Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1193–1197, February 1984
Genetics

Adenovirus early region 1A protein activates transcription of a nonviral gene introduced into mammalian cells by infection or transfection

(gene expression/transcriptional regulation/preproinsulin gene)

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Communicated by Phillip A. Sharp, November 10, 1983

ABSTRACT **Transcription from all early adenovirus promoters is stimulated by a 289 amino acid phosphoprotein encoded in the pre-early transcription unit *E1A*. To determine if this protein could act on a nonviral gene placed on the viral chromosome, adenovirus recombinants were constructed in which the rat preproinsulin I gene, including its promoter region, was substituted in both orientations for *E1A*. Preproinsulin mRNA synthesis from these recombinants was greatly stimulated after infection of line 293 cells, which constitutively express E1A protein, compared to HeLa cells, which do not. Expression of the preproinsulin gene was also greatly stimulated when HeLa cells were coinfected with the recombinants and wild-type adenovirus or a mutant defective in a second E1A protein, but much less so by coinfection with a mutant defective in the 289 amino acid phosphoprotein. Much of the *E1A*-induced preproinsulin mRNA had a 5′ end at the same position as the preproinsulin mRNA isolated from insulinoma cells, but a considerable fraction had 5′ ends mapping heterogeneously within several hundred nucleotides of this site. Preproinsulin mRNA was also detected in 293 cells but not HeLa or HEK cells after transfection of a plasmid containing the preproinsulin gene with no adenovirus sequence. This indicates that there is no *cis*-acting adenovirus sequence required for E1A protein stimulation of preproinsulin transcription. Infection of rat cells with adenovirus did not induce detectable mRNA synthesis from the endogenous preproinsulin I gene. These results demonstrate that the E1A protein can induce expression of a nonviral gene when it is newly introduced into mammalian cells by viral infection or transfection, but it does not induce the endogenous cellular gene.**

A protein encoded in early region 1A (E1A) of the DNA of adenovirus serotype 5 (Ad5) promotes the rapid onset of viral transcription (1–3). However, this protein is not absolutely required for transcription from viral promoters. After infection with d1312, a mutant from which *E1A* has been deleted (4), early transcription becomes fully induced, but at much later times than after infection with wild-type virus (3, 5). Thus, the E1A protein has been said to "facilitate" early transcription (3). Two closely related phosphoproteins are expressed from *E1A* during the early phase of infection (6, 7). Only the larger of these, predicted from the sequence of its mRNA to be 289 amino acids long, is required to facilitate early transcription (8, 9). The mechanism by which this protein (hereafter referred to as the E1A protein) functions is not known.

In an analysis of the induction of early transcription in cells infected with *E1A* mutants, we found that cells infected with one *E1A* mutant and incubated for a time sufficient to allow induction of early transcription did not immediately transcribe a second *E1A* mutant introduced into the same cells by superinfection (5). The second *E1A* mutant recapitulated the delayed activation of its early genes. To explain this delayed *cis*-acting induction of early transcription observed after infection with *E1A* mutants, we suggested that the viral DNA was slowly assembled into stable transcriptional complexes. Stable transcriptional complexes have been demonstrated in experiments *in vitro* with genes transcribed by RNA polymerases II (10) and III (11). Although not yet thoroughly defined, the complexes appear to result from the assembly of several proteins with DNA to form a complex that is stable for many hours and that acts as the substrate for transcription initiation by the RNA polymerase. Since the E1A protein facilitates early transcription, we suggested that it might function by catalyzing the assembly of viral DNA into such stable transcriptional complexes (5).

To test this model of the mechanism of action of the E1A protein, we constructed adenovirus recombinants containing a nonviral gene and asked if the E1A protein could influence transcription of this nonviral gene when it was present on the viral chromosome. We chose the rat preproinsulin I gene because it is well characterized (Fig. 1; refs. 13 and 14) and had been shown to be expressed in cells in culture from a simian virus 40 recombinant containing a convenient cloned restriction fragment from the rat chromosome (15).

MATERIALS AND METHODS

Construction of Rat Preproinsulin I–Ad5 Recombinants. Methods for plasmid construction were as described (16). A 1372-base-pair (bp) *Pvu* II/*Xba* I fragment was isolated from λ Charon 4A-rI1 phage (13) and inserted between the *Pvu* II and *Xba* I sites of the Ad5 sequence in HE3 (17) to generate pHE3-RI-1. pHE3-RI-2 was a clone of the same 1372-bp fragment in the opposite orientation between the *Pvu* II and *Sma* I sites of pHE3. Rat insulin DNA fragments RI-1 and RI-2 were generated by ligating *Eco*RI/*Xba* I-digested pHE3-RI-1 or -2 to *Xba* I-cut Ad5 d1309 DNA followed by transfection into 293 cells (17). Each was plaque-purified, grown into stocks, and titrated by plaque formation, all on 293 cells (18).

Analysis of Preproinsulin I RNA. Cytoplasmic RNA was isolated (19) from infected cells treated with cytosine arabinonucleoside (Sigma) at 20 μg/ml as described (5). S1 nuclease analysis (19, 20) was performed by using a 5′-end-labeled 914-bp *Hin*fI fragment extending from −402 to +411 in the preproinsulin I gene sequence (A. Efstratiadis, personal communication) [+1 equals the proposed cap site (12)]. RNA (100 μg) was hybridized to 1.5 nmol of probe in 50 μl of

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Abbreviations: E1A, early region 1A; bp, base pair(s); Ad5, adenovirus serotype 5.

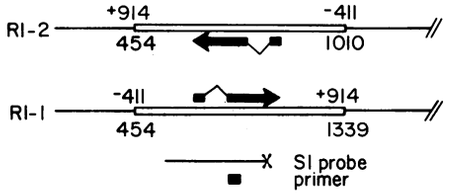

FIG. 1. Map of the left end of the Ad5–preproinsulin I gene recombinants. Ad5 sequence is represented by the lines with nucleotide numbers (12) indicated below the maps. Rat genome sequence is represented by the open box with sequence relative to the insulinoma preproinsulin I mRNA cap site (13) indicated above the line. The map positions of the two preproinsulin I mRNA exons are shown, the arrowhead indicating the 3′ end. The location of the 5′-end-labeled probe used for S1 nuclease analysis and the labeled primer used for primer extension analysis of preproinsulin I mRNA are diagrammed below the map of RI-1.

80% (vol/vol) formamide buffer (19) at 58°C for 12–16 hr and subjected to S1 nuclease analysis as described (21).

For primer extension mapping (22), 5 pmol of a phage M13 clone of the plus strand of the preproinsulin I gene from −160 to +911 and 350 nmol of a fragment from +274 to +356 in 50 μl of reverse transcription buffer (23) were incubated at 100°C for 5 min, then at 68°C for 30 min. Twenty picomoles each of [α^{32}P]dATP (3000 Ci/mmol; 1 Ci = 37 GBq), dTTP, dGTP, dCTP, and 12 units of reverse transcriptase from avian myeloblastosis virus (Life Sciences, St. Petersburg, FL) were added and the mixture was incubated for 30 min at 42°C followed by addition of 100 pmol each of dATP, dTTP, dGTP, and dCTP and incubation for 30 min. The product was digested with *Hae* III and denatured, and the resulting labeled single-stranded fragment from +210 to +356 was isolated by electrophoresis. Seventy nanomoles of this labeled single-stranded fragment was hybridized to 60 μg of oligo(dT)-selected (24) cytoplasmic RNA isolated from HeLa cells 40 hr after coinfection with RI-1 plus Ad5 or RI-2 plus Ad5 in 50 μl of 1 M NaCl/10 mM Tris·HCl, pH 7.0/1 mM EDTA at 68°C for 2 hr and treated with reverse transcriptase as described (25).

Transfection Experiments. The 1372-bp *Pvu* II/*Xba* I fragment isolated from λ Charon 4A-RI-1 (13) was inserted into the *Eco*RI site of pACYC184 (16). Twenty micrograms was used to transfect (26) each 100-nm plate of 80% confluent 293, HeLa, or HEK (human embryonic kidney) cells. RNA was isolated after 44 hr.

RESULTS

Construction of Adenovirus Recombinants Containing the Rat Preproinsulin I Gene. To test the ability of the E1A protein to facilitate transcription of a nonviral gene, two adenovirus recombinants were constructed in which the rat preproinsulin I gene (13, 14) was substituted for the adenovirus *E1A* region (Fig. 1). Rat chromosomal sequence was inserted between nucleotide 454 in the Ad5 sequence (12), 45 nucleotides upstream from the major *E1A* transcription start site (21, 27), and nucleotide 1339 in recombinant RI-1, or nucleotide 1010 in recombinant RI-2. The rat DNA extended from 411 nucleotides upstream from the preproinsulin mRNA cap site to 353 bp downstream from the poly(A) site (refs. 13 and 14; A. Efstratiadis, personal communication). In RI-1 the direction of preproinsulin transcription is the same as *E1A* transcription in wild-type adenovirus, and in RI-2 it is in the opposite direction. The structures of the recombinant genomes was confirmed by restriction enzyme digestion and hybridization of Southern blots (28) of these digests to a ^{32}P-labeled (29) cDNA clone of preproinsulin mRNA (30) (data not shown).

Adenovirus E1A Protein Stimulates Transcription of the Rat Preproinsulin I Gene. To determine if the adenovirus E1A protein could stimulate transcription of the rat gene in the adenovirus recombinants, the production of rat preproinsulin mRNA was compared after infection of HeLa cells and 293 cells by RI-1. Because the *E1A* gene is deleted from RI-1, there was no E1A protein expressed during the HeLa cell infection. In contrast, 293 cells constitutively express the E1A protein from an integrated copy of the left 14% of the Ad5 genome (2) so that the E1A protein was present during infection of 293 cells. Cytoplasmic RNA was assayed for the presence of adenovirus mRNA from early region E3, which is under E1A control (1, 2), and for rat preproinsulin mRNA by hybridization S1 nuclease analysis (19, 20). E3 mRNA was analyzed by using a uniformly labeled M13 clone, which results in several S1 nuclease-protected fragments (5). Rat preproinsulin mRNA was detected by using a 5′-end-labeled fragment of rat chromosomal DNA sequence extending from near the 3′ end of the mRNA to a point upstream of the mRNA 5′ end (Fig. 1). This fragment is expected to generate a labeled S1 nuclease-resistant fragment of 350 nucleotides due to cleavage at the 3′ splice site in a hybrid between preproinsulin mRNA and the chromosomal DNA. As expected for an *E1A* deletion mutant, E3 mRNA was expressed at much higher levels in 293 cells than in HeLa cells (Fig. 2*A*). Of significance here, cytoplasmic RNA containing the rat preproinsulin sequence was also expressed at much higher levels in 293 cells than in HeLa cells (Fig. 2*A*). This result was not due to viral DNA replication and a resulting increased dosage of the preproinsulin gene in 293 cells because in this and subsequent experiments cytosine arabinonucleoside was added to prevent viral DNA replication (5). However, a similar stimulation of RNA synthesis in 293 compared to HeLa cells was also observed when cytosine arabinonucleoside was not added to the infected cells (data not shown). These results are consistent with the hypothesis that the E1A protein can facilitate transcription from the rat pre-

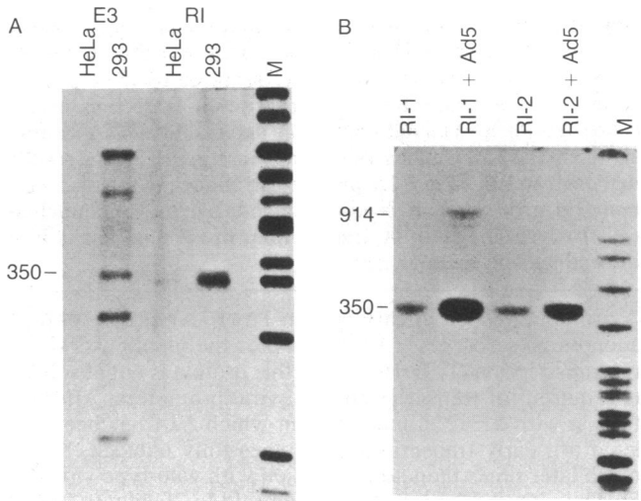

FIG. 2. E1A protein stimulates RNA synthesis from the rat preproinsulin gene in the adenovirus recombinants. (*A*) HeLa or 293 cells were infected at a multiplicity of infection of 10 and cytoplasmic RNA was isolated 36 hr after infection in the presence of cytosine arabinonucleoside. E3 shows an autoradiogram of S1 nuclease-protected fragments from early region 3, using a uniformly labeled probe from 75.9 to 81.0 map units. RI shows an autoradiogram of S1 nuclease-protected fragments generated by using the 5′-end-labeled rat preproinsulin I probe diagrammed in Fig. 1. This probe should generate a fragment of 350 nucleotides from preproinsulin I mRNA. M, marker fragments of known length. (*B*) HeLa cells were infected with RI-1 or RI-2 alone at a multiplicity of infection of 10 or were coinfected with wild-type Ad5 and RI-1 or RI-2 at a multiplicity of infection of 10 each. Cytoplasmic RNA was isolated at 36 hr after infection and S1 nuclease analysis was performed.

proinsulin promoter when it is located on the adenovirus chromosome.

Since HeLa cells are not isogenic with 293 cells, it was possible that the increased production of preproinsulin mRNA in 293 cells compared to HeLa cells was due to some difference between these cells other than the presence or absence of the E1A protein. Therefore, the production of preproinsulin mRNA was assayed in HeLa cells either singly infected with RI-1 or coinfected with RI-1 and wild-type Ad5 to provide the E1A protein. Cytoplasmic RNA containing rat preproinsulin sequence was expressed at a much higher level in the coinfected cells than in cells infected with RI-1 alone (Fig. 2*B*). The only genetic difference between the singly infected and coinfected cells in this experiment was the presence of the intact *E1A* gene in the coinfected cells. Similar results were observed when the preproinsulin I gene was inserted in the opposite orientation in the recombinant RI-2 (Fig. 2*B*). S1 nuclease analysis of nuclear RNA also showed higher concentrations of preproinsulin RNA in coinfected cells (data not shown). Thus, the E1A protein stimulates preproinsulin RNA synthesis by a mechanism that is independent of the orientation of the preproinsulin gene.

Primer Extension Mapping of the 5′ Ends of the *E1A*-Induced Preproinsulin mRNA. Primer extension studies were performed to locate the 5′ ends of the preproinsulin mRNAs induced by the E1A protein. A ^{32}P-labeled fragment extending from +210 to +356 in the preproinsulin I gene sequence (Fig. 1) was hybridized to poly(A)$^+$ RNA isolated from coinfected HeLa cells (Fig. 2*B*) and extended by polymerization with reverse transcriptase (22). Extension to the 5′ end of the preproinsulin mRNA expressed in rat insulinoma cells, which we will call the authentic 5′ end, would yield a 237-nucleotide fragment (13, 15). The major primer-extension product observed with cytoplasmic RNA from both RI-1- and RI-2-infected HeLa cells coinfected with Ad5 was ≈240 nucleotides long (Fig. 3*A*). Thus, a large fraction of the E1A protein-induced preproinsulin mRNA transcribed from both of these recombinants had the authentic 5′ end. In addition, primer extension products longer than 240 nucleotides but shorter than 610 nucleotides were produced from both RI-1 and RI-2, indicating that the E1A protein induced transcription from start sites in rat DNA sequence upstream from the authentic start site. Primer extension products of less than 240 nucleotides were also observed. Many of these fragments may have been due to premature termination of reverse transcription, but it is possible that some of these fragments were reverse transcripts of RNAs initiated downstream from the authentic start site.

The results of these primer extension analyses demonstrate that preproinsulin gene transcription stimulated by the E1A protein was initiated principally in the rat DNA sequence of these adenovirus recombinants, within the promoter region of the preproinsulin I gene. RNA isolated from HeLa cells coinfected with RI-1 and wild-type Ad5 also yielded a primer extension fragment of 800 nucleotides (Fig. 3*A*). This corresponds to a start site at 370 in the adenovirus sequence. This is the major 5′ end of the residual *E1A* transcripts observed after infection with adenovirus mutants that have a deletion of the *E1A* "TATA box" region (21). This RNA probably represents transcription directed by the adenovirus sequence from 1 to 454 which is present in RI-1.

Transcription of the Preproinsulin Gene Is Stimulated by the 289 Amino Acid E1A Protein. Two closely related proteins are expressed from E1A during the early phase of Ad5 infection. These proteins have primary sequences of 289 and 243 amino acids as predicted from the sequence of their mRNAs (6). The 289 amino acid protein has the activity that is chiefly responsible for facilitating viral transcription (8, 9). To determine which of the E1A proteins is responsible for stimulating rat preproinsulin transcription in these recombinants, HeLa cells were infected with RI-2 alone or were coinfected with RI-2 and one of the following: (*i*) wild-type Ad5, (*ii*) Ad5 hr1, which expresses only the 243 amino acid protein (8, 31), or (*iii*) Ad2/5 pm975, which expresses only the 289 amino acid protein (9). Analysis of preproinsulin mRNA revealed that pm975 stimulated production of this mRNA as well as wild-type Ad5 and much more so than hr1 (Fig. 3*B*). Therefore, it is the 289 amino acid protein that is principally responsible for stimulating mRNA production from the rat gene as well as from adenovirus genes.

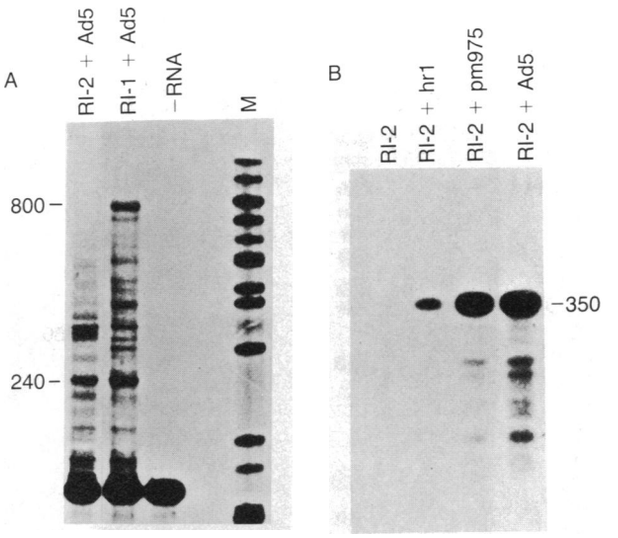

FIG. 3. (*A*) Primer extension analysis of E1A protein-induced preproinsulin transcripts. Poly(A)$^+$ cytoplasmic RNA was isolated from HeLa cells coinfected with wild-type Ad5 and RI-1 or RI-2 at 40 hr after infection, hybridized to the labeled primer shown in Fig. 1, and used as a template for reverse transcriptase. The products were denatured and fractionated by electrophoresis in an 8 M urea/polyacrylamide gel. An autoradiogram of the gel is shown. −RNA, same procedure, with poly(A)$^+$ RNA omitted. (*B*) *E1A* induction of preproinsulin transcription is due chiefly to the 289 amino acid E1A protein. HeLa cells were infected with RI-2 alone or RI-2 plus Ad5 hr1, Ad2/5 pm975, or wild-type Ad5. Cytoplasmic RNA isolated 40 hr after infection was subjected to S1 nuclease analysis with the preproinsulin I probe diagrammed in Fig. 1.

No *cis*-Acting Adenovirus DNA Sequence Is Required for E1A Protein Induction of the Rat Preproinsulin Gene. An experiment was performed to determine if E1A protein induction of the rat preproinsulin gene requires an adenovirus DNA sequence on the same DNA molecule as the rat gene. Such a requirement might be expected if the E1A protein interacted with a specific viral DNA sequence in order to stimulate transcription from the entire DNA molecule. A plasmid clone of the rat preproinsulin I gene that contained no adenovirus DNA sequence was used to transfect 293 cells and HeLa cells. This plasmid contained the same restriction fragment from the rat genome that was used to construct the adenovirus recombinants. Preproinsulin I sequence was detected in cytoplasmic RNA isolated from transfected 293 cells but not from transfected HeLa cells (Fig. 4*A*). It is unlikely that this difference was due to a failure to transfect the HeLa cells. Weeks and Jones (32) found little difference between the transient expression of pSV2CAT (33) in 293 and HeLa cells after transfection. Our HeLa cells can be efficiently transfected with adenovirus DNA and they express Ad2 *E1A* mRNA after transfection of plasmid clones of the left end of the Ad2 genome (unpublished results). A similar experiment in which this plasmid was used to transfect 293 cells and HEK cells [from which 293 cells were derived (18)] also revealed expression of the preproinsulin gene in the 293 cells only (data not shown). These results indicate that no

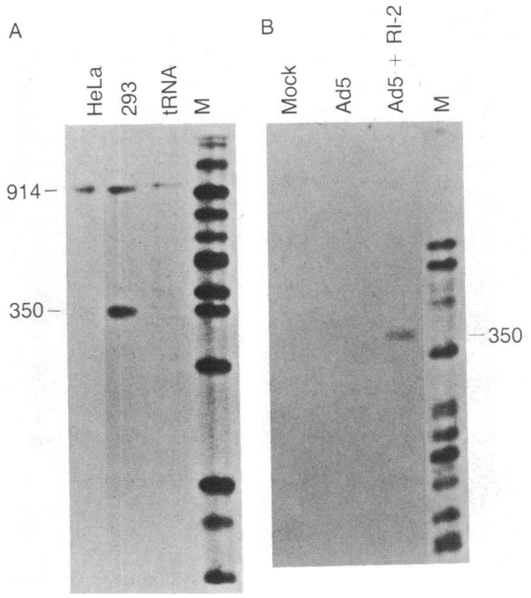

FIG. 4. (*A*) Viral sequences are not required for *E1A* induction of the rat preproinsulin I gene. A plasmid containing rat preproinsulin I gene sequence was used to transfect either HeLa or 293 cells. Cytoplasmic RNA was harvested 48 hr after infection and S1 nuclease analysis was performed with the end-labeled probe diagrammed in Fig. 1. In the tRNA lane, hybridization was to yeast tRNA rather than cytoplasmic RNA. The 914-nucleotide band is due to a small fraction of undigested probe. (*B*) The E1A protein does not induce detectable expression of the endogenous preproinsulin I gene in a rat cell line. Cells of a continuous line of rat embryo fibroblasts were mock-infected, infected with wild-type Ad5, or infected with wild-type Ad5 and RI-2, each at a multiplicity of infection of 10. Cytoplasmic RNA was harvested 40 hr after infection and subjected to S1 nuclease analysis using the end-labeled probe diagrammed in Fig. 1.

viral DNA sequence is required in *cis* for E1A protein activation of preproinsulin gene transcription. Because of the small quantities of preproinsulin mRNA produced in the transfection experiments, it was not possible to map the 5′ ends of the mRNAs by primer extension. Therefore, it is possible that transcription was initiated at other sites in the plasmid than the preproinsulin I promoter region. However, even if this were the case, transcription from these other possible sites would have been greatly stimulated by the E1A protein even though no adenovirus sequence was present on the plasmid.

E1A Protein Does Not Stimulate Expression of the Endogenous Preproinsulin I Gene in Cultured Rat Cells to Detectable Levels. To determine if the E1A protein could stimulate expression of the endogenous preproinsulin I gene in rat cells that are not expressing the gene, wild-type Ad5 was used to infect CREF cells, a continuous line of rat cells (34). Preproinsulin I mRNA could not be detected in the cytoplasmic RNA isolated from these cells (Fig. 4*B*). On the other hand, the message could be detected in CREF cells coinfected with Ad5 and RI-2, indicating that sufficient E1A protein was expressed in these cells to induce transcription of the gene when it was introduced into the cells via the adenovirus recombinant. Thus, the E1A protein can induce transcription of the rat preproinsulin gene when it is introduced into cells by infection or transfection, but it does not induce transcription of the resident preproinsulin I gene in a cultured rat cell.

Delayed Activation of Preproinsulin Gene Expression in the Absence of the E1A Protein. As discussed in the Introduction, the delayed induction of transcription of early adenovirus genes after infection of HeLa cells with E1A mutants and the *cis*-acting nature of this induction suggested to us that the early genes were being slowly assembled into stable active transcription complexes (5, 10, 11). To examine whether the preproinsulin I gene in the adenovirus recombinants might undergo the same process, we analyzed the kinetics of activation of the preproinsulin gene in the absence of E1A protein. HeLa cells were infected with RI-1 alone and the concentration of preproinsulin I mRNA was assayed at 6, 22, and 48 hr after infection (Fig. 5). Just as for early adenovirus genes, the expression of the preproinsulin I gene increased greatly at prolonged times after infection. This delayed expression of the preproinsulin I gene that is observed after infection of HeLa cells with the adenovirus recombinants but not after transfection of HeLa cells with the gene (Fig. 4*A*) may be mediated by the recently described adenovirus transcription enhancing sequence, which maps near the left end of the genome (35). In any case, as for early adenovirus genes, the E1A protein is not absolutely required to reach high rates of transcription of the preproinsulin I gene. Rather, the E1A protein decreases the time required for transcription to reach high rates. As we suggested for early adenovirus genes, this could be explained by the E1A protein catalyzing assembly of the gene into an active stable transcription complex. The prolonged period of infection may overcome the E1A protein requirement for high levels of transcription by allowing sufficient time for the putative transcription complex to form.

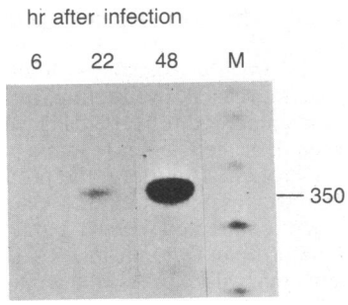

FIG. 5. Analysis of preproinsulin mRNA at prolonged times after infection. HeLa cells were infected with RI-1 at a multiplicity of infection of 10 and cytoplasmic RNA was harvested at 6, 22, and 48 hr after infection. S1 nuclease analysis was performed with the end-labeled preproinsulin probe diagrammed in Fig. 1.

DISCUSSION

The mechanism by which the E1A protein functions is not known. However, a number of relevant experimental results are available. The E1A protein is not absolutely required for high rates of transcription (3, 5). Directly or indirectly it significantly diminishes the time required for high rates of viral transcription to be induced, but, even in its complete absence, high rates of transcription are eventually achieved (3, 5). Two types of observations make it seem unlikely that the E1A protein functions by interacting directly with DNA sequences near the start sites of viral transcription units. First, no significant sequence homologies occur within at least 100 nucleotides of the transcription start site (27). Even the TATA box, the most highly conserved element of RNA polymerase II transcription units, is not common to all the viral transcription units regulated by the E1A protein since it is not found near the E2 early start site (27). Second, the pseudorabies virus immediate early gene product can complement the transcription-inducing activity of the E1A protein when cells are coinfected with pseudorabies virus and an adenovirus *E1A* deletion mutant (36). It seems very unlikely that a protein encoded by an unrelated virus could interact with multiple specific adenovirus sequences.

The experiments presented in this paper directly demonstrate that the E1A protein can stimulate mRNA synthesis

without interacting with specific viral sequences in the proximity of transcription start sites. When a gene completely unrelated to adenovirus, the rat preproinsulin I gene, is inserted into the adenovirus genome, its transcription becomes regulated by the E1A protein similarly to early adenovirus genes. Moreover, this stimulation of transcription is observed when a plasmid containing the preproinsulin I gene is used to transfect cells expressing the E1A protein, even though no adenovirus sequence is present on the plasmid. Thus the E1A protein can stimulate transcription of a gene even when it is completely unlinked to viral sequences.

We were led to perform the experiments presented here by our earlier analysis of adenovirus transcription from *E1A* mutants. These studies demonstrated that in the absence of the E1A protein, induction of adenovirus transcription resulted from a *cis*-acting modification of the viral template (5). This result is inconsistent with a model which proposes that the E1A protein inactivates a cellular repressor of early viral transcription (3). It is difficult to see how the inactivation of a repressor could lead to transcription of some DNA molecules and not others in the same cell. To explain the observed *cis*-acting induction of early transcription, we suggested that the viral DNA was slowly assembled into a stable transcriptional complex of the type demonstrated to be the template of *in vitro* transcription by RNA polymerases III (11) and II (10). Since the E1A protein decreases the time required for induction of early transcription, we suggested that the function of the E1A protein is to catalyze the assembly of such stable transcriptional complexes (5). Furthermore, since the E1A protein facilitates transcription from multiple promoters on the viral genome, we suggested that the process affected the viral DNA molecule as a whole. This led us to ask if the E1A protein would affect transcription from the preproinsulin promoter when it was placed on the viral chromosome.

Whatever the mechanism of E1A protein action, it activates the preproinsulin gene only when that gene is newly introduced into cells by transfection or infection. Transcription of the endogenous gene was not stimulated to detectable levels. This is probably true of most cellular genes, since infection with adenovirus does not cause a generalized increase in the transcription of host sequences (37). The failure of endogenous genes to respond to the E1A protein may be because they are already assembled into stable transcriptional complexes (i.e., chromatin structures), which determine their rates of transcription.

Adenovirus early genes appear to be more receptive to the action of the E1A protein than the preproinsulin gene, since transcription of early adenovirus genes is readily detected at 4–6 hr after infection, whereas *E1A*-induced transcription of the preproinsulin I gene was not detectable until 16 hr after infection by using methods with similar sensitivities (unpublished observation). Furthermore, transcription from viral genes initiates principally at one or a few closely spaced nucleotides (27), whereas transcription of the preproinsulin I gene was less precise, initiating over a region of several hundred bases as well as at the authentic cap site (Fig. 3*A*). Finally, the E1A protein does not induce detectable transcription of the endogenous preproinsulin gene. However, when the adenovirus *E3* transcription unit is integrated into rat fibroblasts transformed by an *E1A* mutant (Ad5 hr1), it is induced by infection of those cells with wild-type Ad5 (unpublished observations). Thus, although a nonviral gene can be activated by the E1A protein, the early adenovirus genes appear to have either a sequence or chromatin structure that makes them particularly responsive to its action.

We thank A. Efstratiadis for providing us with λ Charon 4A-rI1 and the sequence of the rat preproinsulin I gene before publication. We thank Carol Eng for excellent technical assistance and Debra Bomar for typing. This work was supported by a grant from the National Cancer Institute. A.J.B. is supported by an American Cancer Society Faculty Research Award and R.B.G. is supported by a National Institutes of Health New Investigator Award.

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