The Expression Patterns of Cdc25A, Cdc25B, Sox2 and Mnb in Central Nervous System in Early Chicken Embryos*

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ABSTRACT: The sense and antisense digoxigenin-labeled RNA probes of four genes, Cdc25A, Cdc25B, Sox2 and Mnb, were produced by using SP6s and T7 RNA polymerases, respectively, and in vitro transcription. Expression patterns of the four genes were detected by in situ hybridization in HH (Hamburger and Hamilton) stage 10 chick embryos. In general, expression patterns of the four genes were similar. mRNA of the four genes was mostly restricted to the entire CNS (central nervous system). All were confined to an identical region, neural tube, neural groove and caudal neural plate, corresponding to the notochord or spinal cord, but there was some distinction in specific region or in concentration, for example in somites. The overlap in expression at the same developmental stage in the CNS suggests that the four genes may be functional similar or related in CNS development. Expression patterns of the four genes support specific roles of these regulators in the developing CNS. (Key Words: Cdc25A, Cdc25B, Sox2, Mnb, CNS, In situ Hybridization, Chicken, Embryo)

INTRODUCTION

Cell division cycle (Cdc) genes are required for regulation of cell proliferation. However, others factors are necessary for cell differentiation events. To understand the mechanisms that underlie this regulated process, greater knowledge of the molecular control of the acquisition of cell proliferation and differentiation is required. At present, considerable progress has been made in identifying the signals and elucidating the molecular mechanisms that regulate cell proliferation and/or differentiation. However, it is important to investigate these genes at the transcription level and elucidate regulated factors in relation to each other and their roles in embryo development. Cell cycle progression is regulated by the cyclin-dependent kinase (CDK) family. CDK activity throughout the cell cycle is highly regulated by association with cyclins and with inhibitory proteins (Oogood, 2002). CDK activity is also regulated by phosphorylation. The Cdc25 gene was first identified in the fission yeast Schizosaccharomyces pombe as a positive regulator of the G2/M transition in the cell cycle (Russell and Nurse, 1986). Three members of the Cdc25 family have been identified in mammalian cells while only two isoforms (A and B) have been characterized in the chick. The Cdc25 proteins are 300-600 residues in length and can be divided into two regions. The N-terminal regions are highly divergent in sequence. The highly homologous C-terminal regions (~60% pair-wise identity over ~200 amino acids) contain the catalytic functionality of the Cdc25s (Rudolph, 2007). Cdc25 phosphatases serve as key activators of the CDK/ cyclins. The Cdc25 phosphatase family activates CDKs and stimulates cell cycle progression by catalysing removal of CDK inhibitory phosphates (Kumagi and Kornbluth, 1991). The Cdc25 phosphatases can dephosphorylate both phospho-tyrosine and phospho-serine/threonine residues, a property shared with several other dual-specificity phosphatases. The dual-specificity phosphatases are related in reaction mechanism to the tyrosine-specific phosphatases (Perry and Kornbluth, 2007).

Cdc25 is itself regulated by phosphorylation. CDKs and polo-like kinases increase Cdc25 phosphatase activity thus contributing to an amplification loop that ensures the faithful activation of CDKs during cell cycle transitions.
(Powers et al., 2000). Other Cdc25 kinases, including Chk1 (checkpoint kinase 1) and Cds1 (checking DNA synthesis 1) (Blasina et al., 1999), inhibit Cdc25 function by inhibiting phosphatase activity or through generation of a 14–3–3 binding site (Wilker and Yaffe, 2004). Other proteins are also reported to associate or co-distribute with Cdc25, including Ras, p13, Raf-1 and cyclin B (Powers et al., 2000).

Sox2 is a member of the Sox (SRY-related HMG box) gene family that encode transcription factors with a single HMG DNA-binding domain. Sox2 belongs to the Sox B1 subgroup based on homology within and outside the HMG box (Kamachi et al., 2000). Several lines of evidence indicate that Sox2 may act to maintain or preserve developmental potential. Sox2 is a transcription factor that plays multiple critical roles during embryonic development in vertebrates. Sox2 is expressed in cells that retain their ability to proliferate and/or acquire glial fates, whereas it is down-regulated in cells that become postmitotic and differentiate into neurons (Bylund et al., 2003). In all vertebrates studied to date, Sox2 is also a general marker for the very early developing neural plate. The complex expression profile of Sox2 is controlled by multiple regulatory elements, each responsible for directing expression to a specific subset of expression sites (Papanayotou et al., 2008). To date, no single secreted factor or any combination thereof has been found to induce either Sox2 expression or a neural plate in competent cells not normally fated to form part of the neural plate (Papanayotou et al., 2008).

"minibrain" (Mnb) Kinase is a mutant of Drosophila that exhibits a specific and marked size reduction of the optic lobes and central hemispheres of the adult brain (Fischbach and Teichman, 1984). The Mnb gene encodes a Ser/Thr protein kinase that possesses a YXY sequence in the activation loop (Tejedor et al., 1995). Mnb was originally identified as a gene essential to the neuronal proliferation of Drosophila (Miyata and Nishida, 1999). The ortholog of Drosophila Mnb, termed dual specificity tyrosine-phosphorylation regulated kinase IA (Mnb/DYRK1A), was subsequently characterized in many organisms. Similar to Drosophila, Mnb/DYRK1A is involved in the early development of the central nervous system (CNS) of vertebrates (Adayev et al., 2006). The Mnb gene is essential in cell proliferation and neuronal differentiation during postembryonic neurogenesis (Kentrup et al., 1996). DYRKs possess Ser/Thr phosphorylation activity as well as autophosphorylation activity on Tyr residues, suggesting that DYRKs are dual specificity kinases (Kentrup et al., 1996). The kinase activity of DYRK is dependent on the YXY motif in the activation loop (Kentrup et al., 1996), suggesting the existence of a phosphorylation-dependent activation mechanism of DYRK by certain upstream kinases. Thus, from Drosophila to humans, it is suggested that DYRK/Mnb is a key regulator of growth of neuronal cells as it is the case for conventional MAP kinases regulating growth in certain cell types. Although the exact cellular function of the DYRK kinases are yet unknown, it may be very interesting to know the physiological role of this family of protein kinases (Miyata et al., 1999; Yang et al., 2001).

The aim of the present study was to determine the expression patterns of Cdc25A, Cdc25B, Sox2 and Mnb in HH stage 10 (Hamburger and Hamilton, 1951) chicken embryos by in situ hybridization. Gene specific roles were predicted during CNS development.

**MATERIALS AND METHODS**

Preparation of the Digoxigenin-labeled RNA probes for four genes

Total RNA was isolated from chicken embryos using TRIZol reagent (Invitrogen, Shanghai, China). The fragments of Cdc25A, Cdc25B, Sox2 and Mnb genes were obtained by RT-PCR through total RNA of chicken embryos. The primers used were: 5'-TGTCGCCCTCTCGTGAGGTA-3' and 5'-CGGGTCACCTGCGGAAACTA-3' for Cdc25A; 5'-GGAGCCTCTGCCTGCAGGTA-3' and 5'-CAATTAACCCCTGCAATGTGTTGGCC-3' for Cdc25B, obtained from Benazraf et al (Benazraf et al., 2005), 5'-AGGCTCCGCGGTAAATATTAGCA-3' and 5'-GCCGGGAGGTTCGATCCTGGTTC-3' for Sox2; 5'-CTTACTGTTAGGCAATGCACATC-3' and 5'-CTTAGTTTCAATTTATCTCGACGCT-3' for Mnb. Then, the PCR products were subcloned into pGM-T vector (Tiangen, Beijing, China) using the TA cloning system.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Accession no</th>
<th>Primer sequences</th>
<th>Annealing temperature</th>
</tr>
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<tbody>
<tr>
<td>Cdc25A</td>
<td>XM418479</td>
<td>5'-TGTCGCCCTCTCGTGAGGTA-3'</td>
<td>63°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-CGGGTCACCTGCGGAAACTA-3'</td>
<td></td>
</tr>
<tr>
<td>Cdc25B</td>
<td>BM490858</td>
<td>5'-GGAGCCTCTGCCTGCAGGTA-3'</td>
<td>68°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-CAATTAACCCCTGCAATGTGTTGGCC-3'</td>
<td></td>
</tr>
<tr>
<td>Sox2</td>
<td>NM205188</td>
<td>5'-AGGCTCCGCGGTAAATATTAGCA-3'</td>
<td>70°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-GCCGGGAGGTTCGATCCTGGTTC-3'</td>
<td></td>
</tr>
<tr>
<td>Mnb</td>
<td>AJ459381</td>
<td>5'-CTTACTGTTAGGCAATGCACATC-3'</td>
<td>58°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5'-CTTAGTTTCAATTTATCTCGACGCT-3'</td>
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</table>
The recombinant plasmids were used to transform Escherichia coli strain DH5α and chosen by “white-blue plaque selection”. Subsequently, the positive recombinant plasmids were identified by sequencing. The recombinants were linearized with the restriction enzymes Neol and SpeI (TaKaRa, Dalian, China). The linearized recombinant plasmids were used as templates for in vitro transcription. The sense and antisense Digoxigenin-labelled riboprobes of the four genes were produced by using SP6 and T7 RNA polymerase (Roche, Mannheim, Germany), respectively and in vitro transcription by the use of DIG RNA Labeling Kit (SP6/T7) (Roche) following the manufacturer’s instructions.

**Embryos**

Fertile hens’ eggs were incubated at 38°C in a humidified incubator to yield embryos of appropriate stages (HH 10) according to Hamburger and Hamilton (Hamburger and Hamilton, 1951), and isolated embryos from yolk (Mozdzia et al., 2008).

**Whole mount in situ hybridization**

Whole mount in situ hybridization analysis was performed as described by Rex et al. (1997) with minor modifications. Following overnight fixation in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at 4°C, embryos were dehydrated through a series of ethanol solutions (25%, 50%, 75%, and 100%). Embryos were rehydrated and washed twice in PBS and 0.1% Tween-20 (PBST) and once in 6% hydrogen peroxide for 20 min. Embryos were incubated at room temperature in 10 mg/ml proteinase K (pre-warmed to 37°C) for 15 min and then post-fixed in 4% PFA/0.2% glutaraldehyde for 20 min. Following two washes with PBST, embryos were pre-hybridised for 1 h in pre-hybridisation solution, at 65°C. Digoxigenin-labelled RNA probes were added to fresh pre-hybridisation solution and hybridised with the embryos overnight at 70°C. Embryos were washed twice in solution 1 (50% formamide, 5× SSC, pH 4.5) for 30 min at 70°C and three times in solution 2 (50% formamide, 2× SSC, pH 4.5) for 30 min at 65°C. Embryos were then washed three times in TBST (140 mM NaCl, 25 mM KCl, 25 mM Tris pH 7.5, 0.1% Tween-20) and blocked with 10% sheep serum for 2 hr at room temperature to prevent non-specific binding of antibody before overnight incubation at 4°C with alkaline phosphatase-conjugated anti-digoxigenin antibody. Embryos were next washed five times (1 h each) with TBST. Embryos were incubated with 35 μg/ml 4-nitro blue tetrazolium chloride and 15 μg/ml 5-bromo-4-chloro-3-indolyl-phosphate in NTMT (100 mM Tris pH 9.5, 100 mM NaCl, 50 mM MgCl₂, 0.1% Tween-20) at room temperature. After completion of the color reaction, the embryos were washed in PBST (phosphate-buffered saline and 0.1% Tween-20) and photographed with a XTL30 zoom stereo microscope.

**RESULTS**

Expression patterns of the four genes were detected by in situ hybridization in HH stage 10 chick embryos. In general, expression patterns of the four genes were similar, but there was some distinction in specific region or in concentration.

Expression of Cdc25 isoforms in CNS of chicken embryo

Cdc25A mRNA expression was most redundant in rostral head fold according to the staining (Figure 1a). The caudal neural plate was fairly plentiful (Figure 1d), but the middle neural groove showed weak expression (Figure 1b). In addition, the mRNA was weak in somites (Figure 1e).

Cdc25B mRNA expression was also conspicuous in rostral head fold (Figure 1e). Cdc25B was expressed in neuroepithelium of the neural tube and neural groove. Cdc25B mRNA was conspicuous in the closing neural tube (Figure 1f), but tenuous in the caudal neuroepithelium (Figure 1g). Cdc25B mRNA was sufficient in somites (Figure 1h).

Expression of Mnb in CNS of chicken embryo

Mnb mRNA was considerable in the CNS. At HH stage 10, Mnb mRNA was detected at different levels of the closed neural tube: the prospective prosencephalon, mesencephalon, rhombencephalon, and spinal cord (Figure 1i). The mRNA was redundant in rostral head fold (Figure 1j), but it was weak in caudal neural plate (Figure 1l). Moreover, Mnb mRNA was very poor in somites (Figure 1k).

Expression of Sox2 in CNS of chicken embryo

Expression patterns of Sox2 were detected in chick embryo (Figure 1G and H). Sox2 was predominantly expressed in the immature neural epithelium of the entire CNS. In general, Sox2 mRNA was detected in the proliferative neural epithelium. Sox2 expression was also detected in the early neural tube. Sox2 mRNA was specific to the neural tube. Sox2 expressed in a band along the length of most of the CNS and in all compartments of the brain. Sox2 mRNA expression was redundant in rostral head fold according to the staining (Figure 1n). Sox2 was expressed in the CNS and all regions of the brain. The middle neural groove was weak in expression (Figure 1o). In addition, Sox2 expression was not visible in somites (Figure 1m).
Figure 1. Expression patterns of Cdc25A, Cdc25B, Mnb, and Sox2 approximately at stage HH 10 in the chick embryo. A (30x) and B (90x) show expression patterns of Cdc25A. Cdc25A expression was seen in CNS. Cdc25A mRNA expression was most redundant in rostral head fold according to the staining (a). The caudal neural plate was fairly plentiful (d). The middle neural groove showed weak expression (b). In addition, the mRNA was weak in somites (c). C (30x) and D (50x) show expression patterns of Cdc25B. Cdc25B was expressed in neuroepithelium of the neural tube and neural groove. Cdc25B mRNA was tenuous in the caudal neuroepithelium (g). The mRNA was conspicuous in the closing neural tube (i). Cdc25B mRNA was conspicuous in rostral head fold, too (e). Besides, Cdc25B mRNA was sufficient in somites (b). E (30x) and F (75x) show expression patterns of Mnb. Mnb mRNA was considerable in CNS. Mnb expression was detected in the neural tube, the prospective prosencephalon, mesencephalon, rhombencephalon, and spinal cord (i). Mnb mRNA was redundant in rostral head fold (j). The mRNA appears weak in caudal neural plate (l). Mnb expression was very poor in somites (k). G (30x) and H (100x) show expression patterns of Sox2. Sox2 was predominantly expressed in the immature neural epithelium of the entire CNS. Sox2 mRNA was detected in the neural tube. Sox2 expressed in a band along the length of most of the CNS and in all compartments of the brain. Sox2 mRNA expression was redundant in rostral head fold according to the staining (n). The middle neural groove showed weak of expression (o). Sox2 expression was not visible in somites (m). Furthermore, the caudal neural plate was fairly plentiful (p).

DISCUSSION

The patterns of expression of the four genes were roughly similar, notwithstanding that expression patterns had diversity in level in the same region. Whereas mRNA of all four genes was restricted to the entire CNS, only Cdc25A and Cdc25B had weak staining in somites. All of them were confined to an identical region, neural tube, neural groove and caudal neural plate, corresponding to the future notochord or spinal cord. However, expression patterns of the four genes were distinctive in the CNS. The overlap in expression in the same developmental stage in the CNS suggests that the four genes may be functionally similar or related in CNS development.

Cdc25A and Cdc25B

According to the staining of Cdc25A and Cdc25B in situ hybridization, it seems that the expression patterns of Cdc25A and Cdc25B are complementary in HH stage 10 chick embryos (Benazeraf et al., 2006).

Recent evidence suggests that all three isoforms can dephosphorylate Cdc2/cyclin B and play important roles in the G1/S and G2/M transitions of the cell cycle (Mailand et al., 2002; Perry and Kornbluth, 2007). Expression patterns of Cdc25A and Cdc25B were complementary in HH stage 10 in chick embryo CNS. When one Cdc25 is inhibited or deactivated, the other will be activated and replace it. Both Cdc25 phosphatases seem to function as key regulators of the G1-S and G2-M transitions and of mitosis, to spatially and temporally regulate their respective CDK substrates. The two Cdc25 phosphatases appear to cooperate during each stage of the cell cycle to activate the relevant CDK/cyclin complexes. A single Cdc25 protein that possesses characteristics of multiple Cdc25 isoforms, is capable of performing all phosphatase-dependent activation of CDK/cyclin complexes required for normal cell division (Russell et al., 1986). Cdc25A and Cdc25B may possess functional redundancy to regulate cell proliferation in CNS development. However, expression patterns of Cdc25A and Cdc25B are differential, for example, in somites, indicating
that perhaps the redundancy between the different Cdc25 isoforms is limited. Functional redundancy is a universal feature of cell cycle regulators that have evolved from common ancestors to fulfill more specialised functions, but that have kept the ability to carry on most of the functions of the other family members (Boutros et al., 2006).

**Cdc25 and Sox2**

It appears that the expression patterns of Cdc25A and Sox2 were more similar at stage HH 10 in chick embryos. Sox2 mRNA expression was fairly plentiful in the caudal neural plate similarity to Cdc25A, which is different from the report of Uwamogho et al. (1995). Cdc25A mainly activates the CDK2/cyclin E and CDK2/cyclin A complexes during the G1-S transition (Jinno et al., 1994), but also has a role in the G2-M transition by activating CDK1/cyclin B complexes, which are thought to initiate chromosome condensation (Molimari et al., 2000; Boutros et al., 2007). The Cdc25A role is one of controlling cell proliferation regulator by regulating cell cycle progress.

Expression patterns of Cdc25 and Sox2 were overlapping in the CNS in HH stage 10. Sox2 can retain cell proliferation and/or initiate differentiation. Cdc25 participates in cell proliferation by regulating the checkpoint in cell cycle progress. Moreover, expression of both Cdc25A and Sox2 is regulated by STAT3 (signal transducer and activator of transcription).

Through the up-regulation of cell cycle survival genes, STAT3 plays important roles in cell growth, anti-apoptosis, and cell transformation. STAT3 and its transcriptional cofactors are recruited to the promoter of the Cdc25A gene to activate its expression. Myc and STAT3 cooperate to induce the expression of Cdc25A. However, STAT3 also functions as a transcriptional repressor of the Cdc25A gene. STAT3 forms a repressor complex with the retinoblastoma (Rb) tumor suppressor to occupy the Cdc25A promoter and block its induction (Barre et al., 2005). A novel signaling pathway exists during early neural development in which STAT3 directly regulates the Sox2 promoter leading to Sox2 expression. STAT3 and Sox2 are expressed in the same areas of developing neural tissue that suggests that STAT3 is capable of regulating nestin via Sox2 in vivo (Poshay and Gallicano, 2008).

At the beginning, when STAT3 and Myc cooperate to induce the expression of Cdc25A, cells undergo proliferation. Following hydrogen peroxide stimulation, STAT3 forms a repressor complex with the Rb tumor suppressor to occupy the Cdc25A promoter and block its induction. Subsequently, cells go into differentiation. Similarly, Sox2 has roles both in proliferation and differentiation. STAT3 directly regulates the Sox2 promoter leading to Sox2 expression. The high dose of Sox2 can retain cell proliferation. Sox2 activity plays a key role in the regulation of the NOTCH1 signaling pathway in a concentration-dependent manner in retinal progenitor cells (Taranova et al., 2006). Precise regulation of Sox2 dosage is critical for temporal and spatial regulation of retinal progenitor cell differentiation (Taranova et al., 2006). Following cell proliferation, the collection of Sox2 is decreased increasingly, Sox2 cannot retain cell proliferation, and afterwards cells fulfill differentiation events. The two mechanisms of CNS cell proliferation and differentiation corresponds with our results. Transcripts of Cdc25A and Sox2 can be detected both in proliferating and differentiating xameral cells. However, in general, the transcript level in proliferating cells is higher than in differentiating cells. The different functions of Sox2 imply that it regulates a wide range of target genes. Furthermore, depending on cellular context, Sox proteins can sometimes function as activators or repressors, adding to their high versatility (Episkopou, 2005).

**Cdc25 and Mnb**

In general, the expression patterns of Cdc25 and Mnb were analogous according to their staining. However, Mnb expression was not nearly as visible in somites. It was different to Cdc25B. Mnb mRNA was considerable in CNS, which is different to other results (Hammerle et al., 2002).

The Mnb gene is expressed and required in distinct neuroblast proliferation centers during neurogenesis. The Mnb kinases share extensive sequence similarities with kinases involved in the regulation of cell division (Miyata and Nishida, 1999). So, Mnb kinases have an important function in controlling the generation of the correct maturate of neuroblast progeny (Miyata and Nishida, 1999). Structural similarities with CDK suggest that the DYRK family of kinases could be involved in the regulation of cellular proliferation. DYRK1A might control proliferation and maturation events during development (Miyata and Nishida, 1999). Although the overall scheme of neuronal development is quite different in invertebrates and vertebrates, molecular studies on vertebrate neurogenesis have revealed a remarkable evolutionary conservation of the cellular mechanisms of neuronal development (Purves and Lichtman, 1992). Moreover, CDKs are known to regulate cellular proliferation in various species, suggesting a more universal regulatory mechanism (Nigg, 1995).

CDK/cyclin complexes are key complexes in controlling cell cycle progress. Mnb regulation of cell proliferation may be through CDK/cyclin complexes. Mnb may regulate cell proliferation through three pathways. One of these pathways is cyclin. It is reported that cyclin L2 is a novel substrate of DYRK1A. Cyclin L2 contains an N-terminal cyclin domain and a C-terminal arginine/serine-rich domain. Full-length cyclin L2 was associated with the cyclin-dependent kinase PITSLRE (also named CDK11 (57) or Cdc2-like 1 (official gene symbol CDC2L1). DYRK1A may regulate splicing by phosphorylation of cyclin L2 (Graaf et
The structure of cyclin L2 and cyclin D/E/B is similar. It is predicted that Mnb can catalyse cyclin D/E/B/A and participate in cell cycle regulation and cell proliferation. The other pathway is Cdc25. Mnb belongs to a family of dual-specificity protein kinases (DYRK kinases). This family of protein kinases comprises the yeast Yak1p (one of DYRKs subfamilies in Saccharomyces cerevisiae) (Garrett and Broach, 1989) and Pom1p (in Schizosaccharomyces pombe) (Bailer and Pringle, 1997), the Dictosteylum YakA (in Dictosteylum discoideum) (Souza and Kuspa, 1998). Yak1p is a negative regulator of growth (Garrett et al., 1991). YakA regulates G-protein signaling during Dictosteylum growth (Es et al., 2001). The structure of Mnb and CDK are similar (Miyata and Nishida, 1999). Furthermore, DYRKs autophosphorylate their activation loop on an essential tyrosine but phosphorylate their substrates on serine and threonine (Lohchead et al., 2005). Thus, Mnb is inhibited by phosphorylation just like Yak1p and Pom1p. The Mnb-p may inhibit cell proliferation. So, Mnb-p is a negative regulator of growth by phosphorylation. When it is dephosphorylated, Mnb is activated to promote cell proliferation. The mechanisms are the same for CDK, too. Cdc25 can dephosphorylate CDK, then CDK/cyclin is activated, and cause cells to pass a checkpoint and promote cell proliferation. However, Cdc25 phosphatase activities are regulated by Cdc25/cyclin complexes themselves (Boutros et al., 2007). Therefore, activities of the Cdc25 phosphatases may be regulated by Mnb. Another pathway is through STAT3. Previous results indicate that DYRK kinases have a potential to phosphorylate serine 727 in STAT3 protein (Matsuo et al., 2001) and serine phosphorylation of STATs also regulates their transcriptional activities (Wen et al., 1995; Wen and Damell, 1997). Mnb may phosphorylate serine 727 in STAT3 protein and regulates the transcriptional activities of STAT3 similarly to DYRK kinases. Subsequently STAT3 cooperates with Myc or Rb tumor suppressors to regulate Cdc25A promotion, and induces or inhibits Cdc25A, respectively. Thereby, Mnb is involved in cell proliferation and differentiation even by regulating cell cycle progression.

In summary, we produced RNA probes of four genes and detected transcription level by whole mount in situ hybridization in the same developmental stage of the CNS in chick embryos. In general, all four gene expression patterns were similar in CNS, only having some distinction in specific region and in concentration. The results suggest that chicken Cdc25A, Cdc25B, Sox2 and Mnb expression patterns support specific roles of these regulators in CNS development because of gene expression with a spatiotemporal pattern in embryonic development. The overlap in expression in the same developmental stage of the CNS suggests that the four genes may be functional similar or have a regulatory relationship in CNS development (Collignon et al., 1996). The mechanisms of regulating CNS development indicate that the four genes are involved in cell cycle progression and cell migration and differentiation events. Moreover, the four genes may regulate each other coordinately. However, the gene network of regulating CNS development is precise and complicated. The development of the CNS of the chick requires a precise and reproducible pattern of neuroblast proliferation during embryonic neurogenesis. Here, we elucidated the mechanisms only in a morphologic manner. Further investigation is needed to identify molecules involved in CNS development and to elucidate the mechanisms of CNS development.

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REFERENCES


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