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11. A statistical analysis using the $t$ statistic of the mean channel dimensions of samples (1.11 nm) autoclaved for 48 hours versus samples (1.08 nm) autoclaved for 8 hours showed no significant difference ($t = 0.7146$, d.f. = 38; $P > 0.48$).
12. Manganite crystals were large and separated from the todorokite crystals, when viewed by TEM, and showed no evidence of topotactic transformation from birnessite or todorokite.
13. Chemical analysis of todorokite and determination of the oxidation state of manganese was done by the method of J. D. Hem [*Geochim. Cosmochim. Acta* **45**, 1369 (1981)]. In assigning the cations, a 12-oxygen unit cell was assumed. Although the presence of $Mn^{3+}$ in the structure of todorokite has been demonstrated, in this study atomic assignments were done by assuming the presence of only two species, $Mn^{2+}$ and $Mn^{4+}$. Sodium was considered a tunnel cation, whereas magnesium was considered both a tunnel and a structural cation. No chloride or silicon were detected in synthetic todorokite.
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# E1A Transcription Induction: Enhanced Binding of a Factor to Upstream Promoter Sequences

IMRE KOVESDI, RONALD REICHEL, JOSEPH R. NEVINS

**The adenovirus E1A gene product *trans*-activates a number of viral and cellular promoters. The mechanism for this transcriptional induction was investigated with an in vivo exoIII mapping technique to assay for proteins that interact with an E1A-inducible promoter. A protein bound to the early E2 promoter was detected in wild-type infected cells. In the absence of E1A induction, specific interactions at the promoter could not be detected, as indicated by the absence of an exoIII-protected fragment. However, if conditions were established that allowed transcription of the E2 gene in the absence of E1A, the same exoIII protection was observed as was found in the presence of E1A. These results suggest a model in which the efficient utilization of the E2 promoter is mediated by a cellular transcription factor. In the absence of E1A, the interaction can take place, but slowly and inefficiently in comparison with the interaction in the presence of E1A.**

THE PROCESS BY WHICH THE INITIATION of transcription is regulated, while of central importance to many aspects of biology, is not clearly understood. The critical aspects of the process are the protein factors that interact with regulatory sites of promoters and the manner in which the activity of these factors is controlled. A particularly useful system for the study of transcriptional control are the early genes of adenovirus. The regulatory gene that is responsible for this control, the E1A gene, has been identified (*1–3*). A set of five viral promoters are coordinately regulated. In addition, two cellular promoters, hsp70 and β-tubulin, are stimulated by the action of the E1A gene product (*4–6*). Therefore, all of the components of a system of transcriptional control are contained within an early viral infection: genes that respond to positive control, an identified regulatory gene, a set of coordinately controlled genes, and genes in a distinct context (cellular chromosome) that are coordinately controlled.

To gain further insight into the mechanism of E1A-mediated transcription control, we analyzed adenovirus chromatin in infected cells for the presence of proteins in the vicinity of a promoter controlled by E1A—in this case the E2 promoter. We and others have shown that this promoter is inducible by E1A and requires certain upstream sequences for activity (*7–12*). The fact that there exist sequences critical for promoter activity suggests that a protein or proteins may recognize these sequences. To identify such interactions, we used a technique, described by Wu (*13*), in which exonuclease III (exoIII) defines protein-DNA interactions in vivo. The rationale for the procedure in the context of the adenovirus E2 promoter is shown schematically in Fig. 1A. Briefly, nuclei are incubated with a restriction endonuclease for which there is a recognition site in the vicinity of the suspected protein binding site. In the case of the E2 promoter, there is an Eco RI site at −285 relative to the transcription initiation site (+1). Therefore, nuclei are incubated with Eco RI in the presence or absence of exoIII. The DNA is extracted and purified and subsequently digested with S1 nuclease to remove the single-strand tail resulting from exoIII digestion and then digested with a second restriction enzyme, in this case Sst I. The digested DNA is then separated in an agarose gel, transferred to nitrocellulose, and visualized with a probe specific to the sequence adjacent to the Sst I site. Three possible DNA fragments can be detected in this way. An Sst I–Sst I band would be present if the Eco RI digestion was not complete. An Sst I–Eco RI fragment is produced in the absence of exoIII digestion. Finally, if a protein is stably bound in a nonrandom fashion to a specific site on the DNA, then exoIII digestion is stopped and a band is produced that is smaller than the Sst I–Eco RI fragment.

Such an analysis was performed with nuclei from cells infected with wild-type adenovirus 5 (Ad5) for 7 hours, in the presence of arabinosylcytosine to prevent DNA replication (Fig. 1B). In the absence of exoIII, two bands are detected and represent the Sst I–Sst I fragment and the Sst I–Eco RI fragment. In this experiment and most others that have been done, approximately 50 percent of the adenovirus chromatin is cut by Eco RI. As the amount of exoIII added to the nuclei is increased, there is the appearance of lower molecular weight bands. At the highest exoIII concentration, a band of 995 nucleotides predominates. Such a fragment indicates that the exoIII stop is at a position of −85 relative to the E2 transcription initiation site (Fig. 1C). There are intermediate-sized bands, apparently the result of incomplete exoIII digestion since they tend to disappear at higher enzyme

The Rockefeller University, New York, NY 10021

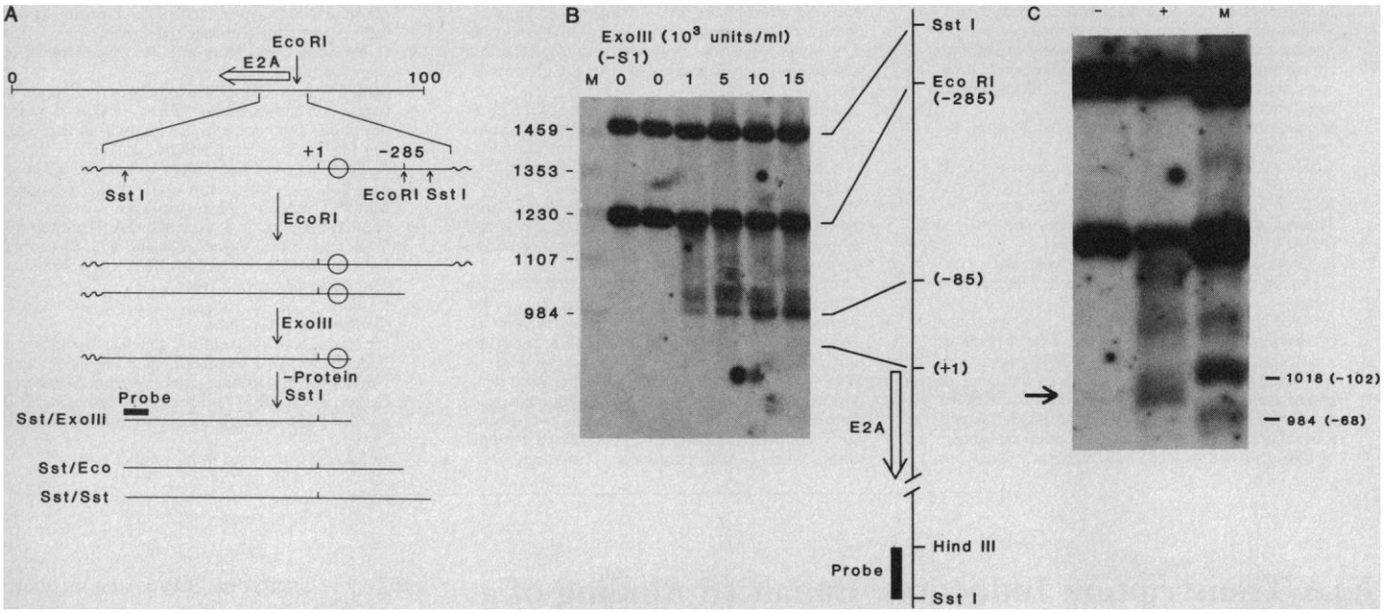


Fig. 1. ExoIII mapping of protein interactions at the adenovirus E2 promoter. (A) Schematic diagram of the adenovirus E2 transcriptional unit and the method for exoIII mapping of protein interactions. There is an Eco RI site located 285 nucleotides upstream from the E2 transcription initiation site. After Eco RI digestion and exoIII digestion, DNA is purified and digested with S1 nuclease and Sst I to generate a defined terminus. The probe is a Sst I–Hind III fragment. (B) ExoIII assay of protein interactions at the E2 promoter in Ad5 wild-type infected HeLa cells. HeLa cells were infected with wild-type Ad5 (1000 particles per cell) and incubated in the presence of arabinosylcytosine (25 μg/ml) for 7 hours. Nuclei were prepared by Dounce homogenization and incubated with Eco RI (6000 U/ml) and the indicated amount of exoIII under conditions described previously for analysis of the *Drosophila* hsp promoter (*13*). After incubation, DNA was extracted and digested with S1 nuclease (4000 U/ml) and Sst I. Samples of the digested DNA (10 μg) were then resolved in 1.4 percent agarose gels and transferred to nitrocellulose; the DNA bands were visualized by hybridization to the Sst I–Hind III probe. (C) An exoIII assay as shown in (B) was electrophoresed beside DNA markers of 1018 nucleotides and 984 nucleotides. The markers were first mixed with an amount of cellular DNA equivalent to that analyzed in the exoIII digestion to control for altered migration. The values in parentheses refer to positions relative to the E2 transcription start site.

concentrations. Whether these stops represent sequences in the DNA that are relatively resistant to digestion or whether they locate loosely bound proteins is not clear.

From the result of Fig. 1, we would conclude that there was a protein bound to the E2 promoter in the region of the sequences critical for activity of this promoter. Because the same sequences that are required for E1A-induced transcription are required for uninduced transcription, the protein detected in the wild-type infection might be a general transcription factor and have nothing to do with E1A regulation. Alternatively, this protein might indeed be important for E1A control. To investigate this question, we analyzed adenovirus chromatin in an infection in the absence of E1A. In this experiment, cells were infected with wild-type Ad5 or with d1312, an E1A deletion mutant (*2*). Nuclei were isolated 7 hours after infection and were analyzed as described in Fig. 1. The results are presented in Fig. 2A. In this experiment only one exoIII concentration was used. Once again, an exoIII stop fragment that mapped to −85 was generated in the wild-type infection. In sharp contrast, no such exoIII stop was observed in the d1312 infection, and in fact no exoIII stop of any kind was observed in the d1312 chromatin. Therefore, we conclude that there is a protein bound to the E2 promoter when the gene is transcribed under the control of E1A, but this protein is not bound in the absence of E1A when the gene is not transcribed.

The data of Fig. 2A are compatible with two mechanisms for E1A transcriptional control. First, the E1A protein could itself be the transcription factor detected by the exoIII assays. A series of indirect results, including the findings that the E1A-inducible promoters can be efficiently activated by heterologous inducers (*14–16*), that there is E1A-independent transcription, the degree of which is a function of the host cell (*17*), and that E1A can activate various unrelated promoters (*5, 6, 18, 19*), argue against DNA sequence recognition although they do not disprove it. Alternatively, E1A might regulate in some manner the activity of cellular transcription factors, which then results in increased binding. To address this question, we analyzed adenovirus chromatin structure in the absence of E1A, but under conditions whereby early genes were transcribed. Specifically, active transcriptional complexes accumulated in a prolonged infection with d1312 (*20*). We therefore assayed for the presence of E2-protein complexes in d1312-infected cells 33 hours after infection or 7 hours after infection. The results of such an experiment are shown in Fig. 2B. There was no exoIII-protected fragment in the 7-hour d1312 sample. However, when the d1312 infection was allowed to proceed for 33 hours, an exoIII stop appeared that again mapped to −85. Thus, the same exoIII-protected site as was found in a wild-type infection was obtained in the complete absence of E1A. If indeed these are the same proteins, then it cannot be E1A, and we suggest that it is a cellular factor.

We and others found earlier that sequences upstream from the E2 transcription initiation site are critical for transcription (*7–12*). However, there is no unique site required for E1A induction; the essential promoter sequences are also those required for E1A stimulation. An examination of the sequence of the E2 promoter in the critical region reveals a 16-nucleotide sequence that is duplicated, except for two residues, in the reverse orientation at position −30 to −75 (Fig. 3). The fact that this sequence is conserved as a duplication in the region essential for activity of the promoter suggests that this is, in fact, the site of binding of a transcriptional factor. Indeed, the location of the upstream copy of the sequence, between positions −60 and −75 relative to the E2 initiation site, is fully consistent with previous deletion mapping experiments.

Specifically, deletion to −79 had no effect on promoter function, whereas deletion to −70 significantly reduced the activity of the promoter. Further deletion to −59 virtually abolished promoter activity. Such results indicate that the proximal element (−30 to −43) is not sufficient by itself for promoter activity, although studies with linker scanning mutants in the E2 promoter suggest that the proximal element is indeed important (*12*). Such a situation is reminiscent of the sequence requirements in the herpes thymidine kinase promoter (*21*). This result and the conservation of the sequence would suggest that a factor interacts with each of these sequences, possibly cooperatively. More precise mapping in vivo, in which deoxyribonuclease digestion or dimethyl sulfate protection is used along with genomic blotting (*22, 23*), should allow such a definition.

It is interesting that the −70 deletion mutant is still inducible by E1A. Perhaps the −70 deletion impairs the binding of a factor to the upstream site but does not totally eliminate binding. This would be consistent with the location of the sequence element discussed above since the −70 deletion removes one-third of this sequence. If the same factor is involved in uninduced and in E1A-induced transcription, then as long as the factor could bind to the site, even inefficiently, there would still be induction if the mechanism of induction increases the amount of the factor. If the entire binding site is removed as is the case with the −59 deletion, there would be no possibility of binding and thus no induction. Therefore, we suggest a process whereby the inducible promoters make use of a cellular transcription factor in the same way that the early SV40 promoter apparently makes use of the SP1 factor (*24, 25*). If such a factor is limiting in the cell, there would be inefficient transcription of these viral promoters. We suggest that the function of E1A is to increase the availability of such a factor so that it then becomes nonlimiting. As a result there is increased transcription of the viral promoters—that is, induction. Such action could involve an increase in the actual amount of the factor or, more likely, a modification that converts an inactive factor to an active factor.

One last point concerning such a model for E1A action deserves comment. It has been suggested that E1A activation counteracts a negative control on the viral promoters. This suggestion was based on the observation that inhibition of protein synthesis either partially relieved a requirement for E1A (*3, 26*) or resulted in a superinduction in the presence of E1A (*27*). Extensive analysis of E1A-inducible promoters has provided no clear evidence for a negative-acting factor (repressor) at the level of DNA recognition. It is more likely that such a negative component is an antagonist of the action of E1A. For instance, if E1A induced a modification that activated a transcriptional factor, the negative component could be an activity that reversed the modification. There are indeed many examples of such regulated systems within the cell (for instance, a kinase and a phosphatase that act on the same substrate), and E1A may simply intercede in such a pathway. The normal role of such a pathway is of interest, one possibility being the control of transcription of certain genes during the cell cycle (*28*).

Our experiments offer some clues to the possible action of the E1A protein in the activation of transcription, but a final resolution of the mechanism will require the actual isolation of the factor involved. Among the unanswered questions are these: Is the pro-

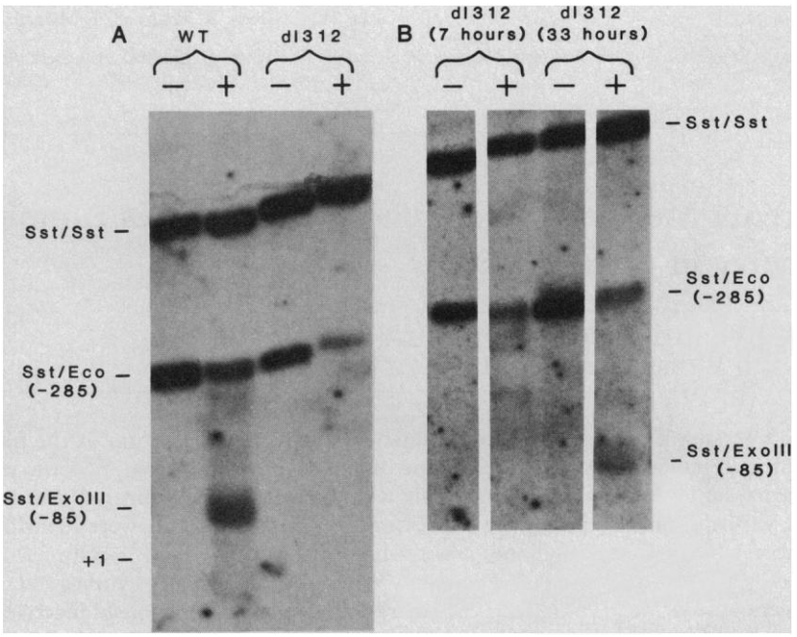


Fig. 2. ExoIII assay of protein interactions at the E2 promoter in dl312 (E1A)-infected HeLa cells. (A) HeLa cells were infected with wild-type Ad5 (WT) or with the E1A deletion mutant dl312, each at 1000 particles per cell, in the presence of arabinosylcytosine for 7 hours. Nuclei were prepared and assayed as described in the legend to Fig. 1. Nuclei were incubated in the presence (+) or absence (−) of exoIII. Where present, the exoIII was used at a concentration of 10,000 units per milliliter. (B) HeLa cells were infected with dl312 (1000 particles per cell) and incubated in the presence of arabinosylcytosine (25 μg/ml) for 7 hours or 33 hours. Nuclei were prepared and assayed as described in the legend to Fig. 1.

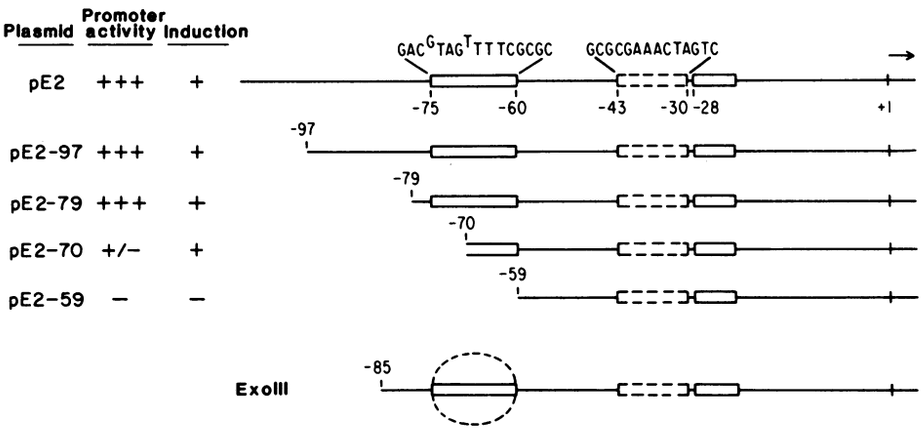


Fig. 3. Schematic diagram of the critical sequence elements in the upstream region of the adenovirus E2 promoter. Depicted is the structure of 5′ deletion mutants in the E2 promoter that have been described (*8*). Indicated at the top is a 16-nucleotide sequence, located between residue −60 and −75 relative to the transcription initiation site, that is duplicated at −30 to −43 (in the reverse orientation). Shown at the left is a summary of previous data (*8, 9*) concerning the activity of these promoter deletions and their ability to respond to E1A stimulation. The approximate position of the exoIII stop that is generated is shown at the bottom.

tein that recognizes the E2 upstream element a cellular protein, and if so, is it present in uninfected cells? If the protein is present before and after the action of E1A, then how is it different as a result of the action of the E1A protein?


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# Adrenal-Mediated Endogenous Metabolites Inhibit Puberty in Female Mice


MILOS NOVOTNY, BOZENA JEMIOLO,* SCOTT HARVEY, DONALD WIESLER, ANNA MARCHLEWSKA-KOJ*



**While assessing a potential role of adrenal glands in the production of the hitherto unidentified puberty-delaying pheromone of female mice, the urinary volatile profiles of normal and adrenalectomized animals were quantitatively compared. Six components, whose concentrations were depressed after adrenalectomy, were identified: 2-heptanone, *trans*-5-hepten-2-one, *trans*-4-hepten-2-one, *n*-pentyl acetate, *cis*-2-penten-1-yl acetate, and 2,5-dimethylpyrazine. When these laboratory-synthesized chemicals were added (in their natural concentrations) to either previously inactive urine from adrenalectomized females or plain water, the biological activity was fully restored.**


BOTH SOCIAL AND ENVIRONMENTAL factors may accelerate or delay the timing of sexual maturation in the female house mouse, *Mus musculus*. The onset of puberty in juvenile female mice is modified by urinary chemical cues (priming pheromones) originating from both males and females. Treatment of juvenile females with the urine of normal males (*1–3*), or its high molecular weight fraction (*4*), causes puberty acceleration, whereas treatment of juvenile females with the excreted urine of intact females that had been housed together for at least 10 days (*5*) substantially delays puberty (*6*). The efficiency of this female-to-female puberty-delaying urinary factor depends on the density and duration of the grouping of the female urine donors (*5, 7, 8*) but not on the donor's age (*7, 9*). Social contact, while necessary for pheromone production, does not appear to enhance the delay of sexual development in juvenile mice (*10*). Changes in gonadal activity, in adrenal gland size, and in the secretion of adrenal hormones, and decreased reproductive performance appear to result from social stress in high-density populations of various rodent species (*11–13*). Studies of Drickamer and co-workers indicate that adrenalectomy (*14*) but not ovariectomy (*15*) abolishes the biological activity of excreted urine to delay puberty in juvenile mice.

On the basis of the suggestion of Christian (*11–13*) and Drickamer and co-workers (*14, 15*) that the puberty-inhibiting effect may be associated with the adrenal function, we designed the series of experiments in which (i) chemical differences between the urinary excretion of normal and adrenalectomized female animals were quantitatively observed; (ii) the urinary volatile substances that differed consistently between the two sample types were subsequently identified and synthesized for both structural verification and biological testing; and (iii) these synthetic pheromone candidates were tested both in mixtures and individually to determine whether their action parallels that of the natural urinary stimuli. These results

Table 1. Mean (in days) of the first vaginal estrus (± SEM) for young female mice painted daily on the external nares with various stimuli or water (control). Means marked with an asterisk are significantly different from means without an asterisk ($P < 0.001$); $F(\text{df } 8267) = 27.15$, $P < 0.005$ (Duncan's new multiple range test).

| Treatment | *n* | Mean day to reach first estrus |
|---|---|---|
| *April* | | |
| Water (control) | 32 | 30.1 (0.3) |
| Urine from intact females caged: | | |
| Singly | 32 | 30.5 (0.2) |
| Group | 32 | 33.9*(0.4) |
| *October* | | |
| Water (control) | 30 | 31.1 (0.3) |
| Urine from ovariectomized females caged: | | |
| Singly | 30 | 31.3 (0.4) |
| Group | 30 | 33.8*(0.5) |
| *September* | | |
| Water (control) | 30 | 29.6 (0.3) |
| Urine from adrenalectomized females caged: | | |
| Singly | 30 | 30.5 (0.3) |
| Group | 30 | 30.4 (0.3) |


Department of Chemistry, Indiana University, Bloomington 47405.

*On leave from the Institute of Zoology, Jagiellonian University, 30-060 Krakow, Poland.