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Molecular cloning of cDNA coding for rat proliferating cell nuclear antigen (PCNA)/cyclin

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The 'proliferating cell nuclear antigen' (PCNA), also known as cyclin, appears at the G_1/S boundary in the cell cycle. Because of its possible relationship with cell proliferation, PCNA/cyclin has been receiving attention. PCNA/cyclin is a non-histone acidic nuclear protein with an apparent mol. wt of 33 000–36 000. The amino acid composition and the sequence of the first 25 amino acids of rabbit PCNA/cyclin are known. Using an oligonucleotide probe corresponding to the sequence of the first five amino acids, a cDNA clone for PCNA/cyclin was isolated from rat thymocyte cDNA library. The cDNA (1195 bases) contains an open reading frame of 813 nucleotides coding for 261 amino acids. The 3'-non-coding region is 312 nucleotides long and contains three putative polyadenylation signals. The mol. wt of rat PCNA/cyclin was calculated to be 28 748. The deduced amino acid sequence and composition of rat PCNA/cyclin are in excellent agreement with the published data. Using the cDNA probe, two species of mRNA (1.1 and 0.98 kb) were detected in rat thymocyte RNA. Southern blot analysis of total human genomic DNA suggests that there is a single gene coding for PCNA/cyclin. The deduced amino acid sequence of rat PCNA/cyclin has a similarity with that of herpes simplex virus type-1 DNA binding protein.

Key words: Cell cycle/DNA binding protein/DNA synthesis/systemic lupus erythematosus

Introduction

The 'proliferating cell nuclear antigen' (PCNA) (Miyachi *et al.*, 1978), also known as cyclin (Bravo *et al.*, 1981; Mathews *et al.*, 1984), which appears in proliferating cells was first recognized by sera drawn from certain patients with systemic lupus erythematosus (Miyachi *et al.*, 1978). Immunofluorescence studies using these anti-PCNA autoantibodies revealed the PCNA/cyclin in a speckled pattern in the nucleus during the early S phase of the cell cycle, as a punctate pattern with foci throughout the nucleus in the late S phase, near the nuclear membrane at the time of maximum DNA synthesis, and again as a punctate pattern at the S/G_2 phase (Takasaki *et al.*, 1981; Celis and Celis, 1985a; Bravo and Macdonald-Bravo, 1985). Furthermore, these immunofluorescence patterns are remarkably similar to those observed in the autoradiograms of [^{3}H]thymidine-labeled nuclei (Celis and Celis, 1985b). Recent studies using DNA synthesis inhibitors such as hydroxyurea (Bravo and Macdonald-Bravo, 1985) and aphidicolin (Macdonald-Bravo and Bravo, 1985) demonstrated that the synthesis of PCNA/cyclin occurs immediately before DNA replication. These observations have led to suggestions that the changes in nuclear distribution of PCNA/cyclin are controlled by DNA synthesis itself or events triggered by DNA replication, that the level of synthesis of PCNA/cyclin is tightly associated with the cell cycle, and that PCNA/cyclin may regulate a pathway necessary for cell proliferation (Takasaki *et al.*, 1981; Celis and Celis, 1985a; Bravo and Macdonald-Bravo, 1985).

PCNA/cyclin is an acidic non-histone nuclear protein with an apparent mol. wt of 33 000–36 000 as determined by SDS–PAGE (Bravo *et al.*, 1981; Mathews *et al.*, 1984; Takasaki *et al.*, 1984). Recently the amino acid composition and the sequence of the first 25 amino acids from the N-terminus of rabbit PCNA were determined (Ogata *et al.*, 1985). Based upon this sequence, as a first step to unravel the relation between the primary structure and the function(s) of PCNA/cyclin, we determined the nucleotide sequence of rat PCNA/cyclin, predicted its complete amino acid sequence and compared the sequence to other DNA binding proteins.

Results

Isolation of cDNA clones

Out of 10 000 colonies screened for PCNA/cyclin-specific sequence, four hybridized with the probe. One of these clones, which hybridized strongest, pCR-1, was subjected to sequence analysis. The restriction map and sequence strategy are shown in Figure 1.

Nucleotide and predicted amino acid sequence of pCR-1 cDNA

The nucleotide and predicted amino acid sequence is presented in Figure 2. The cDNA insert of pCR-1 is 1195 bp long and contains an open reading frame of 813 nucleotides (nucleotides −30 to 783) with the first in-frame methionine being encoded by nucleotides 1–3 as part of Kozak's consensus sequence, −CCACCATG− (Kozak, 1984). Initiation of translation at nucleotide 1 generates an open reading frame of 783 nucleotides

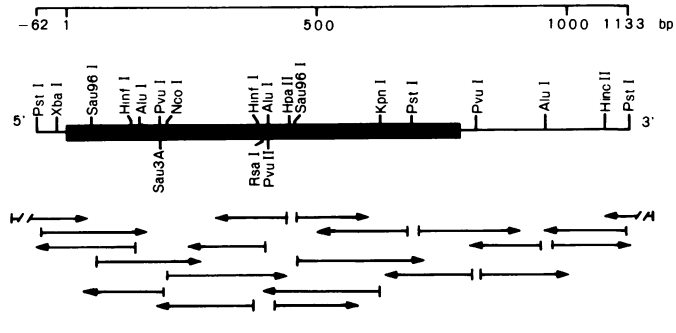

Fig. 1. Restriction map and sequencing strategy for clone pCR-1. Only the restriction sites used for sequence analysis are shown. Horizontal arrows indicate the direction and length of each fragment sequenced. Nucleotides are numbered in the 5' to 3' direction, beginning with the first residue of the initiator codon ATG. The nucleotides on the 5' side of residue 1 are indicated by negative numbers. The protein-coding region of the mRNA is indicated by the thick line. '//' indicates that the labeled end was located on the vector DNA.

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                                                                    TTGTCAGCAAGACCTCGCTCCCCTTACAGTAACTCTCATCTAGACGTCGCAACTCCGCCACC   -1

Met Phe Glu Ala Arg Leu Ile Gln Gly Ser Ile Leu Lys Lys Val Leu Glu Ala Leu Lys Asp Leu Ile Asn Glu    25
ATG TTT GAG GCA CGC CTG ATC CAG GGC TCC ATC CTG AAG AAG GTG CTG GAG GCC CTC AAA GAC CTC ATC AAT GAG    75

Ala Cys Trp Asp Ile Ser Ser Gly Gly Val Asn Leu Gln Ser Met Asp Ser Ser His Val Ser Leu Val Gln Leu    50
GCC TGC TGG GAC ATC AGT TCG GGC GGC GTG AAC CTA CAG AGC ATG GAT TCG TCT CAC GTC TCC TTA GTG CAG CTT   150

Thr Leu Arg Ser Glu Gly Phe Asp Thr Tyr Arg Cys Asp Arg Asn Leu Ala Met Gly Val Asn Leu Thr Ser Met    75
ACT CTG CGC TCC GAA GGC TTC GAC ACA TAC CGC TGC GAT CGC AAC CTG GCC ATG GGC GTG AAC CTC ACC AGC ATG   225

Ser Lys Ile Leu Lys Cys Ala Gly Asn Glu Asp Ile Ile Thr Leu Arg Ala Glu Asp Asn Ala Asp Thr Leu Ala   100
TCC AAA ATT CTA AAA TGT GCT GGT AAT GAA GAC ATC ATT ACA TTA AGG GCT GAA GAT AAT GCT GAT ACC TTA GCA   300

Leu Val Phe Glu Ala Pro Asn Gln Glu Lys Val Ser Asp Tyr Glu Met Lys Leu Met Asp Leu Asp Val Glu Gln   125
CTA GTA TTT GAA GCA CCA AAT CAA GAG AAA GTT TCA GAC TAT GAG ATG AAG TTA ATG GAC TTA GAC GTT GAG CAA   375

Leu Gly Ile Pro Glu Gln Glu Tyr Ser Cys Val Val Lys Met Pro Ser Gly Glu Phe Ala Arg Ile Cys Arg Asp   150
CTT GGA ATC CCA GAA CAG GAG TAC AGC TGC GTA GTA AAG ATG CCA TCT GGT GAA TTT GCA CGT ATA TGC CGG GAC   450

Leu Ser His Ile Gly Asp Ala Val Val Ile Ser Cys Ala Lys Asp Gly Val Lys Phe Ser Ala Ser Gly Glu Leu   175
CTT AGC CAT ATT GGA GAT GCT GTG GTG ATC TCC TGT GCA AAG GAC GGG GTG AAG TTT TCT GCG AGT GGG GAG CTT   525

Gly Asn Gly Asn Ile Lys Leu Ser Gln Thr Ser Asn Val Asp Lys Glu Glu Glu Ala Val Ser Ile Glu Met Asn   200
GGC AAT GGG AAC ATT AAG TTG TCC CAG ACA AGC AAT GTT GAT AAA GAA GAG GAA GCT GTG TCC ATA GAG ATG AAT   600

Glu Pro Val Gln Leu Thr Phe Ala Leu Arg Tyr Leu Asn Phe Phe Thr Lys Ala Thr Pro Leu Ser Pro Thr Val   225
GAG CCA GTT CAG CTA ACT TTT GCT CTG AGG TAC CTG AAC TTT TTC ACA AAA GCC ACT CCA CTG TCT CCT ACA GTA   675

Thr Leu Ser Met Ser Ala Asp Val Pro Leu Val Val Glu Tyr Lys Ile Ala Asp Met Gly His Leu Lys Tyr Tyr   250
ACA CTC AGT ATG TCT GCA GAT GTA CCC CTT GTT GTA GAG TAT AAA ATT GCT GAC ATG GGA CAC TTA AAG TAT TAT   750

Leu Ala Pro Lys Ile Glu Asp Glu Glu Gly Ser  *
TTG GCT CCC AAG ATC GAA GAT GAA GAA GGA TCT TAG GCGTTGCTAG AAATTGAGAA ACTAAACCTT TGACGATCGC TTCTGAG   833

ATG CCAGCGTGTT CTGAGGTCTT TTCTGTCACC AAGTTTGTAC CTGAGTATTC TTAAATATTA AAATAAAATG TAGATATCTT CTGTAAA   923

TAA CCTTTTTTTT CCTCTCCATA ATTTGCTTAC AGAATAAGCT CCAAAGTAAA AACTGGTTTT GTTAACATAA ATGTCTCTGC CTTACAA  1013

ATA CTGGTGATTT TCATAAATGA TCTTGACGCT AAATGCAGTT TTAAGAAATA TTTTTCAATT TAAATAAAGT TAACTGAATT TGA35    1133
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Fig. 2. Nucleotide and predicted amino acid sequence of pCR-1. The nucleotide sequence of the coding strand and the predicted amino acid sequence are shown. Nucleotides are numbered in the 5′ to 3′ direction, beginning with the first residue of the initiator codon ATG. Nucleotides on the 5′ side of residue 1 are designated by negative numbers. Amino acids are numbered from the initiator 'Met'. The numbers of the last amino acid and the last nucleotide in each lines are indicated on the right. Polyadenylation signals in the 3′ non-coding region are underlined. The termination codon, TAG, at nucleotide position 784–786 is indicated by '*'.

Table I. Amino acid compositions of rabbit and rat PCNA/cyclin

	Rabbit	Rat		Rabbit	Rat		Rabbit	Rat
Ala	8.0	7.3	Asp	12.0	6.9	Thr	3.7	4.2
Val	8.3	7.7	Asn		4.6	Ser	5.8	9.2
Met	3.7	3.8	Glu	13.0	8.8	Pro	3.7	3.1
Ile	5.5	5.7	Gln		3.1	Gly	7.7	5.7
Leu	12.0	11.1	His	1.8	1.2	Cys	ND	2.3
Tyr	3.1	2.7	Arg	3.7	3.1	Trp	–	0.4
Phe	3.7	3.1	Lys	3.7	6.1			

For rabbit PCNA (Ogata *et al.*, 1985), the sum of Asp and Asn, and the sum of Glu and Gln were indicated, respectively. The number of amino acids is expressed as the mean number of each amino acid residue per 100 residues. ND and – stand for not detected and not analysed, respectively.

coding for 261 amino acids. The mol. wt of rat PCNA/cyclin was calculated to be 28 748. The 3′-untranslated region is 312 nucleotides long and contains three putative polyadenylation signals at nucleotides 893–898 (ATTAAA), 898–903 (AATAAA) and 1079–1084 (AATAAA). The pCR-1 clone utilized the third signal. Using the cDNA insert as a hybridization probe, two species of mRNA (1.1 and 0.98 kb in size) were detected by Northern blot analysis of rat thymus RNA (Figure 3A), suggesting that one of the first two signals is utilized for the shorter mRNA. The predicted sequence of the first 25 amino acids of pCR-1 matched with the corresponding sequence of rabbit PCNA reported previously (Ogata *et al.*, 1985), except at residue 7. At residue 7, valine in the rabbit protein sequence was replaced by isoleucine in the rat protein sequence. The amino acid composition of the rabbit PCNA (Ogata *et al.*, 1985) and that of the rat agreed well for most amino acids with exception of lysine and serine (Table I).

Southern blot analysis

To estimate the numbers of genes for PCNA/cyclin in rat and human genomes, high mol. wt DNAs from rat liver and human thymocytes were digested with several kinds of restriction enzymes and analyzed by Southern blotting. The nick-translated entire cDNA insert of pCR-1 clone was used as a hybridization probe (see Materials and methods). In *Bam*HI, *Bgl*II, *Hin*dIII and *Pvu*II digests of human genomic DNA, single major bands were detected (Figure 3B and C), suggesting that there is one copy of PCNA/cyclin gene per haploid genome. Single major bands were also observed in rat genomic DNA digested with *Bam*HI, *Ava*II and *Eco*RI (Figure 3B and C). However, several bands were seen with *Bgl*II and *Hin*dIII digests, although the pCR-1 cDNA insert has no recognition sites for these enzymes (Figure 3B). *Bgl*II digestion generated two bands of 17 and 7.5 kb. *Hin*dIII digest gave three bands of 8, 4.6 and 2.7 kb. In longer exposures additional faint band(s) were occasionally seen with both human and rat DNA digested with most of the enzymes used.

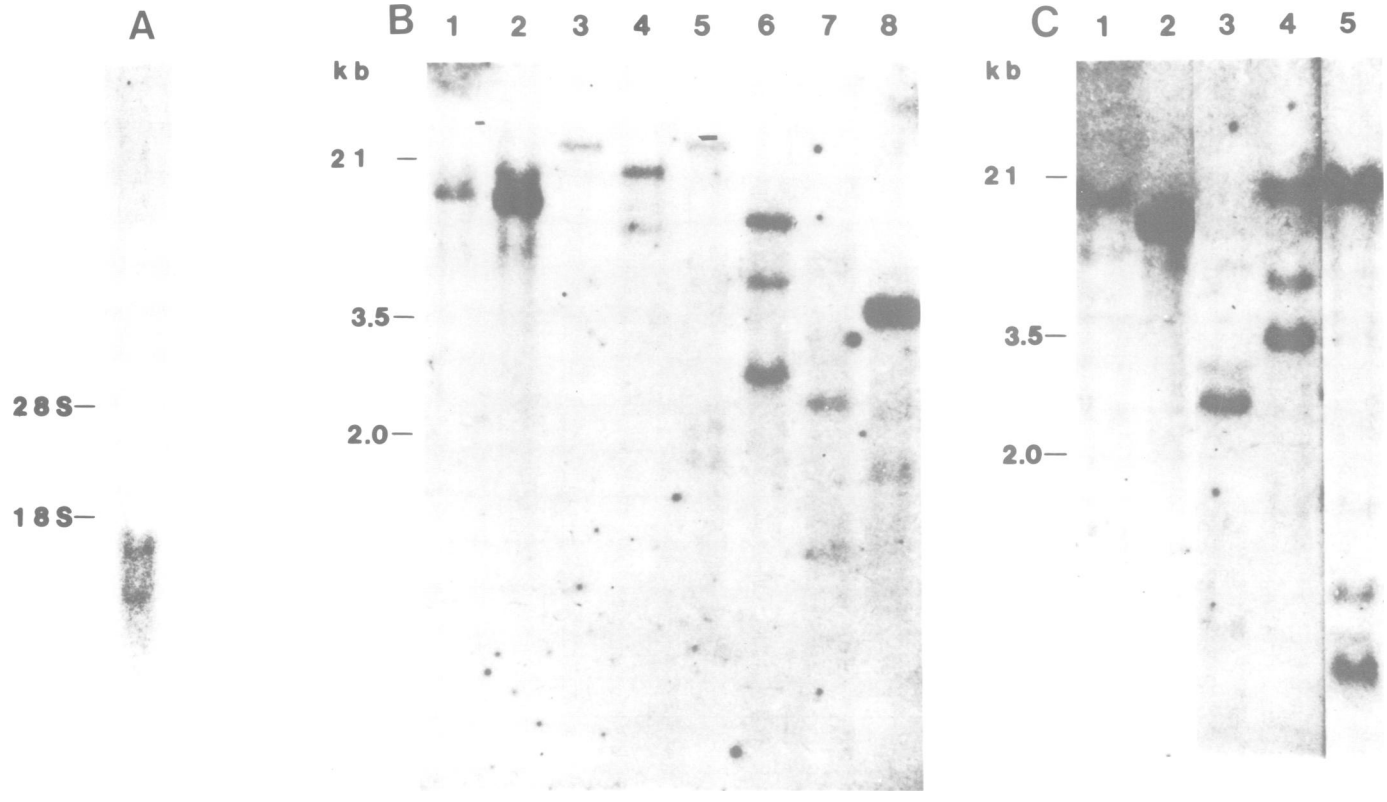

Fig. 3. Blot analysis. (**A**) Northern blot analysis of rat thymocyte PCNA/cyclin mRNA. Blotting was carried out as described in the test. (**B**, **C**) Southern blot analysis of restriction endonuclease-digested high mol. wt DNA prepared from human thymocytes and rat liver. Blottings were carried out as described in the text. **Panel B: lane 1**, human DNA digested by *Bam*HI; **lane 2**, rat DNA digested by *Bam*HI; **lane 3**, human DNA digested by *Bgl*II; **lane 4**, rat DNA digested by *Bgl*II; **lane 5**, human DNA digested by *Hin*dIII; **lane 6**, rat DNA digested by *Hin*dIII; **lane 7**, human DNA digested by *Ava*II; **lane 8**, rat DNA digested by *Ava*II. **Panel C: lane 1**, human DNA digested by *Eco*RI; **lane 2**, rat DNA digested by *Eco*RI; **lane 3**, human DNA digested by *Pvu*II; **lane 4**, rat DNA digested by *Pvu*II; **lane 5**, rat DNA digested by *Xba*I.

Relationship between PCNA/cyclin mRNA induction and the cell cycle in PHA-stimulated lymphocytes

At various time points after PHA stimulation of human peripheral lymphocytes, DNA synthesis, mitotic index (Figure 4A) and the level of PCNA/cyclin mRNA (Figure 4B) were determined. DNA synthesis (as determined by [^{3}H]thymidine incorporation) began to increase 24 h after the onset of PHA stimulation, reached a maximum by 48 h and slightly decreased at 72 h. The mitotic index started to increase at 48 h after the PHA stimulation. These results indicate that majority of cells at 48 h were in the S phase of the cell cycle and at 72 hours entered into the M phase. When the level of PCNA/cyclin mRNA was examined by Northern blot analysis using total RNA from parallel cultures at 0, 48 and 72 h after the onset of PHA stimulation, the PCNA/cyclin mRNA was undetectable at 0 h, but the amount increased at 48 h and decreased at 72 h.

Computer search for related proteins

The nucleotide and deduced amino acid sequence of PCNA/cyclin were compared with those of other proteins which localize to the nucleus. A computer search did not identify genes or proteins which have strong homologies with PCNA/cyclin, although there were some similarities with herpes simplex virus type 1 (HSV-1) DNA binding protein, ICP8, (Quinn and McGeoch, 1985) and with adenovirus type 5 (ad5) E1A protein (Perricaudet *et al.*, 1979). The homology between PCNA/cyclin and HSV-1 DNA binding protein is more extensive than that between PCNA/cyclin and ad5 E1A protein. To achieve the alignment between PCNA/cyclin and ad5 E1A as illustrated in Figure 5, it was necessary to insert a relatively large gap and several small gaps. At the level of nucleotide sequences, there were many more identities between the N-terminal portion of PCNA/cyclin and the HSV-1 DNA binding protein than expected on the basis of amino acid similarities; indeed, 116 out of 270 nucleotides (43%) were identical (Figure 6).

Discussion

The cDNA sequence reported here is judged to encode rat PCNA/cyclin, since the sequence of the first 26 amino acids deduced from the cDNA (MFEARLIQGSILKKVLEALKDLINEA) fits almost exactly with the sequence of the first 25 amino acids (MFEARLVQG?ILKKVLEALKDLINEA) of rabbit PCNA, the amino acid composition derived from the nucleotide sequence also agreed well with the reported amino acid composition of rabbit PCNA (Ogata *et al.*, 1985), and mRNA hybridizable with pCR-1 cDNA was induced at S phase of the cell cycle. The only difference between the sequence of the first 25 amino acids of PCNA/cyclin of rat and that of rabbit was at residue 7, rat PCNA/cyclin contained isoleucine and rabbit PCNA/cyclin contained valine. With regard to the amino acid composition, the rat PCNA/cyclin contained more lysine and serine than rabbit PCNA/cyclin.

In the Northern blot analysis, the pCR-1 probe hybridized with mRNAs of the approximate size of 1.1 and 0.98 kb. Since the PCNA/cyclin cDNA is 1.195 kb, the pCR-1 clone should cover

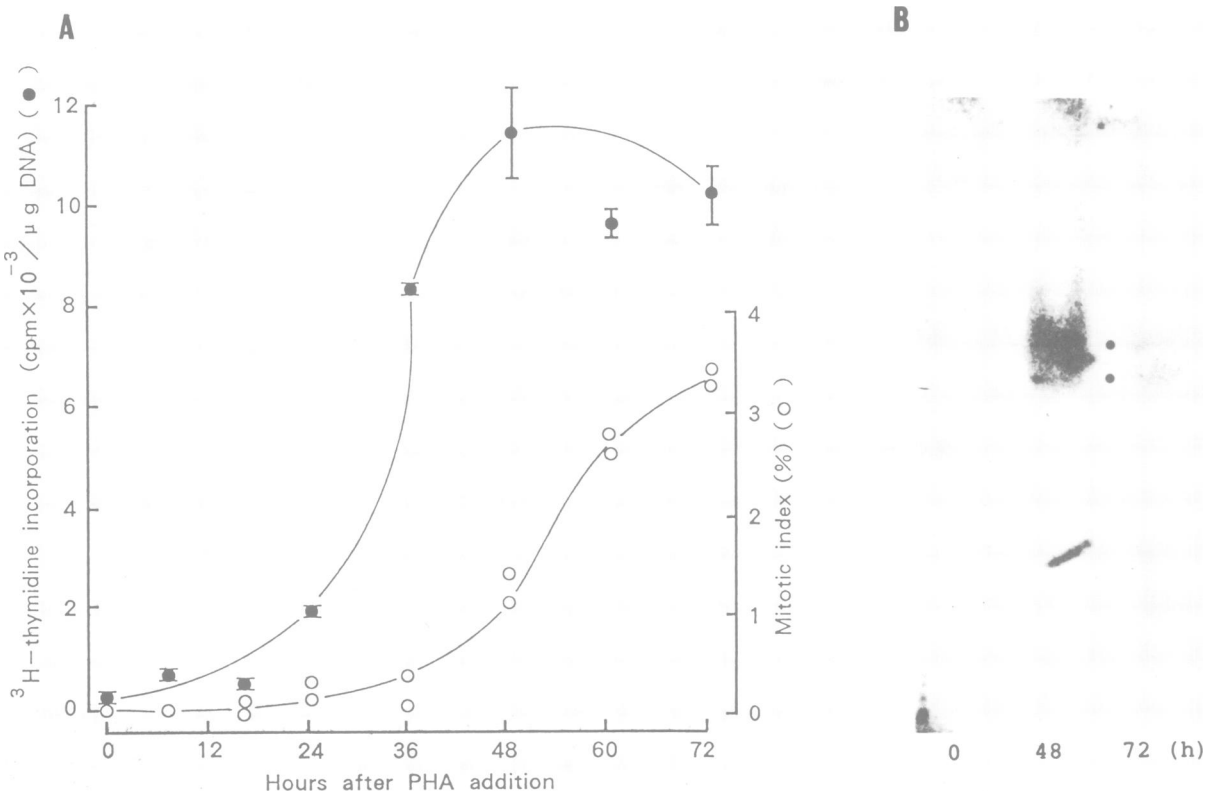

Fig. 4. Relationship between the appearance of PCNA/cyclin mRNA and the cell cycle in PHA-stimulated lymphocytes. (**A**) Time course of the rate of DNA synthesis and mitotic activity. DNA synthesis was determined by pulse-labeling (1 h) with [^{3}H]thymidine as described in Materials and methods. Each point (closed circles) represents mean ± SD from quadruple determinations. Mitotic activity was determined as mitotic index by counting mitotic figures after Feulgen staining of PHA-stimulated cells, as detailed in Materials and methods. Each point (open circles) represents the value calculated from each cytospin preparation by scoring >500 cells. (**B**) Levels of PCNA/cyclin transcripts during PHA stimulation. Total RNA was prepared from lymphocytes at 0, 48 and 72 h after PHA stimulation. Fifteen micrograms of total RNA from each sample were analysed by Northern blot hybridization with ^{32}P-labeled PCR-1 cDNA probe.

most, if not all, of the PCNA/cyclin mRNA sequence. The 5′ end of the cDNA contains a 62-bp non-coding region. This is followed by an active start codon in eukaryotic mRNA, the methionine codon included in the Kozak's consensus sequence (Kozak, 1984). From the initiation codon, ATG (nucleotides 1−3), to the termination codon, TAG (nucleotides 784−786), there were 783 bp of an open reading frame with a capacity to encode 261 amino acids. When the open reading frame was converted to amino acid sequence, the mol. wt was calculated to be 28.7 kd. This is less than the mol. wt of 33 and 36 kd estimated by SDS−PAGE and immunoblotting (Bravo *et al.*, 1981; Mathews *et al.*, 1984; Takasaki *et al.*, 1984). Since the mol. wt of PCNA/cyclin synthesized in the *in vitro* cell-free translation system is same as that of PCNA/cyclin isolated from cells (Sadaie and Mathews, 1986), the post-translational modification cannot account for the discrepancy in the mol. wts. One possible explanation for the discrepancy is that PCNA/cyclin migrates abnormally slowly in SDS−PAGE and the mol. wt may have been overestimated. Occurance of this phenomenon has been observed with several other proteins such as adenovirus E1A and c-*myc* protein (Smart *et al.*, 1981; Gingeras *et al.*, 1982; Persson *et al.*, 1984). The other possibility is that PCNA/cyclin differs slightly between species, since a difference was noted in the amino acid residue 7 between the rat and the rabbit.

Southern hybridization analysis of total human genomic DNA indicates that there is a single gene for PCNA/cyclin. With the rat, however, the interpretation of the Southern hybridization patterns is somewhat complicated. A single band was identified in

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ad5-E1A   MRHIICHGGVITEEMAASLLDQLIEEVLAD-NLPPPSHFEPPTLHELYDLDVTAPEDPNEEAVS
PCNA      MFEARLIQGSILKKVLEA-LKDLINEACWDISSGGVNLQSMDSSH-VSLVQLTLRSEGFDT---
HSV-DBP   VAVAPLVVGLTVESGFEANVAVVVGSRTTGLGGTAVSLK-LTPSHYSSSVYV------------

ad5-E1A   QIFPDSVMLAV-QEGIDLLTF--PP--APGSPEPPHLSRQPEQPEQRALGPVSMPNLVPEVIDL
PCNA      -YRCDR-NLAM---GVNLTSMSKILKCA-GNEDIITL-RAEDNADTLAL-VFEAPN--------
HSV-DBP   -FHGGR-HLDPSTQAPNLTRLCERARRHFGFSDY-TP-RPGDLKHETTG---EALC---------

ad5-E1A   TCHEAGFPPSDD-EDEEGPVSEPEPEPE--PEPEPARPTRRPKMAPA-ILRRPTSPVSRECNSS
PCNA      --------QEKV-SDYEMKLMDLDVEQLGIPEQEYSCVVKMPSGEFARI-CRDLSHIGDA---V
HSV-DBP   ---------ERLGLDPDRALLYLVVTE-GFKE-A-VCI----NNTFLHLGGSDKVTIGGA---E

ad5-E1A   TDSCDSGPSNTPPE-IHPVVPLCPIKPVAVR-VGGRRQAVECIEDLLNEPGQPLDLSCKRPRP
PCNA      VISC-AKDGVK-FSASGELGNGN-IKLSQTSNVDKEEEAVS-IE--MNEPVQ-LTFALRYNFFT
HSV-DBP   VHRI-PVYPLQLFMPDFSRVIAEPFN-ANHRSI-GEKFTYPLLP-FFNRPLNRLLFEAVV--GP

PCNA      KATPLSP-TVTLSMSADVPLVVEYKIADMGHLKYYLAPKIEDEEGS
HSV-DBP   AAVALRCRNVDAVARAAAHLAFDENHEGAALPADITFTAFEASQGK
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Fig. 5. Alignment of amino acid sequences of PCNA/cyclin with amino acid sequences of other proteins. The complete deduced amino acid sequence of rat PCNA/cyclin (1−261), complete deduced amino acid sequence of ad5 E1A (1−243, smaller protein encoded by 12 S mRNA) (Perricaudet *et al.*, 1979), and the residues 46−294 of HSV-1 DNA binding protein (HSV-DBP) (Quinn *et al.*, 1985) were arranged to achieve maximum alignment. The single letter code is used to denote amino acids. '*' denotes identities between amino acid residues. ':' stands for substitutions that occur with greater than average frequency among related proteins (McLachlan, 1971).

each of *Bam*HI, *Ava*II and *Eco*RI digests, suggesting one copy of PCNA/cyclin per haploid genome in the rat. However, several bands of different intensities were seen when other enzymes

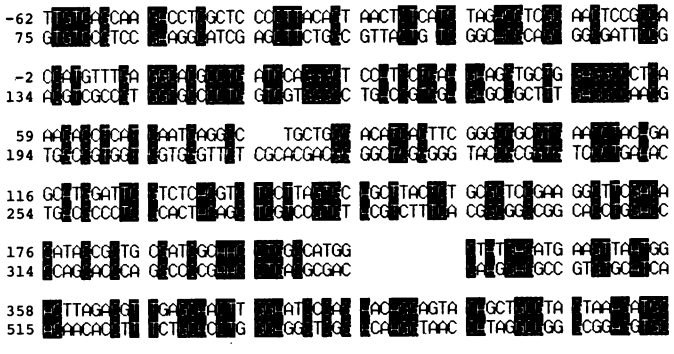

Fig. 6. Nucleotide sequence homologies between rat PCNA/cyclin and HSV-1 DNA binding protein (HSV-DBP) (Quinn *et al.*, 1985). PCNA/cyclin sequence is the upper and HSV-1 DNA binding protein sequence is the lower. Identical residues are shaded. Nucleotides are numbered in the 5′ to 3′ direction, beginning with the first residue of the initiator codon ATG.

(*Bgl*II, *Hin*dIII, *Pvu*II and *Xba*I) were used. With *Pvu*II and *Xba*I, two bands were expected since the cDNA sequence includes one cleavage site, but there were at least three bands. The cDNA contains no cleavage site for *Bgl*II and *Hin*dIII, but there were two and three bands, respectively. There are at least two possible explanations for this results, specifically, the cleavage sites for these enzymes may occur in introns of genomic DNA for PCNA/cyclin, or there could be a family of PCNA/cyclin-related genes.

The deduced amino acid sequence was compared to that of other reported amino acid sequences of DNA binding proteins. The PCNA/cyclin did not contain a sequence similar to the repressor proteins from *Escherichia coli* and bacteriophages which binds to DNA through their side chain in the alpha-helical region (Ohlendorf and Mattews, 1983). Also, no homology was observed between PCNA/cyclin and other proteins such as c-*fos* (Straaten *et al.*, 1983), adenovirus DNA binding protein (Kruijer *et al.*, 1981) and HSV-1 DNA polymerase (Quinn and McGeoch, 1985), and as well as the DNA binding domain of SV40 large T antigen (residue 139−220) (Paucha *et al.*, 1986). The amino acid sequence of ad5 E1A can be aligned relatively well with PCNA/cyclin sequence provided one large gap is inserted between 107 and 108 of the PCNA/cyclin. Human c-*myc* protein sequence (Watt *et al.*, 1983) also had a local similarity with the C-terminus of PCNA/cyclin (data not shown). Weak but detectable homology was observed with the N-terminal one-fifth of infected-cell polypeptide 8 (ICP 8) of HSV-1 (Honess and Roizman, 1973). ICP 8 is a major DNA binding protein encoded by HSV-1 with an approximate mol. wt of 130 kd and is synthesized in HSV-1 infected cells shortly before viral DNA replication (Honess and Roizman, 1974). The involvement of ICP 8 in viral DNA replication is suggested by the fact that mutants with temperature-sensitive lesions in this gene fail to replicate the viral DNA at the non-permissive temperature (Conley *et al.*, 1981; Powell *et al.*, 1981). In this respect, ICP 8 differs from the adenovirus E1A. E1A drives the G_0 arrested rodent cells to the G_1 and S phases, but is not essential for viral DNA replication (Spindler *et al.*, 1985).

Aside from the similarity between the amino acid sequence of ICP 8 and that of PCNA/cyclin, one other similarity is the intranuclear movement of ICP 8 and PCNA/cyclin in association with the DNA replication. Immunofluorescence studies using monoclonal antibody against ICP 8 revealed that ICP 8 moves from the prereplicative site to another nuclear location upon initiation of viral DNA replication (Quinlan *et al.*, 1984). This pattern of ICP 8 movement is similar to the movement of PCNA/cyclin as mentioned in the introduction.

Although direct evidence which demonstrates the involvement of PCNA/cyclin in DNA replication is still lacking, the similarities of PCNA/cyclin with ICP 8 in both the amino acid sequences and the intranuclear movement in association with DNA replication, together with the coincidence of the intranuclear sites of PCNA/cyclin and that of DNA synthesis suggests that PCNA/cyclin plays a role in DNA replication. The availability of PCNA/cyclin cDNA should allow a more detailed characterization of the role that PCNA/cyclin plays in DNA synthesis and replication.

Materials and methods

Materials

All restriction and DNA-modifying enzymes were obtained from Takara Shuzo (Kyoto, Japan) and Toyobo (Osaka, Japan). [α-^{32}P]dCTP and [γ-^{32}P]ATP were obtained from Amersham Japan (Tokyo, Japan) and ICN Radiochemicals (California, USA). Nitrocellulose filters were purchased from Schleicher and Schuell (Dassel, FRG). Nylon membranes were purchased from Micron Separation Inc. (New York, USA).

Preparation of cDNA library

The cDNA library was constructed as described by Moriuchi *et al.*, (1983). Briefly, poly(A)-containing RNA was isolated from W/Fu rat thymocytes and cDNA was synthesized by reverse transcription using an oligo(dT) primer followed by second-strand synthesis using DNA polymerase I. The double-stranded cDNA molecules were treated with S1 nuclease and chromatographed on Sephadex G-50. The cDNA was introduced by the dG/dC-tailing method at the *Pst*I site of pBR322. The hybrid molecules were used to transform competent *E. coli* strain MC1061.

Screening of the cDNA library

After amplication of the plasmid on replica plates that contained chloramphenicol, the colonies were lysed and DNA was denatured and fixed on the nitrocellulose filters (Maniatis *et al.*, 1982). The plasmids containing cDNA were screened with a ^{32}P-labeled oligonucleotide (14-mer) mixture composed of all 32 possible sequence permutations corresponding to N-terminal residues 1−5 (Met-Phe-Glu-Ala-Arg) of rabbit PCNA. For hybridization with the synthetic probe, the filters were prehybridized at 68°C for 4 h in a solution containing 0.9 M NaCl, 0.09 M sodium citrate, 0.2% Ficoll, 0.2% polyvinyl pyrrolidone, 0.2% bovine serum albumin, 0.5% SDS and 100 μg/ml of yeast tRNA. The 5′-labeled tetradecameric oligonucleotide mixture (50 pmol) was added and incubated for 16 h at 38°C. The filters were washed with a solution containing 0.9 M NaCl and 0.09 M sodium citrate for 2 h at room temperature, dried and exposed to Kodak XAR-5 film at −70°C.

DNA sequence analysis

The 5′-end of restriction endonuclease-digested DNA fragments were labeled with ^{32}P using T4 polynucleotide kinase and [γ-^{32}P]ATP. DNA fragments were further digested with endonuclease and electrophoresed. Those labeled with ^{32}P were separated and sequenced by the chemical modification technique (Maxam and Gilbert, 1980).

Southern blot analysis

Total genomic DNA was isolated from inbred Donryu rat liver and human thymocytes. High mol. wt DNA was digested with appropriate restriction enzymes, electrophoresed on 1% agarose gels, transferred to nylon membrane, and hybridized with nick-translated cDNA fragments. Hybridization was performed at 42°C for 16 h in a solution containing 50% formamide, 0.1% Ficoll 400, 0.1% polyvinyl pyrrolidone, 0.1% bovine serum albumin, 5 × SSPE (1 × SSPE = 180 mM NaCl, 10 mM sodium phosphate and 1 mM EDTA), 1% SDS, 5% dextran sulfate, and 200 μg/ml salmon sperm DNA. The filters were washed twice in 2 × SSPE and 0.5% SDS at 65°C and once in 0.2 × SSPE and 0.5% SDS at 65°C. The *Pst*I digest of pCR-1 insert yielded two bands in addition to the plasmid DNA in gel electrophoresis. From each cDNA band, DNA fragments were electroeluted, mixed in an equal molar ratio, nick-translated, and used as a hybridization probe. This cDNA probe covered the entire length of the pCR-1 cDNA insert.

Northern blot analysis

Cytoplasmic RNA was isolated from rat thymocytes. Ten micrograms of RNA were electrophoresed under denaturing conditions on a 1% agarose gel containing 5 mM methyl mercury (Maniatis *et al.*, 1982), and blotted to nylon membrane. The filters were hybridized with nick-translated cDNA in a solution containing 50% formamide, 5 × SSC (1 × SSC = 150 mM NaCl and 15 mM

sodium citrate), 0.1% Ficoll 400, 0.1% polyvinyl pyrrolidone, 0.1% bovine serum albumin, 1% glycine, and 200 μg/ml salmon sperm DNA. The filter was washed with 0.1 × SSC and 0.1% SDS at room temperature.

Mitogen response assay

Human peripheral blood lymphocytes in aliquots were cultured at a concentration of 1.95×10^6 cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin G and 100 μg/ml streptomycin. Each aliquot of lymphocytes was stimulated with phytohemagglutinin-P (PHA) (Sigma, St Louis, MO, USA) at a concentration of 10 μg/ml. At various time points after the onset of PHA stimulation, three aliquots were processed for various analyses. Each aliquot was divided into three groups. In order to determine the rate of DNA synthesis, the first group was incubated with 1 μCi/ml (200 nM) [methyl-^{3}H]thymidine (Amersham) for 1 h. At the end of the hour, cold trichloacetic acid (TCA) was added to stop the labeling. TCA-insoluble material was isolated and used to determine the radioactivity as detailed previously (Koji and Terayama, 1984). The data were expressed as c.p.m./μg DNA. The quantity of isolated DNA was measured using calf thymus DNA as a standard (Giles and Myers, 1965). The second group was cytocentrifuged on to glass slides, stained with Schiff's solution (Merck) and used to determine the mitotic index. The mitotic index was defined as the number of cells with mitotic figures per 100 cells after scoring more than 500 cells per each preparation. The third group was used for preparation of RNA for the Northern blot analysis.

Computer search

A NEC-9801F computer and GENETYX (SDC, Tokyo, Japan) and DNASIS (Hitachi Software Engineering, Tokyo, Japan) programs were used to detect both nucleotide and amino acid sequence homologies with other DNA and proteins, respectively.

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References

Bravo,R. and Macdonald-Bravo,H. (1985) *EMBO J.*, **4**, 655–661.
Bravo,R., Fey,S.J., Bellatin,J.B., Larsen,P.M., Arevalo,J. and Celis,J.E. (1981) *Exp. Cell Res.*, **136**, 311–319.
Celis,J.E. and Celis,A. (1985a) *Proc. Natl. Acad. Sci. USA*, **82**, 3262–3266.
Celis,J.E. and Celis,A. (1985b) *EMBO J.*, **4**, 1187–1192.
Conley,A.J., Knipe,D.M., Jones,P.C. and Roizman,B. (1981) *J. Virol.*, **37**, 191–206.
Giles,K.W. and Myers,A. (1965) *Nature*, **206**, 93.
Gingeras,T.R., Sciaky,D., Gelinas,R.E., Bing-Dong,J., Yen,C.E., Kelly,M.M., Bullock,P.A., Parsons,B.L., O'Neill,K.E. and Roberts,R.J. (1982) *J. Biol. Chem.*, **257**, 13475–13491.
Honess,R.W. and Roizman,B. (1973) *J. Virol.*, **12**, 1347–1365.
Honess,R.W. and Roizman,B. (1974) *J. Virol.*, **14**, 8–19.
Koji,T. and Terayama,H. (1984) *Exp. Cell Res.*, **155**, 359–370.
Kozak,M. (1984) *Nucleic Acids Res.*, **12**, 857–872.
Kruijer,W., van Schaik,F.M.A. and Sussenbach,J.S. (1981) *Nucleic Acids Res.*, **9**, 4439–4457.
Macdonald-Bravo,H. and Bravo,R. (1985) *Exp. Cell Res.*, **156**, 455–461.
McLachlan,A.D. (1971) *J. Mol. Biol.*, **61**, 409–424.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.
Mathews,M.B., Bernstein,R.M., Franza,B.R. and Garrel,J.I. (1984) *Nature*, **309**, 374–376.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499–560.
Miyachi,K., Fritzler,M.J. and Tan,E.M. (1978) *J. Immunol.*, **121**, 2228–2234.
Moriuchi,T., Chang,H.C., Denome,R. and Silver,J. (1983) *Nature*, **301**, 80–82.
Ogata,K., Ogata,Y., Nakamura,R.M. and Tan,E.M. (1985) *J. Immunol.*, **135**, 2623–2627.
Ohlendorf,D.H. and Mathews,B.W. (1983) *Annu. Rev. Biophys. Bioeng.*, **12**, 259–284.
Paucha,E., Kalderon,D., Harvey,R.W. and Smith,A.E. (1986) *J. Virol.*, **57**, 50–64.
Perricaudet,M., Akusjarvi,G., Virtanen,A. and Petterson,U. (1979) *Nature*, **281**, 694–696.
Persson,H., Henninghhausen,L., Taub,R., DeGrado,W. and Leder,P. (1984) *Science*, **225**, 687–693.
Powell,K.L., Littler,E. and Purifoy,D.J.M. (1981) *J. Virol.*, **39**, 844–902.
Quinlan,M.P., Chen,L.B. and Knipe,D.M. (1984) *Cell*, **36**, 857–868.
Quinn,J.P. and McGeoch,D.J. (1985) *Nucleic Acids Res.*, **13**, 8143–8163.
Sadaie,M.R. and Mathews,M.B. (1986) *Exp. Cell Res.*, **163**, 423–433.
Smart,J.E., Lewis,J.B., Mathews,M.B., Harter,M.L. and Anderson,C.W. (1981) *Virology*, **112**, 703–713.
Spindler,K.R., Eng,C.Y. and Berk,A.J. (1985) *J. Virol.*, **53**, 742–750.
Straaten,F., Muller,R., Curran,T., Van Beveren,C. and Verma,I.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3183–3187.
Takasaki,Y., Deng,J.-S. and Tan,E.M. (1981) *J. Exp. Med.*, **154**, 1899–1909.
Takasaki,Y., Fishwild,D. and Tan,E.M. (1984) *J. Exp. Med.*, **159**, 981–992.
Watt,R., Stanton,L.W., Warcu,K.B., Gallo,R.C., Croce,C.M. and Rovera,G. (1983) *Nature*, **303**, 725–728.

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