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IN VITRO TRANSCRIPTION OF A CHROMATIN-LIKE COMPLEX OF MAJOR CORE PROTEIN VII AND DNA OF ADENOVIRUS SEROTYPE 2

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SUMMARY: Major core protein VII of adenovirus serotype 2 (Ad2) is thought to play a role as a histone octamer in eukaryotic cells. We compared the template activity of the VII-DNA complex formed in vitro with that of protein-free DNA. Hybridization assay of in vitro transcripts showed that transcription from regions located in the middle of Ad2 DNA decreased when Ad2 DNA formed a complex with VII. This suggests that the chromatin structure plays a role in regulation of transcription of the adenovirus genome.

Chromatin structure is thought to be important in regulation of transcription in eukaryotic cells. Although the occurrence of local perturbation of chromatin structure has been suggested from changes in sensitivity to DNase I during activation and inactivation of transcription of certain genes (1), no direct evidence that chromatin structure is involved in control of transcription has yet been obtained. Reconstitution of chromatin of particular genes as templates for in vitro transcription should be a good method for demonstrating that chromatin structure is involved in the regulation of transcription. However, reconstitution of chromatin from DNA and histones does not occur at a physiological ionic strength unless certain proteins that facilitate assembly of nucleosomes are present (2-4). Therefore, reconstituted chromatin with the intact native structure cannot easily be obtained.

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Adenoviral genes are expressed in an ordered sequence when host cells are infected with viruses. The first set of genes to be transcribed are early genes, and late genes are expressed only after the virus genome replicates (5). Adenovirus DNA is present in virions forming a chromatin structure (6), and the chromatin structure is maintained in nuclei after infection (7,8). It is, therefore, expected that early genes are preferentially transcribed from the chromatin. Since all the chromatin proteins are encoded by late genes (9-11), newly replicated adenovirus DNA is unlikely to take the native chromatin structure before late genes are transcribed. Thus chromatin structure probably plays an important role in selective transcription of adenoviral genes.

Adenovirus chromatin consists of DNA and three proteins: a major core protein VII, a minor core protein V and polypeptide μ, all of which are encoded by the viral genome. Of these, the major core protein VII, an 18-kDa basic protein, is thought to participate in the formation of a nucleosome-like structure as histone octamers of eukaryotic chromatin (12), and it has been shown to bind to adenovirus DNA forming a complex when simply mixing with the latter under physiological ionic conditions (13). The characteristics of the complex formed *in vitro* are similar to those of chromatin in virions, as judged by physicochemical analyses (13).

In this study we compared the template activities of protein-free DNA and the VII-DNA complex *in vitro*. The patterns of transcription of the two templates were found to be different, suggesting that the chromatin structure regulates Ad2 gene expression.

MATERIALS AND METHODS

Preparation of Ad2 DNA and major core protein VII Ad2 virions were purified by CsCl-centrifugation (14). Virions were disrupted with proteinase K and SDS, and adenovirus DNA was extracted with phenol. Major core protein VII was prepared as

described previously (11,15). Purified VII gave a single band on SDS-polyacrylamide gel electrophoresis (16).

Preparation of lysates of nuclei of Ehrlich ascites tumor cells, in vitro transcription and hybridization assay A nuclear lysate for in vitro transcription was prepared from Ehrlich ascites tumor cells by the method of Dignam et al. (17). Starting from 10 g of fresh cells, we routinely obtained about 10 ml of lysate with a protein concentration of 6-8 mg/ml. The lysate was stable for at least 6 months when stored at -80°C.

In vitro transcription was performed as described previously (18). For the experiments shown in Fig. 2, transcription was carried out at 30°C for 1 h with 0.5 µg of SmaI-digested pSmaF (19) as DNA template in 25 µl of reaction mixture in the presence of [α-^{32}P]UTP (Amersham Corp., 410 Ci/mmol). The ^{32}P-labeled RNA was extracted, separated on 5% polyacrylamide-7 M urea gel and subjected to autoradiography. For transcription of Ad2 DNA, 100 µl of reaction mixture containing 2 µg of template DNA was first incubated at 30°C for 1 h in the absence of ribonucleoside triphosphates and then transcription was performed at 30°C for 10 min in the presence of ribonucleoside triphosphates. The RNA chain was shown to be elongated up to 1 kb under these conditions (22). ^{32}P-labeled transcripts were then extracted, treated with DNase I and incubated with nitrocellulose papers on which Ad2 DNA digested with restriction enzymes had been immobilized by the method of Southern (20). Hybridization was carried out by the method of Thomas (21) except that dextran sulfate was omitted from the hybridization buffer. Autoradiography was done with Kodak XAR-5 X-ray film using an intensifying screen at -80°C.

RESULTS

Formation of the complex with VII and Ad2 DNA

To determine the amount of VII required for formation of a complex with a given amount of Ad2 DNA, we monitored complex formation in mixtures of various amounts of VII and a fixed amount of Ad2 DNA. Complex formation was monitored by determining the migration of DNA in agarose gel. As shown in Fig. 1, the mobility of Ad2 DNA decreased with increase in the amount of VII, indicating that complexes were formed. The results indicated that 0.2 µg of VII was sufficient for formation of a complex with 0.1 µg of Ad2 DNA. The molar ratio of VII to DNA in the complex was about 2,400:1, which was about twice that in native Ad2 chromatin (12). These proportions of VII and DNA were used in all subsequent studies to make the VII-DNA complex.

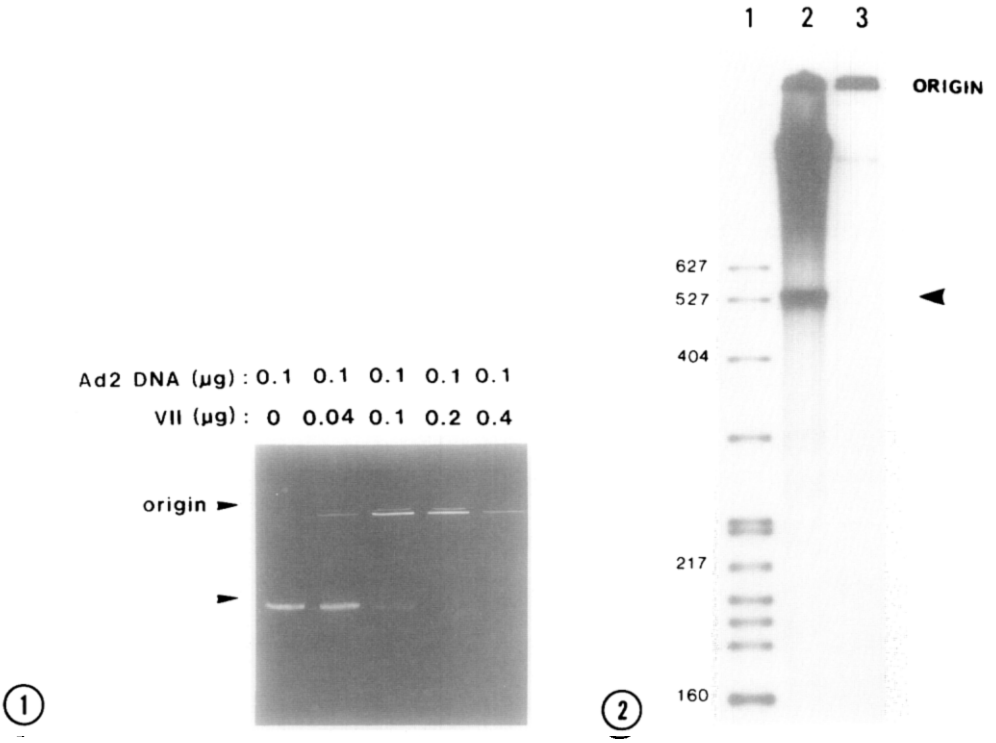

Fig. 1 Agarose gel electrophoresis of the VII-DNA complex. 0.1 μg of Ad2 DNA was mixed with increasing amounts of VII in 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, stood at 0°C for 20 min, and then incubated at 37°C for 5 min. The mixtures were then electrophoresed in 0.8% agarose gel and DNA was stained with ethidium bromide. A photograph of gel stained with ethidium bromide is shown. The position of free Ad2 DNA is indicated by an arrow.

Fig. 2 In vitro transcription of the Ad2 major late gene. SmaI-digested pSmaF was transcribed in a lysate prepared from nuclei of Ehrlich ascites tumor cells and ^{32}P-labeled RNA transcripts were analyzed by 5% polyacrylamide-7 M urea gel electrophoresis. An autoradiograph of the gel is shown. Lane 1, HpaII-digested pBR322 end-labeled with ^{32}P as molecular size markers; lane 2, transcripts from SmaI-digested pSmaF; lane 3, same as lane 2 except that 1 μg/ml of α-amanitin was added during transcription. Sizes are indicated in bases. A 536-base transcript of the Ad2 major late gene is shown by an arrow head.

In vitro transcription of the Ad2 major late gene in nuclear lysates of Ehrlich ascites tumor cells

We next prepared lysates from nuclei of Ehrlich ascites tumor cells. SmaI-digested pSmaF was used as a template to examine the accuracy of transcription in the lysates. When run-off transcripts were analyzed in polyacrylamide gel, the expected RNA band with 536 bases was detected, as shown in Fig. 2, lane 2, and formation of this band was inhibited by α-amanitin

at 1 μg/ml (lane 3). Thus, it was concluded that the Ad2 major late gene was transcribed accurately in the lysates by RNA polymerase II. These lysates were then used for *in vitro* transcription of the VII-DNA complex.

In vitro transcription of the VII-DNA complex

We compared template activity of the VII-DNA complex with that of free DNA. VII and Ad2 DNA were mixed in a ratio of 2:1 by weight, and the resulting complex was used as template for *in vitro* transcription. As shown in Fig. 3(a), the patterns of hybridization of the transcripts derived from the complex and free DNA differed, suggesting that the efficiencies of transcription with the two templates differed locally. Transcription was generally less when the complex was used as template, but the extent of the decrease in transcription differed in different regions of Ad2 DNA. The transcriptions of DNA fragments a, e and f produced by *Xho*I digestion and of b, h and i obtained by *Hin*dIII digestion were suppressed more than those of other DNA fragments of the VII-DNA complex.

The locations of the fragments produced by these two restriction enzymes are shown in Fig. 3(b). Interpretation of these results is difficult, but the results obtained using the two restriction enzymes indicated that transcription of the middle of Ad2 DNA tended to be lower with the VII-DNA complex than with free Ad2 DNA as template.

DISCUSSION

In the present study, we compared the template activity in *in vitro* transcription of Ad2 DNA in its free form and as a complex with the Ad2 major core protein VII. Results showed that transcription of the DNA region located in the middle of the Ad2 genome is more strongly repressed than that of the terminal regions by formation of the complex. The map of transcription

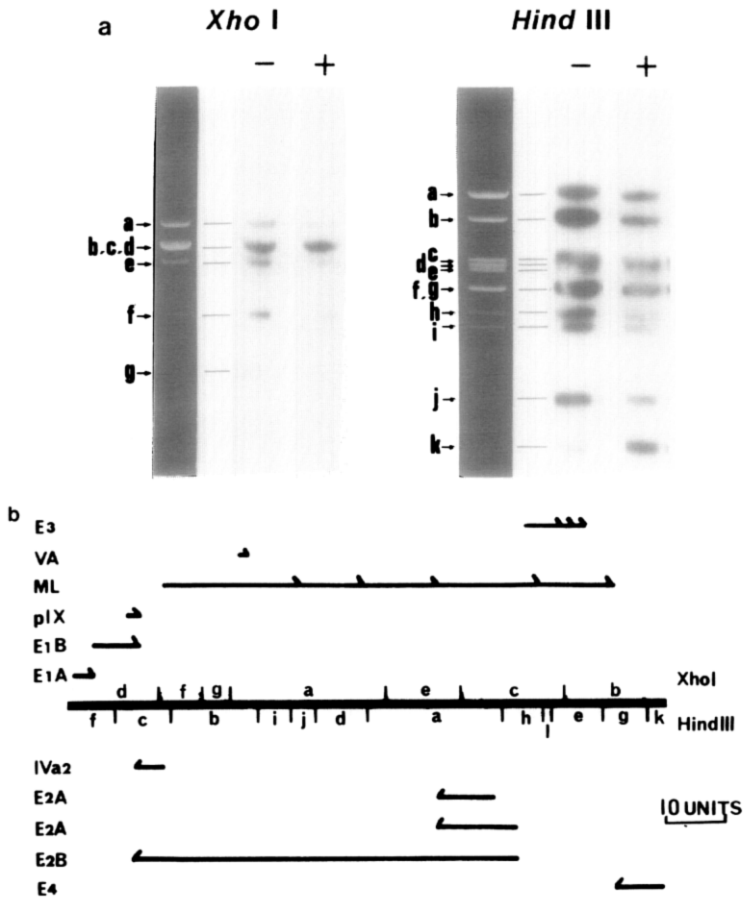

Fig. 3 (a) Hybridization assay of transcripts of the VII-DNA complex and free DNA. The VII-DNA complex or free DNA was transcribed in a nuclear lysate of Ehrlich ascites tumor cells. ^{32}P-labeled transcripts were hybridized to nitrocellulose paper containing Ad2 DNA which had been digested with XhoI or HindIII. Results with each restriction enzyme are shown in three slots. The left slot is a photograph of gel stained with ethidium bromide and the right two slots are autoradiographs of nitrocellulose papers; slots marked - show results with free DNA and slots marked + show results with the VII-DNA complex. The DNA fragments are indicated by alphabetic letters, which correspond to those in Fig. 3(b). (b) Locations of transcription units and cleavage sites of XhoI and HindIII on Ad2 DNA. Transcription units are represented by arrows and putative termination sites of transcription are shown by arrow heads. Cleavage sites for XhoI and HindIII are shown as alphabetic letters above and below the line, respectively. ML and VA mean genes of major late and virus associated RNA, respectively.

units of Ad2 genes in Fig. 3(b) (23) shows that the genes in the regions in which transcription was repressed most were early genes, such as E2A, E2B and E3, and late genes, such as the major late and virus associated RNA genes. The repression of transcription of late genes in the VII-DNA complex seems to be of

particular importance, because, it is expected that Ad2 DNA maintains its chromatin structure when the early genes are transcribed in the early stage of virus infection, but that the chromatin structure is lost when late genes are transcribed after replication of Ad2 DNA. One possibility is that the chromatin structure in the complex with VII and other viral proteins prevents transcription of late genes in the early stage of infection by masking a strong promoter, but that late genes can be transcribed in the newly replicated Ad2 DNA which is free from the chromatin structure. However, the reason why the transcription of early genes such as E2A, E2B and E3 is repressed in the VII-DNA complex is unknown. Possibly, the immediate early genes, E1A and E1B (5), which are located near the left end of Ad2 DNA, can be transcribed actively and their protein products induce transcription of other early genes which would otherwise be inactive. Studies are required on whether the E1A and/or E1B gene product activates transcription of E2A, E2B or E3 when the VII-DNA complex is used as a template for in vitro transcription.

More precise experiments are needed to investigate the possibilities discussed above. In any case, this system is unique and should be useful for further studies on the role of chromatin structure in control of selective gene transcription.

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