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Cloning and characterization of the *Xenopus* cyclin-dependent kinase inhibitor $p27^{XIC1}$

(cell cycle/DNA synthesis)

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ABSTRACT **We have isolated a gene encoding Xic-1, a 27-kDa cyclin-dependent kinase (Cdk) inhibitor from *Xenopus* ovary that shares significant homology with both mammalian CIP1 and Kip1/Kip2. The N- and C-terminal halves of Xic-1 are sufficient for interacting with Cdks and proliferating cell nuclear antigen, respectively. Recombinant Xic-1 inhibits *Xenopus* cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdc2 activities, although with quite different IC_{50} values. Truncation of the N terminus of Xic-1 increases the IC_{50} value for cyclin A/Cdk2 50-fold with no effect on the inhibition of cyclin E/Cdk2 or cyclin B/Cdc2. Xic-1 inhibits both single-stranded and nuclear DNA synthesis in egg extracts, an effect reversed by proliferating cell nuclear antigen or cyclin E/Cdk2, respectively. These results suggest a function for Xic-1 in the control of DNA synthesis by cyclin E/Cdk2.**

Cyclin-dependent kinases (Cdks) are required for a number of key cell cycle transitions and are regulated by checkpoints that ensure particular cell cycle events are initiated only with genomic integrity and after the successful completion of prior events. A form of regulation discovered recently involves Cdk inhibitor proteins (CKIs) that bind to and inhibit a wide variety of Cdk complexes (for review, see ref. 1). The first CKI ($p21^{CIP1}$) was identified by its ability to bind to and inhibit Cdk/cyclin complexes and by its increased expression in senescent cells. In normal cells, Cdk-interacting protein 1 (Cip1) is found in a complex that includes a Cdk and proliferating cell nuclear antigen (PCNA) (2, 3). Cip1 inhibits DNA replication indirectly by binding Cdks and directly by interacting with PCNA (4–6). In addition, Cip1 synthesis is induced by p53, supporting a function for CKIs in checkpoint control of the G_1/S transition (7).

The second member of the CKI family, $p27^{KIP1}$, shares 42% identity in its N terminus with Cip1 and also inhibits Cdc2, Cdk2, and Cdk4 complexes (8–10). Overall, Kip1 and Cip1 appear to respond to different signals, and their levels in stimulated T cells change reciprocally as cells progress through G_1 (8, 9, 11–13). Another inhibitor, Kip2, identified in both human and mouse cells shares striking sequence similarity with Kip1 and Cip1 in the N-terminal Cdk-inhibitory region (14, 15). Both Kip1 and Kip2 lack PCNA binding and have C-terminal regions distinct from Cip1. We are interested in the function of CKIs in *Xenopus* early embryonic development when the cell cycle slows, G_1 and G_2 phases are established, and zygotic transcription begins (16). We report here the characterization of the *Xenopus* CKI, XIC-1 (Xenopus inhibitor of Cdk),‡ which has similarities to both Kip and Cip.

MATERIALS AND METHODS

Cloning of a *Xenopus CIP1/KIP1* Homolog. An internal *Xenopus* CKI fragment was cloned by PCR amplification using degenerate oligonucleotides specific to regions of highly conserved amino acid sequence at the N-terminal end of human CIP1 and KIP1. The 5′ primer 5′-GCC GGA TCC YTD TTY GGD CCD GTD GA-3′ corresponds to the sequence L(F)-FGPVD, and the 3′ primer 5′-GCC GTC GAC RAA RTC RAA RTT CCA-3′ corresponds to the sequence WNFDF. The code for nucleotide mixes is Y = T or C; D = G, A, T, or C; and R = G or A. *Bam*HI and *Sal* I restriction sites (underlined) were generated at the 5′ and 3′ ends of the PCR DNA products, respectively. A total *Xenopus* ovary cDNA library (17) was used as template in PCR reactions containing 50 ng of cDNA, 100 pmol of each primer, dNTPs, and *Taq* polymerase. Samples were subjected to 35 cycles at 94°C for 1 min, 52°C for 30 sec, and 72°C for 30 sec. An amplified fragment of the expected size (≈120 bp) contained an insert homologous to the N terminus of CIP1 and KIP1. The cloned sequence was used to design gene-specific nested primers (cp1/cp2 and np1/np2; see Fig. 1*A*) for subsequent cloning of the full-length gene by rapid amplification of cDNA ends (RACE; refs. 18 and 19). The cloned fragment was radiolabeled and used to screen a total *Xenopus* ovary cDNA library (17); plasmid DNA obtained from both RACE cloning and cDNA library screening revealed identical sequences. Fragments from PCRs were cloned into pGEX-2T (Pharmacia) for production of glutathione *S*-transferase (GST)-tagged fusion proteins.

Baculoviruses expressing *Xenopus* Cdk2 and cyclin A1 or cyclin E1 were constructed according to established procedures, and the cyclin E1/Cdk2 complex was further purified on $p13^{suc1}$-beads to yield a specific activity of 0.7 μmol/min per mg. The coding regions of the *Xenopus* PCNA gene and the human *CDKN1* gene (formerly CIP1/WAF1 gene) were cloned by PCR amplification from a *Xenopus* ovary cDNA library and a human fibroblast cDNA library (Stratagene), respectively, with oligonucleotides designed from published sequences (20, 21).

Assays and DNA Replication Analysis. Samples of each cyclin/Cdk preparation were incubated with various concentrations of inhibitor and assayed as described (22). Maturation-promoting factor and M-phase and interphase egg extracts were prepared as described (22, 23). For phosphorylation experiments, cycloheximide was added with 0.4 mM $CaCl_2$ to prepare the interphase extract. GST–Xic-1 adsorbed to glutathione beads was incubated at 23°C for 30 min in 30 μl of egg extract containing 1 μCi of [γ-^{32}P]ATP per μl. Beads were washed four times and analyzed by SDS/PAGE. For binding

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Abbreviations: Cdk, cyclin-dependent kinase; PCNA, proliferating cell nuclear antigen; CKI, Cdk inhibitor protein; GST, glutathione *S*-transferase; Xic-1, *Xenopus* 27-kDa cyclin-dependent kinase inhibitor; RACE, rapid amplification of cDNA ends; Cip1, Cdk-interacting protein 1.

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‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. U24434).

analysis, 20 μl of beads containing 10 μg of Xic-1 was added to 100 μl of diluted egg extract and incubated with rotation at 4°C for 1 hr. The beads were washed four times, and bound proteins were analyzed by immunoblotting.

For nuclear DNA synthesis assays (24), 200-μl samples of egg extract were supplemented at time zero with 2500 demembranated sperm nuclei per μl and then released from M phase by addition of 0.4 mM $CaCl_2$. Purified GST–Xic-1 was added to 35 nM at the indicated times, and in some experiments cyclin E/Cdk2 was added to 50 nM at 30 min. For single-stranded DNA synthesis assays, high-speed supernatants of *Xenopus* egg extracts (25) (2 mg/ml) and 4 ng of M13mp18 single-stranded DNA were incubated as described (26, 27) in a final 50-μl vol. Various concentrations of recombinant Xic-1 were added; reactions were incubated for 90 min at 30°C and analyzed by 1% agarose gel electrophoresis and trichloroacetic acid precipitation (26).

RESULTS

Isolation of *Xenopus* XIC-1 Gene. We used two degenerate oligonucleotides designed to hybridize with conserved sequences in the N terminus of the human CIP1 and KIP1 coding regions (Fig. 1*A*) to amplify CKI segments of *Xenopus* ovary cDNA by PCR. The full-length XIC-1 gene contains an open reading frame of 210 amino acids, encoding a 23.5-kDa protein (Fig. 1*A*). Northern analysis of *Xenopus* ovary RNA identified a single mRNA of ≈1.8 kb (data not shown). Xic-1 shares 44% identity (73% similarity) with human KIP1 in the conserved N terminus (residues 30–91) and 40% identity (54% similarity) with human CIP1 in the same region (Fig. 1*B*). In addition, Xic-1 shares stretches of homologous sequence in the C terminus with mammalian Kip1 and Kip2, particularly in a recently defined QT domain containing a potential Cdc2 phosphorylation site (refs. 9, 10, and 15; Fig. 1*C*). However, Xic-1 also possesses a short stretch of sequence within its C terminus that resembles a PCNA-binding site defined in human CIP1 (ref. 28; Fig. 1*D*). Overall, Xic-1 represents a class of CKI that has homologous domains from Kip1, Kip2, and Cip1. Xic-1 in egg extract immunoblots or after *in vitro* translation migrated at the 27-kDa region of a gel (data not shown), an electrophoretic mobility similar to that of mammalian Kip1 proteins.

Characterization of Cdk Inhibition by Xic-1. Human CIP1 can be separated into N- and C-terminal domains, which interact with Cdk2 and PCNA, respectively (28–30). We analyzed the functional domains of Xic-1 by constructing various truncated forms of GST-tagged Xic-1. GST–Δ34Xic1 is a fusion protein in which the first 34 amino acids of Xic-1 have been deleted, disrupting one of the most conserved regions in all Cip1/Kip1 proteins (Fig. 1*B*). GST–Xic1-N and GST–Xic1-C encode the entire N terminus (residues 1–96) or C terminus (residues 97–210) of Xic-1, respectively. We incubated egg extracts with various GST-tagged Xic-1 proteins coupled to glutathione-agarose and analyzed bound proteins by immunoblotting. Full-length GST–Xic-1, GST–Δ34Xic-1, and GST–Xic-1-N bound both *Xenopus* Cdk2 and Cdc2 (data not shown). In contrast, GST–Xic-1-C or GST alone did not bind any Cdks. Thus, the sequence motif in Xic-1 required for interacting with Cdks resides in the N terminus.

The H1 kinase activity of *Xenopus* cyclin A/Cdk2, cyclin E/Cdk2, and cyclin B/Cdc2 was measured in the presence of various concentrations of GST–Xic-1. The amount of each complex used in the assay was adjusted to give approximately equal histone H1 kinase activity. As shown in Fig. 2*A*, cyclin E/Cdk2 was the most sensitive to inhibition by GST–Xic-1, with an IC_{50} value of 10 nM. Cyclin B/Cdc2 was the least sensitive to inhibition, with an IC_{50} value of ≈5 μM, and cyclin A/Cdk2 was intermediate, with an IC_{50} value of 100 nM. The fusion protein containing the N terminus alone was capable of

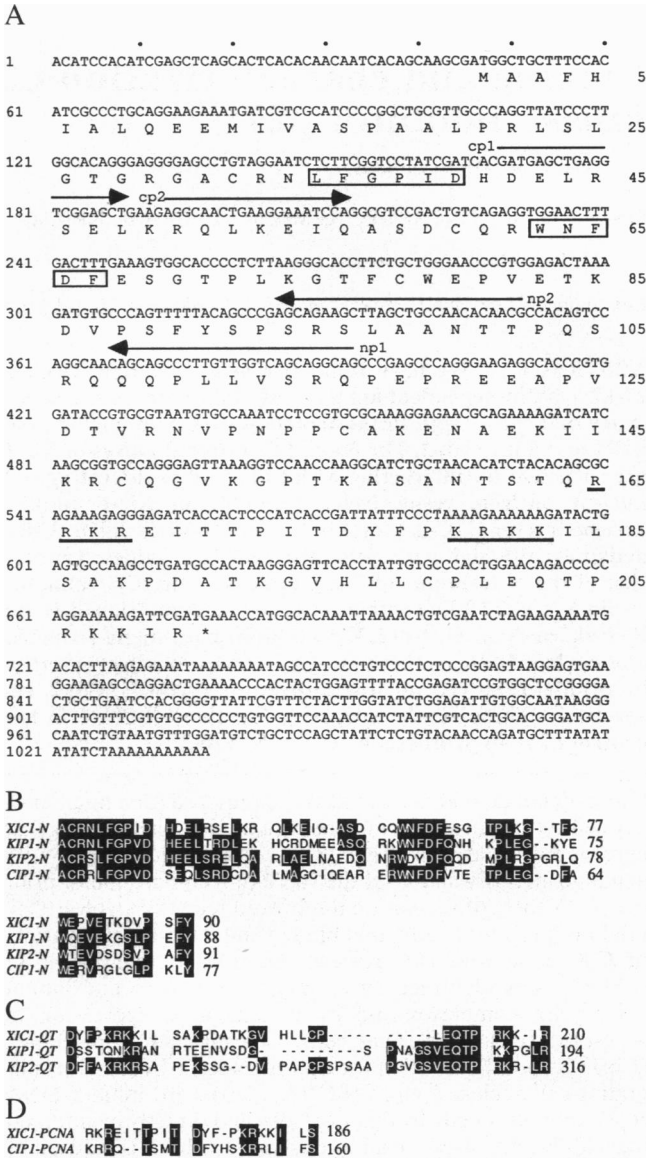

FIG. 1. Sequence analysis of the XIC-1 gene. (*A*) Nucleotide and predicted amino acid sequence of the XIC-1 gene. A predicted open reading frame of 210 amino acids is shown in single-letter code under the nucleotide sequence. Nucleotides are numbered at left, and amino acids are numbered at right. Regions corresponding to sequences used to design 5′ and 3′ degenerate oligonucleotides for PCR amplification are boxed. The arrows indicate nucleotide sequences used to synthesize XIC-1-specific nested primers for 3′ and 5′ RACE cloning and are designated as cp1/cp2 and np1/np2, respectively. A putative bipartite nuclear localization sequence is underlined. An asterisk marks the termination codon. (*B*) Alignment and comparison of the N termini (N) of *Xenopus* Xic-1, and human KIP1, KIP2, and CIP1 sequences. Black boxes indicate identical residues shared by at least two sequences; dashes indicate gaps. (*C*) Alignment of the QT domain (15) in the C terminus of *Xenopus* Xic-1 and human KIP1 and KIP2. (*D*) PCNA-binding site of human CIP1 (28) and a similar region in Xic-1.

inhibiting the three enzyme complexes to the same extent as the full-length protein (Fig. 2*B*). Similar experiments with Xic-1-C at concentrations up to 6 μM showed only a negligible effect on any of the cyclin/Cdk complexes (data not shown). With GST-Δ34Xic-1, there was an almost 50-fold increase in the IC_{50} value for cyclin A/Cdk2, whereas inhibition of cyclin E/Cdk2 or cyclin B/Cdc2 was not affected (Fig. 2*C*). In contrast to inhibition by Xic-1, full-length GST-tagged human CIP1 inhibited cyclin A/Cdk2 more potently than cyclin E/Cdk2 (Fig. 2*D*). Also, CIP1 inhibited cyclin B/Cdc2 10-fold

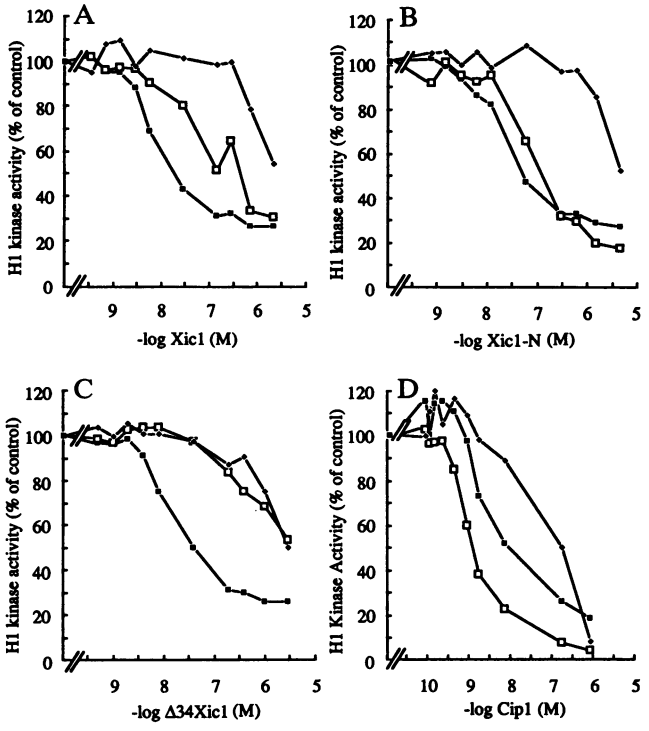

FIG. 2. Inhibition of various cyclin/Cdk complexes by Xic-1 and CIP1. Cdk complexes were incubated with various constructs of GST–Xic-1, GST–CIP1, or with buffer, and histone H1 kinase activity was determined as described. In these experiments the amount of each cyclin/Cdk preparation used in each reaction was adjusted such that 1.5–2 pmol of phosphate was incorporated per min into histone H1. (*A*) Full-length Xic-1. (*B*) Xic-1 N terminus. (*C*) Δ34Xic-1. (*D*) CIP1. ■, Cyclin E1/Cdk2; □, cyclin A1/Cdk2; and ◆, cyclin B/Cdc2.

more potently than Xic-1, although both Cdk inhibitors preferentially inhibited cyclin/Cdk2 complexes (Fig. 2).

The level of Xic-1 remained unchanged in both oocyte and egg cell cycles (data not shown). A recent report (31) has suggested an inhibitor of cyclin B/Cdc2 is involved in the DNA replication checkpoint in *Xenopus* egg extracts. In a similar cycling extract in which the DNA synthesis inhibitor aphidicolin was added to block the extract in interphase, Xic-1 abundance was not changed (data not shown). We conclude that changes in the level of Xic-1 are probably not important in the DNA replication checkpoint. Xic-1 contains several consensus sites for phosphorylation by Cdc2, including a threonine site near the C terminus conserved in human KIP1 and KIP2 (Fig. 1*C*). GST–Xic-1 was incubated with either a metaphase extract or an interphase extract in the presence of [γ-^{32}P]ATP and was phosphorylated only by the M-phase extract (Fig. 3*A*). As shown in Fig. 3*B*, Xic-1 phosphorylation in M phase is on phosphoserine, whereas with cyclin B/Cdc2 it is on phosphothreonine. This result suggests that the enzyme responsible for most of the phosphorylation of Xic-1 in M phase is not cyclin B/Cdc2.

Characterization of DNA Synthesis Inhibition by Xic-1. In *Xenopus* egg extracts, depletion of Cdk2 can block DNA synthesis, suggesting this Cdk plays a major role in regulating DNA synthesis (32, 33). In a recent report, bovine cyclin A and *Xenopus* cyclin E were shown to rescue DNA synthesis inhibition by human CIP1 in *Xenopus* egg extracts, presumably by activating Cdk2 (4). Although there is evidence for a role for cyclin A in DNA synthesis in tissue culture cells (34, 35), cyclin A appears unnecessary for DNA synthesis in *Xenopus* egg extracts (33). During early embryogenesis, cyclin E is more likely than cyclin A to play a role in DNA synthesis (22). We examined nuclear DNA synthesis in cycling egg extracts in the

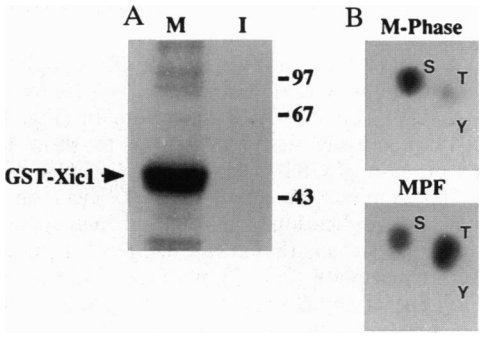

FIG. 3. Phosphorylation of Xic-1 in M phase. (*A*) Phosphorylation of Xic-1 in a M-phase extract. GST–Xic-1 was phosphorylated in a M-phase (lane M) or interphase (lane I) extract, as described. An autoradiogram of the gel is shown. Molecular mass markers (in kDa) are indicated at right. (*B*) Phosphoamino acid (single-letter code) analysis of GST–Xic-1 phosphorylated in M-phase extract or by cyclin B/Cdc2 (maturation-promoting factor; MPF).

presence of recombinant Xic-1. In control extracts DNA synthesis peaked 30–45 min after entry into the cell cycle (Fig. 4*A*), but was delayed for almost an hour by 35 nM GST–Xic-1 (Fig. 4*B*). The delay was dose-dependent, and permanent inhibition of DNA synthesis was observed in the presence of 100 nM GST–Xic-1 (data not shown). These results suggest that GST–Xic-1 efficiently blocks DNA synthesis at a concentration consistent with cyclin E/Cdk2 being the target. To investigate this possibility directly, purified cyclin E/Cdk2 was added to 50 nM 30 min after the addition of GST–Xic-1. Significant DNA synthesis was restored ≈30–40 min after addition of cyclin E/Cdk2 (Fig. 4*C*), which correlated with the length of time required for DNA synthesis to occur in a control extract. Cyclin E/Cdk2 was not added together with GST–Xic-1 at time zero because it could simply titrate out the exogenous GST–Xic-1. This result strongly suggests that cyclin E/Cdk2 is essential for initiation of DNA synthesis. When the same amount of GST–Xic-1 was added 30 min after entry into the cell cycle, DNA synthesis was unaffected (Fig. 4*D*). This result suggests that the activity of cyclin E/Cdk2 is required

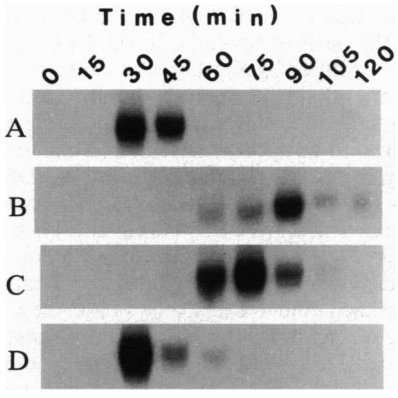

FIG. 4. Inhibition of nuclear DNA synthesis by Xic-1. Cytostatic factor-arrested extracts supplemented with sperm nuclei at time zero were released from metaphase arrest by Ca^{2+} addition. At the indicated times, 5-μl aliquots of the extract were sampled and incubated with 1.0 μCi of [α-^{32}P]dCTP (ICN) for 15 min and assayed for DNA synthesis (24). (*A*) Control cycling extract without GST–Xic-1. (*B*) Xic-1 inhibits nuclear DNA synthesis. At time zero, 35 nM of GST–Xic-1 was added to the extract. (*C*) Effect of cyclin E/Cdk2 on DNA synthesis in the presence of GST–Xic-1. At time zero, 35 nM of GST/Xic-1 was added to the extract, and purified cyclin E/Cdk2 was added 30 min later to a final concentration of 50 nM. (*D*) Xic-1 has no effect after DNA synthesis has been initiated. GST–Xic-1 (35 nM) was added to the extract 30 min after release from metaphase arrest.

only at a very critical time before or during initiation of DNA synthesis.

Human CIP1 inhibits simian virus 40 DNA replication *in vitro* by interacting with and inhibiting PCNA (5, 6). Because Xic-1 has a CIP1-like PCNA-binding domain (Fig. 1*D*), we used the binding assay described above to show that the C-terminal domain of GST–Xic-1 binds to PCNA efficiently (Fig. 5*C*). To examine the effect of GST–Xic-1 on PCNA-dependent DNA replication, we prepared high-speed supernatants from egg extracts that synthesize DNA from a single-stranded DNA template (26, 27), without requiring any Cdk activity. Single-stranded DNA synthesis was inhibited by GST–Xic-1 with an IC_{50} value of ≈350 nM (Fig. 5*A*), and this inhibition was mediated by the C-terminal domain (Fig. 5*B*).The much higher concentration of Xic-1 required for inhibiting complementary vs. nuclear DNA synthesis may be due to the high concentration of endogenous PCNA from the egg extract in the assay, 230 nM (20). In support of this hypothesis, at a 200 nM concentration, GST–Xic-1 reduced DNA synthesis by 35% compared with the control (Fig. 5*A*). When GST–Xic-1 (200 nM) was preincubated with excess purified *Xenopus* PCNA (350 nM) for 10 min before the DNA-synthesis assay, DNA synthesis was 90% of the control (Fig. 5*A*). Exogenous PCNA had no effect on DNA synthesis when added alone. These results indicate that the C-terminal domain of Xic-1 can also inhibit DNA synthesis by specifically interacting with PCNA (Fig. 5 *B* and *C*).

DISCUSSION

In overall sequence similarity and in molecular weight, the *Xenopus* CKI is most similar to the Kip1/Kip2 family and could be the equivalent of mammalian $p27^{KIP1}$. However, the presence of a Cip1-like C-terminal region that binds PCNA and inhibits PCNA-dependent DNA synthesis suggests that this *Xenopus* CKI represents a class of inhibitor, and we have given the gene the name XIC-1. Xic-1 protein may be a primordial form of CKI that in mammals has diverged to give the more specialized Kip1/Kip2 and Cip1 families. Xic-1 might also be a specialized form of CKI present in early embryos, where transcription is absent, and cell cycles are very rapid. Alternatively, mammalian homologs of Xic-1 may exist but have not yet been described.

We propose that cyclin E/Cdk2 is the physiological target for Xic-1 on the basis of several criteria. (*i*) Cyclin E/Cdk2 is most potently inhibited by Xic-1 *in vitro*, and the concentration of Xic-1 (35 nM) that preferentially inhibits cyclin E/Cdk2 *in vitro* also inhibits nuclear DNA synthesis in egg extracts (Fig. 4), an effect overcome by 50 nM cyclin E/Cdk2. (*ii*) In *Xenopus* embryonic cycles cyclin A is not required for DNA synthesis (33), and the cyclin A/Cdk2 complex, implicated in DNA synthesis in fibroblasts (34, 35), does not form in *Xenopus* embryos until the onset of zygotic transcription (22). Cyclin D/Cdk4 is unlikely to be a target for Xic-1 in early embryonic cycles because early embryos lack a G_1 phase, the level of pRb is very low (36), and neither cyclin D nor Cdk4 is present until after the midblastula transition (T. Hunt, personal communication).

We demonstrated that Xic-1 can inhibit nuclear DNA synthesis, presumably by interacting with cyclin E/Cdk2. Xic-1 inhibited only when added early in the process of replication (Fig. 4), and cyclin E/Cdk2 was able to rescue synthesis inhibited by Xic-1 only when added early in the replication process (Fig. 4 and data not shown). This result is consistent with previous studies on inhibition of DNA synthesis in egg extracts with human CIP1 (37), which suggested effects on an initiation step in DNA synthesis that occurs before DNA unwinding but after nuclear assembly. We found that Xic-1 also has the ability to bind PCNA, which correlates with the ability of Xic-1 to inhibit DNA synthesis from a single-stranded template. Our data suggest that Xic-1 has a dual role in regulating DNA synthesis: it prevents entry into S phase by inhibiting Cdks and may directly block ongoing DNA replication by inhibiting PCNA.

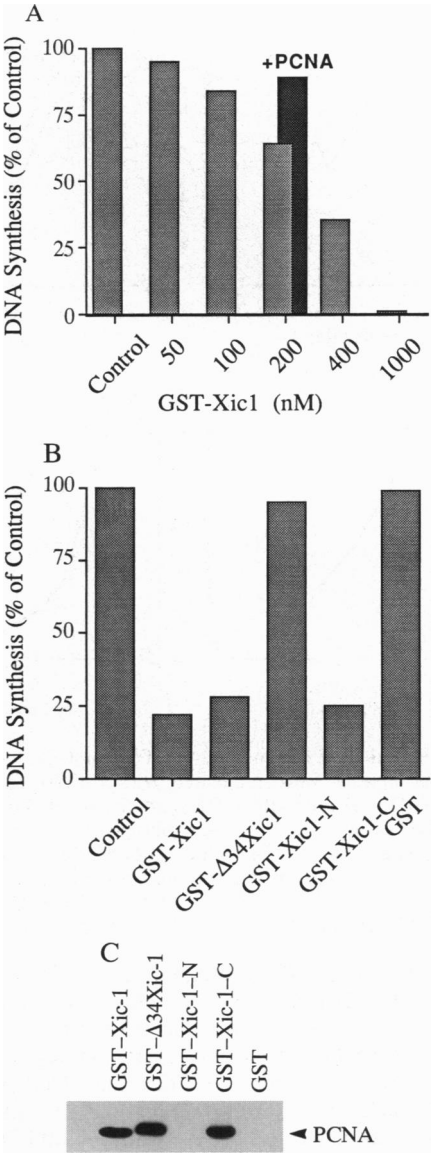

FIG. 5. Inhibition of single-stranded DNA synthesis by Xic-1. High-speed supernatant egg extracts (2 mg/ml) and 4 ng of M13mp18 single-stranded DNA were incubated in described reaction conditions (26) in a final 50-μl vol. Various concentrations of GST–Xic-1 were added as indicated, reactions were incubated for 90 min at 30°C, and products were analyzed by trichloroacetic acid precipitation and Cherenkov counting. In one reaction, as indicated in *A*, 200 nM of GST–Xic-1 was preincubated with 350 nM *Xenopus* PCNA on ice for 10 min before the assay. The amount of DNA synthesis is represented as the percentage of DNA synthesized in a control without the addition of GST–Xic-1. (*A*) Xic-1 inhibits single-stranded DNA synthesis in a dose-dependent manner. (*B*) Single-stranded DNA synthesis is inhibited by various Xic-1 constructs. GST–Xic-1 proteins (400 nM each) were used in the reaction. (*C*) Interaction of PCNA and Xic-1. GST-tagged Xic-1 proteins on glutathione-agarose beads were assessed for ability to bind PCNA in egg extracts as described.

An important question concerns how Xic-1 is regulated. Our data show that Xic-1 is constant during oocyte maturation and in egg extracts that cycle through M and S phases *in vitro*. Similarly, $p27^{KIP1}$ is not subjected to acute transcriptional regulation in mammalian fibroblasts, although some reports have suggested regulation by cAMP and interleukin 2 (11–13). For mammalian $p21^{CIP1}$, it has been suggested that changes in

the stoichiometry of binding may be important for inhibitory effects (38), with inhibition occurring only after a second molecule of Cip1 binds. Whether such a mechanism operates with Xic-1 is not clear at present, but the dose–response curve for inhibition of cyclin/Cdk complexes (Fig. 2) does not show biphasic effects. Another possible mechanism of regulation is via phosphorylation. Xic-1 has several putative Cdc2 phosphorylation sites (Fig. 1), some of which are not present in either Kip1 or Cip1 from mammalian cells. Data in Fig. 3*A* show that ectopic Xic-1 is phosphorylated in M-phase extract but not in interphase by kinases other than cyclin B/Cdc2 (Fig. 3*B*). Several other serine/threonine kinases are active in M-phase extracts, including cyclin E/Cdk2, mitogen-activated protein kinase, S6 kinase (Rsk), and a mitotic epitope kinase. The identity of the kinase(s) responsible for Xic-1 phosphorylation merits further investigation.

Despite the conservation of the Cdk inhibitory domains as a modular element in the N-terminal half (Fig. 1), dose–response curves revealed that human CIP1 and Xic-1 are not identical in their potency for Cdk inhibition, with Cip1 inhibiting cyclin A/Cdk2 more potently than cyclin E/Cdk2. Moreover, a variant of Xic-1 (Δ34Xic-1) demonstrated a markedly reduced inhibition of cyclin A/Cdk2 without change in inhibition of cyclin E/Cdk2 (Fig. 2). This result suggests that the binding site for the two Cdk complexes is different. One of the hallmarks of cell cycle control is the use of the same components under different conditions to generate control points in the cell cycle. It is likely that the roles of Xic-1 and cyclin E/Cdk2 will change during development as the embryonic cell cycle evolves to include G_1 and G_2 phases, regulation by the pRb protein, and periodic vs. constitutive expression of cyclin E. The ability to express a form of Xic-1 relatively specific for cyclin E/Cdk2 should facilitate analysis of the function of cyclin E/Cdk2 in early development.

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