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Authors: Kurokawa, Daisuke, Akasaka, Koji, Mitsunaga-Nakatsubo, Keiko, and Shimada, Hiraku

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Cloning of Cyclin E cDNA of the Sea Urchin, *Hemicentrotus pulcherrimus*

Daisuke Kurokawa, Koji Akasaka, Keiko Mitsunaga-Nakatsubo
and Hiraku Shimada*

Laboratory of Molecular Genetics, Graduate Department of Gene Science,
Faculty of Science, Hiroshima University, Higashi-Hiroshima 739, Japan

ABSTRACT—cDNA encoding maternal cyclin E (HpCycE) has been cloned from the oocyte cDNA library of the sea urchin, *Hemicentrotus pulcherrimus*, by differential screening with a cDNA probe covering the total poly(A)⁺ RNAs of 16 cell-stage embryos and gastrulae. In this communication we describe similarity of amino acid sequences between HpCycE and those of cyclin E from other organisms and maternal origin of HpCycE. The amino acid sequence deduced from the nucleotide sequence of HpCycE cDNA is highly similar to those of human, rat, chicken, *Xenopus*, zebrafish and *Drosophila*, while its similarity to other cyclins is much lower. A gene for HpCycE exists as a single copy in the genome of *H. pulcherrimus*. Northern blotting revealed that the mRNA for HpCycE is maintained at a high level up to the morula stage and thereafter declines.

INTRODUCTION

Cyclins are a group of proteins required for the correct progression of the cell cycle in various organisms. Cyclin E was first isolated from human cells as the cDNA that can restore the yeast CLN mutation (Lew *et al.*, 1991). The amount of human cyclin E is maximal in the late G1 and early S phases, and the cyclin E-cdk2 complex regulates the G1-S transition (Dulic *et al.*, 1992; Koff *et al.*, 1992; Jackson *et al.*, 1995), and transforming-growth-factor- β (TGF- β) prevents the stable assemblage of the cyclin E-cdk2 complex (Koff *et al.*, 1993). In sea urchins, from which cyclin was first detected as a protein that appears and disappears in a cell cycle-dependent manner (Evans *et al.*, 1983), only cDNAs for G2 cyclins have been cloned thus far (Pines and Hunt, 1987), while none of the G1 cyclins such as cyclins C, D and E has been cloned yet.

As part of our efforts to isolate and classify the maternal cDNAs of the sea urchin, we screened the oocyte cDNA library of *Hemicentrotus pulcherrimus* with cDNA probes that cover the total poly(A)⁺ RNAs of 16 cell-stage and gastrula embryos, and isolated cDNA clones that are present in the 16 cell-stage embryo but absent from the gastrula. By the cloning and nucleotide sequencing of these cDNAs, we identified cDNAs encoding cyclins A, B and E.

Since little information is available regarding echinodermal cyclin E, we report here the nucleotide sequence of the *HpCyclinE* cDNA as well as the pattern of its ontogenic accumulation.

MATERIALS AND METHODS

Differential screening of oocyte cDNA library

The total RNA was extracted by the method of Chomczynski and Sacchi (1987) from the 16-cell embryos and from gastrulae of the sea urchin *Hemicentrotus pulcherrimus*, and poly(A)⁺ RNA was isolated from the total RNA using Oligotex-dT30 (Roche, Tokyo, Japan). Digoxigenin (DIG)-labelled cDNA probes representing total poly(A)⁺ RNA of 16-cell embryos and gastrulae were prepared by reverse transcription with Superscript II (Gibco BRL, Rockville, MD, USA). Duplicate filters of the λ gt11 oocyte cDNA library (a gift from Dr. I. Mabuchi of University of Tokyo) were probed with DIG-labelled total cDNA of 16-cell embryo or of gastrula, and 68 plaques with positive signals for the 16-cell probe but negative for the gastrula probe were selected by three rounds of screening. cDNAs isolated from these plaques were amplified by polymerase chain reaction (PCR) with the λ gt11 primer (TaKaRa, Otsu, Japan). Amplified cDNA fragments were digested with *EcoRI* and inserted into pBluescript SK- (Stratagene, La Jolla, CA, USA). The nucleotide sequences of the subcloned fragments were determined by the chain termination method (ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin-Elmer, Foster City, CA, USA) with the pBluescript SK- reverse primer or with the M13-20 primer.

Cloning of *HpCycE* cDNA

Using as a probe the HpCycE fragment that was excised from *HpCycE* clone 1, the cDNA library of unfertilized eggs constructed in λ gt10 vector was screened for a full-length *HpCycE* cDNA. Positive clones were rescued into pBluescript SK-, and the nucleotide sequences were determined by exonuclease III deletion followed by the chain termination procedure using the kit described above.

Genomic Southern hybridization

DNA isolated from the sea urchin sperm was digested by *EcoRI*, *SacI* and *PstI*. The digests were electrophoresed in 0.7% agarose gel, transferred to a nylon membrane (NY13N, Schleicher & Schuell, Dassel, Germany), and hybridized to the *SacI*-digested fragment of *HpCycE* clone 1.

* Corresponding author: Tel. +81-824-24-7446;
FAX. +81-824-24-7498.

GCCTAAGTGAATCATCTGAGTACGATACAAATCAGTCGAACATAAATCATTTTTTCTCAATTTTCGAGCCAAATGTTCAAAGATTGCAT 90
TAGTTCATAGAAATATGATATATTTTTCTTTGATTCAACTCGAAATTTACCTTATACAGCTCATTTTACCGCTTTGGTGTAAAACA 120
TCGTATGTCGTTTGGATATTTGGCGGTAAAGACTAACTCCCGCGGTAGTACTTCGGTCACTGGACGATCTCTTACGCGCCGCTGT 180
GTATGTTTGGGTAGCTCTCTCGCTATACAGCTAGCTTTCCGCGGGAATGACCGACTGAGCAGTATATCTAGAACTTGGCGAGTAGTGC 360
CAGAAGCACGGTAGTAAACTGGTAAATTTGCTGCTCTAATTTCAAAATTTGACCCAGGAAGATTCCAAGACTATAGATATCTACGCGAA 450
GCCAAAGACGATTTCCGCTTCAGCAGAAGATCTACTACTACTAGAACTGTGTGAGAAATCTGTGAAAGACAGGACAAGGCAAGGAAAT 540
M 1
GTCCTGGAGGAGTGGTTCGTTTGAATCAAGACAGGACAATCAGCCGTTAAACAGAGTGCATTTTCAGACGAAAAATAATCTTCCAATGTGTAC 630
S R R S R G R L Q S R Q D N Q P L T E C I S D E N N L P M C T 31
AAGAAAGAGGAAAACACGAGAACAGGACAGCTGGTGTATCAAAGCTGAGGAAGTTCAAAGGAGAAGACAGCAATTTACGATAGAGAA 720
R K R K T R E Q D T T G V S K A E E V Q R R R Q Q F T I E N 61
CCGATGGGTACCGATCTCTGAGTCATCGTCAATAGAAACATCTCTTCTAGTTCCAATGCAAAACCAAGGAACCATCAACTCCTTCAGAAGA 810
R W V P I S E S S S I E T S L L V P M Q T K E P S T P S E E 91
ACTTATGGACACTGCCAATTTGGGTCCACATTCGGTAACCTGTTTCCAGCCCAATGATCAGACAGAGCTTCACCCATCCCTTTGCTACACTG 900
L M D T A N W V T F R N L F P A H V S D R A S P I P L H W 121
GGATGATCTTCCAGAANTGAGCTATAATGACAAGGAAGGAGCCCTCTGCTAACAGGAAGCATGATTGCTTAAAGACTCATCCATCGTT 990
D A D L P E V W T I M T R K E A L C P R K H D C L K S H P S L 151
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G E R M R A I L L D W L I E V C E V Y R L H R E S F Y L A A 181
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K L E E I Y P P K L H E F A Y V T D G A C T D D Q I L D Q E 241
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L I M L M T L L N W D L T P I T V N T W L N A F M Q I C N A E 271
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E I A N R K T N F H F P S Y S T E F V Q V A Q L D V C T 301
CCTAGATACGTTTCAAGTACTTTCAGTACATGATTTGCTGACATGACTGCTTCTCATGTAACCAATGAAGAGTGCATGATGAGTGT 1530
L D I G S M D F D Y S I L A A S A L Y H V T N E E V T L S V 331
AACAGGTTTGAAGTGGGATGACATAGCAGCATGTGTCAGTGGATGTCAACATTTGCCATGACGATCCGTGAAGTGGGCGTGGCTCAGCT 1620
T G L K W D D I A A C V Q W M S T F A M T I R E V G V A Q L 361
CAAGAATTCAGAATATCTATGACAGGATGCACACAACATTCAGACACACTGCTCAAGTTTGAATTTATGGATAAATCCCATGAGAA 1710
K N F K N I Y A G D A H N I Q T H C S S L E L L D K S H E K 391
GCAAAGGCTATTGAGAGGCATCTTGTGTCAGCCCGCTGACAGTGCCTGGTCTGACCCCTCCTCAGAGTGAAGAAGTCAAGAA 1800
Q R L L R E A S C C S P V Q V P G V L T P P Q S D K K S K K 421
GGGAGTTCTATGATAGAGAAGGATATGCCACCAGTCTTGGCCAGCCATATATATCAATGTATTAGCAGTAGGTAGTTTCTTTTTAATGA 1890
G V L * 424
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GAAGGGGGATCAGCATATGATTTTCAACCTCGTCAAGTTGAACTATCACTAAGTGTGTTTCCCTAAAATGAGAGTATGTAATTT 2160
CTCATGTTGCTTCCAATCTATTTGCTTGTGTAATTTTGTAGTATATCGTTCAAACCTGTTTTTAAATCAAAATGTTTATAGT 2250
CCAGGCCATCTTCCATAACAAAATCTTAATTTCAAACCTGATTAATATCAGCGTATCTTCTTTTGTAGATCAGGTCATGCAAAATTTGTA 2340
TACTGATAAATGATTTCAAAATTTTATACACAATTTTGAATACCGATATACAACTAATTTACATGTTTGTGCTCAGCTTCAAGTTT 2430
AATAGGAATATAGTTTTGTCATTGTTGCCGAGAAATATTACTTAAAGTTAGAAAGTTATCATTCTTGTACAATTACAGAAAATTCAAA 2520
GATCAAAAACAGGGTGTCTGTTGAATGTAGACTTAAAAGAGGTTTTTTTTTCACTGAAATATGATGTAATGAGTAAATATGATGAT 2610
ATAATGACATTTGTATTTTCTGAGCTATTTTGTAGCATATAAATATATTTGCCAGAAGATATAAGATATTTGAACTTTGTAAAT 2700
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ATGTTTCTGATGAGTTTGTCTGATTTGTTCTGCTCAGTCTTTGAAATTTTTATATATTGAATAAGTGAATGATACCATTTTCAAAA 5310
AAAAAAAAAAAAAAAAAAAAA 5333

Fig. 1. Nucleotide and amino acid sequence of HpCycE cDNA. The nucleotide sequence was determined from those of Clone 1 and Clone 2. The cyclin box is indicated by an open box and the polyadenylation signal is underlined.

Northern blotting

Five µg of the total RNA extracted from embryos at various developmental stages was electrophoresed in a 1% agarose gel containing formaldehyde and transferred onto a nylon membrane (NY13N, Schleicher & Schuell). *HpCycE* mRNA was probed by digoxigenin-labeled antisense RNA prepared from the *HpCycE* clone 1 using a DIG-RNA labeling kit (Boehringer-Mannheim, Mannheim, Germany), and the hybridized bands were visualized with a DIG Luminescent Detection Kit (Boehringer-Mannheim). Probe hybridizations were performed according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Sixty-eight cDNA clones were isolated by the differential screening of the oocyte cDNA library of the sea urchin *Hemicentrotus pulcherrimus*, with cDNA probes covering the total poly(A)⁺ RNA population of the 16 cell-stage embryo and gastrula. One of them, Clone 1, consists of the nucleotide sequence with a significantly long ORF (open reading frame) containing a cyclin box. Since the ORF of Clone 1 is not completed and lacks a termination codon, we rescreened the cDNA library of unfertilized eggs with Clone 1 as a probe. Clone 2 thus isolated has a significant overlap with Clone 1 in its 5' half but contains a poly(A)⁺ tail at its 3' end. Figure 1 shows the nucleotide sequence constructed from Clone 1 and Clone 2 and the amino acid sequence deduced from it. It consists of 5,333 bp with an ORF of 1,272 bp encoding a polypeptide of 424 amino acids. Its calculated relative molecular mass is 48 kDa. A putative polyadenylation signal is located between +5,238 and +5,283. As Fig. 2 shows, the ORF of Clone 1 contains a stretch of the amino acid sequence highly homologous to the cyclin box of cyclin E of various other animals, with 70.6%, 67.0%, 70.6%, 65.1%, 67.0% and 68.8% sequence identity with human (Koff *et al.*, 1991), rat (EMBL Access. No. P39949), chicken (EMBL Access. No. P49707), *Xenopus* (Rempel *et al.*, 1995), zebrafish (EMBL Access. No. S52288) and *Drosophila* (EMBL Access. No. P54733), respectively.

Since the amino acid sequence of the cyclin box of the cloned ORF is very similar to the cyclin E of other animals and evolutionally well conserved, we concluded that the cDNA

clones we isolated represent a sea urchin homologue of the cyclin E gene, and designated it *HpCycE*. The amino acid sequence similarity of *HpCycE* with other types of cyclins is low (45% with mouse cyclinA and 43% with chicken cyclinA).

To estimate the copy number of the *HpCycE* gene within the *H. pulcherrimus* genome, we Southern blotted the *EcoRI*-, *SacI*- and *PstI*-digests of the sea urchin genomic DNA using DIG-labeled clone1 cDNA as the probe. As shown in Fig. 3, only a single band was detected, suggesting that *HpCycE* is present as a single copy gene in the *H. pulcherrimus* genome.

Figure 4 shows the results of the Northern blotting of the total RNA extracted from various stages of *H. pulcherrimus* embryos with DIG-labeled Clone 1 RNA as the probe. Transcripts of *HpCycE* had accumulated in unfertilized eggs, as expected from their maternal origin. The level of *HpCycE* mRNA remained almost unchanged until the morula stage, but thereafter gradually declined, indicating the possible involvement of cyclin E with rapid cell proliferation during cleavage, while no obvious zygotic expression of the *HpCycE* gene during early embryogenesis was detectable. This is similar to the case of *Drosophila* Cyclin E (Richardson *et al.*, 1993) in that cyclin E (*Dmcyce*) mRNA is supplied maternally, but is rapidly degraded after the completion of cleavage division. Studies of mammalian tissue culture cells reveal that the level of cyclin E is maximal in the late G1 phase and in the early S phase regulating the entry of cells into the S phase (Dulic *et al.*, 1992; Gong *et al.*, 1995, Ohtsubo *et al.*, 1995), while in *Drosophila* embryos, *Dmcyce* is absent in the G1 phase but appears at the onset of the S phase (Richardson *et al.*, 1995). The rapidly proliferating CNS cells that exhibit no obvious G1 stage express cyclin E constitutively (Duronio and O'Farrell, 1995). The ontogenic fluctuation of *HpCycE* mRNA observed in the present study agrees well with the results reported on *Drosophila* embryos and mammalian cells, suggesting that *HpCycE* is also related in a similar way to the cell cycle transition from G1 to S during cleavage. The constitutive presence of cyclin E in the sea urchin cleaving embryo may be related to the lack of G1 at this stage of development.

HpCycE	156:RAILLDWLIEVCEVYRLHRESFYLAADFVDRYLAAKENVPKTKLQLIGITSLFVAAKLEE	216
human	130:.....M.....K....T....Q..F..M.TQ...V..L.....S...I.....	190
rat	131:..V.....M.....K....T....Q..F..M.SQ..II..L.....SA..I.S....	191
chicken	142:..T.....M.....K.Y..T....Q..F..FM.TQQ..V..L.....S...I.....	202
<i>Xenopus</i>	144:.....M.....K....T....Q..F..FM.TQK..I.SR.....I..NV..	208
zebrafish	147:.....M.....K....T....GQ.YF..FM.TQ...L..T.....SC..I...M..	207
<i>Drosophila</i>	255:.....K....T....V..YL.....HVAHK.Q..H.....C.....V..	315
HpCycE	217:IYPPKLHEFAYVTDGACTDDQILDQELIMLMTLNWDLTPITVNTWLNAF	264
human	191:.....Q.....SG.E..TM..MIMKA.K.R.S.L.IVS...VY	238
rat	192:.....Q.....SG.E..TM..M.MKA.K.R.S.L.IVS...VY	239
chicken	203:.....Q.....E.E..SM...IMKA...N.N.L..VS...IY	250
<i>Xenopus</i>	209:.....Q.SFI.....E.E.TRM...IMKD.G.C.S.M.IVS.F.V.	252
zebrafish	208:.....V.Q.....E.D..SM.I.IMKE...S.S.L.PVA...IY	255
<i>Drosophila</i>	316:.....IG.....ERD..NH.K.L.QA.D..IS...ITG..GVY	363

Fig. 2. Comparison of the amino acid sequence of the cyclin box of *HpCycE* with those of other animals. Dots indicate identical amino acids between *HpCycE* and those of other animals.

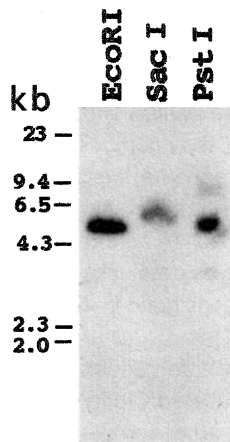


Fig. 3. Southern blot of *H. pulcherrimus* genomic DNA. Two micrograms of sperm DNA was digested with *EcoRI*, *SacI* and *PstI*. *HindIII*-digested λ DNA was used as the DNA size marker.

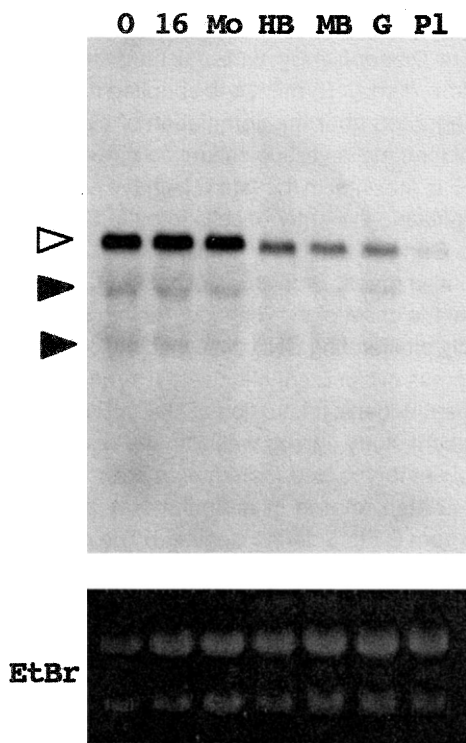


Fig. 4. Ontogenic changes in the accumulation of HpCycE mRNA. Total RNA populations were extracted from sea urchin embryos at various stages, and 10 μ g each was electrophoresed, transferred to a nylon membrane, and hybridized with the antisense RNA probe synthesized from Clone 1. The blank arrowhead indicates HpCycE mRNA, and the solid arrowheads indicate rRNAs. O, unfertilized eggs; 16, 16 cell-stage embryos; Mo, morulae; HB, hatched blastulae; MB, mesenchyme blastulae; G, gastrulae; Pl, pluteus larvae.

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