Growth Regulation of the Expression of Mouse cDNA and Gene Encoding a Serine/Threonine Kinase Related to *Saccharomyces cerevisiae* CDC7 Essential for G1/S Transition

STRUCTURE, CHROMOSOMAL LOCALIZATION, AND EXPRESSION OF MOUSE GENE FOR *S. CEREVISIAE* CDC7-RELATED KINASE*

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Saccharomyces cerevisiae CDC7 encodes a serine/threonine kinase required for G1/S transition of the yeast cells. We previously reported human and Xenopus cDNAs encoding CDC7-related kinases and suggested the possibility that chromosomal replication of higher eukaryotes may be regulated through conserved mechanisms involving Cdc7-related kinases. Here we report a murine cDNA and gene (muCdc7) encoding a serine/threonine kinase related to CDC7. The predicted coding frame for the longest cDNA for muCdc7 consists of 564 amino acids, which shares 46, 77, and 93% identity, respectively, with those of budding yeast, *Xenopus*, and human in kinase conserved domains. The chromosomal gene for muCdc7, located at the band 5E5 on the mouse chromosome 5, consists of 12 exons, and its exon/intron gene for muCdc7 was completed in the absence of ongoing protein synthesis after the addition of 10^6 cpm of DNA probe/ml of hybridization solution. This paper is available on line at http://www.jbc.org

EXPERIMENTAL PROCEDURES

Isolation of cDNA Clones and Determination of Nucleotide Sequences—cDNA clones containing muCdc7 were isolated from mouse testis libraries (a gift from Dr. A. Kikuchi or purchased from CLONTECH). Filters were prehybridized for several hours in 10% formamide, 6 × SSPE, 0.5% SDS, 5 × Denhardt’s solution, 100 μg/ml salmon sperm DNA at 42 °C and were hybridized overnight in the same solution after the addition of 10^6 cpm of DNA probe/ml of hybridization solution. Hybridized filters were washed twice at room temperature in 1 × SSC, 0.5% SDS and once at 42 and 50 °C in 0.1 × SSC, 0.5% SDS for 15 min in each washing prior to autoradiography. DNAs from positive clones were digested with EcoRI and were subcloned into pBluescript vectors.

Southern Hybridization of Genomic DNA—Five μg of mouse genomic DNA, extracted from mouse EL4, NIH3T3, and ES cells, was digested

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with restriction enzymes. The digests were separated on a 0.6% agarose gel, and DNA was transferred to hybond-N nylon membrane (Amersham Pharmacia Biotech), followed by hybridization with a radiolabeled probe, which was generated by T7 RNA polymerase-mediated in vitro transcription on plasmids containing the muCdc7 cDNA (0.4- and 1.4-kb probes). Hybridization under high-stringency conditions was carried out at 68 °C in Quick-hybridization solution (Stratagene) as suggested by the supplier. The membrane was hybridized for 1 h and then washed with vigorous agitation twice at room temperature in 2× SSC, 0.1% SDS and once at 60 °C in 0.1× SSC, 0.1% SDS for 30 min in each washing. The blot was then exposed to an imaging plate, and optical density was measured by a radioimage analyzer (BAS2000, Fujifilm).

**Isolation of Genomic Clones**—Mouse genomic DNA libraries, constructed from adult mouse BALB/c male liver in λ EMBL 3 SP6/T7 vector (CLONTECH) or from 129 mice in αLFXII vector (Stratagene), were screened by the labeled DNA containing a muCdc7 coding region. 1×10⁶ recombinant λ plaques, lifted onto Hybond-N nylon membrane filters (Amersham Pharmacia Biotech), were screened as described in cDNA clone isolation. Restriction maps of the insert DNA were deduced by a partial digestion/end-hybridization method or by a two-dimensional electrophoresis method. In the former method, the insert containing SP6 or T7 promoter site at either end of the fragment, generated by NoI or SacI digestion, was subcloned into the pBluescript KS(−) or pBluescript KS(+) vector (Stratagene). Partial digestions of inserts, digested by NoI or SacI to completion, were partially digested with EcoRI, BamHI, HindIII, or BgIII; electrophoresed; and transferred to Hybond-N membrane (Amersham Pharmacia Biotech), which was hybridized with the 5′-end labeled SP6 or T7 oligonucleotide primer. This way, locations of each restriction site from both ends of the insert could be determined. In the latter method, plasmid DNAs were digested by a first enzyme and electrophoresed on an agarose gel, and the lane containing digested DNA was excised. The excised agarose piece was digested in situ by a second enzyme and was cast in agarose for second dimension electrophoresis. After the run, the gel was stained with ethidium bromide, and fragment patterns were analyzed.

**Chromosomal Localization of muCdc7 Gene** by Fluorescence in Situ Hybridization—DNA containing the muCdc7 gene (a 13-kb insert of clone ES9–2) was labeled with digoxigenin dUTP by nick translation. The labeled probe was combined with sheared mouse DNA and hybridized to metaphase chromosomes derived from mouse embryonic fibroblasts in a solution containing 50% formamide, 10% dextran sulfate to metaphase chromosomes derived from mouse embryonic fibroblasts. Prehybridization and hybridization were carried out at 68 °C in Quick-hybridization solution (Stratagene) as suggested by the supplier. The membrane was hybridized for 1 h and then washed with vigorous agitation twice at room temperature in 2× SSC, 0.1% SDS and once at 60 °C in 0.1× SSC, 0.1% SDS for 30 min in each washing. The blot was then exposed to an imaging plate, and optical density was measured by a radioimage analyzer (BAS2000, Fujifilm).
promoter fusion plasmids were assayed in the mouse interleukin-3-dependent pro-B cell line, Ba/F3, cultured in RPMI medium supplemented with 5% fetal bovine serum and 0.25 ng/ml mouse interleukin 3 (muIL3). For transient transfections, \(2 \times 10^6\) cells were transfected by electroporation (0.2 V, 960 microfarads) with \(2 \mu\)g of a test plasmid together with \(0.1 \mu\)g of pCMVSEAP, which contained the alkaline phosphatase gene driven by the cytomegalovirus promoter and served as an internal control. For assays in proliferating cells, cells were incubated in RPMI medium containing 5% fetal calf serum and 0.25 ng/ml muIL3 after transfection and were harvested after 16 h. For assays in synchronized culture, cells were starved by depletion of the cytokine for 12 h after electroporation, restimulated by the addition of 2.5 ng/ml mouse IL3, and harvested at various times after restimulation for cell cycle analysis and luciferase assays. For cell cycle analysis, cells were washed in ice-cold PBS, fixed in 70% ethanol for at least 24 h, and then treated with RNase A (250 \(\mu\)g/ml). Cellular DNA, stained with propidium iodide (50 \(\mu\)g/ml), was analyzed by a Becton-Dickinson fluorescence-activated cell analyzer. For luciferase assays, cells were lysed by three cycles of freezing-thawing, and soluble extracts were prepared by centrifugation (15,000 rpm for 5 min). Luciferase assays were performed with the luciferase assay reagents (Promega), and light intensity was measured for 30 s on a Berthold luminometer. To correct for the variability in transfection efficiencies, luciferase activity was normalized by alkaline phosphatase activity (Tropix) expressed from cotransfected pCMVSEAP.

**RESULTS**

**Isolation and Characterization of cDNA Encoding muCdc7**—Southern hybridization of genomic DNA from various vertebrates with huCdc7 (a putative human homolog of Cdc7) DNA probe has indicated the presence of a related gene in mouse (18). We have screened a mouse testis cDNA library with a DNA probe containing a part of the coding region of huCdc7, and obtained a clone, NS#1. Nucleotide sequencing of this clone revealed that it encoded a serine/threonine kinase whose primary structure is 93% identical with that of huCdc7 in the kinase conserved domains (see Fig. 1). Therefore, we named this clone muCdc7 (a putative mouse homolog of Cdc7). The isolated clone contained the kinase domains I–VII but lacked the ATG start codon as well as the kinase domains VIII through XI, and translation was terminated in the middle of the kinase insert II. In order to obtain a full-length muCdc7 cDNA, another mouse testis cDNA library was screened by using the isolated muCdc7 and huCdc7 cDNA fragment containing the kinase domains VIII–XI. The 1.0-kb insert of the isolated clone contained the kinase domains I–VII but lacked the ATG start codon as well as the kinase domains VIII through XI, and translation was terminated in the middle of the kinase insert II. In order to obtain a full-length muCdc7 cDNA, another mouse testis cDNA library was screened by using the isolated muCdc7 and huCdc7 cDNA fragment containing the kinase domains VIII–XI. The 1.0-kb insert of the isolated clone contained the kinase domains III–X but was apparently truncated at the EcoRI site present downstream of the domain X. The carboxyl-terminal region of muCdc7 including the kinase domain III–X was identified in the cloned muCdc7 genomic DNA or in 3′-RACE products (see below). The N-terminal coding region as well as 5′-untranslated region were isolated by 5′-RACE using the primers 521 and 519 complementary to the coding region spanning the kinase domain II
The 328-bp DNA fragment generated by 5′-RACE contained a 242-bp coding region initiated from an ATG and 86-bp 5′-untranslated region. A putative full-length muCdc7 cDNA was reconstructed from four DNA fragments (see “Experimental Procedures”), yielding a 1599-bp contiguous open reading frame encoding a 532-amino acid polypeptide (muCdc7#1). Alignment of this sequence with other Cdc7-related kinases indicated that 32 amino acids are missing in the kinase insert II of muCdc7 when compared with huCdc7 (Fig. 1). Therefore, we have conducted reverse transcription-PCR using primers specific to N-terminal and C-terminal portions of muCdc7 as well as those derived from the internal coding region in order to isolate possible alternatively spliced variants. One of them contained the missing 32-amino acid sequence (muCdc7#2). Several other cDNA variants were also isolated that appeared to be generated by alternative splicings at the exons 7–9. Like NS#1 lacking the kinase domains VIII–XI, all of them resulted in premature termination of translation (data not shown). The functional significance of these apparently kinase-negative forms of muCdc7 is currently unknown.

Genomic Southern Analyses of the muCdc7 Gene—Southern hybridization on mouse genomic DNA using the muCdc7 cDNA probe showed distinct DNA patterns, suggesting the presence of a single gene encoding the muCdc7 gene. Genomic DNA isolated from mouse thymoma EL4, ES cells, or fibroblast NIH3T3 yielded an identical pattern with restriction enzymes tested (Fig. 2 and data not shown).

Genomic Organization of muCdc7—The muCdc7 gene was

**Table I**

| Exon/intron no. | Exon size | Sequence at exon/intron junction | Intron length |
|-----------------|-----------|----------------------------------|---------------|
| 1               | 53 bp     | TGA ACC gtaagt caacag CTG CTG    | 0.3 kb        |
| 2               | 185 bp    | TTT CAG gtatgc ccatag GTA TTA   | 3.7 kb        |
| 3               | 84 bp     | GAG AAG gtaagg tcatag GCA CCT   | 0.2 kb        |
| 4               | 136 bp    | TGC AGG gtaagc taatag GGG GCA   | 3.1 kb        |
| 5               | 94 bp     | TGC AGG gtaagc taatag GGG GCA   | 0.2 kb        |
| 6               | 144 bp    | GAA AGA gtaagt tcttag GTA TGC   | 0.6 kb        |
| 7               | 243 bp    | GAA AGA gttctt ttgtag GAG AGA   | 0.6 kb        |
| 8               | 96 bp     | GAA AGA gtaagt ttggag GTT TAC   | 0.8 kb        |
| 9               | 176 bp    | GTC GAC gtaacc tcatag GCG GCA   | 0.8 kb        |
| 10              | 83 bp     | CCA CAG gtaagg tcttag CGA TFG   | 2.3 kb        |
| 11              | 150 bp    | CTT TGG gtaagt ttggag GCA AAT   | 3.4 kb        |
| 12              | 1600 bp   | Consensus                        | 1 kb          |

**Fig. 2.** Southern blot analysis of the muCdc7 gene. Five μg of mouse genomic DNA, isolated from EL4 or NIH3T3, was digested with the restriction enzyme indicated. Fragments fractionated by electrophoresis on a 0.6% agarose gel were transferred to a nylon membrane and were probed with the 32P-labeled muCdc7 RNA probe generated as described under “Experimental Procedures.”

**Fig. 3.** Schematic representation of the exon-intron organization of the muCdc7 gene. The black boxes on cDNA indicate kinase insert sequences. The boxed regions on Genomic indicate the exons. The black and white portions in cDNA and in the boxes on Genomic indicate the coding and 5′- or 3′-untranslated region, respectively. The numbers above Genomic indicate the sizes (in nucleotides) of exons. The introns indicated by downward arrows correspond to the position of a 91 bp insert in a huCdc7 variant (a) and to those of introns of hsk1 (b and c). Locations of restriction sites are indicated by vertical lines on Genomic. E, EcoRI; B, BamHI; H, HindIII; Bg, BglII.
isolated from a mouse genomic library constructed from BALB/C or 129SV in λ EMBL3 SP6/T7 or in the FIX II vector, respectively. Screening these libraries with an EcoRI fragment, derived from the truncated muCdc7 cDNA variants, yielded two overlapping clones, B2-1 and B24-2. Restriction analyses of these clones indicated that they contained the C-terminal two-thirds of the coding frame but lacked the N-terminal coding segment and the promoter region. Screening of the latter library led to isolation of the clone ES9–2. By aligning B2-1, B24-2, and ES9-2, a 24-kb contiguous mouse genomic DNA fragment containing the entire muCdc7 gene could be isolated. These clones were analyzed by restriction mapping, and a composite map of the muCdc7 gene was constructed (Fig. 3). The deduced restriction map of the isolated DNA agreed with the restriction pattern expected from the results of genomic Southern hybridization.

After subcloning of various restriction fragments, exon/intron junctions of muCdc7 were identified by comparison of cDNA with the genomic sequence, and subsequently, all of the exons and introns as well as the upstream promoter region were sequenced (Table I). The 12 exons varied in size from 53 to 1600 nucleotides. The positions of the two introns (indicated by vertical arrows b and c in Fig. 3) of muCdc7 coincide with those of the two introns previously reported for the hsk1 gene, the putative S. pombe homologue of Cdc7 (17). The location of another intron (arrow a in Fig. 3) corresponds to where the 91-nucleotide insertion was found in a variant of huCdc7 cDNA (18). muCdc7#1 (the initial clone isolated) skips the entire exon 8, resulting in a polypeptide lacking the 32 amino acids in the kinase insert II.

Chromosomal Localization of the muCdc7 Gene—The chromosomal assignment of the muCdc7 gene was determined by fluorescence in situ hybridization of genomic clone to normal male mouse metaphase chromosomes. The initial experiment resulted in specific labeling of a medium sized chromosome that was believed to be chromosome 5 on the basis of 4,6-diamidino-2-phenylindole staining. A second experiment was conducted in which a probe, specific for the telomeric region of the chromosome 5, was cohybridized with the same probe. This experiment demonstrated that muCdc7 is located on chromosome 5 (Fig. 4). Measurements of 10 specifically hybridized chromosomes demonstrated that muCdc7 is located at a position...
that is 66% of the distance from the heterochromatic-euchromatic boundary to the telomere of chromosome 5, an area that corresponds to band 5E5.

Identification of muCdc7 Gene Products—Nuclear extracts prepared from mouse spleen, testis, and thymus were analyzed by Western blot using a muCdc7-specific antibody. In all the tissues, a 58-kDa protein was detected in addition to a 43-kDa protein (Fig. 5, lanes 1–3). The former protein comigrated with the muCdc7#1-encoded polypeptide expressed in COS7 cells (Fig. 5, lane 4; the lower band of the doublet expressed by pME18S-HAmuCdc7#1, which represents the nontagged number 1 protein). In testis, there were two additional intense bands of 70 and 40 kDa as well as several other minor bands (Fig. 5, lane 2). None of these bands were detected by preimmune control serum (Fig. 5, lanes 5–7). The results suggest that additional spliced forms may be present in testis.

Expression of muCdc7 Gene in Tissues and during Development—Northern analyses of muCdc7 transcription in various normal mouse tissues showed three transcripts of 4.4, 4.0, and 2.9 kb in size, which could arise from alternative splicing or differential polyadenylation. muCdc7 is transcribed to varied levels in all of the tissues examined (Fig. 6A). It is expressed most abundantly in testis, spleen, and thymus; at a relatively high level in lung, stomach, and brain; and at a lower level in kidney, liver, heart, muscle, and small intestine. This profile of expression is similar to that of huCdc7 (18). It is also efficiently transcribed in rapidly proliferating ES cells. Northern analyses of poly(A)⁺ RNA isolated from mouse embryos of different developmental stages showed four transcripts of 7.5, 4.4, 4.0, and 2.9 kb in length, three of which are identical to those detected in normal tissues (Fig. 6B). Transcription of muCdc7, which is detected all through the development, reaches a maximum at day 11 and gradually decreases up to day 17.

Transcription Initiation Sites of the muCdc7 Gene—The nucleotide sequences of the 2.1-kb region upstream of the ATG translation initiation codon were determined (Fig. 7A). Then, the transcription start sites were determined by primer extension using a 21-nucleotide-long oligonucleotide complementary to a part of the N-terminal coding sequences of the muCdc7 (Fig. 7B). Two clusters of transcription initiation sites were detected at positions +1 to −3 and positions −11 to −14. Furthermore, additional initiation sites were detected at scat-
tered locations further upstream (−129, −199, −224, −279, −339, −369). No consensus TATA box was identified upstream of any of the transcription start sites nor was identified in the entire 2.1-kb segment sequenced. Search for putative transcription factor binding sites revealed the presence of one consensus Sp1 element in a reverse orientation at −52 (5′-GAGGCGTGG-3′) near the two clusters of ATG-proximal transcription start sites. Sp1 is known to guide initiation in some TATA-less promoters (20). A binding site for YY1, also known to determine transcription initiation in some TATA-less promoters (21), was also identified at −1184 to −1169, although its significance is currently unknown. Sequences closely related to E2F binding sites, which are frequently found in cell cycle-regulated promoters, are present at positions −51 to −46, −79 to −73, and −162 to −152 as well as at −625 and −434 (see below).

Promoter Region for muCdc7 Gene—To test the promoter activity of the upstream sequence of muCdc7, the 1.4-kb EcoRI–AatII (−1359 to +73) DNA fragment was inserted into the luciferase reporter plasmid, pGL2-Basic. The resulting plasmid, pGL-FL, produced significant luciferase activity upon transfection into Ba/F3, factor-dependent proB cells, indicating that this fragment contained a functional promoter. In order to localize promoter elements more precisely, series of 5′- and 3′-deletions were constructed and luciferase activities were measured (Fig. 8, A and B). In 5′-deletions, deletion up to nucleotide −522 (ΔKA) led to a decrease in promoter activity by 25%, and further deletion up to −335 (ΔKP) resulted in 75% decrease in promoter activity. Interestingly, further deletion up to −159 (ΔKS) resulted in reactivation of the promoter to the level 50% of pGL-FL. On the other hand, deletions from the 3′-end indicated that the deletion to −158 (ΔHS) led to almost complete loss of the promoter activity (3.2% of that of pGL-FL). Further deletion to −334 (ΔHP) or to −517 (ΔHA) resulted in further decrease of transcription to the background level. The results indicate essential roles of the coding region-proximal 231 bp in muCdc7 expression. They also suggest the presence of positive (−1350 and −335) and negative (−334 and −159) elements controlling muCdc7 expression.

FIG. 8. Transient transfection assays of muCdc7 promoter activity. A, the 1.4-kb EcoRI–AatII DNA fragment was inserted into the luciferase reporter plasmid, pGL2-Basic. Various deletions of the muCdc7 promoter were made as described under “Experimental Procedures.” The square boxes and a circle indicate putative E2F binding sites and an Sp1 binding site, respectively. Upward arrowheads indicate transcription initiation sites. Filled and empty lines indicate DNA regions present or absent, respectively, in each construct. B, the constructs described in A were transfected into proliferating Ba/F3 cells and were harvested at 16 h after transfection. Luciferase activities directed by these plasmids were normalized with the alkaline phosphatase activity of a cotransfected control plasmid.
Growth Regulation of muCdc7 Promoter Activity—We examined the expression of muCdc7 mRNA by Northern blot analysis of synchronized populations of Ba/F3 cells. Ba/F3 cells were arrested at the resting state by factor (muIL3) depletion (Fig. 9A) and harvested at various times after release. muCdc7 mRNA started to increase at 8 h after release and reached a maximum at 10–12 h (Fig. 9B). Flow cytometry analyses of these cells indicated that the increase of muCdc7 mRNA levels coincided with the entry into the S phase. The level of transcription did not change significantly after reaching maximum, although, due to loss of synchrony after the first cycle, we were not able to conclude whether muCdc7 expression alternates during the mitotic cell cycle.

In order to determine the promoter elements involved in growth regulation of the muCdc7 gene, transient transfection assays were conducted with promoter-luciferase fusion plasmids described above. The pGL2-FL containing the 1.4-kb muCdc7 promoter fragment on the vector pGL2-Basic was transfected into Ba/F3 cells, which were then incubated in medium without muIL3 for 12 h and were arrested in the resting state. After the addition of muIL3 to restart the cell cycle, cells were harvested at 2-h intervals. Synchronized entry...
into the cell cycle was confirmed by measuring [3H]thymidine incorporation at each time point (data not shown). The luciferase activity in each extract was normalized by alkaline phosphatase activity directed by a co-transfected plasmid, pCMVSEAP, expressing alkaline phosphatase under the constitutive cytomegalovirus promoter. Luciferase activity from pGL2-FL started to increase at 6–8 h after factor addition, peaked at 10 h, and slightly decreased after that as cells entered G2/M (Fig. 10A). The decrease of the promoter activity after 12 h poststimulation in transient assays may be due to dilution of plasmids during propagation of cells. This result shows that the cis elements responsible for growth regulation of muCdc7 transcription reside in the 1.4-kb EcoRI–AatII fragment. To define the cis elements more precisely, 5’ and 3’ deletions described above were assayed for promoter activity by the same procedure. 5’ deletion up to –522 (ΔKA) increased growth factor responsiveness of the promoter activity, while further 5’ deletion up to –335 (ΔKP) resulted in decreased response of the promoter activity. Two potential E2F binding sites are present at –625 and –434. The E2F site at –434 may be required for full response to growth stimulation, but neither site may be essential for growth regulation of the promoter, since both –522 and –335 deletions can respond to growth signals (Fig. 10B). In fact, the luciferase fusion plasmid containing only the sequences from –158 to +73 (ΔKS), on which a potential Sp1 binding site and three potential E2F binding sites (namely 5’-TTGCGC-3’ (at –49), 5’-TTTCCG-3’ (at –79), and 5’-TCCCGG-3’ (at –162)) are present could respond to growth factor, and the level of expression was even higher than the original 1.4-kb fragment. In contrast, promoter activities, hence growth factor stimulation, was completely lost in 3’-deletions (Fig. 10C), indicating again essential functions of the gene-proximal 231-bp sequence for basic promoter activity and for response to growth signals.

Discussion

Structure of muCdc7 cDNA and Gene—Initiation of the S phase is regulated by a set of serine/threonine kinases. Among them, Cdc7 kinase has been known to be the ultimate triggerer of chromosomal replication in S. cerevisiae (1). In this report, we have isolated and characterized the cDNA and gene for muCdc7 encoding a protein related to S. cerevisiae Cdc7. The predicted longest open reading frame for muCdc7 encodes a 564-amino acid protein that shares identity of 93, 77, and 46% with huCdc7, XeCdc7, and budding yeast Cdc7, respectively, in the kinase conserved domains. In addition to the kinase domains, it contains two stretches of amino acids inserted at the locations conserved in all of the known Cdc7-related kinases (17, 18). Their sequences are 62 and 28% identical with those of huCdc7 and XeCdc7, respectively. Like huCdc7 and XeCdc7, kinase insert I and C-terminal tail sequences, which are found in the yeast genes, are not present in muCdc7. Several cDNA variants were isolated, including ones in which the coding frame is prematurely terminated due to an alternative splicing and one that contains a 32-amino acid deletion within the kinase insert II. The functional significance of these alternatively spliced forms is still not clear.

Only a single copy of the muCdc7 gene was present per haploid mouse genome (Fig. 2). Even under relaxed hybridization conditions, any additional DNA bands that hybridized with the muCdc7 probe were not detected (data not shown). Further experiments are needed to conclude whether any other genes encoding Cdc7-related kinases are present on mammalian genomes. The muCdc7 gene is composed of 12 exons. A survey of the genomic structures of various protein kinases reported so far indicates that locations of introns are scattered throughout the entire kinase domains. However, we noticed that some of the locations of introns are conserved in other kinases. The mouse Cdk5 gene contains introns at the same or very similar locations as introns 3, 5, 6, and 11 of muCdc7 (22). The location of the intron 6 is also shared by the C. elegans cAMP-dependent kinase gene (23). One notable feature is that exon-intron organizations are strikingly conserved in the kinases belonging to the same family. This is most clearly shown by the fact that the locations of all five introns of plant Cdc2 genes are conserved in the murine Cdk5 gene, which contains 10 introns (22–24). Similarly, two introns (introns 3 and 11) of the muCdc7 gene are located at the positions identical to those of the two introns we previously reported for hsk11+, the fission yeast homolog of Cdc7 (17). Locations of other introns include a 91-nucleotide insertion was found in one of the huCdc7 variants (intron 2) (18).

One alternatively spliced form is generated by skipping the eighth exon, resulting in a 32-amino acid deletion in the kinase insert II. This protein was expressed in common in the three tissues examined (Fig. 5). Other splicing variants were also detected at intron 7, all of which resulted in premature translation termination (data not shown). The muCdc7 gene is located on the chromosome 5, an area corresponding to band 5E5,
whereas huCdc7 is located on chromosome 1, band 1p22. The human chromosome 1p22 was previously reported to be syntenic with the mouse chromosome 5 (25). We have not found any potential genetic disorders that could be linked to mammalian Cdc7.

Regulation of muCdc7 Expression in Response to Growth Stimulation—Expression of huCdc7 in a factor-dependent cell line is induced upon growth stimulation of the resting cells by the addition of a growth factor. The increase of muCdc7 transcription occurred at 8–12 h after stimulation, coincident with entry into the S phase, and stayed constant after entry into the cell cycle. Promoter regions for muCdc7 lack apparent TATA box sequence but contain a Sp1 site, as frequently found in growth-regulated promoters (20, 26). Primer extension analyses have shown the presence of multiple transcription initiation sites, locations of which span over a nearly 400-bp region, consistent with absence of TATA box sequence. The transiently expressed reporter protein under the muCdc7 promoter increased after serum stimulation with similar kinetics, and mapping of the promoter elements required for response to growth stimulation revealed that the 231-bp segment containing one Sp1 sequence and two E2F-like sites are sufficient to confer the ability to respond to growth stimulation. Deletion of one of the two distal E2F sites (ΔKP) decreased the level of transcription in both cycling cells as well as in growth-stimulated cells. Expression was recovered by further deletion (ΔKS), suggesting the presence of negative elements between −335 and −159. Expression was completely abolished by deletion of the above 231-bp segment from the 1.4-kb upstream sequence, indicating that this gene-proximal segment is required even for initiation at locations further upstream.

Expression of huCdc7 is down-regulated when differentiation is induced in HL-60 cells by treatment with TPA or cAMP. These results indicate that the level of mammalian Cdc7 transcription is regulated in response to external stimuli, positively by growth signals and negatively by differentiation signals, in keeping with its proposed roles for proliferation of mammalian cells.

Expression of muCdc7 in Tissues and during Development—Three major transcripts, 4.4, 4.0, and 2.9-kb in length, were detected by Northern analysis of muCdc7 transcription in tissues and cell lines. The expression pattern of muCdc7 in various tissues is similar to that of huCdc7 (18). High expression was detected in testis and spleen as well as in rapidly proliferating ES cells. General correlation of muCdc7 expression and active proliferation can be seen, as expected. Both in mice and human, highest expression was detected in testis. In conjunction with genetic data pointing to roles of budding yeast Cdc7 and fission yeast Hsk1 in meiotic processes (27, 28) as well as increased expression of CDC7 and hsk1 in meiosis of the two yeasts, the results may indicate additional conserved meiotic functions of Cdc7-related kinases. Blotting with muCdc7-specific antibody showed that muCdc7 proteins expressed in testis may significantly differ in size from those expressed in other tissues. This may indicate the existence of testis-specific splicing variants that may play distinct roles during meiosis.

muCdc7 is expressed during embryonic development, reaching higher at day 11. Additional transcript, 7.5 kb in length, is detected, although its nature and functions are not known. To address the in vivo functions of mammalian Cdc7 kinase more precisely, we are currently constructing mutant mice lacking the genomic muCdc7 gene.

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