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Temporal and spatial expression patterns of Cdc25 phosphatase isoforms during early *Xenopus* development

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ABSTRACT In early animal development, cell proliferation and differentiation are tightly linked and coordinated. It is important, therefore, to know how the cell cycle is controlled during early development. Cdc25 phosphatases activate cyclin-dependent kinases (Cdks) and thereby promote cell-cycle progression. In *Xenopus laevis,* three isoforms of *cdc25* have been identified, *viz. cdc25A, cdc25B* and *cdc25C*. In this study, we isolated a cDNA encoding a novel *Xenopus* Cdc25 phosphatase (named *cdc25D*). We investigated the temporal and spatial expression patterns of the four *cdc25* isoforms during early *Xenopus* development, using RT-PCR and whole-mount *in situ* hybridization. *cdc25A* and *cdc25C* were expressed both maternally and zygotically, whereas *cdc25B* and *cdc25D* were expressed zygotically. Both *cdc25A* and *cdc25C* were expressed mainly in prospective neural regions, whereas *cdc25B* was expressed preferentially in the central nervous system (CNS), such as the spinal cord and the brain. Interestingly, *cdc25D* was expressed in the epidermal ectoderm of the late-neurula embryo, and in the liver diverticulum endoderm of the mid-tailbud embryo. In agreement with the spatial expression patterns in whole embryos, inhibition of bone morphogenetic protein (BMP), a crucial step for neural induction, induced an upregulation of *cdc25B,* but a downregulation of *cdc25D* in animal cap assays. These results indicate that different *cdc25* isoforms are differently expressed and play different roles during early *Xenopus* development.

KEY WORDS: *cell cycle, Xenopus, cdc25, cdc25D, cell proliferation*

In early animal development, cell-cycle progression and exit from the cell cycle must be precisely controlled, since aberrant cell proliferation results in malformation or hyperplasia. It is important, therefore, to know how the cell cycle is controlled during development. Despite the importance of cell-cycle control in embryogenesis, however, relatively less attention has been paid to the expression patterns of cell-cycle regulators than to those of other factors that are involved in patterning and differentiation. Since amphibian *Xenopus laevis* is one of the most intensely investigated model animals in both developmental biology and cell-cycle control at the molecular level, this animal is highly suitable for studies on the cell-cycle control during embryogenesis.

Over the last three decades, numerous studies have contributed to the understanding of the core mechanisms underlying cell-cycle control. Especially, extensive studies in yeast and mammalian cultured cells have identified many essential regulators that govern cell-cycle progression (Coleman and Dunphy, 1994; Morgan, 1995). Master regulators of cell-cycle progression are the cyclin-dependent kinases (Cdks), which are activated periodically for proper cell-cycle progression (Coleman and Dunphy, 1994; Morgan, 1995). Both Wee1 and Myt1 kinases phosphrorylate and inactivate Cdks to prevent precocious Cdks activation (Coleman and Dunphy, 1994; Morgan, 1995). Cdc25 phosphatases dephospholylate and activate Cdks and thereby promote cell-cycle progression (Coleman and Dunphy, 1994; Morgan, 1995; Boutros *et al*., 2006). While the spatial expression patterns of *cdks*, *cyclins* and *wee1*/*myt1* during early *Xenopus* development have been shown in previous studies (Vernon and Philpott, 2003; Leise and Mueller, 2002), those of

Abbreviations used in this paper: BMP, bone morphogenetic protein; Cdk, cyclin-dependent kinase.

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cdc25 genes have not been described yet.

There are three *cdc25* isoforms, *cdc25A*, *cdc25B* and *cdc25C*, in mammalians and *Xenopus laevis* (Boutros *et al.*, 2006). During early *Xenopus* development, Cdc25C proteins are constantly expressed in both oocytes and early embryos (Hartley *et al.*, 1996), whereas Cdc25A proteins are abundant in cleavage-stage embryos and degraded rapidly at the midblastula transition (MBT) (Kim *et al.*, 1999; Shimuta *et al.*, 2002). While both *cdc25A* and *cdc25C* are expressed in early cleavage-stage embryos, *cdc25B* is not expressed until the MBT (Ueno *et al.*, 2008). After the MBT, however, *cdc25B* is expressed in the neuroectoderm of early neurula embryos, and is essential for primary neurogenesis (Ueno *et al.*, 2008). Although Cdc25 phosphatases are expressed in early *Xenopus* development, their roles in morphogenesis are poorly understood.

In this study, we have isolated a novel *Xenopus cdc25* isoform (termed *Xcdc25D*; GeneBank Accession No. AB601986), and have examined the expression patterns of *cdc25* isoforms (including *Xcdc25D*) during early embryogenesis. The spatial expression patterns of *cdc25A*, *cdc25B*, and *cdc25C* partially overlapped with each other around the time of neurogenesis, but that of *cdc25D* exhibited a quite distinct pattern from those of the other *cdc25* isoforms. Our results provide a framework for future studies on the role of the Cdc25 isoforms in early development.

Results

cDNA cloning of a novel Xenopus cdc25 isoform

We performed BLAST search analysis in order to identify a possible novel *cdc25* isoform(s) of *Xenopus laevis*, and found that some EST clones seem to encode a novel isoform. We then obtained a corresponding plasmid from NIBB (GeneBank Accession No. BJ037611), resequenced it, and found that this plasmid indeed contains a cDNA encoding a novel *cdc25* isoform. Sequence analysis revealed that the predicted translation product of this cDNA has 413 amino acid residues and, like other cdc25 isoforms, consists of an N-terminal regulatory domain (NRD), a C-terminal phosphatase domain (PD), and a short C-terminal regulatory domain (CRD) (Fig. 1A). Neither the NRD nor the CRD of this Cdc25 isoform shares sequence homology with those of conventional *Xenopus* Cdc25 isoforms. However, the PD of this Cdc25 isoform shares 52%, 53%, and 56% identities with the PDs of Cdc25A, Cdc25B, and Cdc25C, respectively (Fig. 1B). Apparently, the PD of this isoform, excluding its inserted sequence, is more similar to that of Zebrafish Cdc25d (Nogare *et al.,* 2007) (Fig. 1B), and indeed, phylogenetic analysis showed that this isoform is most closely related to Zebrafish Cdc25d (Fig. 1C). Thus, hereafter, we call this *Xenopus cdc25* isoform (*X)cdc25D*. A clear orthologue of *cdc25D* was found easily by BLAST in *Xenopus tropicalis* (GenBank Accession No. CR575588), but, interestingly, not in mammalians or chickens.

Temporal expression patterns of cdc25 isoforms during early Xenopus development

By RT-PCR, we first investigated the temporal expression patterns of *cdc25* isoforms during early *Xenopus* development. In agreement

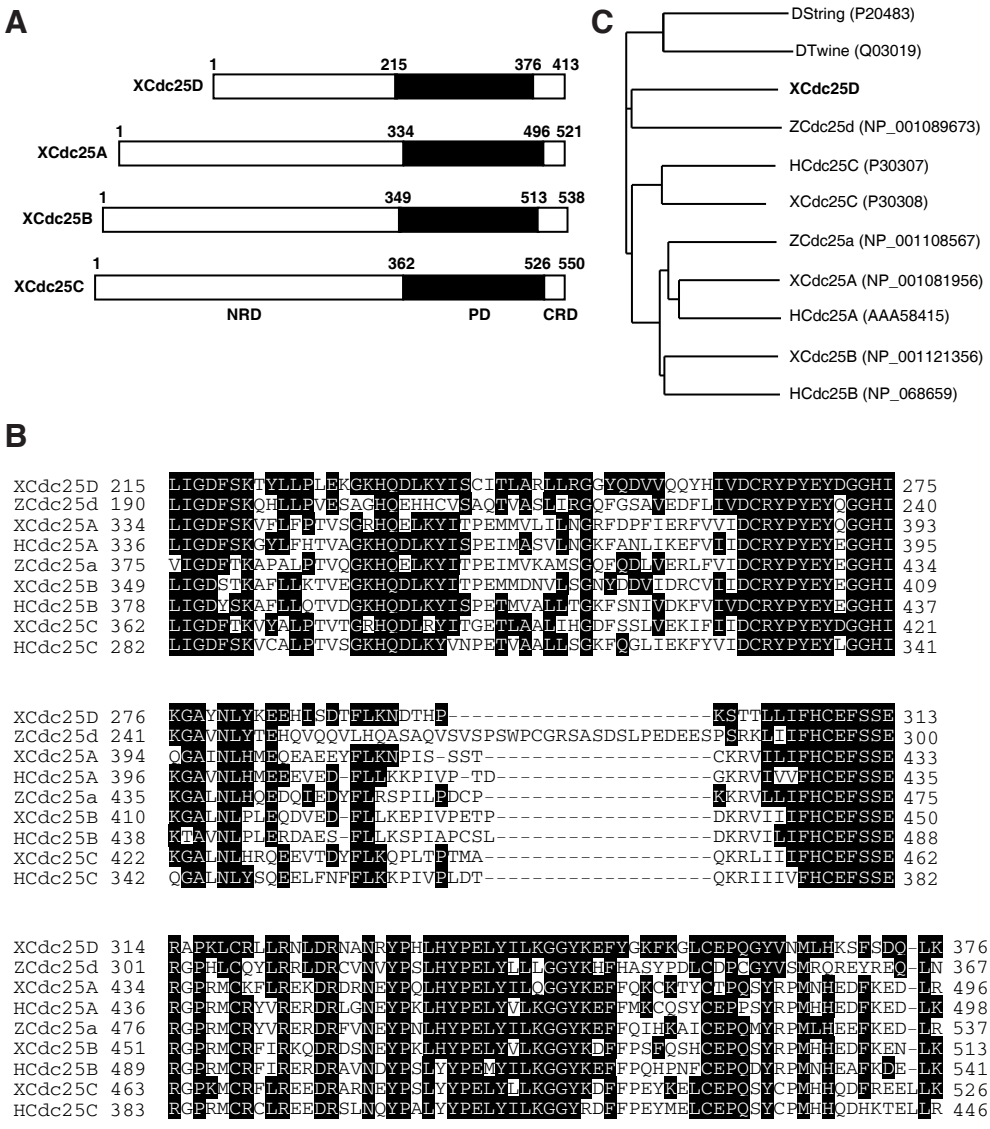

Fig. 1. Sequence analysis of the *Xcdc25D* product. (A) *A schematic representation of* Xenopus *Cdc25 isoforms. Three structural domains, i.e. the NRD, the PD, and the CRD, were defined according to Fauman* et al., *(1998).* **(B)** *Alignment of the PD of Cdc25 proteins. Accession numbers are as in (C). D,* Drosophila Melanogaster*; X,* Xenopus laevis*; H,* Homo sapiens*; Z,* Zebra rerio. **(C)** *Phylogenetic tree of* cdc25 *genes from various species, constructed by using the neighbor-joining method. Accession numbers used are shown.*

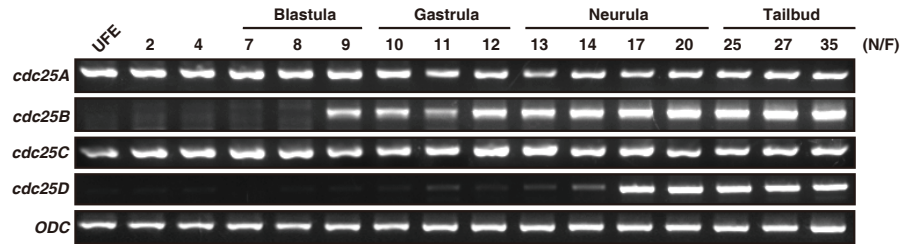

Fig. 2. Temporal expression patterns of *cdc25* isoforms during early *Xenopus* development. *The transcripts of* Xenopus cdc25 *isoforms were analyzed by RT-PCR.* ODC *(*ornithine decarboxylase*) is a loading control. Nieuwkoop-Faber (N/F) stages are shown at the top.*

with previous reports (Kim *et al.*, 1999; Ueno *et al.*, 2008; Hartley *et al.*, 1996), *cdc25A* transcripts were constantly present at the cleavage stages with a slight decrease in its levels at later stages, *cdc25B* transcripts were expressed after the midblastula stage, and *cdc25C* transcripts were constantly present throughout the stages of early embryogenesis (Fig. 2). In contrast, *cdc25D* transcripts were scarcely detected at the preneurula stages, but became detectable during neurulation and persisted at least up to the late tailbud stage (Fig. 2). Thus, *cdc25A* and *cdc25C* are expressed both maternally and zygotically, whereas *cdc25B* and *cdc25D* are expressed zygotically in early *Xenopus* development.

Spatial expression patterns of cdc25 isoforms during early Xenopus development

Although the spatial expression patterns of several cell-cycle regulators, such as *cdks*, *cyclins*, *wee1* and *myt1* isoforms, during early *Xenopus* development have been shown in previous studies (Vernon and Philpott, 2003; Leise and Muller, 2002), those of *cdc25* isoforms have not been reported. We therefore examined the spatial expression patterns of *Xenopus cdc25* isoforms using whole-mount *in situ* hybridization. In these experiments, no specific signals were detected with any of the sense probes (Fig. 3 M,N,O,P). Using an antisense probe, however, *cdc25A* transcripts were detected principally in the animal hemisphere from the blastula to initial gastrula stages (Fig. 3 A,E), and in the anterior neural plate at the early neurula stage (Fig. 3I). At the late neurula stage, *cdc25A* was expressed in the neural fold and the neural crest (Fig. 3Q), and, at the tailbud stage, it was weakly expressed in prospective retinal layers in eye vesicles (Fig 4 A,E,I).

Consistent with the results of RT-PCR (Fig. 2), *cdc25B* transcripts were not detectable in blastula embryos (Fig. 3B). During gastrulation, *cdc25B* transcripts were expressed in both the ectodermal and mesodermal regions (Fig. 3F). As previously reported (Ueno *et al.*, 2008), at the early neurula stage, *cdc25B* transcripts exhibited bilateral expressions in the neural plate and trigeminal ganglions (Fig. 3 J,U), and, at the late neurula stage, they were expressed in the regions undergoing neurogenesis, such as the neural fold and eye anlagen (Fig. 3R). Furthermore, at the tailbud stage, *cdc25B* transcripts were expressed in the central nervous system, such as the spinal cord and the brain, including prosencephalon, mesencephalon and rombencephalon (Fig. 4 B,F); they were also strongly expressed in the prospective retinal layers of eye vesicles (Fig. 4 B,F,J).

cdc25C, like *cdc25A*, was expressed exclusively in the animal hemisphere from the blastula to gastrula stages (Fig. 3 C,G). At the early neurula stage, *cdc25C* transcripts were expressed in the

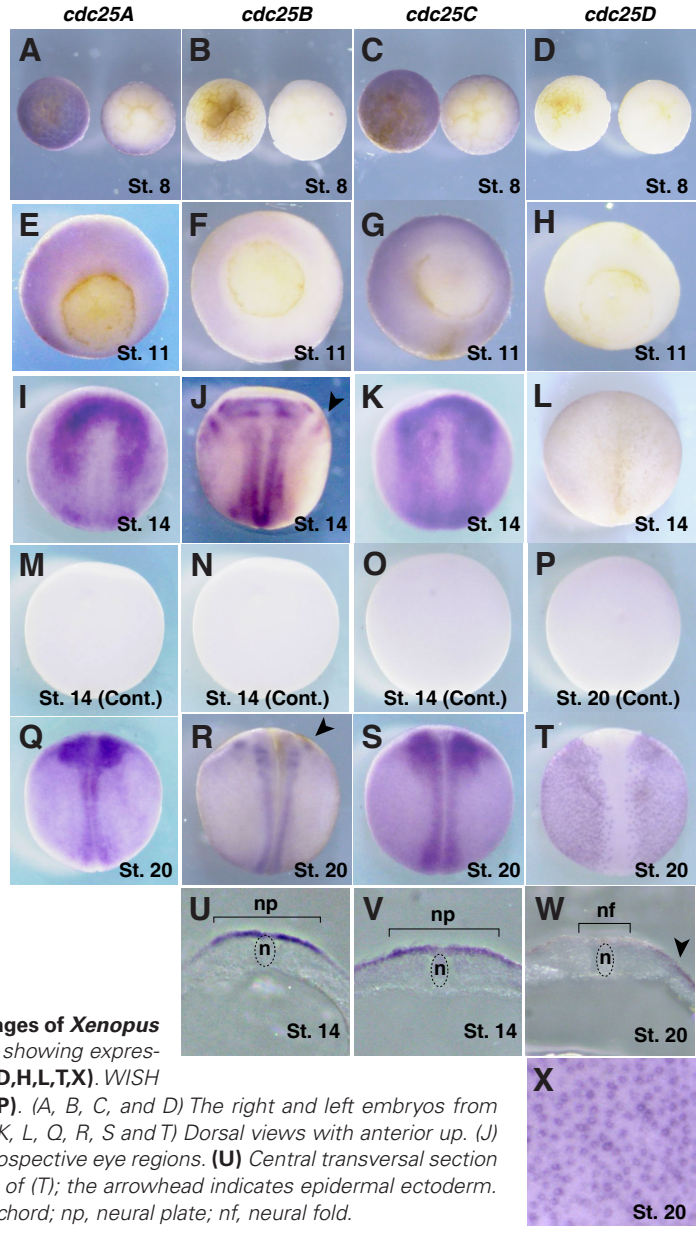

Fig. 3. Spatial expression patterns of *cdc25* isoforms at the indicated stages of *Xenopus* embryos. *Whole-mount* in situ *hybridization (WISH) with antisense probes showing expressions of* cdc25A **(A,E,I,Q)**, cdc25B **(B,F,J,R)**, cdc25C **(C,G,K,S)** *and* cdc25D **(D,H,L,T,X)**. *WISH with control sense* cdc25A, cdc25B, cdc25C *and* cdc25D *probes* **(M,N,O,P)**. *(A, B, C, and D) The right and left embryos from vegetal and animal views, respectively. (E, F, G and H) Vegetal views. (I, J, K, L, Q, R, S and T) Dorsal views with anterior up. (J) The arrowhead indicates trigeminal ganglion. (R) The arrowhead indicates prospective eye regions.* **(U)** *Central transversal section of (J).* **(V)** *Central transversal section of (K).* **(W)** *Central transversal section of (T); the arrowhead indicates epidermal ectoderm. (X) Magnified view of the epidermal ectoderm of (T). Abbreviations: n, notochord; np, neural plate; nf, neural fold.*

neural plate (Fig. 3 K,V), and, at the late neurula stage, they were detected in the neural fold and neural crest regions (Fig. 3S). At the tailbud stage, *cdc25C* transcripts were broadly expressed in the head region (Fig. 4 C,G), particularly in the prospective retinal layers of eye vesicles (Fig. 4K).

Unlike *cdc25A*, *B* and *C* transcripts, *cdc25D* transcripts were undetectable up to the early neurula stage (Fig. 3 D,H,L), consistent with the results of RT-PCR (Fig. 2). Interestingly, at the late neurula stage, however, *cdc25D* transcripts were expressed in the epidermal ectoderm with a punctuate pattern, but not in the neural region (Fig. 3 T,W,X). At the tailbud stage, *cdc25D* transcripts were barely detected in the epidermal ectoderm, but, interestingly, their expression became apparent in the liver diverticulum endoderm (Fig. 4 D,H,L,M).

The effect of BMP inhibition on transcription of cdc25 isoforms

Given their spatially (as well as temporally) restricted expression patterns, expressions of different *cdc25* isoforms would be regulated in different ways during development. In *Xenopus*, the ectodermal cell mass of the mid-blastula embryo (animal cap) is pluripotent, and is useful for addressing the developmental signal(s) (Lamb *et al.*, 1993; Henry *et al.*, 1996). Indeed, *cdc25B* expression in animal caps has been shown to be upregulated by inhibition of bone morphogenetic protein (BMP) (Ueno *et al.*, 2008), a crucial event required for neural induction (Stern, 2005). We therefore investigated whether expression (or transcription) of other *cdc25* isoforms would be affected by BMP inhibition, using animal cap assays and RT-PCR. Consistent with our previous report (Ueno *et al.*, 2008), *cdc25B* transcription in animal caps was readily up-regulated by ectopic expression of *noggin*, a BMP antagonist (Munoz-Sanjuan and Brivanlou, 2002) (Fig. 5). Transcriptions of *cdc25A* and *C* were also up-regulated by *noggin* expression, albeit to slightly lesser extents than *cdc25B* transcription; notably, however, *cdc25D* transcription was down-regulated dose-dependently by *noggin* expression (Fig. 5). Moreover, ectopic expression of *chordin*, another BMP antagonist (Munoz-Sanjuan and Brivanlou, 2002), similarly affected the expression of cdc25 isoforms (Fig. 5). These results were consistent with the spatial expression patterns of the *cdc25* isoforms (Fig. 4). Thus, transcriptions of different *cdc25* isoforms are likely to be differently regulated during the process of neural induction.

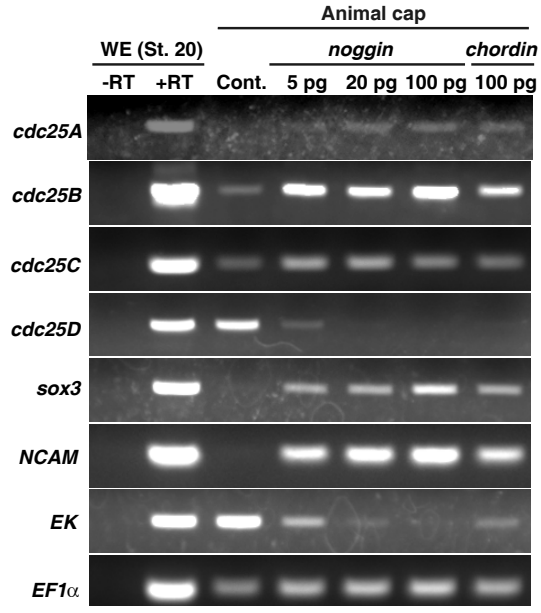

Fig. 5. The effects of BMP inhibition on the expression of *cdc25* isoforms in animal caps. *Animal caps were isolated from late blastula* Xenopus *embryos (st. 9) pre-injected with the indicated amounts of* noggin *or* chordin *mRNA at the one-cell stage. The animal caps were cultured until sibling control embryos reached st. 20 and then analyzed by RT-PCR.* sox3 *and* NCAM *are downstream markers of* noggin *and* chordin. Epidermal keratin *(*EK*) is a marker for epidermis.* EF1α *is a loading control.*

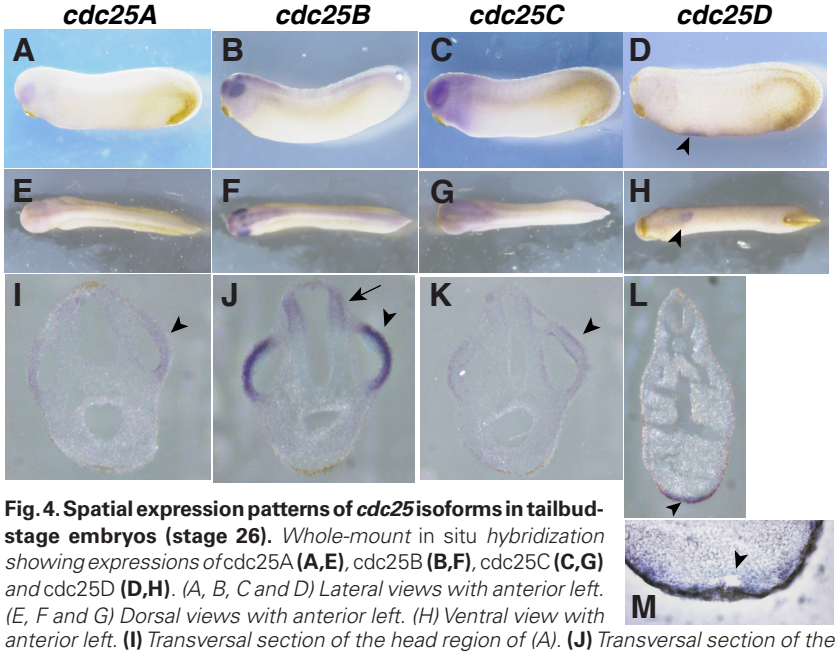

Fig. 4. Spatial expression patterns of *cdc25* isoforms in tailbud-stage embryos (stage 26). *Whole-mount* in situ *hybridization showing expressions of* cdc25A **(A,E)**, cdc25B **(B,F)**, cdc25C **(C,G)** *and* cdc25D **(D,H)**. *(A, B, C and D) Lateral views with anterior left. (E, F and G) Dorsal views with anterior left. (H) Ventral view with anterior left.* **(I)** *Transversal section of the head region of (A).* **(J)** *Transversal section of the head region of (B); the arrow indicates mesencephalon.* **(K)** *Transversal section of the head region of (C). (I, J and K) The arrowhead indicates the eye vesicle.* **(L)** *Transversal section of (H).* **(M)** *Magnified view of the liver diverticulum endoderm of (L). The arrowhead indicates the liver diverticulum. No staining was detected with the sense probe (data not shown).*

Discussion

In this study, we identified a novel isoform of *Xenopus cdc25*, termed *cdc25D*. In addition, we investigated the temporal and spatial expression patterns of all isoforms of *cdc25* phosphatase during early *Xenopus* development, and found that they are different from each other during early development.

In agreement with previous studies (Kim *et al.*, 1999; Hartley *et al.*, 1996), *cdc25A* and *C* transcripts were expressed at the cleavage and early blastula stages, or maternally. In contrast to these, *cdc25B* and *D* transcripts were not expressed maternally. Therefore, Cdc25A and Cdc25C isoforms could contribute to the rapid cell cycles during cleavage stages. Indeed, it has been shown that dominant-negative inhibition of Cdc25A can delay cell cycles in cleaving embryos (Kim *et al.*, 1999). Previous studies showed that Cdc25C protein exists in oocytes (Izumi *et al.*, 1992; Kumagai

and Dunphy,W.G., 1992., Nakajo *et al*., 2000), whereas Cdc25A protein does not (Kim *et al*., 1999; Shimuta *et al*., 2002). Thus, Cdc25C protein seems to be the sole cdc25 isoform expressed in *Xenopus* oocytes, consistent with it being essential for Cdk1 activation during oocyte maturation in *Xenopus* (Izumi *et al*., 1992; Kumagai and Dunphy,W.G., 1992).

It has been shown that cells in the neural region actively proliferate during the neurula stage (Saka and Smith, 2001). Around the neurula stage, *cdc25A*, *B*, and *C* were expressed in the neural region, including the neural plate and the neural fold, and, in animal caps, their expressions were up-regulated by BMP inhibition, which is central to neural induction. Thus, *cdc25A-C* isoforms could promote cell-cycle progression in the neural region. Notably, however, expression of *cdc25B* was more prominent in the neural region, and more readily induced by BMP inhibition in animal caps, than those of *cdc25A* and *C*. These results are consistent with our previous results that Cdc25B contributes significantly to cell proliferation in the neural region (Ueno *et al*., 2008). Previous studies showed that *cdk1* and *cyclin B* transcripts are also preferentially expressed in the neural region (Vernon and Philpott, 2003), whereas *wee1* and *myt1* transcripts, encoding cdk1-inhibitory kinases, are barely expressed in the same region (Leise and Mueller, 2002). Thus, transcriptional regulations of these cell-cycle regulators would also contribute to cell-cycle progression in the neural region.

In contrast to other *cdc25* isoforms, *cdc25D* transcripts were not expressed in the neural region, but were expressed in the epidermal ectoderm of the late-neurula embryos. Moreover, its transcription was suppressed by BMP inhibitors in animal caps. These results suggest that Cdc25D contributes to cell proliferation in non-neural ectoderms. Particularly, the punctuate expression pattern of *cdc25D* in the epidermal ectoderm of late-neurula embryos resembles that of α-*tubulin*, which is a marker of ciliated cells (Deblandre *et al*., 1999). Thus, Cdc25D may have some role(s) in the formation of ciliated cell. Furthermore, and interestingly, at the tailbud stage, *cdc25D* transcripts were expressed in the liver diverticulum endoderm, which is the origin of embryonic liver in *Xenopus* (Nieuwkoop and Faber, 1994). Thus, Cdc25D might also be involved in the formation of embryonic liver.

Xenopus cdc25D has significantly closer homology with zebrafish *cdc25d* than with other conventional *cdc25* isoforms. In zebrafish, however, *cdc25d* is expressed throughout early development and in the restricted ventral mesoderm and nasal placodes of the 24 hpf and 32 hpf embryos (Nogare *et al*., 2007). Therefore, despite their sequence homology, zebrafish *cdc25d* and *Xenopus cdc25D* seem to have different roles in early development. Furthermore, and surprisingly, neither mammalians nor chickens seem to possess the *cdc25D* gene, suggesting that amniotes might have lost it during evolutionary processes. This might be related, however, to the potentially divergent roles of *cdc25D* even between zebrafish and *Xenopus* development.

The existence of distinct *cdc25* isoforms in vertebrates may represent their different roles during developmental processes. This seems to be true for *Xenopus* development but not necessarily for mouse development. Recent studies in mice revealed the functional redundancy of Cdc25 isoforms during development; mice lacking both *cdc25B* and *cdc25C* developed normally, suggesting that *cdc25A* compensates for the loss of *cdc25B* and *cdc25C* (Ferguson *et al*., 2005). In *Xenopus*, however, Cdc25A protein, unlike Cdc25B or C proteins, is largely degraded by Chk1 kinase just after the MBT (Kim *et al*., 1999; Shimuta *et al*., 2002), and thereafter, *cdc25A* is expressed to very limited regions of the late-neurula and tailbud stage embryos. Thus, it appears that, in *Xenopus*, Cdc25A cannot compensate for the function of Cdc25B and Cdc25C.

To summarize, the temporal and spatial expression patterns of different *cdc25* isoforms are different from each other during early *Xenopus* development. In addition, each orthologue of Cdc25 isoforms seems to have at least partially different roles in early development of different species.

Materials and Methods

Cloning of a novel isoform of Xenopus cdc25

The *Xcdc25D* EST was obtained from National Institute for Basic Biology (NIBB) with an identifier XL041a15 (GenBank accession number AB601986). The plasmid was resequenced with ABI PRISM 3100 Genetic Analyzer (applied biosystems, USA). Phylogenetic analysis was performed using the ClustalW program.

Xenopus embryos, in vitro transcription and animal cap assays

Xenopus eggs were artificially fertilized using *Xenopus* testis homogenates and dejellied with 0.1 x Modified Barth's saline (MBS) containing 2% cysteine for 20 minutes. Staging of embryos was done according to Nieuwkoop and Faber (1994). Capped RNAs were synthesized *in vitro* using a MEGAscript SP6 kit (Ambion) and injected into one-cell stage embryos. Injected embryos were cultured in 0.1 x MBS containing 3% Ficoll at 20˚C. Animal cap cells were obtained from stage 8.5 – 9 embryos, and then cultured in 1 x MBS containing 50 μg/ml gentamicin.

RNA extraction and RT-PCR

Total RNAs were extracted from five whole embryos at various stages and from twenty animal cap explants, using TRIzol reagent (invitrogen). The extracted total RNAs corresponding to one embryo or four animal cap explants were used to synthesize cDNAs using oligo-dT_{20} primers and SuperScript III reverse transcriptase (invitrogen). One-fortieth of the reaction products were subjected to PCR (94˚C for 30 sec, 52˚C for 30 sec, 72˚C for 1 min) with TAKARA Ex Taq (TAKARA). The PCR products were confirmed for their identities by sequencing and southern blotting (data not shown). The primer sets (5′ to 3′; U, upstream and D, downstream) and cycles used for PCR were:

cdc25A (27 cycles)
U: GGCCACATACAGGGAGCCATTAACC
D: TAGTTTCTTCAGCCGGCTGTACAGTTC
cdc25B (30 cycles)
U: ACGTGGAAGACTTTCTGCTGAAGGAGC
D: TCTCGCTTGCTCTTGTCTCCGGCCC
cdc25C (27 cycles)
U: GGACACATAAAGGGTGCATTAAACC
D: GCTTCATTATGCGGGCAATCTGTTC
cdc25D (27 cycles)
U: GACGGAGGGCACATTAAGGGAGCCTAC
D: GCTGGATGTTTTTTCAAGACAGTGC
sox3 (27 cycles)
U: GCGCACATGAACGGCTGGACTA
D: GTGTGGGAGGTGATGGCTGGAG

Primer sets for *NCAM* (30 cycles), epidermal keratin (20 cycles), and *EF1*-α (20 cycles) are described in Xenbase (http://www.xenbase.org/common). Under the present RT-PCR conditions, the amounts of the amplified target products were reflecting the amounts of template RNAs (Fig. S1), and the targets were amplified within a linear range of conditions (Fig. S2).

Whole-mount in situ hybridization

Whole-mount *in situ* hybridization was performed according to the previously described methods (Harland, 1991), with slight modifications

described by Takahashi *et al.,* (2006). Open reading frame sequences of *Xenopus cdc25A* (Okazaki *et al.,* 1996), *cdc25B* (Ueno *et al.*, 2008), *cdc25C* (Kumagai and Dunphy, 1992) and *cdc25D* were subcloned into the pBlueScript KS+ plasmid (Agilent technologies). These plasmids were linealized, and digoxigenin-labeled RNA probes in sense and antisense orientations were transcribed using MEGAscript T7 and T3 kits (Ambion), respectively. For sectioning, stained embryos were embedded in gelatin/sucrose (15% cold fish gelatin and 30% sucrose in PBS) and mounted with OCT compound (Tissue-Tek OCT compound, Sakura Finetek, USA), and 40 μm sections were prepared with a cryostat (Jung FrigoCut, Leica, Germany).

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References

BOUTROS, R., DOZIER, C., DUCOMMUN, B (2006). The when and wheres of CDC25 phosphatases. *Curr. Opin. Cell Biol.* 18: 185-191.

BRIVANLOU, A.H., DARNELL, J.E., JR. (2002). Signal transduction and the control of gene expression. *Science* 295: 813-818.

CHEN, M.S., HUROV, J., WHITE, L.S., WOODFORD-THOMAS, T., PIWNICA-WORMS, H (2001). Absence of apparent phenotype in mice lacking Cdc25C protein phosphatase. *Mol. Cell Biol.* 21: 3853-3861.

COLEMAN, T. R., DUNPHY, W. G. (1994). Cdc2 regulatory factors. *Curr. Opin. Cell. Biol.* 6: 877-882.

DEBLANDRE, G.A., WETTSTEIN, D.A., KOYANO-NAKAGAWA, N., KINTNER, C. (1999). A two-step mechanism generates the spacing pattern of the ciliated cells in the skin of *Xenopus* embryos. *Development* 126: 4715-4728.

FERGUSON, A.M., WHITE, L.S., DONOVAN, P.J., PIWNICA-WORMS, H. (2005). Normal cell cycle and checkpoint responses in mice and cells lacking Cdc25B and Cdc25C protein phosphatases. *Mol. Cell Biol.* 25: 2853-2860.

HARTLEY, R.S., REMPEL, R.E., MALLER, J.L. (1996). *In vivo* regulation of the early embryonic cell cycle in *Xenopus. Dev. Biol.* 173: 408-419.

HENRY, G.L., BRIVANLOU, I.H., KESSLER, D.S., HEMMATI-BRIVANLOU, A., MELTON, D.A. (1996). TGF-β signals and a pattern in *Xenopus laevis* endodermal development. *Development.* 122: 1007-1015.

IZUMI, T, WALKER, D.H., MALLER, J.L. (1992). Periodic changes in phosphorylation of the *Xenopus* cdc25 phosphatase regulate its activity. *Mol. Biol. Cell.* 3: 927-939.

KIM, S.H., LI, C., MALLER, J.L., (1999). A maternal form of the phosphatase Cdc25A regulates early embryonic cell cycles in *Xenopus laevis*. *Dev. Biol.* 212: 381-391.

KUMAGAI, A. DUNPHY, W.G. (1992). Regulation of the cdc25 protein during the cell cycle in *Xenopus* extracts. *Cell* 70: 139-151.

LAMB, T.M., KNECHT, A.K., SMITH, W.C., STACHEL, S.E., ECONOMIDES, A.N., STAHL, N., YANCOPOLOUS, G.D., HARLAND, R.M. (1993). Neural induction by the secreted polypeptide noggin. *Science* 262: 713-718.

LEISE, W.L., MUELLER, P.R. (2002). Multiple Cdk1 inhibitory kinases regulate the cell cycle during development. *Dev. Biol.* 249: 156-173.

MORGAN, D. O. (1995). Principles of CDK regulation. *Nature* 374: 131-134.

MUNOZ-SANJUAN, I., BRIVANLOU, A.H. (2002). Neural induction, the default model and embryonic stem cells. *Nat. Rev. Neurosci.* 3: 271-280.

NAKAJO, N., YOSHITOME, S., IWASHITA, J., IIDA, M., UTO, K., UENO, S., OKAMOTO, K. AND SAGATA, N. (2000). Absence of Wee1 ensures the meiotic cell cycle in *Xenopus* oocytes. *Genes Dev.* 14: 328-338.

NIEUWKOOP, P.D., FABER, J. (1994). Normal Table of *Xenopus laevis* (Daudin). Garland Publishing Inc., New York.

NOGARE, D.E., ARGUELLO, A., SAZER, S., LANE, M.E. (2007). Zebrafish cdc25a is expressed during early development and limiting for post-blastoderm cell cycle progression. *Dev. Dyn.* 236: 3427-3435.

SAKA, Y, SMITH, J.C. (2001). Spatial and temporal patterns of cell division during early *Xenopus* embryogenesis. *Dev. Biol.* 229: 307-318.

SHIMUTA, K., NAKAJO, N., UTO, K., HAYANO, Y., OKAZAKI, K., SAGATA, N. (2002). Chk1 is activated transiently and targets Cdc25A for degradation at the *Xenopus* midblastula transition. *EMBO J.* 21: 3694-3703.

STERN, C.D. (2005). Neural induction: old problem, new findings, yet more questions. *Development* 132: 2007-2021.

TAKAHASHI, S., ONUMA, Y., YOKOTA, C., WESTMORELAND, J.J., ASASHIMA, M., WRIGHT, C.V. (2006). Nodal-related gene Xnr5 is amplified in the *Xenopus* genome. *Genesis* 44: 309-321.

UENO, H., NAKAJO, N., WATANABE, M., ISODA, M., SAGATA, N. (2008). FoxM1-driven cell division is required for neuronal differentiation in early *Xenopus* embryos. *Development* 135: 2023-2030.

VERNON, A.E., PHILPOTT, A. (2003). The developmental expression of cell cycle regulators in *Xenopus laevis*. *Gene Expr. Patterns* 3: 179-192.

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