# PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA/cyclin): REVIEW AND SOME NEW FINDINGS

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**PCNA/cyclin was originally described in proliferating mammalian cells as a nuclear protein with an apparent molecular weight of 33,000–36,000, and recently found to be a DNA polymerase-delta auxiliary protein. When the cDNA for PCNA/cyclin was cloned and analyzed, it was found that the protein consisted of 261 amino acids with a calculated molecular weight of 28,700 and the amino acid sequence was well conserved during evolution both in animal and plant kingdoms. The highly homologous nature of PCNA/cyclin suggests that the protein plays an essential role in DNA replication in eukaryotes.**

Miyachi *et al.* (23) recognized that some sera drawn from patients with systemic lupus erythematosus (SLE) reacts with some nuclei of lymphocytes in lymph nodes, spleen and thymus, but not with nuclei of renal tubules, glomerulus and hepatic cells. This observation led them to conclude that these sera reacted with an antigen which appears only in proliferating cells and named the antigen as "proliferating cell nuclear antigen (PCNA)". PCNA appears in nuclei of mitogen-stimulated peripheral blood lymphocytes as well as in nuclei of cell lines maintained *in vitro* such as Hep-2, Ehrlich ascites tumor cells and baby hamster kidney cells. The presence of autoantibodies against PCNA was detected only in 3 sera out of 70 SLE sera and none in sera drawn from 30 rheumatoid arthritis, 33 with Sjögren's syndrome, 10 with progressive systemic sclerosis, 19 with polymyositis, and 26 normal controls. In the three patients with SLE who had anti-PCNA autoantibodies, Miyachi *et al.* (23) were unable to detect any unusual clinical characteristics.

The PCNA was extractable from tissue culture cells or rabbit thymus with physiological saline and was found to be distinct from other previously known antigen-autoantibody systems such as Sm antigen, nuclear RNP, SS-A/Ro, and SS-B/La (39). The isolated PCNA was inactivated as an antigen at 56°C for 30 min. Later it was found that PCNA was identical to "cyclin" (20) which was found as a distinct protein that appeared in the S-phase of various cells *in vitro* (2). More recently it was found that PCNA/cyclin has properties similar, if not identical, to a DNA polymerase-delta auxiliary protein (3, 32). This auxiliary protein stimulates the activity of DNA polymerase-delta, but not that of DNA polymerase-alpha (40).

## CHEMISTRY OF PCNA/cyclin

PCNA/cyclin is an acidic non-histone nuclear protein with an isoelectric point of

4.8. The apparent molecular weight of PCNA/cyclin is 33,000–36,000 D as determined by SDS-polyacrylamide gel electrophoresis (2, 20, 39). Recently the amino acid composition and the first 25 amino acid sequence from the N-terminus of rabbit PCNA were determined (27). Using an oligo-nucleotide probe corresponding to the first 5 amino acid sequence, a cDNA clone for PCNA/cyclin (pCR-1) was isolated from rat thymocyte cDNA library (21, 24).

The cDNA insert of pCR-1 is 1195 base pairs long (Fig. 1) and the 5′ end of the cDNA contains 62 bp non-coding region. This is followed by an active start codon in eukaryote mRNA, the methionine codon included in the Kozak's consensus sequence (16). From the initiation codon, ATG (nucleotides 1–3), to the termination codon, TAG (nucleotides 784–786), there were 783 base pairs 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 molecular weight was calculated to be 28.7 kD (Fig. 1). 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).

Using the pCR-1 cDNA insert as a hybridization probe, two species of mRNA (1.1 kb and 0.98 kb in size) were detected by Northern blot analysis of rat thymus cytoplasmic RNA, suggesting that one of the first two signals is utilized for the shorter mRNA and the pCR-1 clone utilized the third signal. Since the approximate sizes of PCNA/cyclin mRNA (1.1 and 0.98 kb) were similar to that of PCNA/cyclin cDNA (1.195 kb), pCR-1 clone should cover most, if not all, of the PCNA/cyclin mRNA sequence.

To estimate the numbers of genes for PCNA/cyclin in rat and human genomes, high molecular weight 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. A single major band was observed with rat genomic DNA digested with Bam HI, Ava II and Eco RI. However, several bands were seen with Bgl II and Hind III digests, although pCR-1 cDNA insert had no recognition sites by these enzymes. Bgl II digestion generated two bands of 17 kb and 7.5 kb. Hind III-digest gave three bands of 8 kb, 4.6 kb, and 2.7 kb. The interpretation of the Southern hybridization patterns is somewhat complicated. A single band was identified in 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 (Bgl II, Hind III, Pvu II, and Xba I) were used. With Pvu II and Xba I, two bands were expected since the cDNA sequence include one cleavage site, but there were at least three bands. The cDNA contains no cleavage site for Bgl II and Hind III, but there were two and three bands, respectively. There are at least two possible explanations for these results: specifically, the cleavage sites for these enzymes can occur in introns of genomic DNA for PCNA/cyclin, or there could be a family of PCNA/cyclin-related genes. The Southern hybridization analysis of total human genomic DNA using the pCR-1 as the probe indicates that there is a single gene for PCNA/cyclin. In Bam HI, Hind III and Pvu II digests of human genomic DNA, single major bands were also detected. In longer exposures additional faint band(s) were occasionally seen with both human and rat DNA digested by most of the enzymes used. Using the pCR-1, the gene for PCNA was assigned to a long arm of human chromosome 2 at

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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 ACT 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 GTT 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 TCA 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. 1. 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′ and 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 line are indicated on the right. Polyadenylation signals in the 3′ noncoding region is underlined. The termination codon, TAG, at nucleotide position 784–786 is indicated by "*"

2q33 (44).

The first 25 predicted amino acid sequence of pCR-1 matched with the reported first 25 amino acids sequence of rabbit PCNA (27), except the residue 7. At the residue 7, valine in the rabbit protein sequence was isoleucine in the rat protein sequence. The amino acid composition of the rabbit PCNA (27) and that of the rat agreed well for most amino acids with exception of lysine and serine (Table 1).

Soon after the cDNA sequence of rat PCNA was reported, Almendral *et al.* (1) obtained independently a cDNA clone of human PCNA. When the cDNA was sequenced, it was found that its open reading frame also would encode for a protein with 261 amino acids and differed from that of rat in only four amino acids. The rat 7th amino acid Ile was Val in man; at 33rd, Gly was Ser; at 190th Ser was Thr; and at 216th, Pro was Ser. The homology of PCNA between different mammals and the ability of anti-PCNA serum to react with all proliferating cells from mammals and plants so far examined suggested that PCNA is highly conserved through evolution.

The calculated molecular weight of rat PCNA/cyclin was 28.7 kD. This is less than the molecular weight of 33 kD and 36 kD estimated by SDS-polyacrylamide gel electrophoresis and immunoblotting (2, 20, 39). Since, the molecular weight of PCNA/cyclin synthesized in the *in vitro* cell-free translation system is the same as that of PCNA/cyclin isolated from cells (34), the post-translational modification can not account for the discrepancy with the molecular weights. One possible explanation for the discrepancy is that PCNA/cyclin migrates abnormally slow in SDS-polyacrylamide gel electrophoresis and the molecular weight may have been overestimated. Occurrence of this phenomenon has been observed with several other proteins such as adenovirus ElA and c-myc protein (10, 30, 35). The other possibility is that PCNA/cyclin differs slightly between species, since the difference was noted with the amino acid residue 7 between the rat and the rabbit.

The deduced amino acid sequence was compared to that of other reported amino acid sequence of DNA binding proteins. The PCNA/cyclin did not contain a sequence similar to the repressor proteins from E. coli and bacteriophages which bind to DNA through their side chain in the alpha-helical region (28). No homology was also observed between PCNA/cyclin and other proteins such as c-fos (37), adenovirus

TABLE 1. *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. Number of amino acids are expressed as the mean number of each amino acid residue per 100 residues. ND and — stand for not detected and not analyzed, respectively.

DNA binding protein (17) and HSV-1 DNA polymerase (33), and as well as DNA binding domain of SV 40 large T antigen (residue 139–220) (29). The amino acid sequence of adenovirus 5 ElA can be aligned relatively well with PCNA/cyclin sequence provided one large gap was inserted between 107 and 108 of the PCNA/cyclin. Human c-myc protein sequence (43) also had a local similarity with C-terminus of PCNA/cyclin (data not shown). Almendral *et al.* (1) noticed that a domain (amino acids 66–80) has a similarity with the alpha-helix-turn-alpha-helix putative DNA-binding domain of several proteins. Weak but detectable homology was observed with N-terminal one-fifth of infected-cell polypeptide 8 (ICP 8) of HSV-1 (13). ICP 8 is a major DNA-binding protein encoded by HSV-1 with an approximate molecular weight of 130 kD and is synthesized in HSV-1 infected cells shortly before viral DNA replication (14). The involvement of ICP 8 in the viral DNA replication is suggested by the fact that mutants with temperature-sensitive lesions in this gene failed to replicate the viral DNA at the nonpermissive temperature (8, 31). In this respect, ICP 8 differs from the adenovirus ElA. ElA drives the $G_0$ arrested rodent cells to the $G_1$ and S phases, but is not essential for the viral DNA replication (36).

## CELLULAR DISTRIBUTION OF PCNA/cyclin

Immunohistochemical localization of PCNA/cyclin has been performed using either anti-PCNA/cyclin autoantibodies obtained from SLE patients or mouse monoclonal antibody against PCNA/cyclin. The distribution of PCNA/cyclin varies considerably depending upon the fixative used during the immunohistochemical procedures.

Immunofluorescence studies using either cold methanol or ethanol as fixatives and anti-PCNA/cyclin autoantibodies have revealed that the PCNA/cyclin is found as a speckled pattern in nucleus during the early S phase of the cell cycle, as punctuated pattern with foci throughout the nucleus in the late S phase, near the nuclear membrane at the time of maximum DNA synthesis, again as punctuated pattern at the $S/G_2$ phase (4, 5, 39). At the end of the S phase little or no staining was observed (4). None of these investigators reported the staining of cytoplasm before or after the S phase.

These immunofluorescence patterns are remarkably similar to those observed in autoradiogram of [$^3$H]thymidine-labeled nuclei (6). Recent studies using DNA synthesis inhibitors such as hydroxyurea (4) and aphidicolin (18) demonstrated that the synthesis of PCNA/cyclin precedes 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 (4, 5, 39).

Some of these investigators have noticed that there is some discrepancy betwen the biochemical information and the immunohistochemical pattern of staining. Celis *et al.* (7) noticed that, although there is a significant synthesis of PCNA/cyclin during G1 phase and mitosis, only comparatively weak staining is detected with the anti-PCNA/cyclin autoantibody. To further prove this phenomenon, Celis and Celis (5) reacted enucleated transformed human amnion cells (cytoplast) which are known to

synthesize PCNA/cyclin with the autoantibody against PCNA/cyclin and found that the cytoplasms were stained only weakly. They suggested that the antibody may not recognize all forms of PCNA/cyclin in fixed cells. Bravo and Macdonald-Bravo (4) also investigated this phenomenon by pulse-labeling for 30 min with [$^{35}$S] methionine mouse 3T3 cells which had been treated with hydroxyurea for 12 hr. The cells were then chased 2 to 10 hr in the absence of hydroxyurea in complete medium containing 10 times the normal concentration of methionine. They found that [$^{35}$S] labeled PCNA/cyclin persisted for the duration as identified by the 2-D gel electrophoresis. Based upon this observation, Bravo and Macdonald-Bravo (4) suggested that PCNA/cyclin present at the end of S phase is interacting with other macromolecule that render it inaccessible to the antibody. The fact that the anti-PCNA/cyclin autoantibody failed to react with the cytoplasmic PCNA/cyclin was utilized as a control system when Madsen *et al.* (19) studied the migration of nuclear protein-antibody complexes from cytoplasm into nucleus. They found that the antibody gained an ability to migrate into nucleus when the antibody was complexed with nuclear antigen, whereas the anti-PCNA/cyclin failed to do so.

We have also investigated the distribution of PCNA/cyclin in various cells and tissues (26). For the past twenty years, it has been our practice that at any time when a new antigen is to be localized immunohistochemically we search for a fixation condition which will preserve maximally the antigenicity of the antigen and tissue morphology. In practice we usually subdivide fixatives into three categories, i.e. organic, aldehyde and heavy metals. It was found that for the localization of PCNA/cyclin in mammalian tissues, aldehyde fixatives were best suited. Fixation with either 4% formaldehyde in phosphate buffer or periodate-lysine-paraformaldehyde (PLP) (22) retained maximum reactivity with PCNA/cyclin when anti-PCNA/cyclin autoantibody was used (42). The tissues fixed with organic fixatives such as cold acetone, cold ethanol and cold methanol retain a minimum of antigenicity and the heavy metal fixatives such as osmium tetroxide and potassium permanganate failed to preserve any antigenicity. With cells *in vitro*, a brief fixation with mild formaldehyde fixation followed by organic fixation worked best.

Recently we investigated the mode of PCNA/cyclin in regenerating rat livers. In this study, male albino Wistar rats weighing about 300 gm were partially hepatectomized according to the method of Higgins and Anderson (11). Some of rats were sham operated. Some of the partially hepatectomized rats received an injection of bromodeoxyuridine (20 mg/Kg b.w.) intravenously 20 min before sacrifice. A fixed number of the rats were killed at about 4 hr intervals. At the time of sacrifice, livers were dissected out, cut into 5 × 5 × 2 mm blocks and fixed in PLP containing 0.5% formaldehyde in 0.1 M phosphate buffer, pH 7.4, for 4 hr at 4°C with gentle agitation. The tissue blocks were washed with a solution of gum-sucrose (12) over-night at 4°C. The tissue blocks were then processed for immunohistochemical analysis using the indirect peroxidase labeled antibody method (25). For the localization of PCNA/cyclin, mouse monoclonal antibody against PCNA/cyclin (a gift from Dr. E. Tan, La Jolla, CA, USA) was used as the first antibody and for the localization of bromodeoxyuridine, mouse monoclonal antibody against bromodeoxyuridine (B & D, Mountain View, CA, USA). As the second antibody, peroxidase labeled sheep anti-mouse immunoglobulin (Amersham Japan, Tokyo, Japan) was used. Mouse monoclonal antibody against human leucocyte common antigen was used as control sera.

PCNA/cyclin and bromodeoxyuridine were detectable in nuclei of only one or two hepatocytes per low power view in livers of the sham operated rats and of those rats which had been partially hepatectomized within 15 hr. The PCNA/cyclin appeared first in the cytoplasm of hepatocytes which were situated near the portal triads at 16 hr after the partial hepatectomy (Fig. 2). At 21 hr following partial hepatectomy, PCNA/cyclin was found in the nuclei of most hepatocytes near the portal triads (Fig. 3). At 25 hr after the partial hepatectomy, the nuclei of most hepatocytes were void of the immunorective PCNA/cyclin, however in some hepatocytes, PCNA/cyclin was found focally near bile canaliculi in or near the Golgi area (Fig. 4). The time of appearance of bromodeoxyuridine in the nuclei of hepatocytes of partially hepatectomized rats was 2–3 hr after the appearance of PCNA/cyclin.

The spermatogonia in the rat and mouse testis stained most strongly for PCNA/cyclin among tissues we examined. In the small intestine of the mouse, nuclei of cells in the crypts usually contained PCNA/cyclin. In the intestine of mice which received an intravenous injection of [$^3$H]thymidine 30 min before sacrifice, PCNA/cyclin was found in the nuclei of cells in the crypt as usual. When these immunohistochemically stained sections were subjected to radioautography, all cells which contained silver grain also contained PCNA/cyclin. However, not all cells which contained PCNA/cyclin contained silver grain. These cells containing both silver grain and PCNA/cyclin were situated several cell distances from the bottom of the crypt and those containing only PCNA/cyclin were situated at the bottom of the

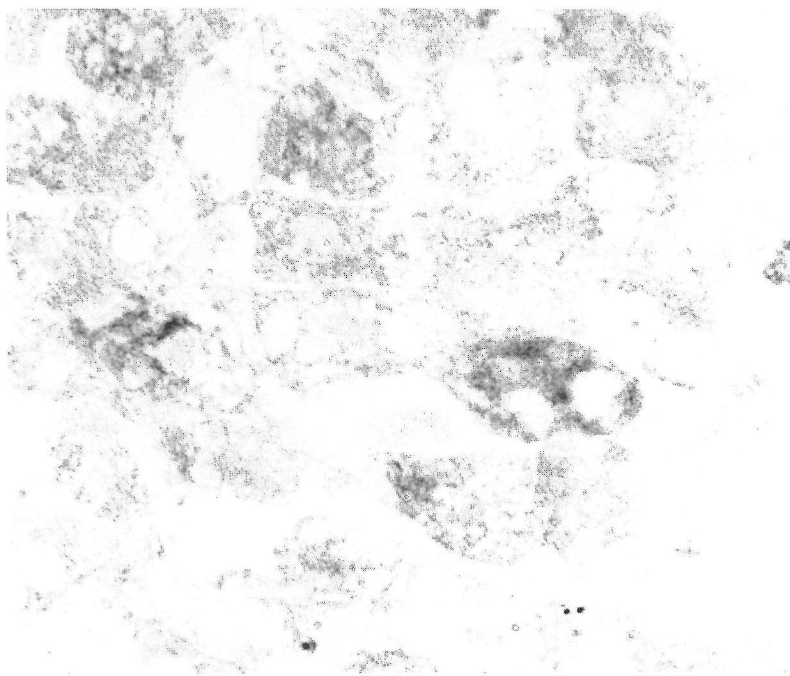

Fig. 2. Rat liver, 16 hr after partially hepatectomy. PCNA/cyclin was localized in the hepatocyte cytoplasm.

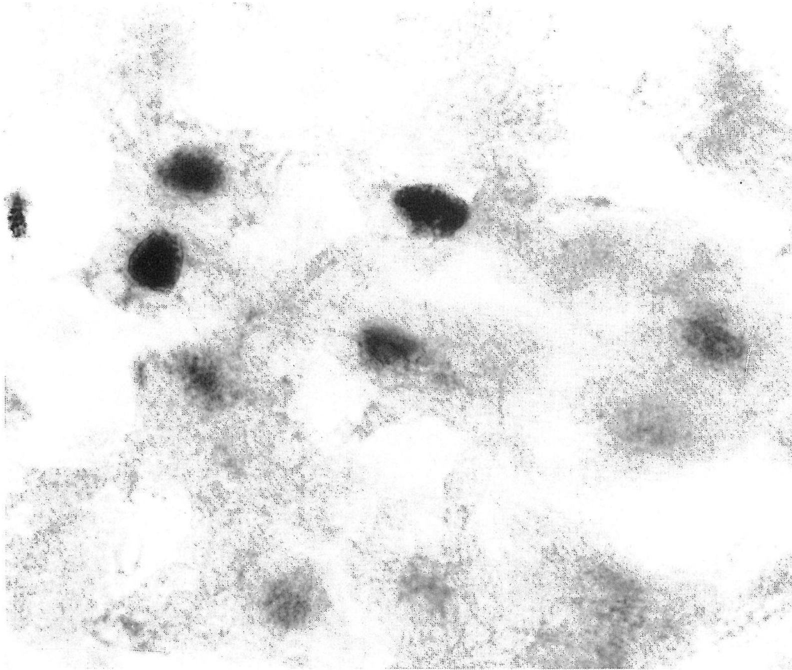

FIG. 3. Rat liver, 21 hr after partially hepatectomy. PCNA/cyclin was localized in the hepatocyte nuclei.

crypt as well as the middle of the villi and slightly above the cluster of cells which contained both PCNA/cyclin and silver grains. When proliferation of cells *in vitro* was arrested by metabolic manipulation, the DNA synthesis ceased within 12 hr, but the immunoreactive PCNA/cyclin persisted in the nuclei as long as another 4 days. An interesting observation on the distribution of immunoreactive PCNA/cyclin was made with neoplastic tissues taken at the time of surgery. It has been our constant finding that most of adenomas as well as adenocarcinomas such as gastric carcinoma and gall bladder carcinoma react rather poorly with the autoimmune antibody against PCNA/cyclin, whereas neoplastic cells of hemopoietic origin such as lymphoma and leukemic cells react strongly. The reason why the adenomas and adenocarcinomas react poorly has not been elucidated, however, when these tumors are transplanted subcutaneously to nude mice, the reactivity of the tumor with the antibody increased dramatically.

If PCNA/cyclin is an essential protein for proliferating cells, we reasoned that PCNA/cyclin should be present in all proliferating cells including that of the plant kingdom. Daidoji *et al.* (9) have demonstrated the presence of proteins which reacted with human autoantibody against PCNA/cyclin in proliferating cells of soybean root tips as well as that in onion root tips. When the immunoreactive protein was identified by the immunoblotting method, the antigen was slightly smaller than that found in animal cells. PCNA/cyclin appear not only in the growing points of the onion root

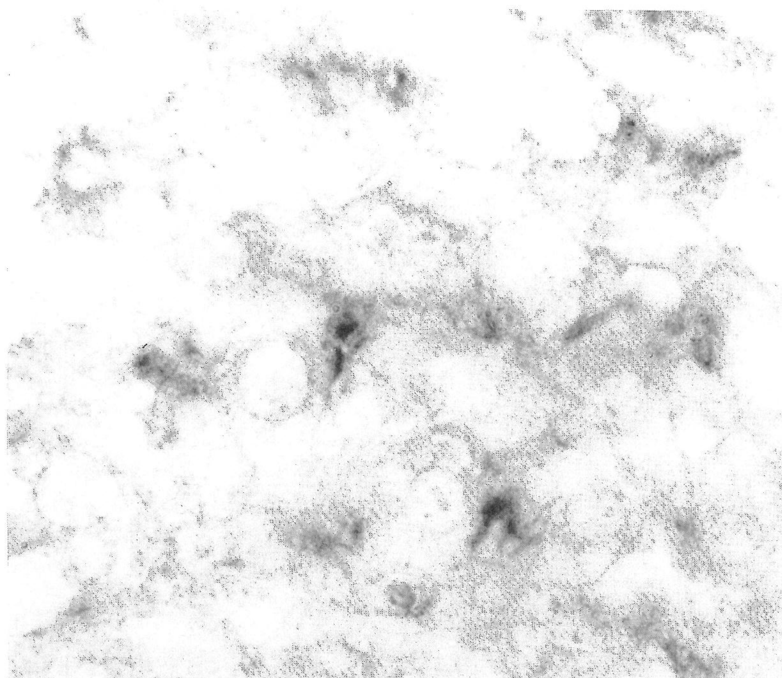

FIG. 4. Rat liver, 25 hr after partial hepatectomy. PCNA/cyclin was localized in the hepatocyte Golgi area.

tip, but also in other cells which cover the root tip. In Southern blot analysis rat PCNA/cyclin cDNA probe hybridized with homologous sequences in genomic DNAs from rice, soybean, tobacco and red pepper. When the PCNA/cyclin-related molecular clone (pCJ-1) isolated from rice DNA was used as a probe for RNA blot analysis, the probe hybridized with a 1.2 kilobases transcript in RNA from rice root tips and shoots (38). Strong conservation of the gene and the protein for PCNA/cyclin among animal and plant kingdoms suggests the essential role of this protein in DNA replication.

The presence of PCNA/cyclin both in the nuclei of animal as well as plant proliferating cells is an indication of the important rôle that PCNA/cyclin plays in the process of cell proliferation. The results of our study on the site of DNA synthesis and the sites of PCNA/cyclin, was slightly different that of Celis and Celis (6) where they almost an identical distribution of immunoreactive PCNA/cyclin and silver grains in the nuclei of proliferating cells *in vitro*, although they did not perform both the immunohistochemical procedure and radioautography on the same sample. This difference is presumably the result of the differences in the amount of antigenicity of PCNA/cyclin retained in proliferating cells after fixation. Immunohistochemically, when formaldehyde containing fixatives were used, PCNA/cyclin was first found in cytoplasm where other nuclear proteins were produced. PCNA/cyclin then migrated into nuclei. Finally, PCNA/cyclin was localized to the Golgi region of cytoplasm.

With our study, at what exact stage of mitosis PCNA/cyclin re-entered cytoplasm could not be defined. We presume it was during the prophase since little or no PCNA/cyclin was associated with metaphase chromosomes.

The difference in the intracellular distribution of PCNA/cyclin when organic fixative and formaldehyde fixatives are used points to an interesting speculation. In order for PCNA/cyclin to be extractable by organic fixatives from cytoplasm and not from nucleus, PCNA/cyclin must have been in complex with some specific structure in nucleus. The fact that when cells were subjected to DNAse treatment, there was a drastic reduction in immunohistochemical staining suggests that they may be in complex with DNA. From our observation together with that of Celis and Celis (6), the sites of [$^3$H]thymidine uptake coincides with the location in methanol fixed cells, PCNA/cyclin may be in complex with DNA in a substrate-enzyme like manner rather than on DNA as an activator of certain genes. In this regard, Toschi and Bravo (41) observed that nuclei of formaldehyde fixed quiescent human fibroblast contained some PCNA/cyclin which could readily be released when the cells were permeated with Triton X-100 and suggested that PCNA/cyclin is loosely attached in the nuclei. An UV irradiation of the cells resulted in an appearance of PCNA/cyclin in the nuclei, some of which could not be released with high salt wash and thus suggests that PCNA/cyclin may exist free in nucleoplasma in the cell nuclei, as a presynthetic complex and as a replicative complex.

The availability of full-length PCNA/cyclin cDNA and information on the site of PCNA/cycline in proliferating cells should allow a more detailed characterization of the role that PCNA/cyclin plays in the physiology of cells. In fact, more recently Jaskulski *et al.* (15) reported that when Balb/c3T3 were exposed to antisense oligodeoxynucleotides to PCNA/cyclin, both DNA synthesis and mitosis were completely supressed. This report together with others indicate that PCNA/cyclin is important in cellular DNA synthesis and proliferation.

## ACKNOWLEDGEMENTS

We thank Drs. Shun-ichi Hirose, Yoshinari Yakasaki, Hideyuki Daidoji, Keiji Matsumoto (Juntendo University, Tokyo, Japan) and Kaoru Abe (National Cancer Center, Tokyo, Japan) for encouragement and Mr. Mitsuyoshi Sekino for technical assistance. This work was supported in part by research grants from the Ministry of Education, Science and Culture of Japan (60015095, 61480134, 60304050, 60480156, 61010069), by the Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare (60-34, 59-1) and by a Tokai Medical Research Grant.

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