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*Review Letter*

# Cyclin (PCNA, auxiliary protein of DNA polymerase δ) is a central component of the pathway(s) leading to DNA replication and cell division

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Cyclin, also known as PCNA or the auxiliary protein of mammalian DNA polymerase δ, is a stable cell cycle regulated (synthesized mainly in S-phase) nuclear protein of apparent $M_r$ 36 000 whose rate of synthesis correlates directly with the proliferative state of normal cultured cells and tissues. Cyclin (PCNA) is absent or present in very low amounts in normal non-dividing cells and tissues, but it is synthesized in variable amounts by proliferating cells of both normal and transformed origin. All available information indicates that this ubiquitous and tightly regulated DNA replication protein is a central component of the pathway(s) leading to DNA replication and cell division.

Cell proliferation; Cell cycle; Late mitogenic event; Mitogenic response; DNA replication; Cancer

## 1. INTRODUCTION

The cell cycle of eukaryotic cells is divided into periods based on two landmarks, cell division at mitosis (M) and replicative DNA synthesis in S-phase. The gaps separating mitosis from S-phase and the latter from mitosis have been termed $G_1$ and $G_2$, respectively ([1] and references therein; fig.1). Normal, non-dividing cells are found in a resting stage ($G_0$; [1] and references therein) and can be stimulated to enter the cell cycle by the action of external signal molecules such as polypeptide growth factors, hormones and pharmacological agents ([2] and references therein). Alone, or in combination, these mitogenic agents trigger an orderly sequence of events that culminate in replicative DNA synthesis and ultimately in cell division. Control mechanisms involved in the choice of daughter cells to either cycle or become quiescent are thought to operate mainly in $G_1$ as resting cells have a $G_1$ content of DNA ([1] and references therein). At present, little is known concerning the identity of cellular protein(s) that may be com-

This article is dedicated to the memory of Professor Osvaldo Cori, who introduced one of us (J.E.C.) to biochemistry

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*Abbreviations:* IEF, isoelectric focusing; PCNA, proliferating cell nuclear antigen. *Note:* Cyclin (PCNA) is different from the cyclin protein described by Hunt et al. in sea urchin eggs [(1983) Cell 33, 389–396]

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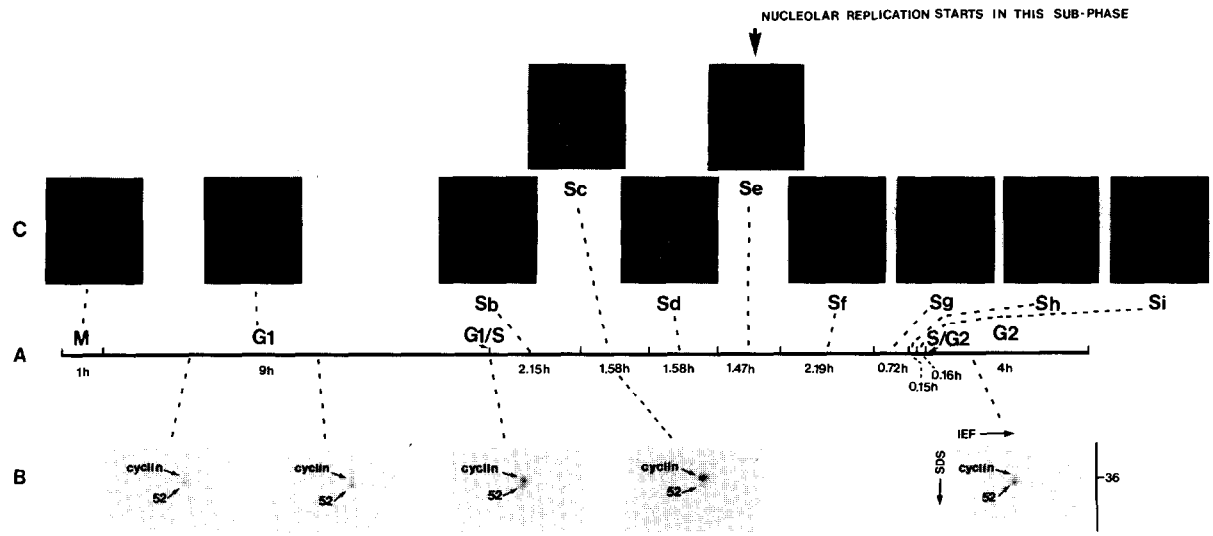


Fig.1. Synthesis and sequence of cyclin (PCNA) antigen distribution during the cell cycle of transformed human amnion cells (AMA). (A) Duration of the cell cycle phases. (B) Synthesis of cyclin (PCNA) at various stages of the cell cycle. Protein IEF 52 corresponds to a tropomyosin [5,67]. Only the pertinent region of the gel is shown. (C) Sequence of cyclin (PCNA) antigen distribution as deduced by indirect immunofluorescence using PCNA autoantibodies specific for cyclin. Patterns of cyclin (PCNA) antigen distribution are now known to represent actual patterns of DNA replication [47,48,54]. Mitotic, $G_1$ and $G_2$ cells contain cyclin (PCNA) [8] but this form is not recognized by the autoantibodies in methanol fixed cells. Cyclin (PCNA) is a stable protein [30]. Modified from Celis and Celis [46,47].

ponents of the pathway(s) that control progression from $G_1$ to S. The rate of synthesis of these proteins however is expected to increase substantially near the $G_1$/S transition border of the cell cycle.

## 2. CYCLIN (PCNA, AUXILIARY PROTEIN OF DNA POLYMERASE $\delta$): A TIGHTLY REGULATED CELL CYCLE PROTEIN WITH A ROLE IN CHROMOSOMAL DNA REPLICATION

Studies by Bravo and Celis [3] using high-resolution two-dimensional gel electrophoresis were amongst the first to search for cell cycle phase specific proteins in mammalian cells. Their studies, which analyzed global patterns of gene expression throughout the cell cycle of HeLa cells identified an acidic nuclear protein of apparent $M_r$ 36 000 (IEF 49 in the HeLa protein catalogue [4]), later termed cyclin [5,6], that was preferentially synthesized during S-phase ([3,7–10]; see also fig.1). The rate of synthesis of this nuclear protein [11] has been shown to correlate directly with the proliferative state of normal cultured cells and tissues of various species. Cyclin (PCNA) is absent or present in very low amounts in normal non-dividing cultured cells (fig.2A) and tissues, but it is synthesized in variable amounts by proliferating cells of both normal and transformed origin ([3–34]; see also fig.2B). Cyclin (PCNA) synthesis seems to be regulated at the level of transcription as suggested by in vitro translation experiments [34] and dot hybridization studies using the cloned gene [35]. Independently, Tan and co-workers [36–44] characterized autoantibodies found in the sera from a small percentage of patients with systemic lupus erythematosus (SLE). These autoantibodies were shown to stain the nucleus of proliferating cells and to react with an acidic polypeptide of $M_r$ 36 000 [39,41–43] that was termed proliferating cell nuclear antigen (PCNA). In 1984, Mathews and colleagues [26] showed that cyclin and PCNA were identical.

Immunofluorescence studies using PCNA autoantibodies specific for cyclin showed that only S-phase cells reacted with these antibodies [8,10,23, 30,34,36,37,39–53] (fig.1), and that various pat-

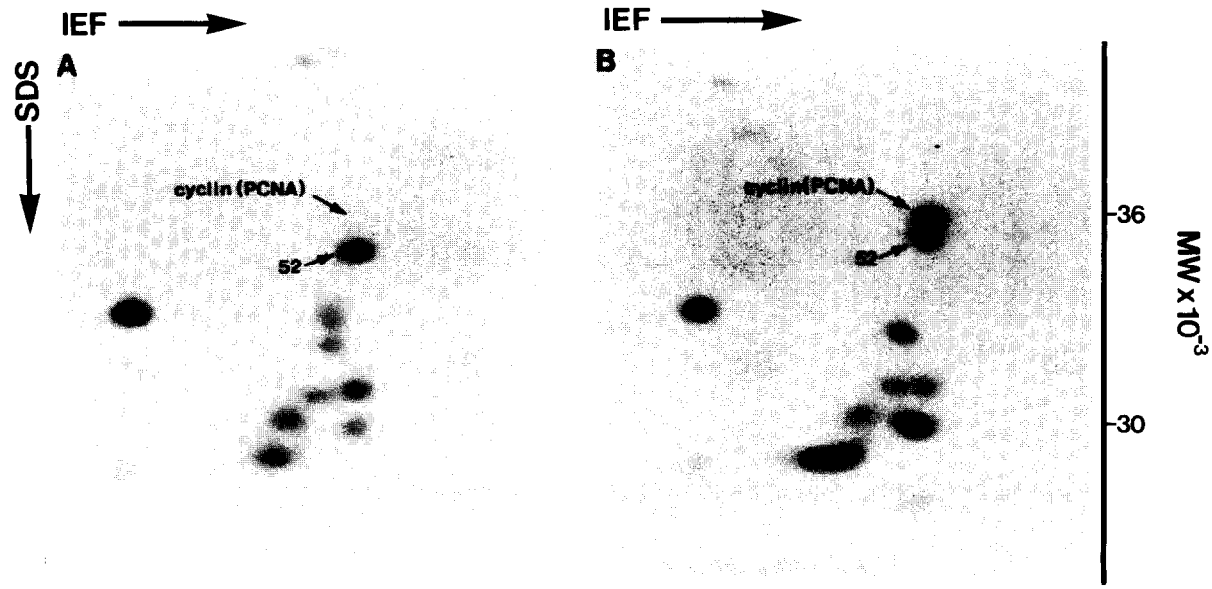


Fig.2. Cyclin (PCNA) synthesis in (A) quiescent (96 h in DMEM containing 0.5% sera) and (B) SV40 transformed human MRC-5 fibroblasts. Protein IEF 52 corresponds to a tropomyosin [5,67].

terns of cyclin (PCNA) antigen distribution subdivided S-phase [8,10,30,34,46–49,51–54] (fig.1). Moreover, many of the cyclin (PCNA) patterns were shown to mimic topographical patterns of DNA synthesis [8,10,34,47,48,51,52,54] (fig. 3A). In particular, late patterns of nucleolar DNA replication as determined by [$^3$H]thymidine autoradiography (fig.3Ba) could be superimposed with immunofluorescence patterns of cyclin (PCNA) antigen distribution ([8,10,34,46,48]; fig.3Bb). These, as well as other observations ([16,17,22,49] and references therein) argued strongly for a role of this protein in chromosomal DNA replication. A role for cyclin (PCNA) in nucleotide excision DNA repair has also been proposed [53].

Recently, immunofluorescence and biochemical data have firmly established a role for cyclin (PCNA) in DNA replication. First, Nakamura and colleagues [54] have shown a complete colocalization of cyclin (PCNA) antigen and DNA polymerase $\alpha$ with replicating replicon clusters as visualized with BrdUrd antibodies, and second, work by two laboratories identified cyclin (PCNA) as the auxiliary protein of mammalian DNA polymerase $\delta$ [9,55–57]. Cyclin (PCNA) has no detectable DNA polymerase, primase, ATPase or nuclease activity, but it is able to stimulate the activity of the DNA polymerase $\delta$ core enzyme up to a few hundred-fold when using templates with low template/primer ratios [55]. Cyclin (PCNA) has no effect on the activity of calf thymus DNA polymerase $\alpha$ when using similar template/primers having long stretches of single-stranded DNA [55]. Recent studies by Prelich et al. [56] have further shown that cyclin (PCNA) may function in the elongation stage of SV40 DNA replication in vitro. Cyclin (PCNA) synthesis is activated by the adenovirus E1A gene concomitant with induction of cellular DNA replication [58].

Interestingly, many of the properties of cyclin (PCNA) resemble those of the $\beta$-subunit of *E. coli* DNA polymerase III holoenzyme ([59,60] and references therein). Both proteins are required to replicate templates with low template/primer ratios and exist as dimers of identical subunits. These observations as well as the fact that cyclin (PCNA) is found at the sites of DNA replication throughout S-phase [47,48,54] argue strongly for a role of this protein in chromosomal DNA replication. A role for DNA polymerase $\delta$ [55,61–64] in replicative DNA synthesis is also evident [55,57], implying that DNA polymerase $\alpha$ is not the sole polymerase involved in eukaryotic DNA replication. Analysis of the rate of synthesis of core polymerase $\delta$ throughout the cell cycle should reveal if this protein is regulated in a similar

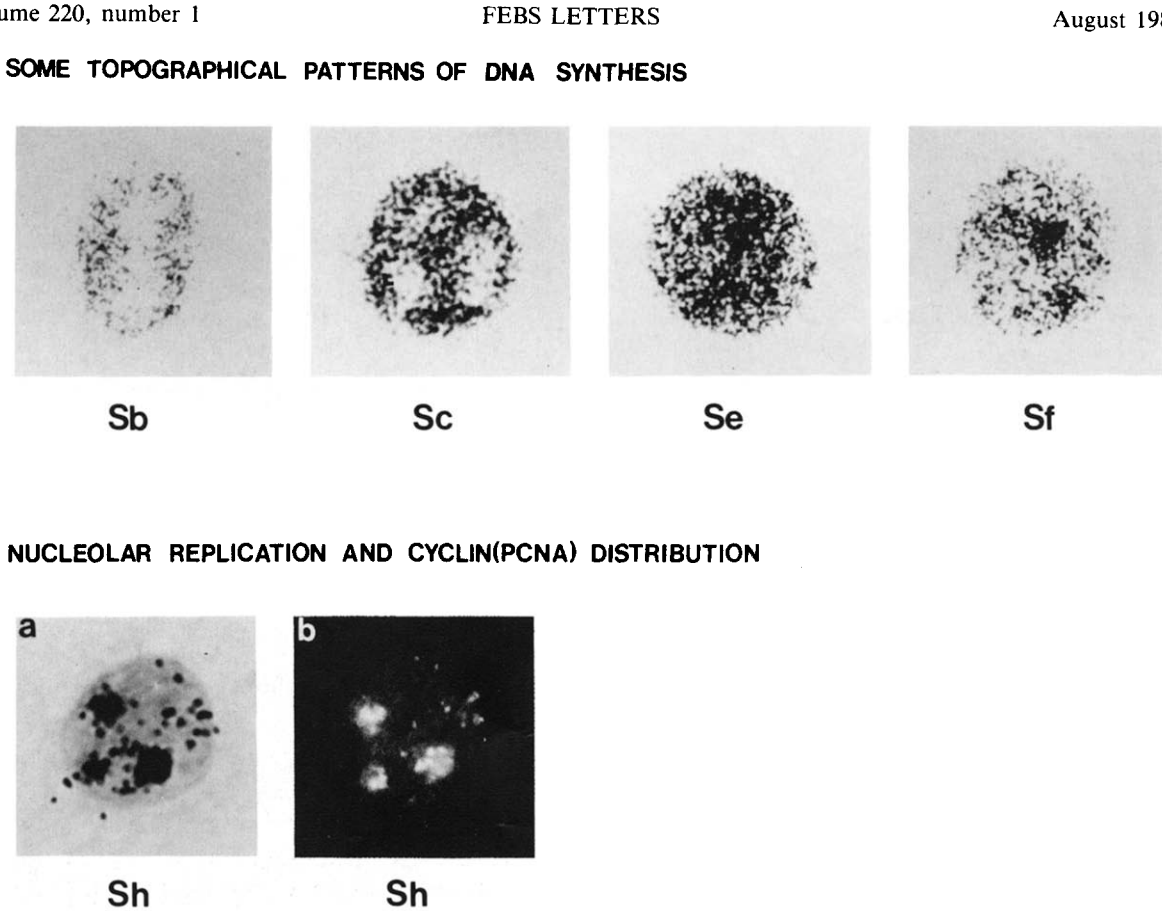


Fig.3. (A) Topographical patterns of DNA synthesis. African green monkey kidney BS-C-1 cells were labelled for 3 h with 4 $\mu$Ci/ml of [*methyl*-$^3$H]thymidine. Autoradiograms were exposed for 24 h. Nucleolar replication is seen in Se and Sf [48]. Similar results have been observed in AMA cells [47], but the autoradiographic patterns are clearer in BS-C-1 cells as these exhibit a very flat morphology. (B) Autoradiography (a, [*methyl*-$^3$H]thymidine incorporation) and immunofluorescence (b, PCNA autoantibodies) of BS-C-1 cells depicting pattern Sh (see also fig.1). From Madsen and Celis [48].

fashion as cyclin (PCNA). Nevertheless, a re-evaluation of the polymerases involved in eukaryotic DNA replication seems necessary in view of the above observations.

## 3. CYCLIN (PCNA) IS A CENTRAL COMPONENT OF THE PATHWAY(S) LEADING TO DNA REPLICATION AND CELL DIVISION

Analysis of proliferating cultured cells from various species using two-dimensional gel electrophoresis, immunofluorescence with PCNA (cyclin) autoantibodies, or both, have shown that cyclin (PCNA) is a ubiquitous protein. Cyclin (PCNA) has so far been detected in proliferating cultured cells (S-phase cells) from the following vertebrate species: aves, bat, bovine, dog, dolphin, goat, hamster, human (fig.2B), mink, monkey, mouse, pisces, potoroo, rabbit and rat ([22,23,25, 29,45] and references therein). Two variant forms of cyclin (PCNA) have been observed: one more acidic variant present in human, hamster, potoroo and rat, and the other more basic present in mouse ([13,22] and references therein). The mouse protein corresponds to IEF 51f in the mouse protein catalogue [65]. Normal proliferating human cultured cells that have been shown to synthesize cyclin (PCNA) include epidermal basal keratinocytes [19,20], some epithelial amniotic fluid cells

([15]; Celis, J.E. and Bravo, R., unpublished observations), various types of fibroblasts ([15,16]; Celis, J.E., unpublished observations) and lymphocytes stimulated with phytohemagglutinin (Celis, J.E., unpublished observations; see also [37]). Cyclin (PCNA) synthesis declines or ceases as cell division and DNA synthesis stop [16]. Interestingly, proliferating repair deficient *Xeroderma pigmentosum* cells (XP8LO, group A; XP126LO, group F; XPITE, group C) synthesize normal levels of cyclin (PCNA) suggesting that the defect(s) is (are) unrelated to this protein, (Celis, J.E., unpublished observations).

As mentioned above, normal non-proliferating cultured cells and tissues synthesize very low levels of cyclin (PCNA) (fig.2A). Addition of serum or platelet-derived growth factor (PDGF) to quiescent 3T3 cells has been shown to result in an increased synthesis of cyclin (PCNA) late in the mitogenic response, just about the time at which DNA synthesis begins [17,18,31]. Similar results have been observed in quiescent MRC-5 human fibroblasts [33] and goat sinovial cells stimulated with 20% fetal calf sera (Celis, J.E., unpublished observations). Fig. 4A–C shows indirect immunofluorescence micrographs of quiescent (fig.4A; 96 h in DMEM containing 0.5% sera) and serum-stimulated human MRC-5 fibroblasts [fixed 18 (fig.4B) and 22 h (fig.4C) after release, S-phase] reacted with PCNA autoantibodies specific for cyclin. Recently, Sabath et al. [32] identified a protein of $M_r$ 36 000 (most likely cyclin (PCNA)) that was synthesized at high rates during late $G_1$ and the $G_1$ to S-phase transition in T-helper line L2 stimulated with recombinant human interleukin 2 (rIL-2). Synthesis of this protein was inhibited by the potassium channel blocker quinine. So far, DNA replication in vivo has never been observed in the absence of cyclin (PCNA) synthesis, suggesting that expression of this protein is an obligatory event in the $G_1$ to S transition of the cell cycle. Cyclin (PCNA) synthesis however can take place in vivo in the presence of DNA synthesis inhibitors [30].

Further evidence suggesting that cyclin (PCNA) may be a central component of the pathway leading to DNA replication and cell division comes from the study of adult and newborn human and mouse tissues, respectively. Most normal human adult tissues analyzed by two-dimensional gel electrophoresis and silver staining exhibit very low or undetectable levels of cyclin (PCNA) (Celis, J.E. et al., unpublished observations). Tissues so far analyzed include: aorta, bladder, cavum oris, cerebellar cortex, cerebral cortex, cornea, ductus

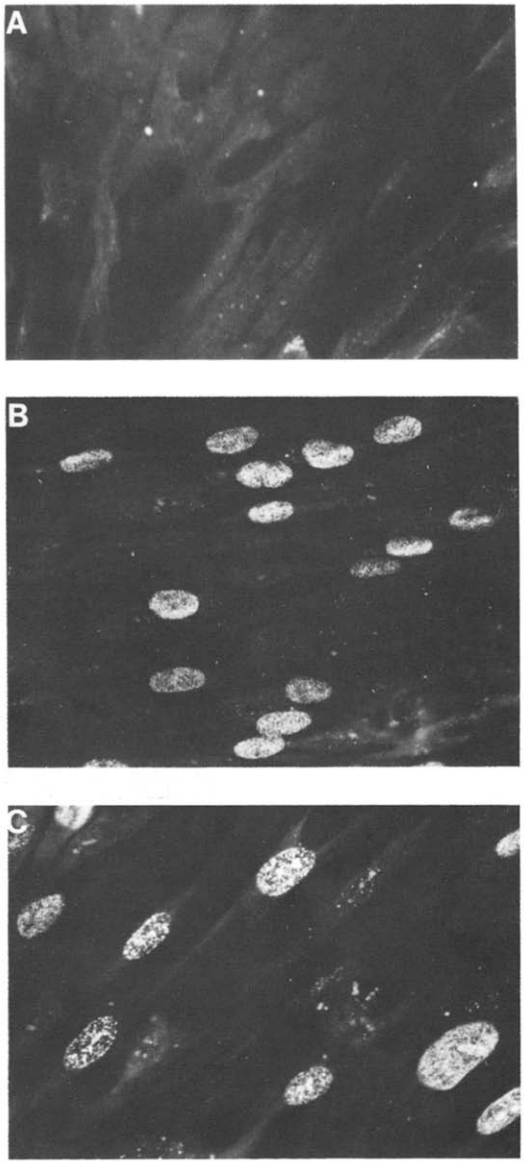


Fig.4. Indirect immunofluorescence with PCNA autoantibodies of quiescent and serum-stimulated human MRC-5 fibroblasts. (A) Quiescent (96 h in DMEM containing 0.5% sera). (B) 18 h after serum stimulation. (C) 22 h after serum stimulation. Nucleolar staining can be clearly seen in some cells in (C).

deferens, epididymis, fat tissue, heart muscle, kidney cortex, kidney medulla, larynx, lung, mammary gland, medulla oblongata, mesencephalon, palate, pharynx, posterior eye polus, prostata, rectum, skeletal muscle, skin, submaxillary glands, thyroid gland, trachea, ureter, uterus, veins and vesicula seminalis. The fraction of dividing cells present in some of these tissues is too low to be detected by silver staining. Many newborn mouse tissues (gut, spleen, kidney, liver, lung), on the other hand, exhibit high levels of cyclin (PCNA) ([5,66]; Celis, J.E., unpublished observations), suggesting that this protein has a central role in cell proliferation in all cell types.

## 4. CONCLUDING REMARKS

Cyclin (PCNA) has emerged as a novel central component of the pathway(s) that control cell proliferation in vertebrates. This ubiquitous and tightly regulated cell cycle specific protein plays a role in chromosomal DNA replication and it is a late obligatory component [2] of the mitogenic response. Further studies using in vitro DNA replication systems as well as microinjection of synchronized cells may help unraveling the precise function of this protein in DNA replication and cell cycle progression. These studies may have important repercussions in cancer chemotherapy. Finally, the recent molecular cloning of cyclin (PCNA) [35,68] should greatly facilite studies concerning regulatory aspects of this protein.

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