Jpn. J. Cancer Res. 83, 609–617, June 1992

Activation of the Mouse Proliferating Cell Nuclear Antigen Gene Promoter by Adenovirus Type 12 E1A Proteins

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A plasmid carrying the 5′-flanking region (−1584 to +47 with respect to the transcription initiation site) of the mouse proliferating cell nuclear antigen (PCNA) gene was fused with the chloramphenicol acetyltransferase (CAT) gene, and then cotransfected into mouse N18TG2 cells with expression plasmids for the adenovirus type 12 E1 genes. Expression of E1A gene products elevated the CAT expression by 5- to 9-fold, but expression of the E1B gene product did not. RNase protection analysis revealed that the activation of the PCNA gene promoter by E1A was at the transcription step. Both the 13S E1A and the 12S E1A activated the PCNA gene promoter, indicating that the activation domain of E1A resides in a common region(s) of 13S and 12S E1A products. The major target region of E1A was mapped within the 68 base-pair region (−21 to +47) of the PCNA gene, which includes consensus sequences for transcription factors PEA3 and E2F, although the upstream region (−83 to −21) including ATF(CREB)-binding consensus had an additional effect in the transactivation.

Key words: Proliferating cell nuclear antigen — Adenovirus E1A — Promoter — CAT-assay — Transactivation

The proliferating cell nuclear antigen (PCNA) is a co-factor of DNA polymerase δ[1] and is necessary for simian virus 40 DNA replication[2] as well as cellular proliferation.[3,4] The amino acid sequence of this protein has been highly conserved during evolution.[5] Homologs have been identified in mammals,[6-9] plants,[10] insects,[11] amphibia,[12] yeast[13] and even bacteriophage T4.[14] Although the mammalian PCNA protein and mRNA levels change relatively little during the cell cycle,[4,15] they can be induced upon growth stimulation of quiescent cells by agents such as serum, growth factors and viral infection.[6,7,16,17] Since the induction of PCNA mRNA levels by these agents requires protein synthesis and occurs after a delay of several hours,[6,17] the PCNA gene is a so-called late growth-regulated gene, like the thymidine kinase gene and other genes coding for proteins of the DNA synthesizing machinery.[18] Expression of the human PCNA gene in relation to cell cycle progression is under the control of complex regulatory mechanisms.[18]

The adenovirus E1A gene products are able to transactivate five adenovirus early genes and a number of cellular genes.[19] Most of the positive transcriptional effects of the E1A protein were mapped to the so-called conserved region 3 which is unique to the 13S E1A protein.[20] However, a virus expressing only the 12S E1A mRNA, which lacks the conserved region 3, can induce expression of cellular genes such as PCNA,[16] hsp70,[21] cdc2[22] and brain creatine kinase.[23] An adenovirus type 2 (Ad2) E1-responsive element of human PCNA gene was mapped in the 85 base-pair (bp) region (−87 to −2).[24] Recently the target region has been narrowed down to the transcription factor ATF(CREB)-binding consensus sequence located at approximately −50 nucleotide position.[25] Previously, we cloned the mouse PCNA gene and analyzed its nucleotide sequence.[9] By a transient expression assay of chloramphenicol acetyltransferase (CAT), the promoter of this gene was localized within a 200 bp region upstream of the transcription initiation site, where several putative transcriptional regulatory elements were found.[9]

In the present study, expression plasmids for each of adenovirus type 12 (Ad12) E1 gene products were constructed in the same plasmid backbone to determine which of the E1 gene products is responsible for the transactivation. The data indicate that both the 13S E1A product and the 12S E1A product can transactivate the PCNA gene promoter. The major target region of the activation by E1A was localized in the region between −21 and +47 of the mouse PCNA gene promoter that contains transcription factors PEA3- and E2F-binding consensus sequences surrounding the transcription initiation site.

MATERIALS AND METHODS

Plasmid constructions The *Bam*HI-*Eco*RI fragment containing Ad12 E1A promoter and E1A gene was isolated from the plasmid gAE1A,[26] then inserted into the

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*Bam*HI and *Eco*RI sites of the plasmid pUC19 to obtain plasmid pAd12E1A. The plasmid pABA[26] was digested with *Bam*HI, then partially with *Eco*RI. The 2.8 kb fragment containing Ad12 E1A promoter and E1B gene was isolated. This fragment was inserted into *Bam*HI and *Eco*RI sites of the pUC19 to obtain plasmid pAd12E1B. The plasmid pNeo13SE1A[27] was digested with *Eco*RI and *Bam*HI. The 1.5 kb fragment containing Ad12 E1A promoter and E1A 13S cDNA was isolated. This fragment was inserted into *Bam*HI and *Eco*RI sites of the pUC19 to obtain plasmid pAd12-13SE1A. The plasmid pNeo12SE1A[27] was digested with *Eco*RI and *Bam*HI. The 1.4 kb fragment containing Ad12 E1A promoter and E1A 12S cDNA was isolated. This fragment was inserted into *Bam*HI and *Eco*RI sites of the pUC19 to obtain the plasmid pAd12-12SE1A.

The plasmid pE2CAT(pEC)[28] contains Ad5 E2 gene promoter (−285 to +40) placed upstream of the CAT gene. A series of 5′-end unidirectional deletion derivatives of mouse PCNA gene promoter were constructed as described.[9] Deletion end points of the mutants are summarized in Fig. 1. All of these mutants contain the PCNA gene sequence up to position +47 that is placed upstream of the CAT gene. The plasmid p5′−89-mgPCNACAT[9] was digested with *Sac*I and *Pvu*II. The 290 bp fragment was isolated, then inserted into *Sac*I and *Sma*I sites of Bluescript SK(−) to obtain plasmid pSPmPCNA, which was used to prepare the RNA probe for RNase protection analysis.

Cell culture, DNA transfection and CAT assay Mouse NIH/3T3 cells were cultured in Dulbecco's modified minimal essential medium (DMEM) supplemented with 10% (v/v) calf serum. Mouse neuroblastoma N18TG2 cells[29, 30] were cultured in DMEM supplemented with 10% (v/v) fetal calf serum. The DEAE-dextran method with chloroquine treatment[31] was used for DNA transfection into N18TG2 cells at 20 h after seeding 8×10^5 cells per 60 mm dish. Each transfection contained 2 μg of reporter plasmid DNA together with 5 μg of the effector plasmid DNA per dish. Cells were harvested at 48 h after transfection. Cell extracts were prepared and CAT activity in 100 μg protein of the extract was measured as described previously.[32] To quantify the CAT activity, radioactive spots corresponding to acetylated chloramphenicols were taken from the thin layer plate, and radioactivity was measured in a toluene-based scintillation cocktail using a Beckman scintillation counter. CAT activities were normalized to protein amounts, which were determined by Bio-Rad protein assay.[33] Although experiments were repeated multiple times to avoid errors caused by fluctuation in DNA-transfection efficiencies, the relatively high efficiency of DNA-transfection with N18TG2 cells enabled us to obtain reproducible results.

Determination of the transcription initiation site by a primer extension method RNA was extracted from NIH/3T3 cells by an acid-guanidinium thiocyanate-phenol-chloroform extraction method.[34] Poly(A^+)RNA

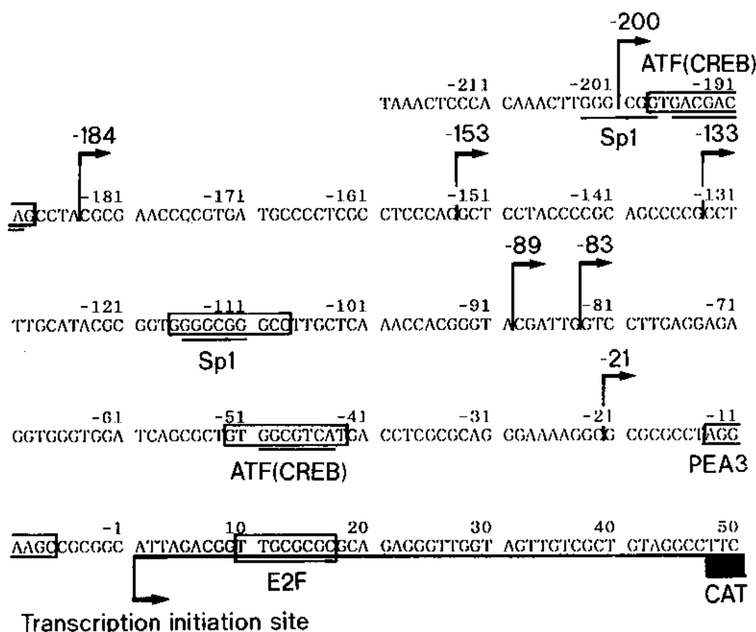

Fig. 1. Nucleotide sequence in and around mouse PCNA gene promoter. Nucleotide +1 denotes the transcription initiation site which is indicated by an arrow, and residues preceding it are indicated by negative numbers. The first exon is indicated by an underline. The numbered vertical lines with arrows indicate positions of the 5′-break points of 5′-deletion mutants. These mutants contain the CAT gene inserted at position +47 as indicated. The sequence which matches 9 out of 10 residues of the Sp1-binding consensus sequence[43] is enclosed with a solid box and the 5′-GGGCGG sequences are underlined. The decanucleotide sequences which are similar to the promoter element found in the DNA polymerase β gene[33, 44] are enclosed with solid boxes. The regions that match ATF(CREB)-binding consensus sequences are underlined. Binding consensus sequences for transcription factors PEA3[38] and E2F[39] are also indicated.

was purified using an oligo(dT)-Sepharose column. The 35mer primer (5′-TCAAACATGGTGGCGGAGT-TGTGGCGACTAGATGA-3′) was chemically synthesized so as to have the sequence complementary to the region containing the ATG initiation codon (nucleotide positions +118 to +152 with respect to the determined CAP site) of the PCNA gene. The primer was 5′-end-labeled by using T4 polynucleotide kinase and [γ-^{32}P]-ATP. The primer was then hybridized with 20 μg of poly(A^+)RNA and extended using avian myeloblastosis virus reverse transcriptase for 90 min. The product was analyzed by electrophoresis in a 6% polyacrylamide gel under denaturating conditions, followed by autoradiography.

RNase protection analysis Total cellular RNA was extracted from DNA-transfected N18TG2 cells by the guanidinium HCl-CsCl gradient method.[35] The isolated RNA was treated with DNase I in the presence of 100 U of human placental RNase inhibitor to remove contaminating plasmid DNA.[34]

RNase protection assay was carried out as described elsewhere.[34] Plasmid pSPmPCNA was linearized with *Sal*I and transcribed with T7 RNA polymerase in the presence of [α-^{32}P]UTP to obtain an RNA probe of 330 nucleotides. Ten μg of N18TG2 cell total RNA or yeast tRNA was mixed with 5×10^5 cpm of RNA probe in a solution containing 80% formamide, 40 m*M* piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES; pH 6.4), 0.4 *M* NaCl and 1 m*M* EDTA. The solution was heated at 85°C for 10 min, then hybridizations were carried out at 42°C for 16 h. RNase digestions were performed by incubation at 37°C for 15 or 45 min after addition of a solution containing 0.3 *M* NaCl, 10 m*M* Tris-HCl (pH 7.4), 1 m*M* EDTA, 2 μg/ml RNase T1, and 40 μg/ml RNase A. RNases were inactivated by proteinase K digestion followed by phenol-chloroform extraction. The protected fragments were applied to a 6% polyacrylamide gel containing 8 *M* urea. ^{32}P-Labeled pUC19 *Msp*I fragments were used as size markers.

RESULTS

Effects of Ad12 E1A and E1B on PCNA promoter activity in N18TG2 cells To examine the effects of E1A and E1B, the plasmid p5′−1584mgPCNACAT carrying mouse PCNA gene regulatory region fused with the CAT gene was cotransfected with either E1A or E1B expression plasmid into mouse N18TG2 cells. The CAT expression level was determined by measuring CAT activity in the cell extracts prepared at 48 h after transfection.

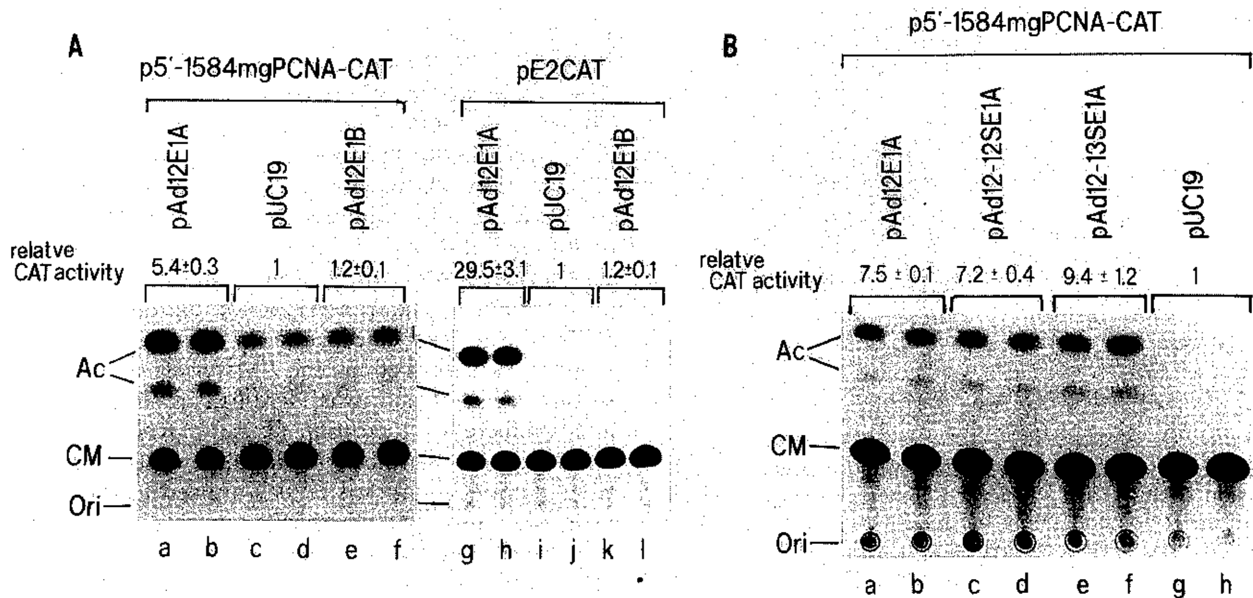

Fig. 2. Effects of Ad12 E1A and E1B on the function of PCNA gene promoter. Reporter plasmids (indicated at the top; 2 μg each) were cotransfected with 5 μg each of effector plasmids (indicated in each lane) into N18TG2 cells. At 48 h after transfection, cell extracts were prepared to measure CAT activity as described in "Materials and Methods." Values of CAT expression (averages ± standard deviations) are expressed relative to the value of each reporter plasmid with the pUC19. The sets of two adjacent lanes represent duplicate independent transfections. Acetylated and non-acetylated forms of [^{14}C]chloramphenicol are marked by Ac and CM, respectively.

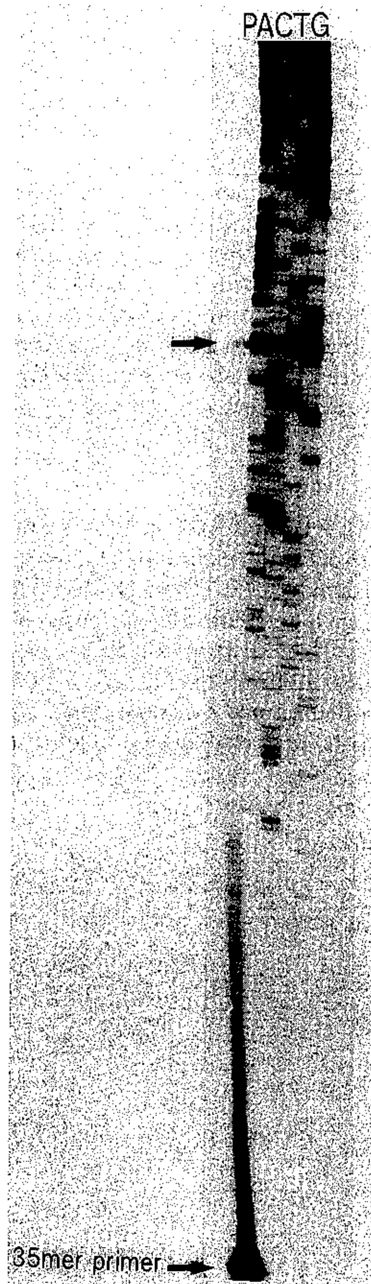

Fig. 3. Mapping of the transcription initiation site by a primer extension analysis. The ^{32}P-labeled 35mer primer complementary to the region containing the ATG initiation codon of the mouse PCNA gene was hybridized with poly(A^+) RNA isolated from NIH/3T3 cells and then extended by reverse transcriptase. To align the extended products with the genomic DNA sequence, dideoxy-sequencing reaction was performed using [^{35}S]dCTP and the same 35mer primer (lanes A, C, T and G). The products of the sequencing reaction and primer extension reaction (lane P) were analyzed by electrophoresis in the same gel under denaturing conditions, followed by autoradiography.

Examples of the experimental results are shown in Fig. 2. Cotransfection of E1A expression plasmid caused a 5- to 9-fold increase of the CAT expression in comparison with those in cells cotransfected with the vector plasmid pUC19 (Fig. 2A, lanes a to d; Fig. 2B, lanes a, b, g and h; Fig. 5, lanes a to d; Fig. 6, lower panel). In contrast, cotransfection of the E1B expression plasmid showed no effect (Fig. 2A, lanes c to f). As noted previously,[28, 36] the expression of E1A extensively stimulated the adenovirus E2 promoter activity, while the expression of E1B showed no significant effect (Fig. 2A, lanes g to l). Cotransfection of E1A and E1B expression plasmids along with the plasmid p5′−1584mgPCNACAT showed no enhancement of the E1A effect by E1B on the PCNA gene promoter activity (data not shown).

Both 13S and 12S E1A's can transactivate the PCNA gene promoter Two kinds of alternatively spliced mRNAs of 12S and 13S are expressed from E1A gene during the early phase of adenovirus infection. The E1A protein coded by the Ad12-13S mRNA consists of 266 amino acid residues,[37] while the E1A protein coded by the Ad12-12S mRNA consists of 235 amino acid residues. The latter is identical to the 13S E1A product except for the deletion of 31 residues, including most of the so-called conserved domain 3.[37]

To determine which of the E1A gene products is responsible for the transactivation of the PCNA gene promoter, each of the 13S and 12S E1A cDNAs was placed under the control of E1A promoter and cotransfected with the plasmid, p5′−1584mgPCNACAT into N18TG2 cells. As shown in Fig. 2B, both 12S E1A and 13S E1A expression plasmids stimulated the PCNA gene promoter activity. The 13S E1A expression plasmid showed a slightly higher extent of stimulation than the 12S one (Fig. 2B, lanes c to f). The results indicate that the transactivation function of E1A resides in the common domain(s) of 13 S and 12S E1A products.

E1A products stimulate the transcription from the PCNA gene promoter The transcription initiation site of mouse PCNA gene was determined by the primer extension method as described in the "Materials and Methods" section. A single site was identified as shown in Fig. 3 and numbered +1 position (Fig. 1). RNase protection analysis was performed to determine whether the proper transcription initiation site was utilized in the transient expression assay and whether the increase in CAT activity observed upon transfection of the E1A-producing plasmid was due to increase in the mRNA level.

Total RNA was isolated from N18TG2 cells at 48 h after transfection and hybridized to an excess amount of uniformly labeled antisense RNA probe shown in Fig. 4A. After digestion with RNase A and RNase T1, protected fragments of nearly the appropriate size, as predicted from the primer extension analysis, were detected

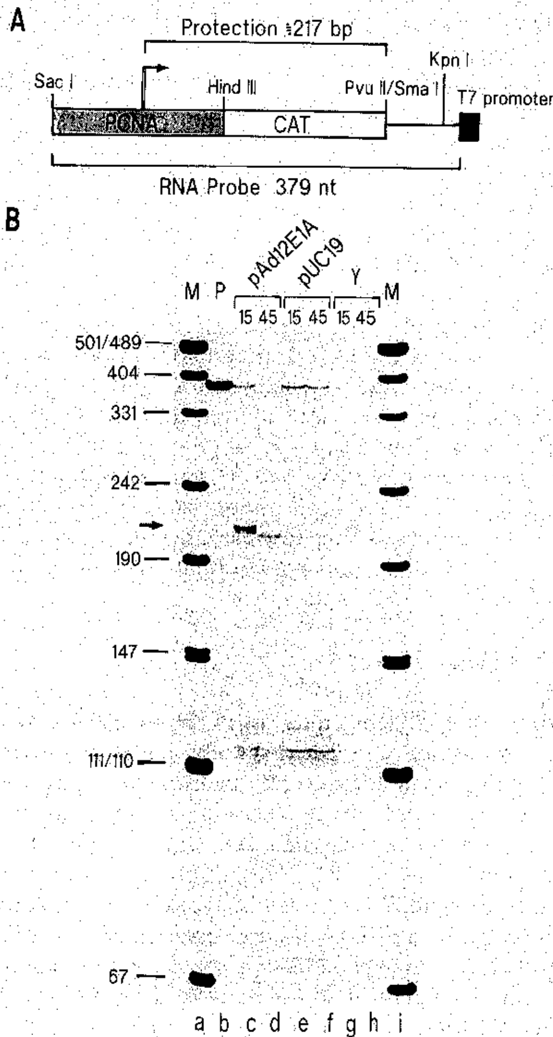

Fig. 4. RNase protection analysis. (A) Probe: Plasmid pSPmPCNA was linearized with *Sal*I, and T7 RNA polymerase was used to synthesize an RNA probe of 379 nucleotides (nt). The expected protection fragment was about 217 nt. (B) RNase protection analysis of RNA extracted from N18TG2 cells transfected with 2 μg each of p5′−1584mgPCNACAT and 5 μg each of effector plasmids indicated at the top (lanes c to f). Similar amounts of total RNA were recovered from cells cotransfected with each combination of plasmids. Lanes a and i, DNA size markers; lane b, undigested probe; lanes c, e and g, digested with RNases for 15 min at 37°C; lanes d, f and h, digested with RNases for 45 min at 37°C; lanes g and h, RNase treatment in the presence of yeast tRNA. The arrow indicates RNase-protected fragments.

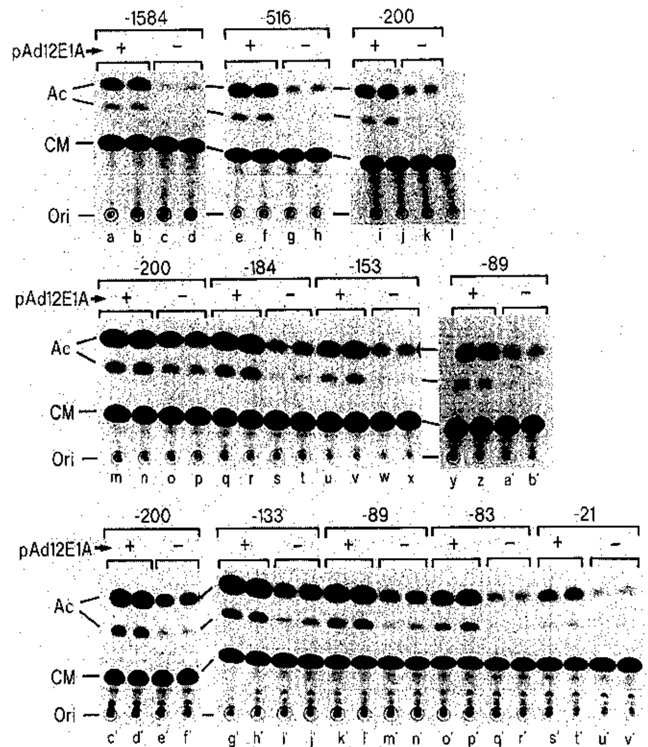

Fig. 5. Mapping of the target region in the PCNA gene for activation by E1A products. Two μg each of the indicated 5′-deletion mutants with 5 μg each of pAd12E1A(+) or pUC19(−) was transfected into N18TG2 cells. At 48 h after transfection, cell extracts were prepared to measure CAT activity. The sets of two adjacent lanes represent duplicated independent transfections.

(Fig. 4B). The intensity of radioactive bands shown in Fig. 4B was quantified by densitometry. Cotransfection of the p5′−1584mgPCNACAT plasmid with E1A-producing plasmid resulted in about 12-fold increase of the protected fragments (Fig. 4B, lanes c to f). The extent of increase was in a similar range to that determined by measurement of CAT activity. These results suggest that the E1A products stimulate transcription from the PCNA gene promoter.

Mapping of the target region of the E1A products A set of 5′-deletion mutants of the mouse PCNA gene was constructed in the previous[9] and the present studies. Each of these deletion derivatives was cotransfected into N18TG2 cells with either the E1A-producing plasmid or the control vector plasmid. The CAT expression level was measured at 48 h after transfection (Fig. 5). Quantitative data from the experiment shown in Fig. 5 and two additional experiments not shown are summarized in Fig. 6. The CAT expression of each deletion derivative is shown in the upper panel and the extent of the stimulation by E1A-producing plasmid is shown in the lower panel.

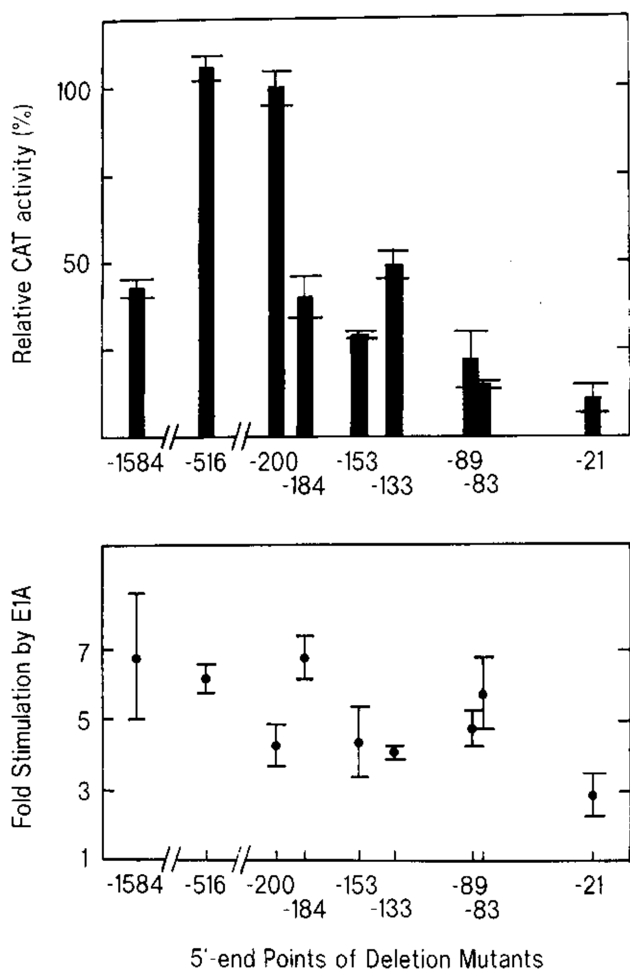

Fig. 6. Quantitative analysis of CAT expression of 5′-deletion mutants of the PCNA gene with and without E1A-expressing plasmid. The radioactivities of spots corresponding to acetylated [^{14}C]chloramphenicols on thin-layer chromatographic plates shown in Fig. 5 and others not shown were quantified. Upper panel, averages and standard deviations obtained from three independent experiments using 5′-end deletion derivatives without E1A-expressing plasmid shown as fractions of CAT expression with p5′−200mgPCNACAT. The averaged 100% value was 10,280 cpm. Lower panel, average stimulations (fold) of 5′-end deletion derivatives by E1A-expressing plasmid.

A deletion from position −1584 to −516 showed a significant increase of the CAT expression, suggesting the existence of a negative regulatory element in this region. A deletion from position −516 to −200 caused no significant change in the CAT expression level, indicating that this region does not contain any sequences affecting the PCNA gene expression, at least in N18TG2 cells. A further deletion to position −184 that removed one of the sequences similar to the ATF(CREB)-binding consensus caused reduction of the CAT expression by 60% (Fig. 6, upper panel).

Deletions to position −133 did not show any significant effects on the CAT expression. However, the deletion from −133 to −89 or −83 that removed the putative Sp1-binding consensus sequence further reduced the CAT-expression to 20% (Fig. 6, upper panel). A further deletion to position −21 that removed another sequence similar to the ATF(CREB)-binding consensus reduced the CAT-expression to 10%.

Four- to seven-fold transactivation by E1A products was observed with deletion mutants up to position −83 (Fig. 6, lower panel). The deletion derivative whose break point was at −21 still showed three-fold activation of its promoter activity by E1A (Fig. 6, lower). Therefore, the major target region of E1A-induced activation is concluded to be in the region downstream of position −21, although the upstream region between −83 and −21 may have some additional roles in the transactivation.

DISCUSSION

Nucleotide sequence analysis of mouse PCNA gene in our previous study revealed several sequences similar to the binding sites for several mammalian transcription factors.[9] Analysis with deletion mutants in mouse N18TG2 cells has suggested that one Sp1-binding consensus and two ATF(CREB)-binding consensus sequences are required for the maximum promoter activity. Transactivations by E1A products were observed with all deletion mutants of the PCNA gene promoter examined, although the extent of stimulation varied between 3- and 9-fold. Removal of sequences between −83 and −21 reduced the E1A response, but did not abrogate it, suggesting that the main E1A responsive element resides in the region downstream of position −21.

In the region between −21 and +47 of the PCNA gene, there are two sequences similar to the transcription factor-binding sites. One of them, starting at position −13, matches 6 out of 7 nucleotides of the PEA3-binding sequence, 5′-AGGAAGT.[38] Binding sites for PEA1 and PEA3, the polyomavirus enhancer binding proteins, are primary components of the polyomavirus late transcription initiator element and have been found in and around the transcription initiation sites of many cellular and viral promoters lacking TATA motifs.[38]

Another site starting at position +10 is very similar to the transcription factor E2F-binding consensus sequence, 5′-TTTCGCGC.[39] Although the sequence in the PCNA gene matches 7 out of 8 nucleotides of the consensus sequence, it should be noted that E2F can, in fact, bind to several divergent sequences from this consensus such as

5′-GATCGCGC of the c-*myc* gene, 5′-CTGCGCGC of the c-*myb* gene, and 5′-GTTCGCGC of the DHFR gene.[40] Interestingly, the E2F-binding site of the DHFR gene is located at the immediate 3′ side of the major transcription initiation site.[41] This E2F site has been proved to be required for efficient expression of the DHFR gene *in vivo*.[41] Judging from the arrangement of the PEA3-binding sequence, E2F-binding sequence and the transcription initiation site, these two putative binding sequences may consist of the transcriptional initiator element of the mouse PCNA gene. Further analysis with internal deletions or deletions from the 3′-terminus would be necessary to clarify the function of these two putative transcription factor-binding sites.

Both 13S E1A and 12S E1A transactivated the PCNA gene promoter function, indicating that the conserved domain 3 is dispensable for the transactivation. This is consistent with the previous report that the N-terminal region common to both the 13S and 12S E1A proteins is sufficient for the induction of synthesis of PCNA by adenovirus infection.[16, 25] A number of recent studies have indicated that the conserved domains 1 and 2 of E1A protein are responsible for E2F-dependent transactivation of a number of viral and cellular genes as well as for the dissociation of E2F from complexes with other cellular proteins such as cyclin A protein or retinoblastoma (Rb) gene product.[42] Therefore, the release of E2F from such complexes by E1A might be the most probable mechanism for the activation of the mouse PCNA gene promoter.

Although the major target of the transactivation by E1A was mapped in the region downstream of −21, the region between −83 and −21 also has an additional role in the transactivation, since the constructs having this region showed significantly higher response to E1A than those without it. A likely candidate is the ATF(CREB)-binding consensus at −52. Interestingly, the sequence matches 8 out of 10 nucleotides of the palindromic sequence located in the mouse DNA polymerase β gene promoter,[33] which is also activated by both 13S and 12S E1A. We have mapped the E1A-responsive region of the DNA polymerase β gene on this palindromic sequence (our unpublished data). Recently, Morris and Mathews[25] reported that the transactivation of the human PCNA promoter by E1A in HeLa cells is mediated by the ATF(CREB)-binding consensus sequence. They described the presence of sites resembling the E2F-binding sequence at positions −47 (5′-CGTTGCGA) and −18 (5′-GCTTGCGG) of the human PCNA gene.[24] However, these sequences match only four out of eight bases of the E2F-binding consensus sequence, 5′-TTTCGCGC.[39] Furthermore, no additional E2F-binding site-like sequences exist in the downstream region of the transcription initiation site where the E2F-binding sequence was found in the mouse PCNA gene. Therefore the difference in the major target sequences of E1A between mouse and human PCNA genes might reflect the difference in the E2F-binding consensus sequence in their promoters. Another possibility is the difference in the cell lines used for the DNA transfection. It should be noted that the activation of E2F by E1A in HeLa cells is likely to be obscured by the related functions of the HPV E7 protein that is constitutively expressed in this cell line.

ACKNOWLEDGMENTS

We are grateful to Dr. K. Nagashima (Aichi Cancer Center Research Institute) for providing the plasmid pE2CAT. This study was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

(Received January 29, 1992/Accepted March 24, 1992)

REFERENCES

1) Fairman, M. P. DNA polymerase δ and PCNA: actions and interactions. *J. Cell Sci.*, **95**, 1–4 (1990).
2) Tsurimoto, T., Melendy, T. and Stillman, B. Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin. *Nature*, **346**, 534–539 (1990).
3) Jaskulski, D., Deriel, J. K., Mercer, W. E., Calabretta, B. and Baserga, R. Inhibition of cellular proliferation by antisense oligodeoxynucleotides to PCNA cyclin. *Science*, **240**, 1544–1546 (1988).
4) Liu, Y. C., Marraccino, R. L., Keng, P. C., Bambara, R. A., Lord, E. M., Chou, W. G. and Zain, S. B. Requirement for proliferating cell nuclear antigen expression during stages of the Chinese hamster ovary cell cycle. *Biochemistry*, **28**, 2967–2974 (1989).
5) Moriuchi, T. Proliferating cell nuclear antigen (PCNA): a nuclear protein engaged in eukaryotic DNA replication for one billion years. *Med. Sci. Res.*, **18**, 911–915 (1990).
6) Almendral, J. M., Huebsch, D., Blundel, P. A., MacDonald-Bravo, H. and Bravo, D. Cloning and sequence of a human nuclear protein cyclin: homology with DNA-binding proteins. *Proc. Natl. Acad. Sci. USA*, **84**, 1575–1579 (1987).
7) Matsumoto, K., Moriuchi, T., Koji, T. and Nakane, P. M. Molecular cloning of cDNA coding for rat proliferating cell nuclear antigen (PCNA/cyclin). *EMBO J.*, **6**, 637–642 (1987).
8) Travali, S., Ku, D.-H., Rizzo, M. G., Ottavio, L., Baserga,

R. and Calabretta, B. Structure of the human gene for the proliferating cell nuclear antigen. *J. Biol. Chem.*, **264**, 7466–7472 (1989).
9) Yamaguchi, M., Hayashi, Y., Hirose, F., Matsuoka, S., Moriuchi, T., Shiroishi, T., Moriwaki, K. and Matsukage, A. Molecular cloning and structural analysis of mouse gene and pseudogenes for proliferating cell nuclear antigen. *Nucleic Acids Res.*, **19**, 2403–2410 (1991).
10) Suzuka, I., Hata, S., Matsuoka, M., Kosugi, S. and Hashimoto, J. Highly conserved structure of proliferating cell nuclear antigen (DNA polymerase δ auxiliary protein) gene in plants. *Eur. J. Biochem.*, **195**, 571–575 (1991).
11) Yamaguchi, M., Nishida, Y., Moriuchi, T., Hirose, F., Hui, C.-c., Suzuki, Y. and Matsukage, A. *Drosophila* proliferating cell nuclear antigen (cyclin) gene: structure, expression during development, and specific binding of homeodomain proteins to its 5′-flanking region. *Mol. Cell. Biol.*, **19**, 872–879 (1990).
12) Leivovici, M., Gusse, M., Bravo, R. and Mechali, M. Characterization and developmental expression of *Xenopus* proliferating cell nuclear antigen (PCNA). *Dev. Biol.*, **141**, 183–192 (1990).
13) Bauer, G. A. and Burgers, P. M. J. Molecular cloning, structure and expression of the yeast proliferating cell nuclear antigen. *Nucleic Acids Res.*, **18**, 261–265 (1990).
14) Tsurimoto, T. and Stillman, B. Functions of replication factor C and proliferating cell nuclear antigen: functional similarity of DNA polymerase accessory proteins from human cells and bacteriophage T4. *Proc. Natl. Acad. Sci. USA*, **87**, 1023–1027 (1990).
15) Morris, G. F. and Mathews, M. D. Regulation of proliferating cell nuclear antigen during the cell cycle. *J. Biol. Chem.*, **264**, 13856–13864 (1989).
16) Zerler, B., Roberts, R., Mathews, M. B. and Moran, E. Different functional domains of the adenovirus E1A gene are involved in regulation of host cell cycle products. *Mol. Cell. Biol.*, **7**, 821–829 (1987).
17) Juskulski, D., Gatti, C., Travali, S., Calabretta, B. and Baserga, R. Regulation of the proliferating cell nuclear antigen cyclin and thymidine kinase mRNA levels by growth factors. *J. Biol. Chem.*, **263**, 10175–10179 (1988).
18) Baserga, R. Growth regulation of the PCNA gene. *J. Cell Sci.*, **98**, 433–436 (1991).
19) Flint, J. and Shenk, T. Adenovirus E1A protein paradigm viral transactivator. *Annu. Rev. Genet.*, **23**, 141–161 (1989).
20) Moran, E. and Mathews, M. B. Multiple functional domains in the adenovirus E1a gene. *Cell*, **48**, 177–178 (1987).
21) Simon, M., Kitchner, K., Kao, H. T., Weber, L., Voellmy, R., Heintz, N. and Nevins, J. Selective induction of human heat shock gene transcription by the adenovirus E1A gene products, including the 12S E1A product. *Mol. Cell. Biol.*, **7**, 2884–2890 (1987).
22) Dretta, G., Beach, D. and Moran, E. Synthesis of p34, the mammalian homolog of the yeast cdc2^{+}/CDC28 protein kinase, is stimulated during adenovirus-induced proliferation of primary baby rat kidney cells. *Oncogene*, **2**, 553–557 (1988).
23) Kaddurah-Daouk, R., Lillie, J. W., Daouk, G. H., Green, M. R., Kingston, R. and Schimmel, P. Induction of a cellular enzyme for energy metabolism by transforming domains of adenovirus E1a. *Mol. Cell. Biol.*, **10**, 1476–1483 (1990).
24) Morris, G. E. and Mathews, M. B. Analysis of the proliferating cell nuclear antigen promoter and its response to adenovirus early region 1. *J. Biol. Chem.*, **265**, 16116–16125 (1990).
25) Morris, G. F. and Mathews, M. B. The adenovirus E1A transforming protein activates the proliferating cell nuclear antigen promoter via an activating transcription factor site. *J. Virol.*, **65**, 6397–6406 (1991).
26) Shiroki, K., Saito, I., Maruyama, K., Fukui, Y., Imatani, Y., Oda, K. and Shimojo, H. Expression of adenovirus type 12 early region 1 in KB cells transformed by recombinants containing the gene. *J. Virol.*, **45**, 1074–1082 (1983).
27) Shiroki, K. and Toth, M. Activation of the human beta interferon gene by the adenovirus type 12 E1B gene. *J. Virol.*, **62**, 325–330 (1988).
28) Imperiole, M. J., Hart, R. P. and Nevins, J. R. An enhancer-like element in the adenovirus E2 promoter contains sequence essential for uninduced and E1A-induced transcription. *Proc. Natl. Acad. Sci. USA*, **82**, 381–385 (1985).
29) Minna, J., Glazer, D. and Nirenberg, M. Genetic dissection of neural properties using somatic cell hybrids. *Nature*, **235**, 225–231 (1972).
30) Yamaguchi, M., Hayashi, Y., Ajiro, K. and Matsukage, A. Cell-type-specific expression of mouse DNA polymerase β-gene is regulated by silencer elements. *J. Cell. Physiol.*, **141**, 431–436 (1989).
31) Benerji, J., Olson, L. and Schaffner, W. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain gene. *Cell*, **33**, 729–740 (1983).
32) Gorman, C. M., Moffat, L. F. and Howard, B. H. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.*, **2**, 1044–1051 (1982).
33) Yamaguchi, M., Hayashi, Y. and Matsukage, A. Mouse DNA polymerase β gene promoter: fine mapping and involvement of Sp1-like mouse transcription factor in its function. *Nucleic Acids Res.*, **16**, 8773–8787 (1988).
34) Sambrook, J., Fritsch, E. F. and Maniatis, T. "Molecular Cloning: A Laboratory Manual," 2nd Ed., pp. 1.38–1.43, 7.14, 7.78 and 7.79 (1989). Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
35) Chirgwin, J. M., Prybyla, A. E., MacDonald, R. J. and Rutter, W. J. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, **18**, 5294–5299 (1979).

36) Imperiale, M. L., Feldman, L. T. and Nevins, J. R. Activation of gene expression by adenovirus and herpesvirus regulatory genes acting in trans and by a cis-acting adenovirus enhancer element. *Cell,* **35,** 127–136 (1983).
37) Brathwaite, A. W., Nelson, C. C. and Bellett, A. J. D. E1a revisited: the case for multiple cooperative trans-activation domains. *New Biologist,* **3,** 18–26 (1991).
38) Yoo, W., Martin, M. E. and Folk, W. R. PEA1 and PEA3 enhancer elements are primary components of the polyomavirus late transcription initiator element. *J. Virol.,* **65,** 5391–5400 (1991).
39) Jones, N. C., Rigby, P. W. J. and Ziff, E. B. Trans-acting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumor viruses. *Genes Dev.,* **2,** 267–281 (1988).
40) Mudryj, M., Hiebert, S. W. and Nevins, J. R. A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. *EMBO J.,* **9,** 2179–2184 (1990).
41) Blake, M. C. and Azizkhan, J. C. Transcription factor E2F is required for efficient expression of the hamster dihydrofolate reductase gene *in vitro* and *in vivo. Mol. Cell. Biol.,* **9,** 4994–5002 (1989).
42) Nevins, J. R. Transcriptional activation by viral regulatory proteins. *Trends Biol. Sci.,* **16,** 435–438 (1991).
43) Dynan, W. S., Sazer, S., Tjian, R. and Schimke, R. T. Transcription factor Sp1 recognizes a DNA sequence in the mouse dihydrofolate reductase promoter. *Nature,* **319,** 246–248 (1986).
44) Widen, S. G., Kedar, P. and Wilson, S. H. Human β-polymerase gene: structure of the 5′-flanking region and active promoter. *J. Biol. Chem.,* **263,** 16992–16998 (1988).