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Nucleotide Sequence of a cDNA Coding for Cyclophilin of the Sea Urchin *Hemicentrotus pulcherrimus*

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ABSTRACT—We present the nucleotide sequence of a cDNA coding for cyclophilin homologue of the sea urchin *Hemicentrotus pulcherrimus*. The 1,755-nucleotide sequence contains a 492-bp open reading frame corresponding to a translation product of 164 amino acids. Comparison of the deduced amino acid sequence with the previous data shows a high degree of conservation (~80% homology). Southern blot analysis of genomic DNA suggests the presence of a multi-gene for sea urchin cyclophilin. Northern blot analysis indicates a mRNA size of ~3 kb and that message is accumulated at blastula stage.

INTRODUCTION

Cyclophilin is an abundant cytosolic protein that has the activity of peptidyl prolyl *cis-trans* isomerase in almost all organisms (Fischer *et al.*, 1989; Handschumacher *et al.*, 1984; Takahashi *et al.*, 1989). It has been known that peptidyl prolyl *cis-trans* isomerase involves in protein folding (Fischer and Bang, 1985; Lang *et al.*, 1987). In human immune system, cyclophilin has a role of the receptor of the immunosuppressant, cyclosporin A (Handschumacher *et al.*, 1984). The cyclophilin-cyclosporin A complex interferes the production of cytokines in T-cell (Koletsky *et al.,* 1986) by inhibiting calcineurin, a calcium- and calmodulin-dependent protein phosphatase (Liu *et al.*, 1991). Complementary DNAs of cyclophilin have been isolated from various organisms and deduced amino acid sequences are highly conserved (de Martin and Philipson, 1990; Haendler *et al.*, 1987; Hasel and Sutcliffe, 1990; Stamnes *et al.*, 1991), indicating that cyclophilin is one of the essential protein for living cells. However specific function and intrinsic ligand of cyclophilin have not been determined yet. The analysis of cyclophilin in embryonic development would lead to determine physiological function of it in the cell and/or organ.

In the present study, we report cloning and analysis of a cDNA coding for cyclophilin homologue in the sea urchin *Hemicentrotus pulcherrimus*. We have also demonstrated the temporal expression of the message during early embryonic development. A remarkable accumulation of cyclophilin message was occurred during gastrulation in sea urchin development.

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MATERIALS AND METHODS

Screening of cDNA library and DNA sequencing

A cDNA library was constructed in λgt11 from poly(A)$^+$ RNA isolated from early pluteus larva of the sea urchin *H. pulcherrimus*. Filters containing 20,000 plaques of the library were screened with the mouse cyclophilin homologue (Ohta, K., unpublished data). Single positive clone was isolated and subcloned into pUC119 vector for further analysis.

Nucleotide sequences were determined by the dideoxy chain termination method (Sanger *et al.*, 1977) with the Sequenase Kit (United States Biochemical Co.) using [α-^{35}S] dATP.

Southern blot analysis

Restricted digests of genomic DNA isolated from testis of mature sea urchin were fractionated on a 0.8% agarose gel, transferred overnight to a Hybond-N+ membrane (Amersham International plc). The cDNA insert, HPCyp-1, was labeled with [α-^{32}P] dCTP to specific activities of approximately 10^9 cpm/μg. The filter was prehybridized at 65°C in 5×Denhardt's solution, 0.5% SDS, 0.9 M NaCl, 0.05 M NaH_2PO_4, 0.005 M EDTA, pH 7.5 and 100 μg/ml denatured salmon sperm DNA. Then it was hybridized overnight in the same solution to radiolabeled probe. The filter was subsequently washed at 65°C in 0.1 ×SSC and 0.1% SDS.

Northern blot analysis

Total RNA was isolated from unfertilized eggs and embryos of the sea urchin (Chomczynski and Sacchi, 1987), separated by agarose gel electrophoresis containing formaldehyde and transferred to a Hybond-N+ membrane. HPCyp-1 was labeled with [α-^{32}P] dCTP and used as a probe. Hybridization was performed with the same procedure as in southern blot analysis except for use of 1 mg/ml of yeast tRNA instead of denatured salmon sperm DNA. Final washing was done with 0.2×SSC and 0.1% SDS at 65°C.

RESULTS AND DISCUSSION

A cDNA of sea urchin *H. pulcherrimus* was isolated from λ phage cDNA library constructed from early pluteus larva poly(A)$^+$ RNA using a mouse cyclophilin homologue as a probe. The nucleotide sequence of the cDNA designated HPCyp-1 was determined by the dideoxy chain termination method as shown in Figure 1. The cDNA is 1,775 bp in

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      5'- TCAAAATTGCTCTTTTTGCTGTGTTTCGACGTCAGTTTGCAACTGTCAGT   -1
ATGGCTAAACCTCAAGTTTTCTTCGACCTTCAAGCCAATGGCGAGAATCTTGGAAGAATA   60
 M  A  K  P  Q  V  F  F  D  L  Q  A  N  G  E  N  L  G  R  I    20
GTTATGGAGCTTAGGGCCGATGTAGTTCCCAAGACTGCTGAGAACTTCCGTGCCCTGTGC  120
 V  M  E  L  R  A  D  V  V  P  K  T  A  E  N  F  R  A  L  C    40
ACTGGGGAGAAGGGCTTCGGCTACAAGGGATCTACTTTCCATCGTGTCATCCCAGGGTTC  180
 T  G  E  K  G  F  G  Y  K  G  S  T  F  H  R  V  I  P  G  F    60
ATGTGCCAAGGCGGAGACTTCACTAGGCACAACGGCACTGGTGGAAAAAGCATCTACGGA  240
 M  C  Q  G  G  D  F  T  R  H  N  G  T  G  G  K  S  I  Y  G    80
GAGAAGTTTGCTGATGAGAACTTCACTCTGAAGCACACTCAACCAGGAATCCTGTCAATG  300
 E  K  F  A  D  E  N  F  T  L  K  H  T  Q  P  G  I  L  S  M   100
GCCAACGCTGGAGTCAACACCAATGGATCTCAATTTTTCATCTGCACAGCAGTGACCTCT  360
 A  N  A  G  V  N  T  N  G  S  Q  F  F  I  C  T  A  V  T  S   120
TGGCTCGATGGAAAGCATGTAGTCTTTGGCGCAGTGACTCAAGGCCTTGACATCATTAAG  420
 W  L  D  G  K  H  V  V  F  G  A  V  T  Q  G  L  D  I  I  K   140
AAGGTTGAGAGTTATGGAGCGACAGCGGCAAAACCAGTAAGAAGATCACGATTGCCGAC  480
 K  V  E  S  Y  G  S  D  S  G  K  T  S  K  K  I  T  I  A  D   160
TGTGGCCAGCTGTAAATCAACGAAATTCAAAATATTGGTCTAGTCTAATATCATCAGACC  540
 C  G  Q  L  *                                                 164
TATTTGTTAAGTTTTATCTCATGTAGTAGCAGCATGTGATGTTGAATATACTGGTTTTGT  600
ACAAGATGGTTCTCTTCTGATTTTTTTTTTTTAATTGTGTAGATGTGTTGAATAACTTGTT  660
GAACCCGCAGTTGAAATGAGTAAAAATTTGTATTTCAAAATAGTCTGGTTGCATCAGATA  720
GATAGAGAAGTACTATGCATATGGTAAAAAATAATCCTTGGTCCCAATGTAATTTTGATA  780
AGAAATGATCAGGAAGCAGATGGTGTTTGAGATCGCATAGTGATATGCTTTCTTTGTGCG  840
TCACATTAATGTCATTGAGTTTCCATTTCTATTCAAGGCAGCACACCAAGCCGATCAACC  900
ATTTCTTCACTTAATGCTTTTTATACTTCGTACATATTATGAATACATGTATCTATAATG  960
GTTAGAACTGCTACGTTGGTCTAAAGCCACTTTTTCAGATTCTTCACTCTTATCACTGTC 1020
CTGCACTCTTCTCATTACAACACAAACACTGGCCCTTCTGCTCTCCAAAGAGAGATGGGC 1080
CCATTTTGGCACCTTTCTTTAAATATAGACTGGAAGTAGTTGTGGTAAATAAAAGATAAT 1140
GTTCTGAATCAAGATCCTAGACCGATTCCAAAATACAAAAACGGATAAGAAATTGAGCAT 1200
CTTTGTGCAGCGTAGATTGTATCTGTGCGTCTCTAGTATGGAGCAGAATTTCATGTTGTG 1260
TAGAATATATATTCCATTATATTAATTGGAGAATACTTGAATTTCCTTTTTGGGGGAGGG 1320
GGGTCATTTGCCTTCTGTGAAGGTGAGTGTAAATACAGATCATTTTTTTTCTAGCTGAGA 1380
TGGTGGGGCATCTGTTTACCCAGCGACAATCATTCATGTGAGTTGTGAAATGCAATTTCT 1440
GCTCATAATTCATAAGCAGTTCATGTTTATTTTCTGAGATTCTTATTTGTATAATTTGGA 1500
TAAAGATTTGGTTTGACACTTTGACAGCTTAGCAAGTCAGTGGGATTTTGAGACCTTTTT 1560
TTCATATGGTTCAACCAGAATATTGTGAAATCGATCATGATCTTTGAGAACAATACCTGA 1620
AGCTGTTTTCCCTTAAATGTTTTTAAACAATTGGCATGCAGATTGTCTTGTTGACATAAG 1680
TGAAGAAATCAATATTTTTTCTTTA -3'                                1705
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Fig. 1. Nucleotide and predicted amino acid sequences of sea urchin *Hemicentrotus pulcherrimus* cyclophilin. The complete sequence ot the HPCyp-1 cloned using the mouse cyclophilin homologue as a probe is indicated. The consensus signal for polyadenylation is underlined.

length. Fifty base pairs of 5'-untranslated region are followed by a start codon ATG lying in a favorable context for a translation initiation according to the Kozak's criteria (Kozak, 1981). An open reading frame of 492 bp codes for 164 amino acids to yield a protein of estimated molecular weight of 17,677. A stop codon TAA occurs at position 493. The non-coding region of 3'-end of the cDNA is composed 1,210 bp including polyadenylation site (AATAAA) (Proudfoot, 1991). But poly(A)$^+$ sequence is not contained downstream of this polyadenylation signal in the clone. Therefore this polyadenylation signal in the HPCyp-1 sequence we cloned may be not functional for polyadenylation.

The deduced amino acid sequence shows a high degree of homology to cyclophilin family except for the N- and C-terminal 20 to 30 amino acids (Fig. 2). HPCyp-1 product has 78.7%, 77.4%, 80.1% and 75.3% homology to human T-cell cyclophilin (Haendler *et al.*, 1987), mouse

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                     10        20        30        40        50
Hp-CYP   .MAKPQVFFDLQANGENLGRIVMELRADVVPKTAENFRALCTGEKGFGYKG
Hu-CYP   .-VN-T----IAVD--P---VSF--F--K-----------S----------
Mo-CYP   .-VN-T----IT-DD-P---VSF--F--K-----------S----------
Ff-CYP   MSTL-R----MT-DN-P----------S-----------------------
Ye-CYP   ...MSNC---VI---QP-----FK-FD--------A----------Y--A-

         60        70        80        90        100       110
STFHRVIPGFMCQGGDFTRHNGTGGKSIYGEKFADENFTLKHTQPGILSMANAGVNTNGS
-C---I---------------------------E----I----G----------P-----
-S---I-------------------R-------E----I----G----------P-----
-I------N---------N-----------N--P----E----GS---------A-----
--------Q--L-------G-------------P----A---NK--L-------P-----

         120       130       140       150       160
QFFICTAVTSWLDGKHVVFGAVTQGLDIIKKVESYGSDSGKTSKKITIADCGQL.
-------K-E----------K-KE-MN-VEAM-RF--RN---------------E
-------K-E----------K-KE-MN-VEAM-RF--RN---------S-----.
------VK-A---N------E-VE---VV--I-----Q--------IV-NS-S-.
----T-V--P----------E--E-M-VV-----L--N--A-RAR-V-DK--TV.
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Fig. 2. Comparison of amino acid sequences of *H. pulcherrimus* cyclophilin (Hp-CYP), human T-cell cyclophilin (Hu-CYP), mouse cyclophilin (Mo-CYP), *Drosophila* cyclophilin-1 (Ff-CYP) and *Schizosaccharomyces pombe* cyclophilin (Ye-CYP). Numbering of the amino acids starts with the Met of the Hp-CYP. Dash indicates the same amino acid residue as Hp-CYP and dot indicates sequence gap introduced for maximum homology.

cyclophilin (Hasel and Sutcliffe, 1990), *Drosophila* cyclophilin-1 (Stamnes *et al.*, 1991) and *Schizosaccharomyces pombe* cyclophilin (de Martin and Philipson, 1990), respectively. Comparison with the sequence deduced from a *H. pulcherrimus* cyclophilin cDNA, HPCyp-1, shows that the three consensus sequences, NGTGGKSIYG, LSMANAGPNTNGSQFF and WLDGKHVVFG (Koser *et al.*, 1990) are conserved in the protein except the discrepancy in the second sequence; Pro-125 is replaced with Val in *H. pulcherrimus* protein. The single conserved tryptophan and the four cysteine residues are found in the predicted amino acid sequence.

In order to determine the different sequences that code for cyclophilin of *H. pulcherrimus*, genomic southern analysis was done under the high-stringency condition using the insert of the clone as a probe. Several bands were revealed in the blot (Fig. 3a). This result suggests the presence of additional sequences homologous to HPCyp-1. Same result was obtained using the probe derived from the *Eco*RI-*Pvu*II fragment of the cDNA insert (data not shown). Because this fragment contains part of the complete coding region and 50 bp of 5'-untranslated region, the hybridization pattern actually represents cyclophilin sequence. In this study, it is unknown whether these sequences reflect functional genes or pseudogenes.

Northern blot analysis was performed under the high-stringency condition using HPCyp-1 as a probe. The embryonic stages used for the assay were unfertilized egg, early blastula, mesenchyme blastula, mid-gastrula and prism larva. A band of ~3,000 nucleotides is revealed (Fig. 3b). The mRNA is not identified in unfertilized eggs and cleavage stage embryos. The cyclophilin message is originally accumulated at early blastula stage and then the expression level of mRNA is slightly increased. Thereafter a remarkable accumulation of the message is identified during gastrulation in *H. pulcherrimus* embryos.

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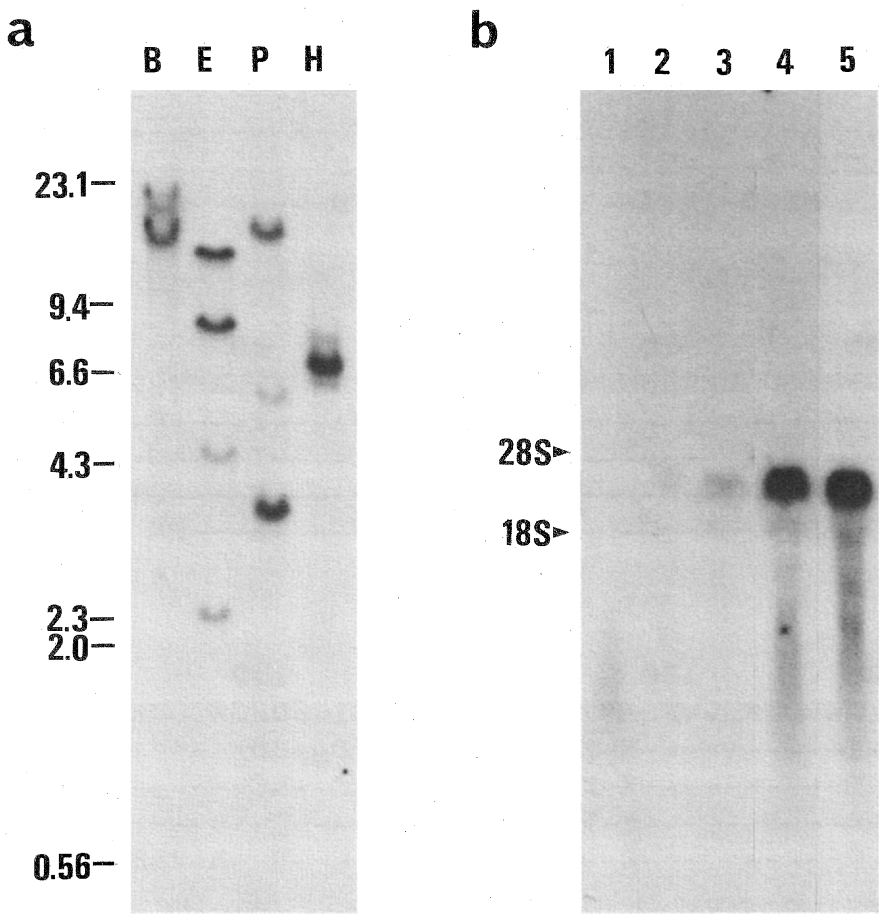

Fig. 3. (a) Genomic Southern blot analysis with a cDNA clone coding for sea urchin cyclophilin homologue. Genomic DNA was isolated from an adult *H. pulcherrimus* and 10 μg of DNA were digested with *Bam*HI (B), *Eco*RI (E), *Pst*I (P) and *Hin*dIII (H). The DNA subjected to electrophoresis, transferred to a nylon membrane. The blots were hybridized to HPCyp-1. (b) Accumulation of the sea urchin cyclophilin message during embryonic development. Total RNA was isolated form unfertilized eggs (1), early blastula (2), mesenchyme blastula (3), mid-gastrula (4) and prism larva (5). Twenty micrograms of total RNA were subjected for electrophoresis and transferred to a nylon membrane. The blots were hybridized to HPCyp-1.

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