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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 SP6 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 more 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 (Kumagai 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

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(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 (Papanayotoul 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 (Papanayotoul 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 Technau, 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 1A* (*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 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′-TGTCCGGCCTCCTGGTGA-3′ and 5′-CGGGTGACCTGGAAAACTA-3′ for *Cdc25A*; 5′-GGAGCCCTGCGTGCCGAG-3′ and 5′-CAATTAACCC TCACTAAAGGGTCCTGGTGTTTGCC-3′ for *Cdc25B* according to Benazeraf et al (Benazeraf et al., 2006); 5'-AGGCTCCGGCGGTAATAATAGCA-3' and 5'-GCGGGAGGTTTCAGCTCGGTTTC-3′ for *Sox2*; 5'-CTCTACTGGTAACCAAGGCAATC-3' and 5'-CTTAGTTTCAGTTTAGTCACGAGCT-3' for *Mnb*. Then, the PCR products were subcloned into pGM-T vector (TIANGEN, Beijing, China) using the TA cloning system.

Table 1. The parameters of four genes for PCR

Gene symbol	Accession no	Primer sequences	Annealing temperature
Cdc25A	XM418479	5′-TGTCCGGCCTCCTGGTGA-3′ 5′-CGGGTGACCTGGAAAACTA-3′	63°C
Cdc25B	BM490858	5′-GGAGCCCTGCGTGCCGAG-3′ 5′-CAATTAACCCTCACTAAAGGGTCCTGGTGTTTGCC-3′	68°C
Sox2	NM205188	5'-AGGCTCCGGCGGTAATAATAGCA-3' 5'-GCGGGAGGTTTCAGCTCGGTTTC-3'	70°C
Mnb	AJ459381	5'-CTCTACTGGTAACCAAGGCAATC-3' 5'-CTTAGTTTCAGTTTAGTCACGAGCT-3'	58°C

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 *Nco*I and *Spe*I (TaKaRa, Dalian, China). The linearized recombinant plasmids were used as templates for *in vitro* transcription. The sense and antisense Digoxigenin-labeled 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 (Mozdziak 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% para-formaldehyde (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% gluteraldehyde 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 2hr at room temperature to prevent nonspecific 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 $MgCl_2$, 0.1% Tween-20) at room temperature. After completion of the color reaction, the embryos were washed in PBT (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 1c).

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). The caudal neural plate was fairly plentiful (Figure 1p). In addition, *Sox2* expression was not visible in somites (Figure 1m).

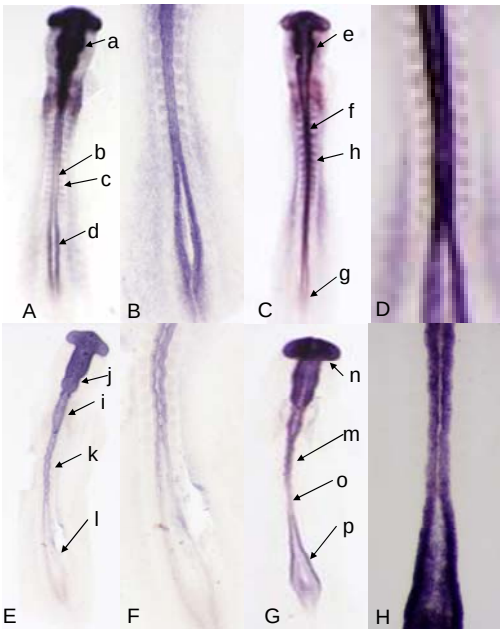

Figure 1. Expression patterns of *Cdc25A*, *Cdc25B*, *Mnb*, and *Sox2* approximately at stage HH 10 in the chick embryo. A (30×) and B (90×) show expression patterns of *Cdc25A*. *Cdc25A* expression was seen in CNS. *Cdc25A* mRNA expression was most redundant in rostral head fold according to the staining (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 (30×) and D (50×) 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 (f). *Cdc25B* mRNA was conspicuous in rostral head fold, too (e). Besides, *Cdc25B* mRNA was sufficient in somites (h). E (30×) and F (75×) 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 (30×) and H (100×) 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 similaly to *Cdc25A*, which is different from the report of Uwanogho 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 (Molinari 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 and 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 C*dc25A* 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 which suggests that STAT3 is capable of regulating nestin via Sox2 *in vivo* (Foshay 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 fulfil 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 xamaterol 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 mitosis 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 complexs are key complexs in controlling cell cycle progress. *Mnb* regulatation of cell proliferation may be through CDK/cyclin complexs. *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

al., 2004). The structure of cylcin L2 and cyclin D/E/B is similar. It is predicted that *Mnb* can catalysis 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*) (Bahler and Pringle, 1998), the Dictyostelium YakA (in *Dictyostelium discoideum*) (Souza and Kuspa, 1998). Yak1p is a negative regulator of growth (Garrett et al., 1991). YakA regulates G-protein signaling during *Dictyostelium* growth (Es et al., 2001). The structure of *Mnb* and CDK is similar (Miyata and Nishida, 1999). Furthermore, DYRKs autophosphorylate their activation loop on an essential tyrosine but phosphorylate their substrates on serine and threonine (Lochhead 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 CDK/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 serine727 in STAT3 protein (Matsuo et al., 2001) and serine phosphorylation of STATs also regulates their transcriptional activities (Wen et al., 1995; Wen and Darnell, 1997). Mnb may phosphorylate serine727 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 progress.

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 spatio-temporal 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 progress and cell proliferation 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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