), in which cells coexpressing *Nolz-1* and Isl1 could be found (Fig. 4*G*). The expression of *Nolz-1* mRNA was decreased in the differentiated mantle zone. Double labeling showed that *Nolz-1*-positive cells coexpressed TuJ1, a marker of early differentiating neurons (27), in the SVZ (Fig. 4*I* and *J*), suggesting that *Nolz-1* was expressed in early differentiating neurons. Second, in contrast to the LGE, *Nolz-1* was nearly absent in the MGE (Fig.

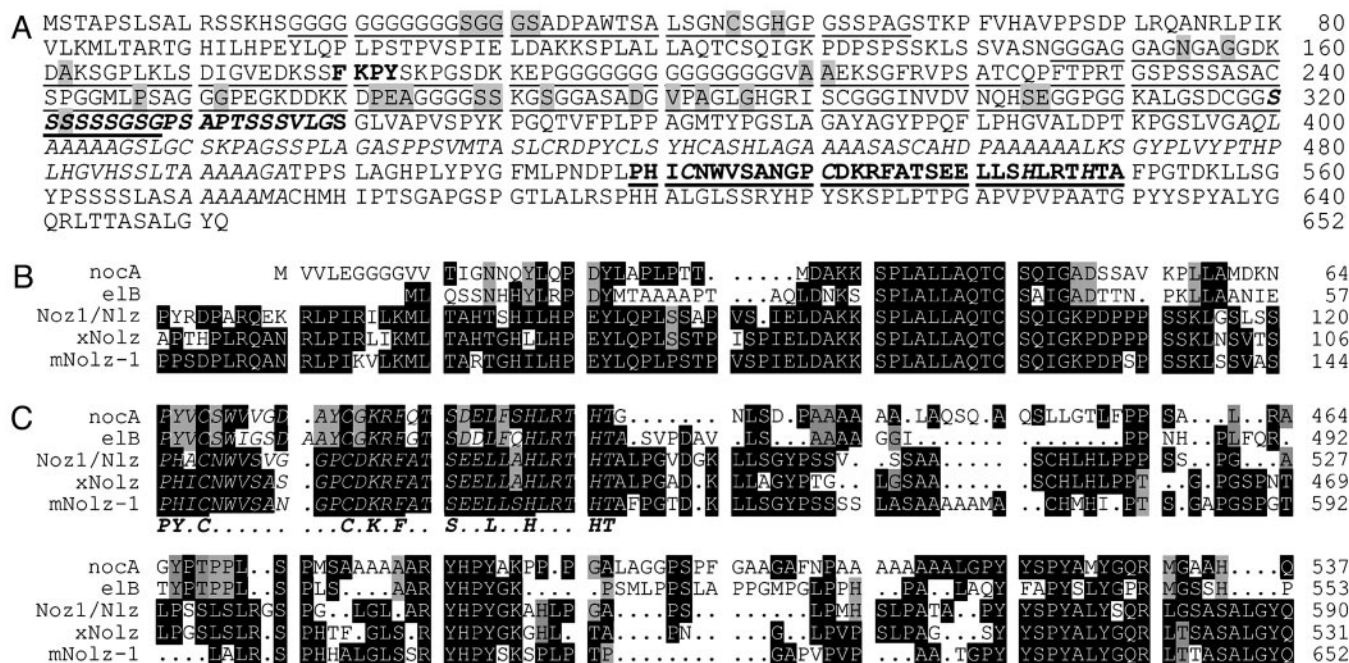


Fig. 1. (A) The deduced amino acid sequences of mNolz-1. The following domains are marked: glycine (G)-rich domains (17–56 and 146–328; underlined); serine (S)-rich domain (320–340; italic and bold); alanine (A)-rich domains (398–496 and 570–576; italic); Grochu binding sequence (bold); C₂H₂ zinc-finger motif (bold and underlined); Zn²⁺ binding motif [two cysteine (C) and two histidine (H)]; bold, italic, and underlined; amino acids that differ from hNolz-1 (shading). (B and C) Amino acid sequence alignments of the *nocA/eIB* family in amino-terminal (B) and carboxyl-terminal (C) domains showing high homology among the family members. The identical residues are indicated by black and shaded boxes. The zinc-finger motifs are in italic, and the consensus residues are shown in bold below (C). The sequences of mNolz-1 shown in B and C are identical for rNolz-1 and hNolz-1.

4 B and C). Notably, *Nolz-1* was highly expressed in the SVZ in which retinaldehyde dehydrogenase-3 (Raldh-3), a synthetic enzyme of RA, was also expressed (Fig. 4 E and F) (28). *Nolz-1* mRNA was not detectable in the dorsal telencephalon.

At E18–E20, the LGE and the MGE were fused to form a single bulge of ganglionic eminence that became the developing striatum. High levels of *Nolz-1* mRNA were maintained in the SVZ of the ganglionic eminence except the dorsalmost region, which lacked *Nolz-1* expression (Fig. 5A). *Nolz-1* was still not detectable in the VZ. Double labeling of *Nolz-1* mRNA and DARPP-32 protein, a marker for striatal projection neurons (29), showed that many DARPP-32-immunoreactive neurons coexpressed *Nolz-1* mRNA in the mantle zone of E18 and E20 striatum (Fig. 4H). In addition to the developing striatum, *Nolz-1* mRNA was expressed in a number of developing brain regions, including the medial septum, superior colliculus, and hindbrain (Fig. 5B).

Developmental Regulation of *Nolz-1* in the Striatum. After birth, the expression of *Nolz-1* mRNA in the striatum was gradually down-regulated from postnatal day 0 and 14 to adulthood (Fig. 6A–C). In the adult striatum, the levels of *Nolz-1* mRNA were

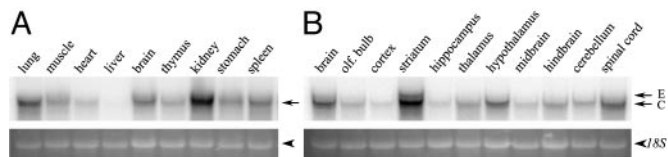


Fig. 2. Northern blots of *Nolz-1* transcripts in E20 rat embryo. Shown is expression of *Nolz-1* in different organ tissues of E20 rat embryo (A) and in different regions of E20 rat brain (B). Note that a doublet of transcripts consisting of the E and C forms are detected in the striatum.

very low in the dorsal striatum, but low levels of *Nolz-1* expression remained in the nucleus accumbens and high levels remained in the olfactory tubercle (Fig. 6C). Interestingly, the Northern blotting analysis indicated that there were dynamic changes of *Nolz-1* transcripts during striatal development (Fig.

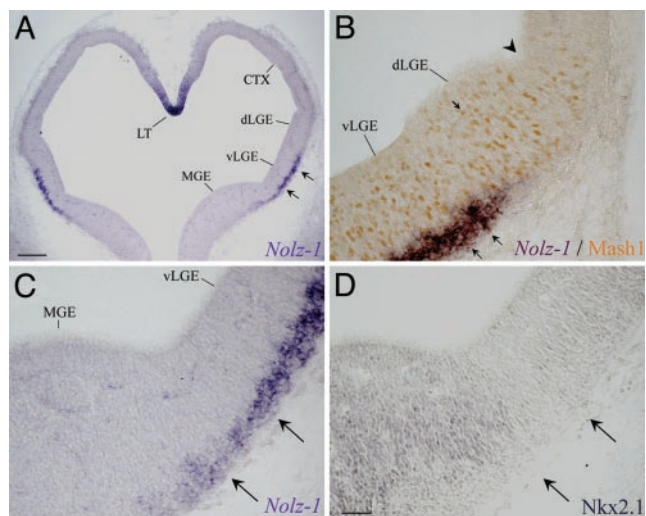


Fig. 3. Expression of *Nolz-1* mRNA in E13 rat telencephalon. (A) *Nolz-1* mRNA (double arrows) is expressed in the lateral part of ventral LGE (vLGE). (B) *Nolz-1* is not expressed in the dorsal LGE (dLGE) containing Mash1-positive nuclei (arrow), nor is it expressed in the Nkx2.1-positive MGE (D) or the cortical primordium (CTX). The arrowhead in B indicates the border between the dLGE and CTX. The sections in C and D are adjacent sections, and the two arrows point to the *Nolz-1*-positive streak slightly extending into the Nkx2.1-poor region in the MGE. LT, lamina terminalis. (Scale bars: A, 200 μ m; D for B–D, 50 μ m.)

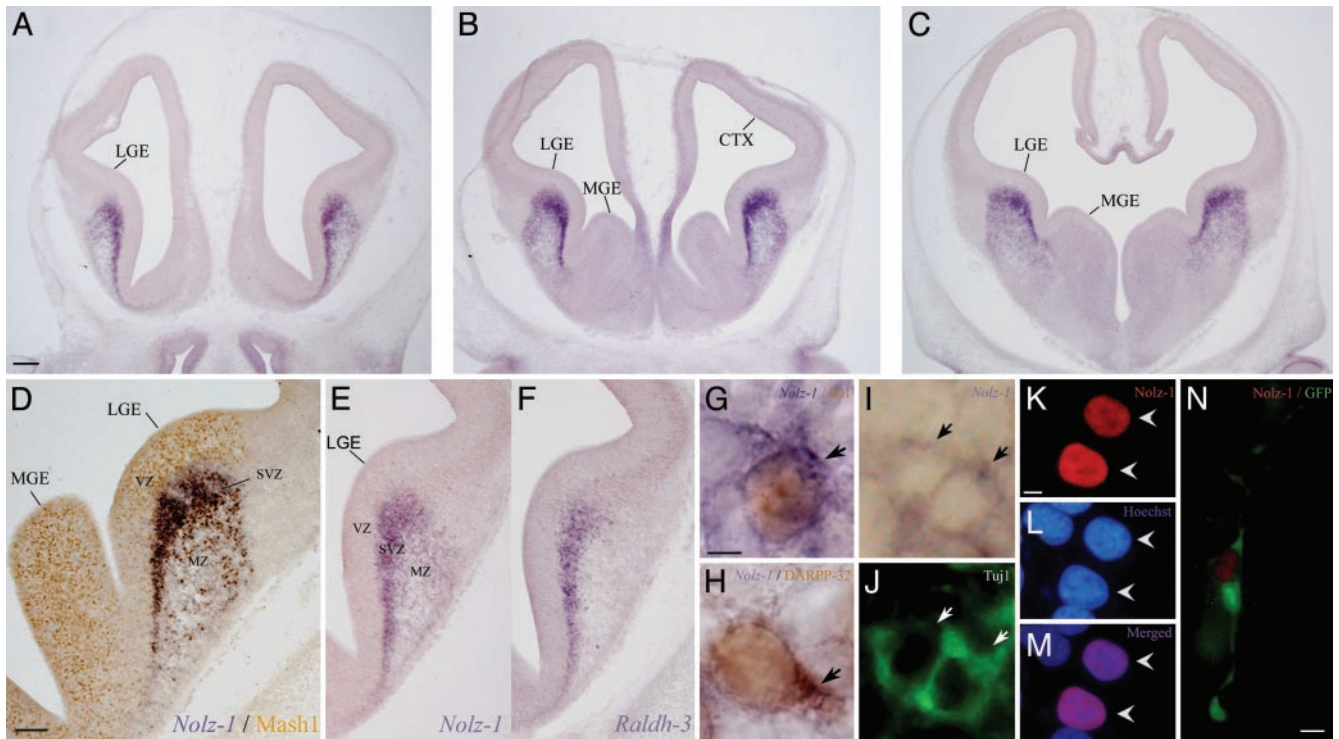


Fig. 4. Expression of *Nolz-1* mRNA in E15 rat telencephalon. (A–C) *Nolz-1* mRNA is primarily expressed in the LGE at rostral (A), middle (B), and caudal (C) levels. (D) Double labeling of *Nolz-1* mRNA and MASH1 protein. *Nolz-1* is highly expressed in the SVZ but is not expressed in the MASH1-positive ventricular zone (VZ, brown nuclei). *Nolz-1* expression is decreased in the mantle zone (MZ). (E and F) Adjacent sections showing spatial correspondence of *Nolz-1* and *Raldh-3* mRNA expression in the SVZ of LGE. (G) Coexpression of *Nolz-1* mRNA (purple cytosolic ring) and *Isl1* protein (brown nucleus) in early differentiating striatal cells (arrow) in the SVZ of LGE. (H) Double labeling of *Nolz-1* mRNA (purple cytosolic ring) and DARPP-32 protein (brown nucleus and cytosol) in E20 striatal neurons (arrow, dark brown cytosol). (I and J) Coexpression of *Nolz-1* mRNA (I) and TuJ1 protein (J) in differentiating neurons (arrows) in SVZ of LGE. (K–M) Nuclear localization of FLAG-tagged *Nolz-1* protein in HEK 293T cells (K–M, arrowheads) and mouse E13.5 forebrain (N). (K) FLAG immunostaining. (L) Hoechst 33258 nuclear staining. (M) Merged images of K and L. (N) A cotransfected GFP-positive cell with neuronal morphology in the brain shows nuclear localization of tagged *Nolz-1* protein (red nucleus). (Scale bars: A for A–C, 200 μ m; D for D–F, 200 μ m; G for G–J, 2 μ m; K for K–M, 5 μ m; N, 5 μ m.)

6D). At postnatal day 0 striatum, instead of the doublet transcripts found at the E20 striatum, a triplet of transcripts was detected, i.e., in addition to the major \approx 3.5-kb transcript, two transcripts (\approx 4 kb and \approx 3 kb) with lower expression levels were found. Similar results were obtained by using another probe (nucleotides 1240–1627 of RIKEN clone 41-22401-M18) that recognized partial coding sequences in the second exon of *Nolz-1* gene (data not shown). It was notable that the expression of these two transcripts was under different temporal control. The \approx 4-kb transcript was transiently present in the embryonic striatum from E15 to postnatal day 0 (Figs. 6D and 7A), possibly representing

an embryonic isoform of *Nolz-1* transcript [embryonic (E) form]. By contrast, the \approx 3-kb transcript was not significantly detected in the striatum until after birth, which might represent a post-

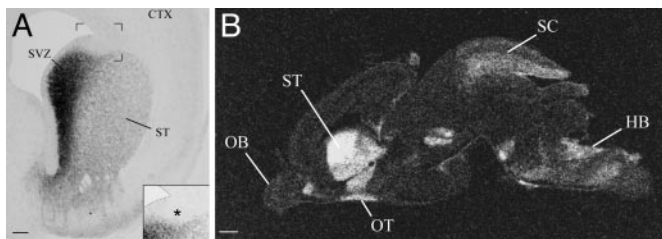


Fig. 5. Expression of *Nolz-1* mRNA in E20 rat forebrain. (A) *Nolz-1* mRNA is highly expressed in the SVZ of the developing striatum (ST) except the dorsal cap, which lacks *Nolz-1* (asterisk). The bracketed region is shown at high magnification in *Inset*. (B) Film autoradiogram of a parasagittal section showing enriched expression of *Nolz-1* mRNA in the striatum of E20 rat brain with 35 S-labeled probes. CTX, cortex; HB, hindbrain; OB, olfactory bulb; OT, olfactory tubercle; SC, superior colliculus. (Scale bars: A, 200 μ m; B, 500 μ m.)

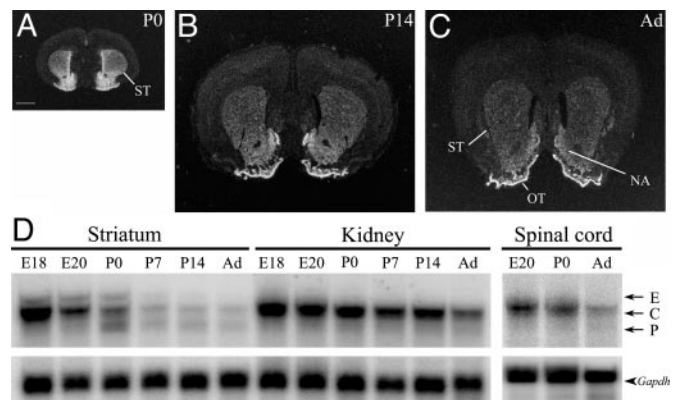


Fig. 6. Developmental regulation of *Nolz-1* mRNA in the striatum during development. Expression of *Nolz-1* mRNA is gradually decreased from postnatal day 0 (A) and postnatal day 14 (B) to adult (Ad) striatum (ST; C) as assayed by *in situ* hybridization with 35 S-labeled probes. The *Nolz-1* expression remains in the nucleus accumbens (NA) and olfactory tubercle (OT) at adulthood. (D) Dynamic regulation of different *Nolz-1* transcripts in the striatum during development. A triplet of transcripts consisting of the E, C, and P forms are present only in the postnatal day 0 striatum. No such complex regulation of *Nolz-1* transcripts is detected in the development of the kidney and the spinal cord where *Nolz-1* expression is also abundant. (Scale bar: A for A–C, 1 mm.)

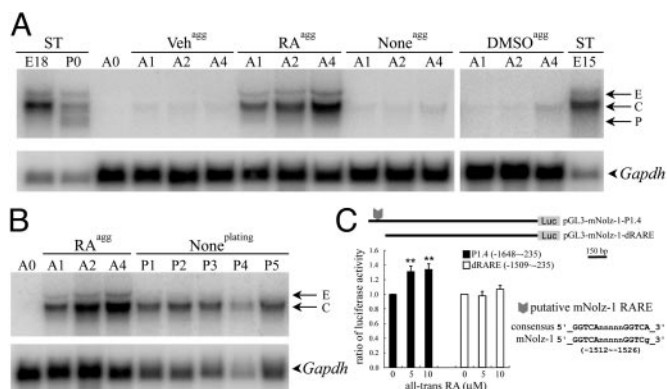


Fig. 7. Regulation of *Nolz-1* in P19 cells and the LGE culture. (A) A doublet of *Nolz-1* transcripts similar to the E and C forms found in E15, E18, and postnatal day 0 striatum (ST) are induced in aggregated (agg) P19 cells with RA treatment (RA^{agg}). Little or no *Nolz-1* induction is found with vehicle (Veh^{agg}) or DMSO (DMSO^{agg}) treatments, or without any treatment (None^{agg}). (B) After the cell aggregates are dissociated for plating and cultured without RA (None^{plating}), only the C form remains in the dissociated cells. (C) The pGL3-mNolz-1-P1.4 luciferase reporter gene vector was transfected into E16 rat LGE explant culture. All-trans RA increases the luciferase activity by 1.3-fold. This inductive effect is abolished when the RARE-containing region is deleted from the construct (pGL3-mNolz-1-dRARE). **, $P < 0.01$, Student's *t* test. A0–A4, 0–4 days after cell aggregation; P1–P5, 1–5 days after dissociating cell aggregates for dissociated cells culture.

natal isoform (P form; Fig. 6D). The major ≈ 3.5 -kb transcript [constitutive (C) form] appeared constitutively through striatal development, albeit with gradually decreasing expression level during postnatal maturation. No such complex regulation of different *Nolz-1* transcripts was observed in the development of the spinal cord and the kidney (Fig. 6D).

Subcellular Distribution of Nolz-1 Protein. To study the subcellular distribution of Nolz-1 protein, we expressed a FLAG epitope-tagged Nolz-1 recombinant protein in HEK293T cells. The FLAG-tagged recombinant protein was immunocytochemically detected in the nuclei of transfected HEK293T cells (Fig. 4K–M). A similar result of nuclear localization was obtained in the transfected P19 embryonal carcinoma cells (data not shown). To further confirm these results in a physiologically relevant context, we electroporated the expression plasmids into E13.5 mouse forebrains *in vivo* (21). Consistently, the tagged mNolz-1 protein was detected in the nucleus of transfected cells in the forebrain (Fig. 4N).

Regulation of Nolz-1 in Neural Induction of P19 Cells and in the LGE. We used the P19 cell line as a paradigm for neural differentiation to study whether *Nolz-1* was associated with neural induction and differentiation. Little *Nolz-1* mRNA was detected in the naive P19 cells. On the neural induction of aggregated P19 cells by RA, a doublet of *Nolz-1* transcripts similar to striatal E and C forms were induced (Fig. 7A and B). After dissociating the inductive cell aggregates to facilitate neural differentiation, only a single transcript similar to the striatal C form was detected (Fig. 7B). Little *Nolz-1* was induced with the DMSO treatment, which promoted P19 cell differentiation into nonneural cells (Fig. 7A) (30). These findings suggest that the induction of striatal E form transcript may require aggregation of cells in the presence of activated RA signaling, a situation that may occur in the SVZ of LGE where cells are tightly packed and *Raldh-3* is highly expressed (Fig. 4E and F).

The analysis of the genomic sequence of *mNolz-1* indicated that a putative direct repeat 5 (DR5) RARE was present at nucleotides -1512 to -1526 upstream of the first ATG trans-

lation start site (Fig. 7C). We then transfected into E16 rat LGE explant culture a luciferase reporter gene driven by an ≈ 1.4 -kb *Nolz-1* promoter region including the putative RARE (nucleotides -235 to -1648). The results showed that all-trans RA induced 1.3-fold increases of luciferase activity ($5 \mu\text{M}$, 1.31 ± 0.07 , $P < 0.01$, $n = 12$; $10 \mu\text{M}$, 1.34 ± 0.08 , $P < 0.01$, $n = 12$; Fig. 7C). Notably, deletion of the putative RARE-containing region (nucleotides -1648 to -1508) from the construct abolished the RA inductive effects, demonstrating the specificity of RA effect ($5 \mu\text{M}$, 0.98 ± 0.06 , $n = 8$; $10 \mu\text{M}$, 1.07 ± 0.05 , $n = 9$). These results suggest that retinoid signaling may regulate *Nolz-1* expression in the developing striatum. Although the activation of the *Nolz-1* reporter gene in the LGE by the exogenous RA was small, it is possible that high levels of endogenous RA in the LGE might have prevented further increases of *Nolz-1* reporter gene activity by the exogenous RA (31). Alternatively, RA may regulate only the E form of *Nolz-1* transcripts in the LGE, and the weak inductive effect may reflect the minor representation of the E form in the entire *Nolz-1* transcripts of the LGE (Fig. 7A).

Discussion

Despite the importance of the striatal system in neurological function (1), the developmental process by which striatal neurons acquire their special neurochemical properties and establish specific neural connectivity with other brain regions remains elusive. In an attempt to explore this issue, we searched for genes that are preferentially expressed in the developing striatum. We reasoned that developmentally regulated striatum-enriched genes may be involved in neural development and plasticity of the striatal system. The zinc-finger gene *Nolz-1* that we identified represents an interesting molecule for understanding the molecular mechanisms underlying striatal development and function.

***Nolz-1* as a Previously Uncharacterized Mammalian Member of the *nocA/elB/tlp-1* Family.** A salient feature of the Nolz-1 protein is that it contains an atypical C_2H_2 zinc-finger motif. In contrast to the typical C_2H_2 zinc-finger motif, X (2)-Cys-X (2, 4)-Cys-X (12)-His-X (3–5)-His, in which the two cysteine residues are spaced by conserved two or four amino acid residues (32), the two cysteine residues are spaced by eight residues in Nolz-1. This atypical arrangement of zinc-finger motif is conserved in the *nocA/elB/tlp-1* family (22–26). The mNolz-1 protein contained five stretches of alanine residues and a serine-rich domain, all of which were also found in *nocA* protein (23). The alignments of *nocA*, *elB*, *Noz1/Nlz*, *xNolz*, and *mNolz-1* indicated that, in addition to the zinc-finger motif-containing carboxyl terminus, a domain of ≈ 79 aa in the amino terminus was also highly conserved, suggesting that these two domains may be of functional significance.

***Nolz-1* as a Developmental Marker for Differentiating Striatal Progenitors.** Our study showed that *Nolz-1* mRNA was highly expressed in the SVZ, a transitional zone between the ventricular and mantle zones. This expression pattern of *Nolz-1* classifies it into a group of striatum-enriched genes that are primarily expressed in the SVZ of the ganglionic eminences, including *Dlx5*, *Brn4*, *RAR-alpha*, *SCIP*, *EphA4*, and *Raldh-3* (7, 28). The finding that *Nolz-1* mRNA was coexpressed with the early postmitotic neuronal markers TuJ1 and Isl1 suggests that *Nolz-1* is likely to be expressed by striatal neurons at early phases of differentiation (9, 27, 33). *Nolz-1* is selectively expressed in the LGE, together with a small group of *Nolz-1*-positive cells extending ventromedially into the MGE at caudal levels. This nearly selective expression of *Nolz-1* in the LGE distinguishes *Nolz-1* from other striatum-enriched genes such as *Dlx1*, *Dlx2*, *Mash1*, and *Gsh2* that are generally expressed in both the LGE

and MGE (5–7). The LGE contains progenitors that give rise to striatal projection neurons and GABAergic interneurons destined for the olfactory bulb and the cerebral cortex (34). The expression levels of *Nolz-1* mRNA were, however, low in the developing olfactory bulb and cerebral cortex. Moreover, *Nolz-1* was not expressed in the most dorsal part of the SVZ in which progenitors of olfactory bulb interneurons resided (35). The MGE, which is the main source of GABAergic interneurons tangentially migrating to the striatum and the cerebral cortex (34), also lacked *Nolz-1* expression. Therefore, *Nolz-1* may be primarily expressed by differentiating progenitors of striatal projection neurons. Consistent with this hypothesis, *Nolz-1* was colocalized with DARPP-32, a marker for striatal projection neurons (29). *Nolz-1* may prove to be a useful marker for identifying differentiating striatal progenitors.

Potential Involvement of *Nolz-1* Gene in Neural Development of the Mammalian Telencephalon. The possibility that *Nolz-1* is involved in regulating mammalian neural development is consistent with the functional study of *Drosophila nocA*. Mutation of *l(2)35Ba/nocA* results in hypertrophy of the embryonic supraesophageal ganglion (*Drosophila* brain). This hypertrophy is due to incorporation of nonneural cells in the procephalic lobe into the supraesophageal ganglion, and many of the misplaced nonneural cells become neurons (23). Mutation of *tlp-1* affects asymmetric cell fate determination and morphogenesis of tail (22), whereas *elB* is involved in cell fate specification of tracheal branches (24). The zebrafish *Noz1/Nlz* is expressed mainly in the posterior parts

of the brain, and it is inducible by the RA signaling (25, 26). Consistent with the reports that Tlp-1 and EIB proteins are nuclear transcription regulators (22, 24), the transfected *Nolz-1* protein was localized in the nucleus. Notably, EIB protein can associate with Grouch protein to function as transcriptional repressor (24), and its Grouch interacting motif FKPY is conserved in *Nolz-1* protein. It is possible that *Nolz-1* may also function as transcriptional repressor.

The developing telencephalon is compartmentally organized into three progenitor domains including the dorsal cortex and the ventral LGE and MGE. The boundaries between these three domains have been shown to be specified and maintained by the balanced activity of domain-specific genes (36–38). The specific expression of *Nolz-1* in the LGE domain and the possibility that *Nolz-1* may function as transcriptional repressor suggest that *Nolz-1* may regulate the formation and maintenance of the LGE domain by interacting with other domain-specific genes in the developing telencephalon. Future study of *Nolz-1* function may provide insight into the genetic programs underlying development of the telencephalon.

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