

UV Inducibility of Rat Proliferating Cell Nuclear Antigen Gene Promoter

Hsueh-Wei Chang, Yi-Chyi Lai, Ching-Yang Cheng, Jih-Lin Ho, Sheue-Ting Ding, and Yin-Chang Liu*

Department of Life Science, National Tsing-Hua University, Hsin-Chu, Taiwan 30043

Abstract Proliferating cell nuclear antigen (PCNA), also known as a cofactor of DNA polymerase δ , is required for eukaryotic cell DNA synthesis and nucleotide excision repair. Expression of PCNA gene is growth-regulated and UV inducible. In our previous study, we have observed that the rat PCNA promoter has the serum responsiveness. In this study, we demonstrate its UV inducibility in CHO.K1 cells. The UV induction of the rat PCNA promoter activity was dose-dependent in the cells synchronized at different phases. In addition, the sequences of the promoter responsible for the UV inducibility were delimited to the region between nucleotides -70 and $+125$, which contains an AP-1 site and a downstream proximal ATF/CRE site. While mutation of the AP-1 site abrogated the UV inducibility, mutation of the ATF/CRE site enhanced the UV inducibility, suggesting that the two sites play different roles in the UV induction of the promoter. In addition, the role of p53 in the UV induction of rat PCNA promoter was investigated. We found that exogenous p53 was unable to mimic the UV irradiation to induce rat PCNA promoter and that the UV induction of the rat PCNA promoter was seen in p53 deficient cells. Therefore, it is unlikely that the UV induction of the rat PCNA promoter is p53 dependent. *J. Cell. Biochem.* 73:423–432, 1999. © 1999 Wiley-Liss, Inc.

Key words: UV induction; PCNA promoter; AP-1; ATF/CRE; p53

Proliferating cell nuclear antigen (PCNA), also known as an auxiliary factor of DNA polymerase δ , is required in both DNA synthesis and DNA excision repair in eucaryotes [Miyachi et al., 1978; Mathews et al., 1984; Prelich et al., 1987; Jaskulsk et al., 1988a; Liu et al., 1989a; Shivji et al., 1992]. As an analogue of β subunit of DNA polymerase III of *E. coli*, PCNA forms a trimeric ring around the DNA strands to assist DNA polymerase δ in the processivity of DNA replication [Krishna et al., 1994]. In addition to its interactions with components of DNA synthesis machinery, PCNA also interacts with molecules involved in regulation of cell cycle progression such as cyclin D, p21^{WAF1}, and gadd45 [Xiong et al., 1992; Matsuoka et al., 1994; Smith et al., 1994]. These interactions suggest that the level of PCNA expression is a potential target of all growth related processes including cell proliferation, transformation, dif-

ferentiation, senescence, DNA repair, and apoptosis. Extensive evidence has indicated that expression of PCNA is growth regulated, i.e., high expression in dividing cells or tissues and very low in their quiescent counterparts [Jaskulski et al., 1988b; Liu and Bambara, 1989b; Liu et al., 1993]. In addition, PCNA expression may be induced by viral transformation or UV irradiation [Zerler et al., 1987; Hall et al., 1993; Zeng et al., 1994]. Control of the PCNA expression is at the transcriptional and/or post-transcriptional level and may vary with different cell types [Morris and Mathews, 1989; Chang et al., 1990; Baserga, 1991]. We have found that the rat PCNA promoter is serum responsive [Liu et al., 1995]. The rat PCNA promoter, like other mammalian PCNA promoters, does not have a typical TATAA box in the region near the transcription initiation site. The full activity of the rat PCNA promoter (or other mammalian PCNA promoters) is in the region between nucleotides -240 and $+120$ with reference to the transcription initiation site. When the upstream region of the promoter is trimmed down to -70 , the activity decreases to about 40%, however, the serum responsiveness remains. This short promoter has an AP-1 site (TGGGTCA) at $-64/-58$ and a proximal down-

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*Correspondence to: Dr. Yin-Chang Liu, Department of Life Science, National Tsing-Hua University, Hsin-Chu, Taiwan 30043. E-mail: lslyc@life.nthu.edu.tw

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stream ATF/CRE site (TGACGCCA) at -51/-44. The respective region of human promoter contains an E1A responsive element [Labrie et al., 1993]. In this study, we have demonstrated the UV inducibility of the rat PCNA promoter. The region that responds to UV irradiation has been defined by a series of deletion. Our study indicate that the AP-1 and ATF/CRE sites in this region play differential roles in the UV induction of the rat PCNA promoter. Furthermore, p53 is probably not involved in the UV induction of rat PCNA promoter.

MATERIALS AND METHODS

Plasmids

Plasmid d693-pCAT, a derivative of pBasic-CAT vector (Promega, Madison, WI), contained the rat PCNA promoter sequence between nucleotides -693 and +125 [Liu et al., 1995]. Plasmid pC53-SN, a human wild-type p53 expression vector and plasmid PRE-pCAT, a vector with p53 responsive element, were provided by Drs. J.L. Hwang and Y.-S. Lin (Academia Sinica, Taipei), respectively. Plasmid pCMV-Neo, an empty vector of pC53-SN (without p53 expression), was obtained from Dr. S.Y. Ng.

Site-Directed Mutagenesis of AP-1 and ATF/CRE of PCNA Promoter Mutants

Construction of AP-1 mutant. Site-specific mutation at the AP-1 site (-64/-58) of the rat PCNA promoter d240 (see Fig. 4A), was constructed by the method of overlap extension polymerase chain reaction (OE-PCR) [Higuchi et al., 1988]. The four primers used in OE-PCR were listed in the followings and their AP-1 mutated sequences were underlined: -240f: 5'-CCTGCAGAACATGGAAACCACAGC-3', mAPr: 5'-CAGCGCCATATGACCCACCTCTCCT-3', mAPf: 5'-GGTGGGTCATATGGCGCTGTGACGC-3', and 125r: 5'-GCGACGTCTAGATGAGAG-3'. The original AP-1 sequences were changed into a Nde I site to facilitate our cloning process. The PCR products of -240f/mAPr and mAPf/125r, made individually, were pooled together and used as templates for OE-PCR reaction using primers -240f and 125r. The OE-PCR products were subcloned into pBasic-CAT (Promega).

Construction of ATF/CRE mutant. Site-specific mutation of ATF/CRE site (-51/-44) of the rat PCNA promoter was constructed by the megaprimering PCR method as described [Sarkar and Sommer, 1990]. In theory, one of the double

DNA strands of the first PCR product is used as a primer for the next PCR. Thus, as above, we designed a primer with ATF/CRE sequence altered; this primer, named mATFr, had sequence as 5'-AGGTTGTGCATATGCAGCGCTGACC-3' in which the original ATF/CRE sequence was altered to a Nde I site. This reverse primer and the primer -193f (5'-GGAAGC-TTGGCGGTGACGACAGCCTACG-3') were used in a PCR. The PCR product with about 150 bp was subsequently mixed and used as a megaprimer for a new PCR with primer 125R (5'-GCGACGGTC-GACTGAGAGTTACTG-3') to generate a fragment with 318 bp. This product was co-digested with restriction enzyme Hind III and Sal I and then subcloned into the pBasic-CAT vector. Both AP-1 mutant and ATF/CRE mutant constructs were verified by DNA sequence analyses.

Establishment and Maintenance of Cell Line, Cell Synchronization

CHO.K1 cells, a Chinese hamster ovary cell line from ATCC, were routinely cultured in McCoy's 5A medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 µg/ml streptomycin. All cells were kept at 37°C in a humidified atmosphere containing 5% CO₂. To obtain the stable transfectants of wild-type (or mutant) rat PCNA promoter, cells were co-transfected with PCNA promoter and pCMV-Neo. Cells which survived in medium containing antibiotics G418 were collected as stable transfectants. For obtaining cells synchronized at G₀ stage, cells (in 70-80% confluence) were serum starved for 48 h as described previously [Liu et al., 1995]. To synchronize cells at early S phase, the G₀ cells were grown in the medium containing 10% FCS and 1 mM hydroxyurea for 24 h according to [Johnson et al., 1993]. To synchronize cells at G₂ stage, the early S phase cells were grown in the medium containing the 7.5 µg/ml Hoechst 33342 (Sigma, St. Louis, MO) for a subsequent 8 h according to [Johnson et al., 1993].

UV Treatment

Cells were washed with phosphate-buffered saline and, with the covers off, placed in marked areas in the tissue culture hood, which had been pre-calibrated for the required dose of UV using the germicidal lamp (254 nm) with the aid of a UV radiometer (UVP, San Gabriel, CA). The media that were removed prior to irradiation were added again after UV exposure, and

the cells were harvested for analysis at certain intervals. Control cells received mock treatment without UV exposure.

Western Blot Analysis

Western blot analysis of PCNA was done according to the standard protocol. For example, CHO.K1 cell crude extracts were prepared with freeze and thaw method. Aliquots of crude extract containing 19 μ g for each sample were used for protein separation on 12.5% SDS-PAGE. Protein blot was prepared by transferring proteins in gel to membrane. PCNA protein in the blot was recognized by the mouse monoclonal antibody to PCNA (Ab-1, Oncogene Science, Manhasset, NY). Then goat anti-mouse IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) was used for subsequent reaction. To detect the complexes, ECL detection kit (Amersham, Arlington Heights, IL) was used according to the instruction of the manufacturer.

DNA Transfection and CAT Assays

The transfection (stable or transient) was performed with liposome-mediated method using LipofectAMINE[™] (Gibco, Grand Island, NY) according to the manufacturer's manual. Briefly, DNA (e.g., 1 μ g per 60-mm dish) in 0.1 ml of serum-free McCoy's 5A medium was mixed with 6–10 μ l of lipofectamine reagent dissolved in 0.1 ml serum-free medium. Then, the mixed solution was incubated for the formation of DNA-liposome complex at room temperature for 30 min. Cells grown to 70% confluence were treated with these reaction solution in the presence of serum-free medium for 24 h. Subsequently, the cells were grown in serum containing medium until confluence. CAT activity was measured and quantified according to Gorman et al. [1982]. Cells were harvested and lysed by the Reporter Lysis Buffer (Promega) at certain intervals as described in the figure legends. Protein concentration of the cell lysate was determined by BCA* protein assay (Pierce, Rockford, IL). After thin-layer chromatography, acetylated chloramphenicols were quantitated by using a PhosphoImager (Molecular Dynamics, Sunnyvale, CA). CAT activities of different lysates were normalized by protein concentration or β -galactosidase activity (if co-transfected with pCMV- β -gal for transfection efficiency control). As we were aware that the pCMV promoter is slightly serum responsive

[Liu et al., 1995] and may be also UV inducible, we repeated the transient expression experiments (5–6 times) to dilute the weight of transfection efficiency variation on our data.

RESULTS

UV Induction of PCNA Expression and Construction of Stable Clones of Rat PCNA Promoter

Previous investigators have seen that the expression of PCNA, at the mRNA or protein level, can be induced by UV irradiation [Hall et al., 1993; Zeng et al., 1994]. The same induction was also observed by us in human and rodent cells as shown in Figure 1. Since the UV inducibility of PCNA promoter has not yet been reported, we decided to proceed the study. To facilitate our study, we obtained stable transfectants of the rat PCNA promoter by co-transfecting a rat PCNA promoter construct, d693-pCAT and a neomycin resistance plasmid, pCMV-Neo, into CHO.K1 cells. For convenience, the stable transfectants are referred to as d693-pCAT.K1 cells henceforth. Previously in our transient expression study, we have shown that d693-pCAT is responsive to serum stimulation [Liu et al., 1995]. The promoter construct d693-pCAT contains the sequences between the nucleotides –693 and +125. We found that the promoter in the stable transfectants preserved the character of serum responsiveness as shown in Figure 2. Thus, we assumed that insertion of the promoter construct into the host genome during stable transfection had little alteration on the property of the promoter. Hence, the use of the stable transfectants is appropriate for further characterization of the rat PCNA promoter.

UV Inducibility of the Rat PCNA Promoter

Initially, we used quiescent cells to study the UV effect on the rat PCNA promoter. The d693-pCAT.K1 cells were synchronized at G₀ stage by serum deprivation. After receiving a certain dose of UV irradiation at 254 nm, cells were allowed to recover in the serum-free medium (to avoid the effect of serum stimulation) for a period of time before harvest for CAT activity assay. In contrast to that in the untreated cells, the PCNA promoter activity in the treated cells increased as cells recovered from the treatment (Fig. 3), reaching 6.7 ± 0.5 -fold induction at 24 h. We also performed a similar experiment ex-

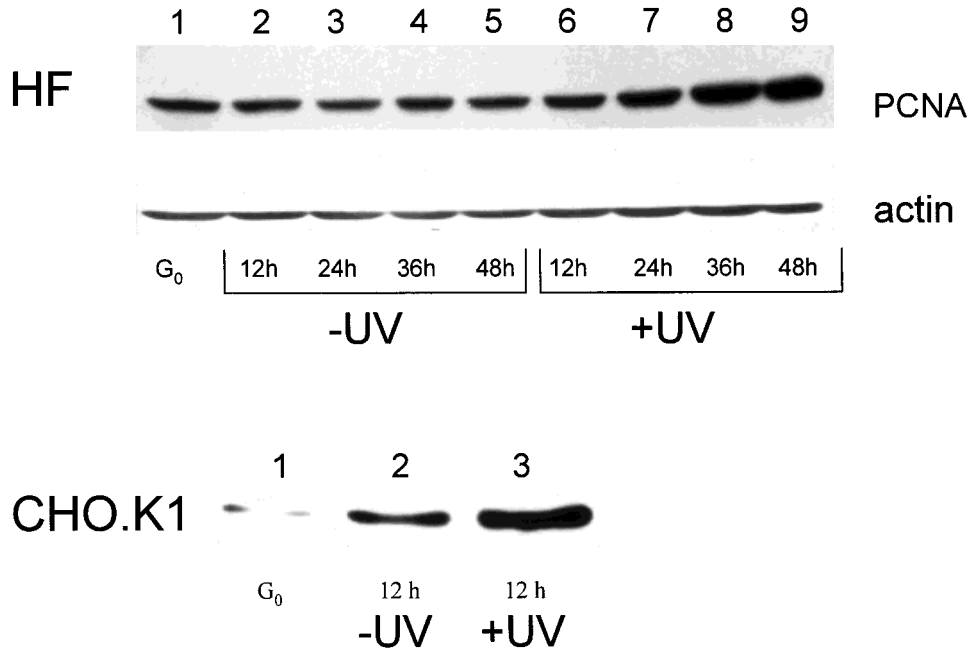


Fig. 1. UV induction of PCNA expression detected by Western blot analysis. Top: Human fibroblast cells were grown to complete confluence to reach G₀ state as described in [Johnson et al., 1993]. The quiescent cells (**lane 1**) were treated (**lanes 6–9**) or untreated (**lanes 2–5**) with UV (25 J/m²) followed by recovery in fresh medium (with serum) for the indicated intervals. Each lane contained equal amount of protein (about 60 µg) as indicated by the approximately equal level of β-actin in each lane.

Bottom: CHO.K1 cells were made quiescent by growing in serum deprived medium for 48 h (**lane 1**) before the treatment (**lane 3**) or without the treatment (**lane 2**) of UV (25 J/m²) irradiation followed by recovery in complete medium for the indicated intervals. In these experiments, the untreated cells actually received mock treatment, i.e., otherwise the same treatment except UV exposure.

cept that the UV treated (or untreated) cells were recovered in serum containing medium. The UV induction was also detectable with the induction fold of 5.0 ± 1.0 at 24 h after recovery. Since the cells recovered in serum containing medium appeared healthier, the experiments described henceforth were performed under this condition. Next, we performed the dose effect experiment. The cells were exposed to UV at doses ranging from 8 to 100 J/m². The UV induction fold of the promoter activity showed a dose-dependent fashion reaching a peak induction at the doses between 25 and 50 J/m² (Fig. 4A). As a control of UV dose, the level of heat shock protein 70 was measured. Since the experiments just described were performed with G₀ cells, to see if cell cycle played any role in the UV induction of PCNA promoter activity, we performed the similar experiment with cells synchronized at different stages. The d693-pCAT.K1 cells were synchronized at the early S phase with hydroxyurea or at G₂ with Hoechst 33342 according to the protocols described in [Johnson et al., 1993] prior to UV irradiation and the subsequent procedures for analysis.

The UV induction was seen with these cells in a dose dependent manner, although the induction fold and optimal dose may vary (see Fig. 4B,C). For comparison, results with different phase cells were summarized in Figure 4D, indicating that the UV induction of PCNA promoter may vary with cell cycle.

Localization of UV Inducibility in Rat PCNA Promoter and Differential Roles of AP-1 and ATF/CRE Sites in UV Inducibility

To localize the sequences of the rat PCNA promoter responsible for the UV inducibility, we tested three different promoter constructs with 5' end deletion of varying length (Table I). All these three PCNA promoter constructs showed similar UV inducibility (Table I). These data suggest that the sequences responsible for UV inducibility are within the shortest promoter which has nucleotides between -70 and +125. This region contains an AP-1 like site (-64/-58, TGGGTCA vs. consensus TGACTCA) and a nearby ATF/CRE like site (-51/-44, TGACGCCA vs. consensus TGACGTCA; Fig. 5A). These two sites were found to be protected

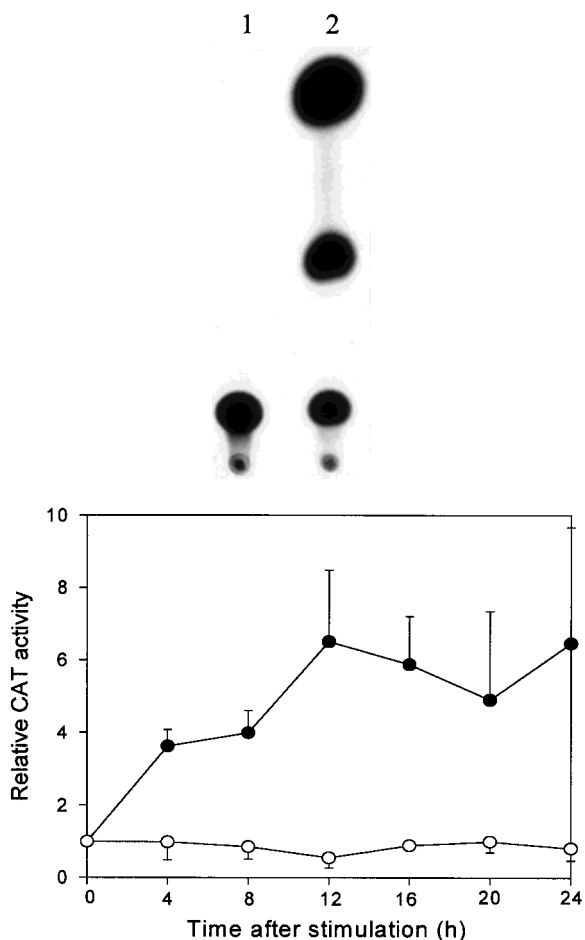


Fig. 2. Serum responsiveness of rat PCNA promoter in stably transfected cells. Top: CAT activity in PCNA promoter stably transfected cells (lane 2) and in parental cells (lane 1). Bottom: Kinetics of CAT activity after serum stimulation of quiescent stable transfectants, d693-pCAT.K1. The promoter activity in the serum-stimulated cells and in the unstimulated cells was indicated as filled-circle (●) and open-circle (○), respectively. Each data point was the mean of at least three individual experiments and the respective standard deviation.

by nuclear factors in DNase I footprinting analysis (data not shown). To test the roles of the cis elements in the UV inducibility, site-specific mutations at the AP-1 or ATF/CRE sites were performed. Construction of the two mutants was done by using an overlapping PCR strategy [Higuchi et al., 1988] or a megaprimer PCR method [Sarkar and Sommer, 1990] as described in Materials and Methods. The site-directed mutant promoters were confirmed by DNA sequence analyses. Stable transfectants of the mutant promoters were obtained and treated with UV as described earlier. The results shown in Figure 4B indicate that the AP-1 mutant lost UV inducibility, but surprisingly,

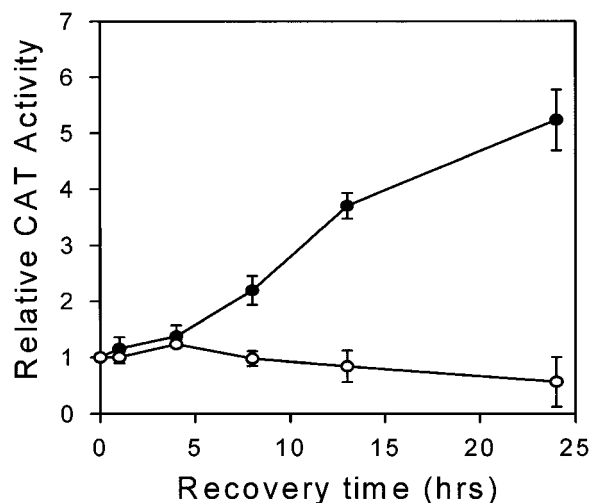
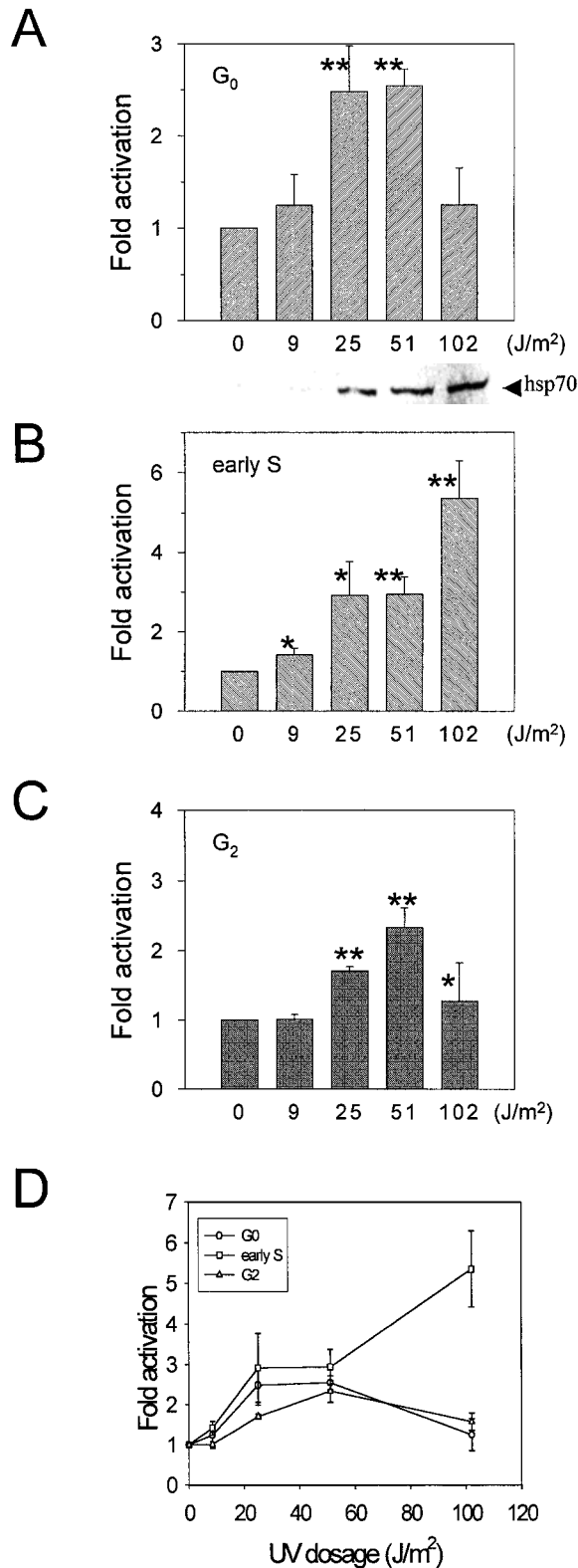


Fig. 3. UV induction of the rat PCNA promoter in stably transfected cell. Stable transfectants, d693-pCAT.K1 cells, were synchronized at G₀ stage by serum starvation before UV irradiation. Cells were UV irradiated (●) at dose of 25 J/m². Control cells (○) received mock treatment. After UV treatment (or nontreatment), cells were recovered in serum free medium for the indicated intervals before harvest for CAT activity assay. Each data point was the mean of at least three individual experiments and the respective standard deviation.

the ATF mutant showed an opposite result; it had more UV inducibility than even the wild-type. Apparently, the AP-1 and ATF/CRE sites play different roles in UV induction of the promoter. In contrast, these two sites may play the same roles in serum responsiveness as both AP-1 and ATF/CRE mutants were no longer responsive to serum stimulation (see Fig. 5C). In addition, double mutants at both AP-1 and ATF/CRE sites behaved similarly as AP-1 mutant (data not shown). Taken together, these results implied that, AP-1 site serves as a positive regulator, while ATF/CRE site acts as an attenuator in UV induction of rat PCNA promoter.

UV Inducibility of Rat PCNA Promoter is p53 Independent

Since the p53 tumor suppressor gene has been implicated as a guardian for genomic integrity, the level of p53 may be induced in UV irradiation [Hall et al., 1993]. A low level of p53 can transactivate human PCNA promoter activity [Shivakumar et al., 1995; Morris et al., 1996] possibly via the p53 consensus sequences located between nucleotides -238 and -199. Though no corresponding p53 response elements are detected in mouse or rat PCNA promoters, it remains to be known whether p53



plays any role in the UV induction of rat PCNA promoter. To test the possibility, we first performed the experiment to see if rat PCNA promoter activity could be activated by p53. We transiently transfected the wild type human p53 expression vector into the d693-pCAT.K1 cells to see if the expression of p53 could mimic the UV induction of PCNA promoter activity. The results are summarized in Table II, indicating that the rat PCNA promoter activity was either unaffected or inhibited by p53 at the transfected doses of p53 expression vector ranging from 0.25 to 5 μ g. In contrast, p53 was able to trans-activate PRE-pCAT, a p53 responsive element [Hsu et al., 1995] (Table II). Thus, p53 was unable to mimic the UV induction. Furthermore, if the UV induction of the rat PCNA promoter was due to induction of p53 in cells, such induction would be undetectable with cells in which p53 is deficient. Hence, we performed the UV irradiation experiment with HeLa cells. The p53 level in HeLa cells is deficient because of the presence of the E6 oncoprotein of HPV. As shown in Figure 5, the UV induction of the rat PCNA promoter was still seen with HeLa cells. Moreover, the p53 was not detectable in UV treated HeLa cells. Therefore, the UV induction of rat PCNA promoter activity is unlikely p53 dependent.

DISCUSSION

Although it has been reported that PCNA expression at mRNA or protein level is UV inducible, there is lack of documentation about UV inducibility of PCNA promoter. In this re-

Fig. 4. Dose dependence of UV induction of the rat PCNA promoter in G₀, early S or G₂ phase cells. Stable transfectants of the rat PCNA promoter, d693-pCAT.K1 cells, were synchronized at G₀ (A), early S stage (B), or G₂ (C) according to the procedures described in the section of Materials and Methods prior to UV treatment. Following UV treatment, cells were recovered in serum containing medium for 24 h before harvest for CAT activity assay. The change of CAT activity in cells due to UV was indicated as fold activation. The fold activation is the ratio of the CAT activity in UV irradiated cells versus those in untreated cells. Thus, the effect due to serum supplement was normalized. Each data point was the mean of at least three individual experiments and the respective standard deviation. Significant changes (i.e., $P < 0.05$ or $P < 0.01$, by Student *t*-test) were indicated by * or **, respectively. A, bottom: Western blot analysis of hsp70 in the d693-pCAT.K1 cells. The measurement of hsp70 was used as the control of UV dose received by the cells. For comparison, the above results were summarized in D. The symbols of open circle, open square and open triangle represent G₀, early S stage and G₂, respectively.

TABLE I. Localization of UV Inducibility in Rat PCNA Promoter^a

Promoter region	Relative CAT activity U.V.		Fold activation (+/-)
	-	+	
-693 _____ +125	241.0 ± 24.2	477.0 ± 22.7	~2.0
-240 _____ +119	183.0 ± 30.2	426.3 ± 25.3	~2.3
-70 _____ +125	56.0 ± 21.9	114.7 ± 9.0	~2.1
none	1.0	1.0	1.0

^aRat promoter was transiently transfected into CHO.K1 cells. Promoter activity was assayed in lysate of the transfected cells after exposure to UV (25.6 J/m²) and recovery for 24 h in serum containing medium. Symbols: -, without UV treatment; +, with UV treatment. Fold activation is the ratio of activities between + and - UV treatment. Each datum of activity was the mean value obtained from at least three independent experiments, and the standard deviation was indicated.

TABLE II. Inhibitory Effect of p53 on the Rat PCNA Promoter Activity

pC53-SN (μg)	Fold activation of	
	d693-pCAT ^a	PRE-pCAT ^b
0	1.00	1.00
0.1	0.87 ± 0.04	N.D.
0.25	0.83 ± 0.06	30.35 ± 11.56
0.5	0.86 ± 0.04	24.56 ± 2.10
1	0.48 ± 0.05	11.00 ± 3.97
5	0.11 ± 0.08	1.60 ± 0.15

^aHuman wild-type p53 expression vector, pC53-SN at variable dose was transiently transfected to d693-pCAT.K1 cells. The d693-pCAT.K1 cells were stable transfectants of rat PCNA promoter d693-pCAT in CHO.K1 cells. In each transfection (per 100-mm dish), total amount of DNA was made up to 10 μg with pCMV-Neo.

^bA p53 responsive promoter, PRE-pCAT, at a fixed amount (3 μg), was co-transfected with pC53-SN at variable dose into CHO.K1 cells. Total DNA in each transfection was also control as constant as in previous case. Following transfection, cells were harvested for CAT activity assay after 24 h recovery. CAT activity in the cells was indicated as relative level with the activity in cells untransfected with pC53-SN as 1.0. Data were presented as the mean of at least three individual experiments and the respective standard deviation. N.D., not determined.

port, we investigated the UV inducibility of the rat PCNA promoter and the cis-elements responsible for this UV inducibility. We first verified the UV induction of PCNA protein in both human and rodent cells (Fig. 1). Then we proceeded the construction of stable transfectants of the rat PCNA promoter. The advantages of using stable transfectants include the avoiding the variation of transfection efficiency in transient expression experiments and simplifying the experimental procedures. To ensure that the nature of the promoter was not altered due to the plasmid insertion during stable transfection, we showed the serum responsiveness of rat PCNA promoter in stable transfectants (Fig. 2). We then showed the UV inducibility of the

rat PCNA promoter in kinetics (Fig. 3). The UV induction was seen in cells of different phases, however, the optimal doses for UV induction of PCNA promoter seemed to vary with cell cycle (Fig. 4). Apparently, more systematic study is necessary to reach the conclusion in a quantitative manner. However, it is worth mentioning that in contrast to typical cell lines, e.g., NIH3T3, the cells in our study (i.e., CHO.K1) do not arrest at G₁ following UV irradiation. Although tumor suppressor protein p53 of the cells is induced by UV treatment and appears functional, p21^{WAF1} protein is undetectable in UV treated (or untreated) cells (our unpublished results). P21 is p53 inducible and is a general cyclin-dependent protein kinase inhibitor [El-Deiry et al., 1993]. Hence, the roles of potential cell cycle regulators in the UV response of PCNA promoter remain elusive. We then made efforts to define the region responsible for UV inducibility and found that the small sequence between nucleotides -70 and +125 remained UV inducible (Table I). The region of rat PCNA promoter has an AP-1 and an ATF/CRE site. The analogous region in the human PCNA promoter is responsible for the E1A induction [Morris and Mathews, 1989] and the ATF/CRE site is crucial to both basal activity and E1A activation [Labrie et al., 1993]. Hence, we mutated the AP-1 and/or ATF/CRE sites and studied their effects. While mutations at the AP-1 or ATF/CRE sites abrogated the serum responsiveness, the two mutants behaved differently toward UV irradiation. The AP-1 mutant decreased but ATF/CRE mutant increased UV inducibility (Fig. 5), implicating that the AP-1 site is involved in positive regulation and the ATF/CRE site in negative regulation. This unique characteristic of rat PCNA promoter may be associated with the proximity of the AP-1 and the ATF/CRE sites. Our prelimi-

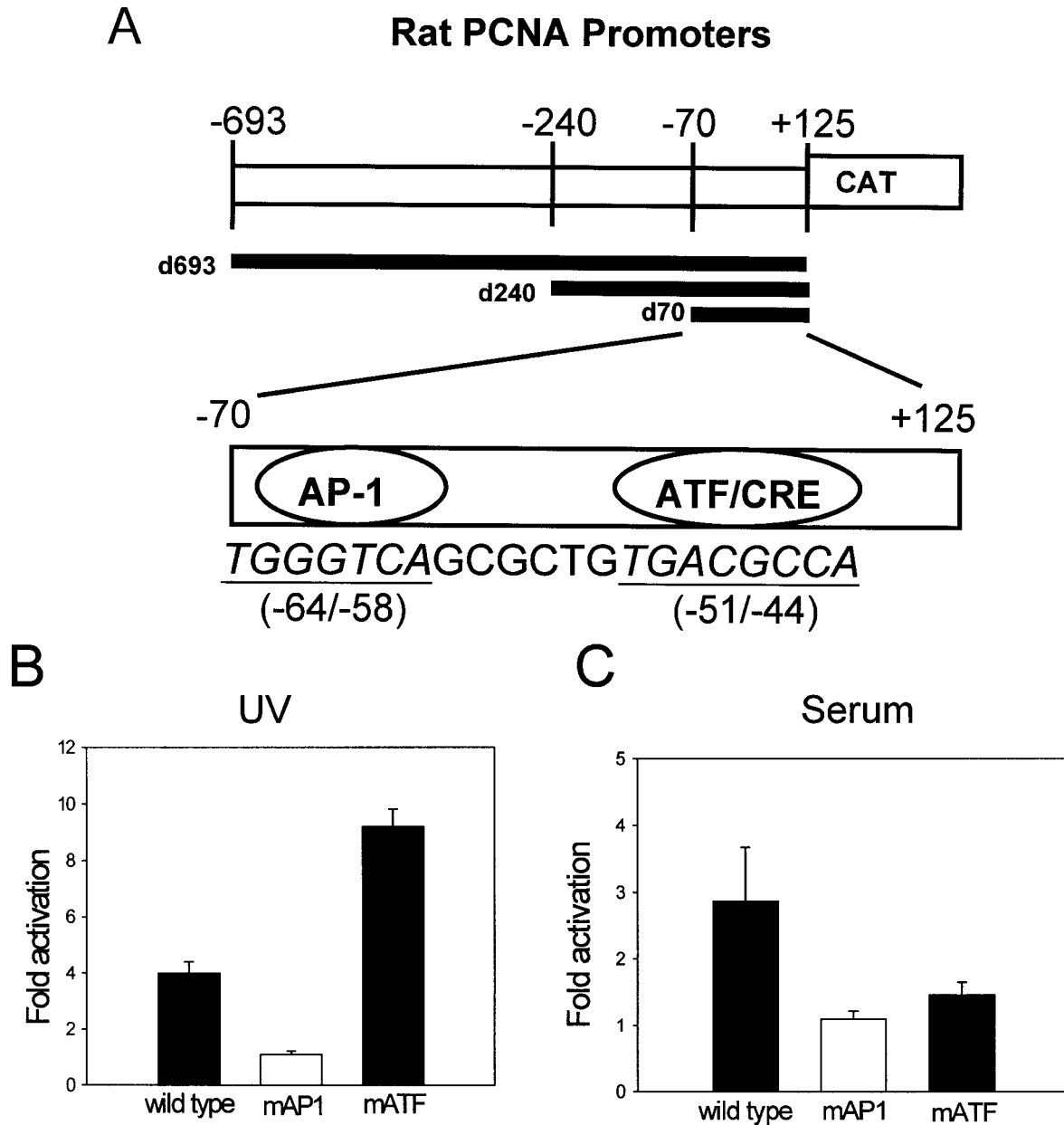


Fig. 5. Effect of AP-1 and ATF/CRE mutations on UV inducibility of PCNA promoter. **A:** Schematic illustration of the rat PCNA promoter and location of AP-1 and ATF/CRE sites. **B:** Differential effects of AP-1 and ATF/CRE mutants on UV induction of the rat PCNA promoter. Cells stably transfected with the wild-type or the AP-1 or ATF/CRE mutant PCNA promoter were synchronized at G₀, and then exposed to UV (50 J/m²) and recovered in

serum containing medium for 24 h before harvest for CAT assay. **C:** Mutation of AP-1 or ATF/CRE sites of the rat PCNA promoter abolishes serum responsiveness. Stable transfectants of wild-type or AP-1 (or ATF/CRE) mutant promoters were synchronized at G₀, and then serum-stimulated for 24 h before harvest for CAT assay. The change of CAT activity was indicated as fold induction as described in the legend of Figure 4.

nary results suggest "cross-talk" between the two sites. When a rat promoter related oligonucleotide with both the AP-1 and the ATF/CRE sites was used as a DNA probe in a gel mobility shift assay, protein:DNA complexes were formed with nuclear extracts from CHO.K1 or Hela cells, however, these com-

plexes were totally abrogated with oligomers containing either the AP-1 or ATF/CRE site. Thus, the distance (6 base pairs) and/or sequences between the ATF/CRE and AP-1 sites may play some role in regulation of rat PCNA promoter. These are currently under investigation. Regarding the differential roles played by

AP-1 and ATF/CRE sites in UV induction of rat PCNA promoter, we thought of two models. One invokes certain transcription repressor(s) which may appear and bind to ATF/CRE site during UV irradiation. The other assumes that a limiting level of ATF-like transcription factor is competed between c-Jun and CREB. Transcription factor ATF-2, besides its nature to associate with another ATF/CREB, has the potential to form a heterodimer with c-Jun which binds to AP-1 site. These hypotheses await further examination. Finally, we investigated the role of p53 in UV inducibility of PCNA promoter. Our results indicated that while the exogenous p53 was able to transactivate a p53 responsive promoter, it failed to activate the rat PCNA promoter (Table II), suggesting that p53 is unable to mimic the UV irradiation to induce the rat PCNA promoter. Furthermore, the UV induction of rat PCNA promoter was observed even with the p53 deficient cell line; e.g., Hela (Fig. 6). Thus, the UV inducibility of rat PCNA promoter is unlikely p53 dependent. This is consistent with the fact that the rat PCNA promoter

lacks a p53 consensus sequence. In contrast to the rodent promoter, the human PCNA promoter has a p53 binding site and is transactivated by wild-type p53 at a low expression level (at the transfecting dose of p53 expression vector $\leq 1 \mu\text{g}$). Therefore, it is possible that p53 may be involved in the induction of human PCNA promoter by UV or other DNA damaging agents. This possibility is currently under our examination.

In conclusion, the rat PCNA promoter is UV inducible. The UV inducibility of rat PCNA promoter is differentially mediated via the AP-1 and ATF/CRE sites and is p53 independent.

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REFERENCES

- Baserga R. 1991. Growth regulation of the PCNA gene. *J Cell Sci* 98:433–436.
- Chang CD, Ottavio L, Travali S, Lipson KE, Baserga R. 1990. Transcriptional and posttranscriptional regulation of the proliferating cell nuclear antigen gene. *Mol Cell Biol* 10:3289–3286.
- El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. 1993. *WAF1*, a potential mediator of p53 tumor suppression. *Cell* 75:817–825.
- Gorman CM, Moffat LM, Howard BH. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol Cell Biol* 2:1044–1051.
- Hall PA, Mckee PH, Menage HDP, Dover R, Lane DP. 1993. High-levels of p53 protein in UV-irradiated normal human skin. *Oncogene* 8:203–207.
- Higuchi R, Krummel B, Saiki JK. 1988. A general method of in vitro preparation and specific mutagenesis of DNA fragments—Study of protein and DNA interactions. *Nucl Acids Res* 16:7351–7367.
- Hsu YS, Tang FM, Liu WL, Chuang JY, Lai MY, Lin YS. 1995. Transcriptional regulation by p53—Functional interactions among multiple regulatory domains. *J Biol Chem* 270:6966–6974.
- Jaskulski D, DeRiel K, Mercer WE, Calabrett B, Baserga R. 1988a. Inhibition of cellular proliferation by antisense oligodeoxynucleotides to PCNA cyclin. *Science* 240:1544–1546.
- Jaskulski D, Gatti C, Travali S, Calabretta B, Baserga R. 1988b. Regulation of the proliferating cell nuclear antigen cyclin and thymidine kinase mRNA levels by growth factors. *J Biol Chem* 263:10175–10179.
- Johnson RT, Downes CS, Meyn RE. 1993. The synchronization of mammalian cells. In: Fantes P, Brooks R, editors. *The cell cycle—A practical approach*. Oxford University Press: IRL Press. p 1–24.

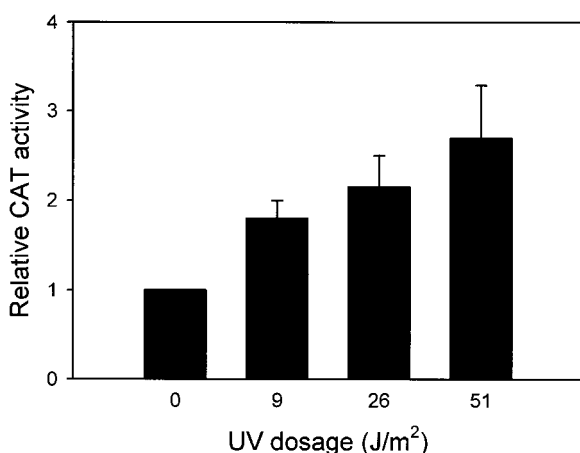


Fig. 6. UV induction of the rat PCNA promoter is p53 independent. Hela, a p53 deficient cell line (about 10^6 cells in 60 mm dish) was transiently co-transfected with the rat PCNA promoter (d693-pCAT, 10 μg) and pCMV- β -gal (2 μg). Following transfection, cells were synchronized at G₀ and UV irradiated at the indicated dose and recovered 24 h before harvest for CAT assay. The CAT activity was normalized with β -galactosidase activity and was presented as relative level with the activity in non-irradiated cells as 1.0. Data were presented as the mean of at least three individual experiments and the respective standard deviation. Note: although it remains to be known whether the CMV promoter was UV responsive (i.e., it may not be suitable for transfection efficiency control), similar results were obtained if protein amount was used for normalization. In addition, the repetition of the experiment (five to six times) was performed to dilute the weight of variation of transfection efficiency on the data.

- Krishna TSR, Kong XP, Gary S, Burgers PM, Kuriyan J. 1994. Crystallization of proliferating cell nuclear antigen (PCNA) from *Saccharomyces cerevisiae*. *Cell* 79:1233-1243.
- Labrie C, Morris GF, Mathews MB. 1993. A complex promoter element mediates transactivation of the human proliferating cell nuclear antigen promoter by the 243-residue adenovirus E1A oncoprotein. *Mol Cell Biol* 13:1697-1707.
- Liu YC, Marraccino RL, Keng PC, Bambara RA, Lord EM, Chou WG, Zain SB. 1989a. Requirement for proliferating cell nuclear antigen expression during stages of the Chinese hamster ovary cell cycle. *Biochemistry* 28:2967-2974.
- Liu YC, Bambara RA. 1989b. Gene expression of PCNA cyclin in adult tissues and the R3230Ac mammary tumor of rat. *Biophys Biochem Res Commun* 161:873-882.
- Liu YC, Liu WL, Ding ST, Cheng HM, Cheng JT. 1995. Serum responsiveness of the rat PCNA promoter. *Exp Cell Res* 218:87-95.
- Liu YW, Chang KJ, Liu YC. 1993. DNA methylation is not involved in growth-regulation of gene expression of proliferating cell nuclear antigen. *Exp Cell Res* 208:479-484.
- Mathews MB, Bernsstein RM, Franza JBR, Garrels JI. 1984. Identity of the proliferating cell nuclear antigen and cyclin. *Nature* 309:374-376.
- Matsuoka S, Yamaguchi M, Matsukage A. 1994. D-type cyclin-binding regions of proliferating cell nuclear antigen. *J Biol Chem* 269:11030-11036.
- Miyachi K, Fritzler MJ, Tan EM. 1978. Autoantibody to a nuclear antigen in proliferating cells. *J Immunol* 121:2228-2234.
- Morris GF, Mathews MB. 1989. Regulation of proliferating cell nuclear antigen during the cell cycle. *J Biol Chem* 264:13856-13864.
- Morris GF, Bischoff JR, Mathews MB. 1996. Transcriptional activation of the human proliferating cell nuclear antigen promoter by p53. *Proc Natl Acad Sci USA* 93:895-899.
- Prelich G, Kostura M, Marshak DR, Mathews MB, Stillman B. 1987. The cell cycle regulated proliferating cell nuclear antigen is required for SV40 DNA replication in vitro. *Nature* 326:471-475.
- Sarkar G, Sommer SS. 1990. The megaprimer method of site-directed mutagenesis. *Biotechniques* 8:404-407.
- Shivakumar CV, Brown DR, Deb S, Deb SP. 1995. Wild-type human p53 transactivates the human proliferating cell nuclear antigen promoter. *Mol Cell Biol* 15:6785-6793.
- Shivji MKK, Kenny MK, Wood RD. 1992. Proliferating cell nuclear antigen is required for DNA excision repair. *Cell* 69:367-374.
- Smith ML, Chen I, Bae I, Chen C, Gilmer T, Kastan MB, O'Connor PM, Formace AJ. 1994. Interaction of the p53-regulated protein gadd45 with proliferating cell nuclear antigen. *Science* 266:1376-1380.
- Xiong Y, Zhang H, Beach D. 1992. D-type cyclins associate with multiple protein-kinases and the DNA-replication and repair factor PCNA. *Cell* 71:505-514.
- Zeng X, Jiang Y, Zang S, Hao H, Lee MYWT. 1994. DNA polymerase-delta is involved in the cellular response to UV damage in human cells. *J Biol Chem* 269:13748-13751.
- Zerler B, Roberts RJ, Mathews MB, Moran E. 1987. Different functional domains of the adenovirus E1A gene are involved in regulation of host cell cycle products. *Mol Cell Biol* 7:821-829.