MOLECULAR AND CELLULAR BIOLOGY, Dec. 1986, p. 4149–4154
0270-7306/86/124149-06$02.00/0

Vol. 6, No. 12

Adenovirus 2 Peptide IX Gene Is Expressed Only on Replicated DNA Molecules

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Received 12 May 1986/Accepted 25 August 1986

Transcription of the adenovirus type 2 peptide IX (pIX) gene was examined in transient expression assays. When a nonreplicating plasmid DNA containing the pIX gene was introduced into HeLa cells by the DEAE-dextran method, no pIX gene transcript was detected. In contrast, efficient transcription was observed in the cells transfected with a replicating plasmid containing the pIX gene. Adenovirus early genes did not affect the level of transcription of the pIX gene on either a nonreplicating or a replicating plasmid. Inhibition of plasmid replication with cytosine arabinoside prevented transcription of the pIX gene. By quantitative analysis of the amount of the pIX gene and its transcript in transfected cells, it was concluded that active transcription of the pIX gene occurred only on DNA molecules replicated in the cell.

The adenovirus has served as an excellent model for many studies of the regulation of gene expression in eucaryotic cells. At least nine transcription units within the adenovirus type 2 (Ad2) genome that code for proteins have been identified (2). Among these transcription units, the E1a gene is expressed first after virus infection. The E1a gene products are thought to be required not only for the positive regulation of expression of other adenovirus genes (4, 5, 10, 12, 13, 21) but also for activating (26, 27) and, in some cases, repressing transcription of cellular genes (23) and other viral genes (5, 30).

The Ad2 peptide IX (pIX) gene is one of the intermediate-stage viral genes and does not contain any introns (1). This gene codes for a small protein which is a structural component of the virion (22). Its expression begins between 6 and 8 h after infection, i.e., about the same time as viral DNA replication begins. It is evident that the pIX gene promoter is the last promoter to be activated (2, 17, 25, 31), leading one to postulate that pIX gene expression might be controlled by the functions of early genes. Therefore, this gene would serve as an excellent model not only for studies on the regulatory mechanisms of gene expression, but also for studies on the mechanisms of the early-to-late gene switch during viral infection. Of the several genes that are expressed before the pIX gene, however, it is not known which is required directly for the control of pIX gene expression.

Recently, the possibility has been raised by Crossland and Raskas (9) that viral DNA replication is required for activation of the pIX gene, although evidence of pIX gene expression has also been found in cells infected in the presence of cytosine arabinoside (ara-C), an inhibitor of DNA replication (17, 22). However, it is evident that the maximal level of pIX gene expression is obtained at late times after viral DNA replication has begun (2, 17, 25, 31). Therefore, it is likely that structural changes of the viral genome might be involved in control of pIX gene expression. To understand the regulatory mechanism of expression of the Ad2 pIX gene, we analyzed its transcription in transient expression assays and showed that efficient transcription of the pIX gene occurred only on DNA molecules replicated in cells.

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MATERIALS AND METHODS

Cell culture. HeLa cells were maintained in Eagle minimal essential medium (Nissui, Japan) supplemented with 10% fetal bovine serum (Gibco Laboratories). HeLa cells were seeded the day before transfection. At the time of transfection, they were about 30% confluent.

Plasmid construction. pHindG (Fig. 1) was constructed by inserting the *Hin*dIII G fragment of Ad2 DNA into the *Hin*dIII and *Pvu*II sites of pBR322 DNA. The plasmid contains the entire E1a gene and the 5′ end of the E1b gene. pHindC (Fig. 1) has been described previously (19). pSVIX (Fig. 1) was constructed by inserting the simian virus 40 (SV40) early region (*Hpa*II-*Bam*HI fragment) into the *Cla*I and *Sma*I sites of pHindC. pSV was constructed by inserting the SV40 early region into the *Cla*I and *Bam*HI sites of pBR322 DNA. pE2 and pE4 contain the entire Ad2 E2 and E4 genes, respectively (kindly provided by H. Handa).

Transfection of cells. Transfection was done by the DEAE-dextran method (3, 18) with slight modifications. The cells were washed twice with 10 ml and once with 5 ml of TBS (25 mM Tris hydrochloride, 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.6 mM Na_2HPO_4, pH 7.5). For transfection of one plate (75 cm^2), 12 μg of DNA was added to 5.5 ml of TBS and mixed well at room temperature. A 0.6-ml amount of a solution of DEAE-dextran (25 mg/ml in TBS, M_r 5 × 10^5; Pharmacia) was added to the DNA solution with gentle mixing. The DNA sample was added to the plate, and the plate was kept at 37°C for 4 h with occasional tilting. The DNA was then removed and the plate was washed once with 10 ml of TBS. The cells were grown at 37°C in 20 ml of tissue culture medium.

Analysis of RNA by single-strand nuclease protection mapping. The cells were harvested 48 h after the addition of DNA and washed twice with 5 ml of cold phosphate-buffered saline and once with 1 ml of hypotonic buffer (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.5], 1.5 mM $MgCl_2$, 10 mM KCl). The cells were homogenized in 0.5 ml of hypotonic buffer with 0.2% Nonidet P-40 after 15 min of swelling on ice. After the cells were spun at 1,500 × *g* for 5 min, the supernatant was used for preparation of total cytoplasmic RNA. In some experiments, the nuclear pellet was washed once with 2 ml of

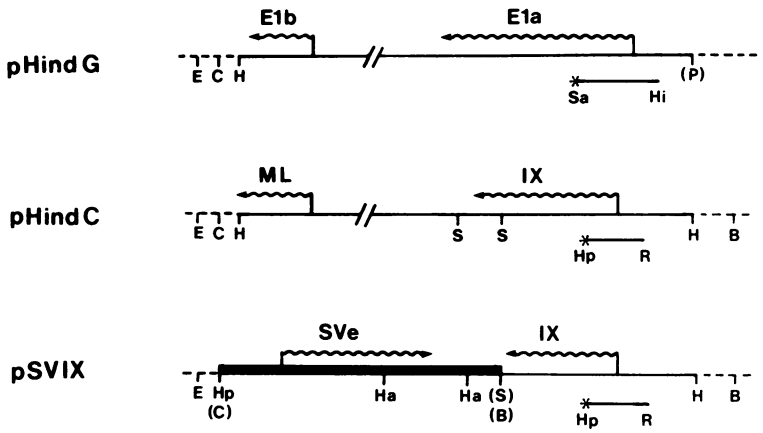

FIG. 1. Plasmid construction. pBR322 sequences are represented by a dashed line. Ad2 DNA inserts are represented by a narrow line. SV40 early sequences are represented by a thick line. The transcription direction of each gene is represented by a wavy arrow. The probes for P1 mapping are also shown by narrow lines beneath each map; the asterisk indicates the labeled end. Abbreviations: E, *Eco*RI; C, *Cla*I; H, *Hin*dIII; B, *Bam*HI; P, *Pvu*II; S, *Sma*I; Hp, *Hpa*II; Ha, *Hpa*I; R, *Rsa*I; Sa, *Sau*3A; Hi, *Hin*fI.

hypotonic buffer and used for preparation of DNA. RNA (50 μg) was hybridized with a single-stranded DNA probe labeled at the 5′ end. The probes used were 176 nucleotides of the *Rsa*I-*Hpa*II fragment for the pIX gene probe and 246 nucleotides of the *Hin*fI-*Sau*3AI fragment for the E1a gene probe. Hybridization and P1 nuclease digestion were done as described previously (19). The digestion products were electrophoresed on a 10% polyacrylamide–8 M urea gel. The E1a and pIX transcripts initiated correctly from their cap sites should give 132- and 94-nucleotide bands, respectively, after P1 nuclease digestion.

Quantitative analysis of DNA. Plasmid DNA was extracted from a Hirt supernatant (11) and digested with *Eco*RI or *Eco*RI and *Dpn*I. The DNA digest was electrophoresed on a 1% agarose gel and transferred to a nitrocellulose filter (Millipore Corp.), and the filter was hybridized with the probes (24). The region corresponding to each band was cut out from the filter and counted in a liquid scintillation counter.

RESULTS

To address the question of how pIX gene expression is regulated, first we introduced the pHindC plasmid, which contains the entire region of the pIX gene and the 5′ end of the major late gene, alone into HeLa cells by the DEAE-dextran method. No obvious pIX transcripts initiated from the cap site were observed in the cells transfected with this plasmid (Fig. 2, lane a). To see whether an Ad2 early gene was required for activation of pIX gene transcription, effects of cotransfection with pHindG and the pE2 or pE4 plasmid on the expression of the pIX gene were examined (lanes b, c, and d). However, we failed to detect the pIX transcript even in the presence of the early genes. In this case, it is possible that the cotransfected early genes did not produce enough products to activate pIX gene transcription or that other early gene products, such as E1b or E3, are also required. However, these possibilities were ruled out by the observation that infecting the transfected cells with Ad2 6 or 15 h before harvest also failed to activate pIX gene transcription (data not shown). We could detect the E1a gene transcript but not the pIX gene transcript in the cells transfected with pHindC and pHindG. The E2, E4, and SV40 early genes were expressed in the transfected cells (data not shown). Therefore, failure to detect any transcripts of the pIX gene might be due simply to the weakness of the pIX gene promoter compared with the E1a gene promoter, although both genes showed almost equal template activities in an in vitro transcription system (data not shown). Since less than 12 μg of total plasmid DNA was used for transfection under our conditions, we next constructed a replicating plasmid, pSVIX, containing the replication origin and the entire early gene of SV40 (Fig. 1). Transfection of cells with pSVIX would give higher amounts of the pIX gene DNA than with pHindC. Simultaneously, effects of DNA replication on pIX gene transcription could be examined by using pSVIX. The cells transfected with pSVIX DNA now synthesized a large amount of pIX transcripts even in the absence of the Ad2 early genes (Fig. 2, lane e). On the other hand, cotransfection of the early genes did not affect the transcription level of the pIX gene on the pSVIX plasmid (Fig. 2, lanes e through

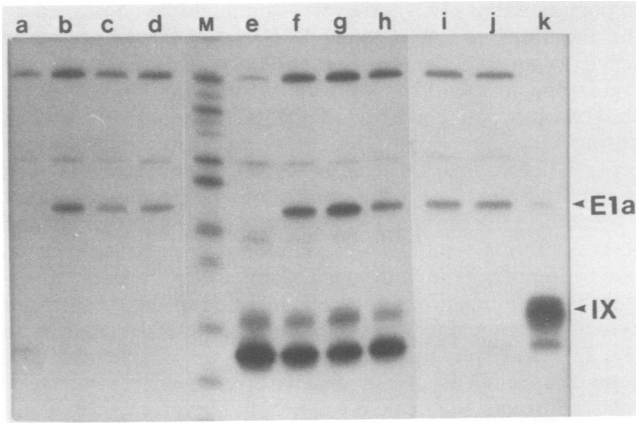

FIG. 2. P1 nuclease analysis of pIX- and E1a-specific transcripts from HeLa cells transfected with plasmids. Lanes: RNAs from cells transfected with pHindC (a), pHindC and pHindG (b and i), pHindC, pHindG, and pE2 (c), pHindC, pHindG, and pE4 (d), pSVIX (e), pSVIX and pHindG (f), pSVIX, pHindG, and pE2 (g), pSVIX, pHindG, and pE4 (h), and pHindC, pHindG, and pSV (j). A 4-μg amount of each plasmid was used, and the total amount of DNA was adjusted to 12 μg with pBR322 DNA. Lane k, RNA from late-stage (15-h) Ad2-infected HeLa cells. The bands protected with the pIX- and E1a-specific transcripts are indicated.

h). Because the cells cotransfected with pHindC and pSV DNA did not produce pIX gene transcripts (Fig. 2, lane j), the possibility that the early gene products of SV40 DNA might activate transcription of the pIX gene was ruled out. As pHindC but not pSVIX contains the entire pIX gene region, it is also possible that the pIX gene product might inhibit its own transcription. However, the cells transfected with pAdIX, a nonreplicating plasmid containing only the truncated pIX gene region, did not produce pIX transcripts. These results strongly suggest that transcription of the pIX gene occurs on replicating but not on nonreplicating plasmids in transient expression assays and also that at least the E1a, E2, and E4 genes do not affect transcription of the pIX gene.

Before characterizing further the mechanism of pIX gene transcription on pSVIX plasmid DNA, we first examined whether transcription of the pIX gene observed above correlated with DNA replication in transient expression assays. Addition of ara-C clearly prevented transcription of the pIX gene in the cells transfected with pSVIX DNA (Fig. 3, lane c). Moreover, the cells transfected with pSV^{d}IX, which has had the *Hpa*I fragment in the SV40 early region deleted and therefore cannot produce intact T antigen, failed to produce the pIX gene transcripts (lane d). In contrast, the pIX gene on the pSV^{d}IX plasmid was efficiently transcribed by adding intact T antigen through cotransfection with pSV (lane e). These results clearly indicate that pIX gene transcription is dependent on DNA replication.

Recent studies have shown that SV40 DNA does not replicate efficiently in HeLa cells, although it does replicate efficiently in 293 cells, which are a cell line transformed with the E1a gene (15, 16). This enhanced level of DNA replication in 293 cells might be achieved through activation of cellular topoisomerase I by E1a gene products, as demonstrated by Chow and Pearson (7). To determine whether the observed effect on pIX gene transcription was due solely to an increase of gene dose through DNA replication or whether expression of the pIX gene occurs on replicated but not on unreplicated DNA within the cells, we prepared a Hirt supernatant (11) from cells transfected with pHindC or pSVIX DNA and measured the amount of pIX DNA by a Southern blot hybridization analysis (24). In these experiments, DNA was digested with the restriction endonuclease *Dpn*I to distinguish between unreplicated and replicated molecules (this enzyme only cleaves DNA methylated at the adenine residue in its recognition sequence). A large fraction of pSVIX DNA sequences were resistant to digestion with the enzyme (Fig. 4, lanes c and d), although pHindC and pHindG were completely digested (lanes a and b), indicating that the undigested pSVIX DNA was the molecule that had been replicated in the cells. In contrast, pSVIX DNA from cells treated with ara-C was completely digested with the enzyme (lanes e and f). There was about five times as much undigested pSVIX (replicated DNA) as digested pSVIX DNA (input DNA) (compare lanes c and d). (We repeated the same kind of experiment several times and found that the amount of replicated pSVIX DNA was not more than 10 times that of input pSVIX DNA.) To rule out the possibility

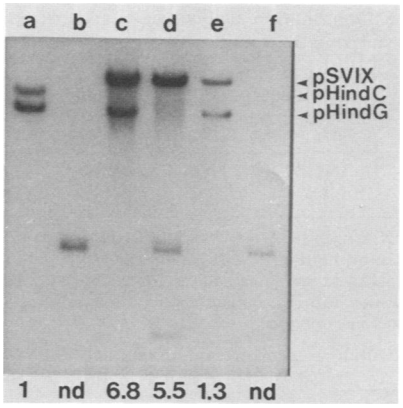

FIG. 4. Southern blot analysis of transfected DNA. A Hirt supernatant was prepared from half of the cells used for preparation of the RNA samples used in Fig. 3, lanes a to c. Lanes a and b, c and d, and e and f correspond to lanes a, b, and c in Fig. 3, respectively. DNA was digested with *Eco*RI (a, c, and e) or *Eco*RI and *Dpn*I (b, d, and f). The probes used were the 5′-end-labeled 1.1-kb *Sau*3A fragment of pSVIX (containing the SV40 early region) in addition to those used in the P1 analysis. The numbers shown below each lane are the relative amounts of pHindC (lanes 1 and 2) and pSVIX (lanes 3 to 6) DNA calculated as described in Materials and Methods. Each value was normalized to pHindG DNA as the standard. nd, Not detected.

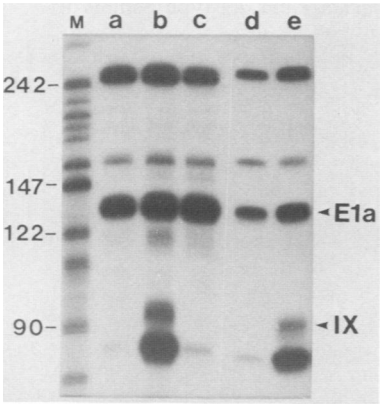

FIG. 3. Correlation between pIX gene transcription and DNA replication. Lanes: RNAs from cells transfected with pHindC (a), pSVIX (b and c), pSV^{d}IX (d), and pSV^{d}IX and pSV (e) in the presence of pHindG. Ara-C was added at 30 μg/ml 24 h after the addition of DNA in lane c. Transfection and RNA analysis were as described in the text, except that 6 μg of each plasmid was used. The total amount of DNA was adjusted to 12 μg with pBR322 DNA. Lane M, Size makers; sizes are indicated in nucleotides.

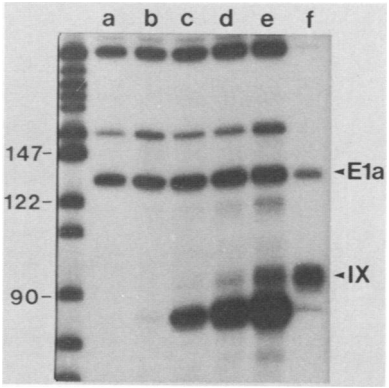

FIG. 5. DNA dose and transcription level. Lanes: RNAs from cells transfected with pHindC (lane a, 1 μg; lane b, 10 μg) and pSVIX (lane c, 0.1 μg; lane d, 0.5 μg; lane e, 2 μg) in the presence of 2 μg of pHindG. The total amount of DNA was adjusted to 12 μg with pBR322 DNA. Lane f is RNA from late-stage (15-h) Ad2-infected HeLa cells.

TABLE 1. Quantitative analysis of pIX transcript and DNA

pIX[a]	Relative level in transfections with:				
	pHindC (μg)		pSVIX (μg)		
	1	10	0.1	0.5	2.0
Transcript	ND[b]	ND	3.1	12.4	57.7
DNA	1	12	0.8	6.0	21.1

[a] For the pIX transcript, RNA was quantitated by densitometry of the autoradiogram shown in Fig. 5. The results are expressed in arbitrary units. DNA was quantitated as described in Materials and Methods from the autoradiogram shown in Fig. 5. The results are expressed as relative value standardized to values obtained with E1a DNA.

[b] ND, Not detected.

that DNA unincorporated in the cells may have contaminated the Hirt preparation, a similar experiment was carried out on total DNA purified from isolated nuclei. The results, however, were identical (data not shown; see Fig. 6). Additionally, almost equal levels of DNA replication were observed in the cells transfected with pSVIX in the absence of pHindG (data not shown).

To analyze further the relationship between RNA level and DNA replication level, increasing amounts of pHindC DNA or pSVIX DNA were transfected into HeLa cells. pIX transcripts, however, were not observed in detectable amounts in the cells transfected with 10 μg of pHindC DNA (Fig. 5, lane b), while the cells transfected with only 0.1 μg of pSVIX DNA synthesized detectable amounts of pIX gene transcripts (lane c). Under these conditions, the pIX transcripts increased in parallel with the increasing amounts of pSVIX DNA (Table 1). Quantitation of pIX DNA showed that the relative amount of pIX DNA in the cells transfected with 0.1 μg of pSVIX DNA was only about one-tenth of that in the cells transfected with 10 μg of pHindC DNA. In this experiment, the amount of pIX DNA within the cells increased in parallel with increasing amounts of input DNA. Therefore, these results strongly suggest that efficient transcription of the pIX gene occurs only on DNA molecules which have been replicated in transfected cells.

Finally, to determine whether pIX gene transcription occurs only during each round of DNA replication, we analyzed the kinetics of pIX gene expression after transfection and the effect of adding ara-C. If pIX gene transcription occurs only on replicating DNA molecules, addition of ara-C at any time after transfection should prevent subsequent transcription of the pIX gene. The E1a gene transcripts were detected just 24 h after transfection and increased slowly up to 48 h (Fig. 6). In contrast, pIX gene transcripts were not detected until 30 h and increased dramatically at least until 48 h. The time of appearance of the pIX gene transcripts was nearly the same as that of the replicated DNA molecules. Ara-C completely inhibited the replication of pSVIX DNA (Fig. 7). Nevertheless, the amount of pIX gene transcripts continued to increase after the addition of ara-C. The rate of increase for the pIX transcript was almost coincident with that of replication of pSVIX DNA. These results indicate that pIX gene transcription does not necessarily require DNA replication but occurs on replicated DNA molecules.

DISCUSSION

In this report we showed that transcription of the Ad2 pIX gene requires prior DNA replication in transient expression assays. Our conclusion was consistent with the observations made by Crossland and Raskas (9). A similar observation has been made by Treisman et al. (29), that transcription of the human α-globin but not β-globin gene was about 50-fold higher in transient expression assays when the gene was contained on replicating than on nonreplicating plasmids. However, since they did not estimate the gene dose in the transfected cells, it is not clear whether transcription of the α-globin gene is also coupled directly with DNA replication.

It is well known that control of gene expression is frequently linked to DNA replication in both procaryotic and eucaryotic cell viruses. The early-to-late gene switch in transcription appears to depend on DNA replication. In adenoviruses, it has been shown that the major late gene was transcribed differently on replicated DNA and on unreplicated DNA (28). On the other hand, the late gene of SV40 can be activated by T antigen in the absence of viral DNA replication (14), although it had been previously proposed that DNA replication is required for expression of late genes in SV40 (8). Interestingly, recent evidence has shown that expression of T antigen was repressed by viral DNA replication (15, 16). This inverse correlation between transcription and DNA replication was also observed for the *Saccharomyces cerevisiae* mating type gene (20).

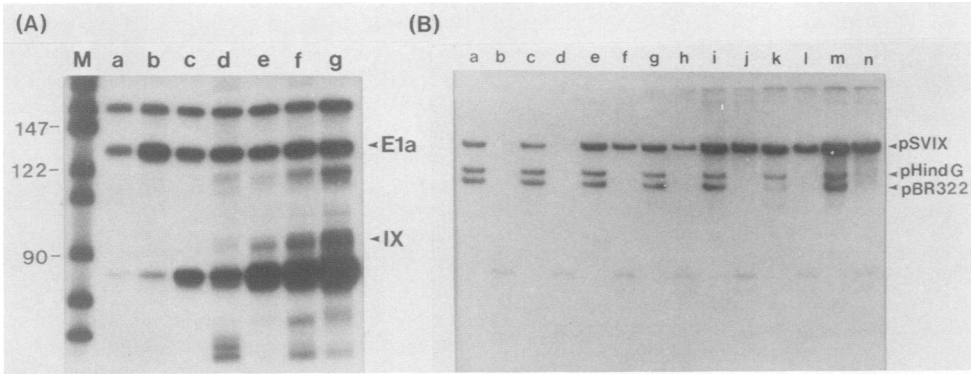

FIG. 6. (A) Kinetics of expression of pIX and E1a genes. Cells were transfected with 4 μg each of pHindG, pSVIX, and pBR322. The cells were harvested at 24 (a), 30 (c), 36 (e), and 48 h (g, b, d, and f) after addition of DNA. Lanes b, d, and f, Cells treated with ara-C at 24, 30, and 36 h, respectively. Transfection and P1 analysis were done as described in the legend to Fig. 2. (B) Southern blot analysis of transfected DNA. DNA was prepared from purified nuclei (see Materials and Methods) and digested with *Eco*RI (a, c, e, g, i, k, and m) or with *Eco*RI and *Dpn*I (b, d, f, h, j, l, and n). Lanes a and b, c and d, e and f, g and h, k and l, and m and n correspond to lanes a, b, c, d, e, f, and g, respectively, in panel A. Gel electrophoresis and Southern hybridization were done as described in the legend to Fig. 4, except that the 5'-end-labeled 1.4-kb *Sau*3A fragment of pBR322 DNA was also used as a probe.

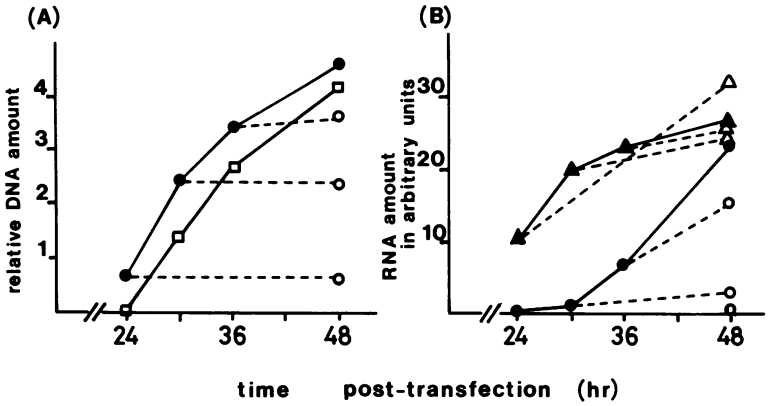

FIG. 7. Quantitation of pIX DNA (A) and transcripts (B). (A) Amount of pIX DNA estimated from the autoradiogram shown in Fig. 6B as described in Materials and Methods and was normalized to E1a DNA as the standard. The total amount of pSVIX DNA was determined in cells harvested at the indicated time after transfection. Symbols: ●, cells grown without ara-C; ○, cells grown in the presence of ara-C; □, *Dpn*I-undigested pSVIX DNA (replicated DNA). (B) Amount of transcripts estimated by densitometry of the autoradiogram shown in Fig. 6A. Symbols: pIX (○, ●) and E1a (△, ▲) transcripts from cells harvested at the indicated times (○, △) and from cells grown in the presence of ara-C (●, ▲).

How is DNA replication involved in the activation of transcription of the pIX gene? What is the difference between unreplicated and replicated DNA molecules? As the plasmid DNAs used in this study were methylated at an adenine residue, adenine methylation of DNA might inhibit transcription of the pIX gene. This was not the case, however, because cells transfected with pHindC cloned in Dam$^-$ bacteria did not synthesize pIX gene transcripts (data not shown). Additionally, DNA methylation at an adenine residue has never been observed in eucaryotes.

Another possibility is that activation of the pIX gene promoter probably depends on changes in the physical structure of the template during DNA replication. Recently, Cereghini and Yaniv (6) have demonstrated that plasmid DNA introduced into cells by DNA-mediated transfection is rapidly assembled into a typical nucleosome structure and that the local nucleosome structure differs between nonreplicating and postreplicated chromatin. This observation supports the idea that an alteration in the distribution of nucleosomes might be essential for exposing the pIX gene promoter to the action of RNA polymerase II or its transcription factor(s). During replication, their accessibility of the pIX gene promoter may be ensured through the structural changes of the DNA template.

The results obtained in this study showed that Ad2 early gene products did not appear to be required for activation of pIX gene transcription in transient expression assays. However, it is possible that they may be involved in control of expression of the pIX gene within virus-infected cells. The early gene product(s) might act as a negative regulatory factor for the pIX gene. Moreover, this repression might be exerted through a structural change(s) in the viral genome after or during DNA replication.

ACKNOWLEDGMENTS

We thank H. Saiga and M. Nomoto for helpful discussion, M. Muramatsu, H. Saiga, and S. Goto for their comments on the manuscript, H. Handa for providing plasmids, and M. Oka for skillful technical assistance.

LITERATURE CITED

1. **Alestrom, P., G. Akusjarvi, M. Perricaudet, M. B. Mathews, D. F. Klessig, and U. Pettersson.** 1980. The gene for polypeptide IX of adenovirus type 2 and its unspliced messenger RNA. Cell **19**:671–681.
2. **Baker, C. C., and E. B. Ziff.** 1981. Promoters and heterogenous 5′ termini of the messenger RNAs of adenovirus serotype 2. J. Mol. Biol. **159**:189–221.
3. **Banerji, J., L. Olson, and W. Schaffner.** 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell **33**:729–740.
4. **Berk, A. J., F. T. Lee, J. Williams, and P. A. Sharp.** 1979. Pre-early adenovirus-5 gene product regulates synthesis of early viral messenger RNAs. Cell **17**:937–944.
5. **Borrelli, E., R. Hen, and P. Chambon.** 1984. The adenovirus-2-E1a products repress stimulation of transcription by enhancers. Nature (London) **312**:608–612.
6. **Cereghini, S., and M. Yaniv.** 1984. Assembly of transfected DNA into chromatin: structural changes in the origin-promoter-enhancer region upon replication. EMBO J. **3**:1243–1253.
7. **Chow, K. C., and G. D. Pearson.** 1985. Adenovirus infection elevates levels of cellular topoisomerase I. Proc. Natl. Acad. Sci. USA **82**:2247–2251.
8. **Contreras, R., D. Gheytsen, J. Knowland, A. van de Voode, and W. Fires.** 1982. Evidence for the direct involvement of DNA replication origin in synthesis of late SV40 RNA. Nature (London) **300**:500–505.
9. **Crossland, L. D., and H. J. J. Raskas.** 1983. Identification of adenovirus genes that required template replication for expression. Virology **46**:737–748.
10. **Green, M. R., R. Treisman, and T. Maniatis.** 1983. Transcriptional activation of cloned human β-globin genes by viral immediate-early gene products. Cell **35**:137–148.
11. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
12. **Hoeffler, W. K., and R. G. Roeder.** 1985. Enhancement of RNA polymerase III transcription by the E1A gene products of adenovirus. Cell **41**:955–963.
13. **Jones, N., and T. Shenk.** 1979. An adenovirus type 5 early gene function regulates expression of other early genes. Proc. Natl. Acad. Sci. USA **76**:3665–3669.
14. **Keller, J. M., and J. C. Alwine.** 1984. Activation of the SV40 late promoter: direct effects of T antigen in the absence of viral DNA replication. Cell **36**:381–389.
15. **Lebkowski, J. S., S. Clancy, and M. P. Calos.** 1985. Simian virus 40 replication in adenovirus-transformed human cells antagonizes gene expression. Nature (London) **317**:169–171.
16. **Lewis, E. D., and J. L. Manley.** 1985. Repression of simian virus 40 early transcription by viral DNA replication in human 293 cells. Nature (London) **317**:172–175.

17. **Lewis, J. B., H. Esche, J. E. Smart, B. W. Stillman, M. L. Harter, and M. B. Mathew.** 1979. Organization and expression of the left third of the genome of adenovirus. Cold Spring Harbor Symp. Quant. Biol. **44**:493–503.
18. **Lopata, M. A., D. W. Cleaveland, and B. Sollner-Webb.** 1984. High level transient expression of a chloramphenicol acetyl transferase gene by DEAE-dextran mediated DNA transfection coupled with a dimethyl sulfoxide or glycerol shock treatment. Nucleic Acids Res. **12**:5707–5717.
19. **Matsui, T.** 1982. In vitro accurate initiation of transcription on the adenovirus type 2 IVa2 gene which does not contain a TATA box. Nucleic Acids Res. **10**:7089–7101.
20. **Miller, A. M., and A. Nasmyth.** 1984. Role of DNA replication in the repression of silent mating type loci in yeast. Nature (London) **312**:247–251.
21. **Nevins, J. R.** 1981. Mechanisms of activation of early viral transcription by the adenovirus E1a gene product. Cell **29**:913–919.
22. **Persson, H., V. Petterson, and M. B. Mathews.** 1978. Synthesis of a structural adenovirus polypeptide in the absence of viral DNA replication. Virology **90**:67–79.
23. **Schrier, P. I., R. Bernards, R. J. Vaessen, A. Houweling, and A. J. van der Eb.** 1983. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature (London) **305**:771–775.
24. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
25. **Spector, D. J., M. McGrogan, and H. J. J. Raskas.** 1978. Regulation of the appearance of cytoplasmic RNAs from region 1 of the adenovirus 2 genome. J. Mol. Biol. **126**:395–414.
26. **Stein, R., and E. B. Ziff.** 1984. HeLa cell β-tubulin gene transcription is stimulated by adenovirus 5 in parallel with viral early genes by an E1a-dependent mechanism. Mol. Cell. Biol. **4**:2792–2801.
27. **Svenson, C., and G. Akusjarvi.** 1984. Adeno2 early region 1A stimulates expression of both viral and cellular genes. EMBO J. **3**:789–794.
28. **Thomas, G. P., and M. B. Mathews.** 1980. DNA replication and the early to late transition in adenovirus infection. Cell **22**:523–533.
29. **Treisman, R., M. R. Green, and T. Maniatis.** 1983. cis and trans activation of globin gene transcription in transient assays. Proc. Natl. Acad. Sci. USA **80**:7428–7432.
30. **Velcich, A., and E. Ziff.** 1985. Adenovirus E1a proteins repress transcription from the SV40 early promoter. Cell **40**:705–716.
31. **Wilson, M. C., and J. E. Darnell, Jr.** 1981. Control of messenger RNA concentration by differential cytoplasmic half-life. Adenovirus messenger RNAs from transcription units 1A and 1B. J. Mol. Biol. **148**:231–251.