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# Repression of Insulin Gene Expression by Adenovirus Type 5 E1a Proteins

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**Insulin gene transcription relies on enhancer and promoter elements which are active in pancreatic beta cells. We showed that adenovirus type 5 infection of HIT T-15 cells, a transformed hamster beta cell line, represses insulin gene transcription and mRNA levels. Using expression plasmids transiently introduced into HIT T-15 cells, we showed that adenovirus type 5 E1a transcription regulatory proteins repress insulin enhancer-promoter element activity as assayed with a surrogate xanthine-guanine phosphoribosyltransferase gene. We relate E1a repression of the insulin gene to other examples of repression of enhancer-dependent genes by E1a and discuss the possible relationship of this repression to insulin gene regulation.**

The adenovirus type 5 (Ad5) E1a gene products are the major adenovirus transcription regulators and are the first viral proteins synthesized during early adenoviral infection. Transcription of the E1a gene produces 13S and 12S mRNAs, which encode two closely related proteins, which are, respectively, 289 and 243 amino acids long (15, 42). These proteins are amino and carboxy coterminal, but the larger protein contains a unique internal peptide 46 amino acids long. A functional distinction between these proteins lies in the ability of the 289-amino-acid protein to activate early adenoviral gene expression under conditions in which the 243-amino-acid protein is unable to do so (35, 58). As well as inducing viral transcription, E1a proteins stimulate transcription from specific cellular genes, including the 70-kilodalton heat shock protein (39) and beta-tubulin (46) genes. In contrast to their differing transcription activation potential, both E1a proteins can immortalize primary cells (35, 43, 58) and repress the activities of viral transcription enhancer elements, including those of simian virus 40 (SV40) (7, 53) and polyomavirus (7, 51), and the cellular immunoglobulin heavy-chain enhancer (21).

The regulatory properties of the E1a proteins may be analogous to the activities of particular cellular transcription factors. Just as E1a proteins regulate transcription from viral promoters, "E1a-like" factors may function in the selective expression of tissue-specific genes in differentiated cells. In these cells, a single gene may account for a large fraction of the total gene expression in one cell type yet be expressed in undetectable levels in other cell types. The predominant control of tissue-specific gene expression is at the level of transcription initiation (9) and involves *trans*-acting factors which repress (29, 40) or stimulate (11, 12, 34) cellular gene activity in differentiated cells. Since the E1a proteins exhibit these same activities in their control of viral gene expression, we studied how the E1a proteins affect transcription from the well-characterized cellular insulin promoter, which is active in pancreatic beta cells.

Several lines of evidence, including gene transfer into mouse embryos (20), have demonstrated that tissue-specific insulin gene expression in pancreatic beta cells is regulated by 5′-flanking insulin gene sequences (10, 20, 55). Transcription of the insulin gene is controlled by functionally distinguishable *cis*-acting enhancer and promoter elements (10). We report here that Ad5 infection inhibits insulin gene expression in HIT T-15 pancreatic beta cells and that this repression may be reproduced by unique E1a products expressed from recombinant plasmids.

## MATERIALS AND METHODS

**Cells and viruses.** HIT T-15 cells were the generous gift of R. F. Santerre (44). *dl*327, an Ad5 early region EIII deletion mutant, and the E1a cDNA reconstruction mutants *dl*347 and *dl*348 (58) were generous gifts of T. Shenk.

**Infected-cell RNA extraction and analysis.** HIT T-15 cells at 20 to 30% confluency (100-mm dishes) were infected with *dl*327 at a high multiplicity of infection (2,000 particles per cell) as described previously (46). Cytoplasmic RNA was isolated, poly(A)$^+$ RNA was selected, and steady-state mRNA levels were analyzed by Northern blot analysis (53). Insulin mRNA levels were analyzed with pI47, a rat cDNA clone containing rat insulin I gene DNA sequences from 256 to 534 base pairs (bp) downstream from the transcription start site (54). These sequences are greater than 85% homologous to the hamster insulin transcript (3). The beta-actin probe was the human beta-actin cDNA clone pHFβA-1 (18). The Ad5 E1a probe was pA-6 (0 to 4.5 map units). The probes were nick translated with [$^{32}$P]dATP to approximately $2 \times 10^8$ cpm per μg (53).

**Nuclear runoff assays.** Nuclear runoff assays were performed as previously described (53) with $2 \times 10^7$ nuclei per incubation. Approximately $10^7$ cpm of each [$^{32}$P]RNA runoff product was hybridized to the following DNAs (5 μg each) applied to nitrocellulose filters: pBR322; probe for E1a, a single-stranded M-13 clone of Ad2 DNA (0 to 5.8 map units); probe for E2, a single-stranded M-13 clone of Ad2 DNA (61 to 63 map units); probe for E4, a single-stranded M-13 clone of Ad2 DNA (89.7 to 93 map units); probe for insulin, pI47, a rat insulin I cDNA clone; probe for beta-actin, pHFβA-1, a human beta-actin cDNA clone (18); and probe for beta-tubulin, D-β1, a human beta-tubulin cDNA clone (19).

**Plasmid transfections and XGPT assays.** Calcium phosphate coprecipitates containing plasmid DNA were added to HIT T-15 cells and to HeLa cells at 30% confluence in

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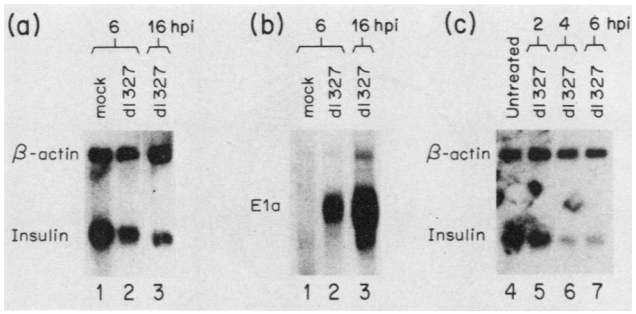


FIG. 1. Effect of *dl*327 infection on insulin mRNA levels in HIT T-15 cells. Northern blot analyses were performed on poly(A)$^+$ cytoplasmic RNAs from mock-infected cells isolated 6 hpi (lanes 1); *dl*327-infected cells isolated 6 hpi (lanes 2); *dl*327-infected cells isolated 16 hpi (lanes 3); untreated cells (lane 4); *dl*327-infected cells isolated 2 hpi (lane 5); *dl*327-infected cells isolated 4 hpi (lane 6); and *dl*327-infected cells isolated 6 hpi (lane 7). In panels a and c, the Northern blots were hybridized with beta-actin and insulin probes. In panel b, the probes from panel a were released by a high-temperature wash, and the filter was hybridized with the E1a probe. HIT T-15 cells were infected with *dl*327 at a high multiplicity of infection (2,000 particles per cell) as described previously (46). Cytoplasmic RNA was isolated, poly(A)$^+$ RNA was selected, and steady-state mRNA levels were analyzed by Northern blot analysis (53) as described in Materials and Methods.

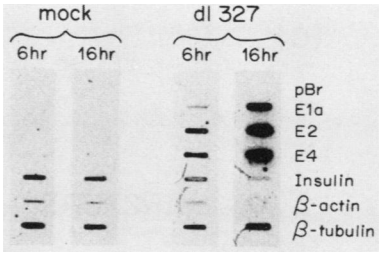


FIG. 2. Inhibition of insulin transcription by *dl*327 infection of HIT T-15 cells. HIT T-15 cells were mock infected or infected with *dl*327 at 2,000 particles per cell, nuclei were isolated at 6 and 16 hpi, and nuclear runoff transcription analyses were performed as described in Materials and Methods. pBr, pBR322.

100-mm dishes (53). Cells were exposed to the DNA precipitates for 16 h, washed twice with phosphate-buffered saline, and incubated in fresh medium for 24 h. Extracts were prepared by sonication and centrifugation in 50 μl (per 100-mm plate) of a buffer consisting of 15% glycerol, 20 mM Tris (pH 7.5), and 10 mM dithiothreitol (8). Xanthine-guanine phosphoribosyltransferase (XGPT) assays contained, in a final volume of 50 μl, 50 mM Tris (pH 8.5), 100 mM KCl, 5 mM $MgCl_2$, 2 mM dithiothreitol, 1 mM phosphoribosyl pyrophosphate, 100 μg of bovine serum albumin, and 180 μM $^{14}$C-labeled xanthine (55 mCi/mmol; ICN Pharmaceuticals Inc.). A 20-μl amount of the cell extract was added to each sample and incubated for 2 h at 37°C. After a single extraction with phenol-chloroform (1:1), 40 μl of the sample was applied to a thin-layer chromatography plate (Polygram CEL 300 PEI; Brinkmann Instruments, Inc.). The plates were soaked in methanol for 5 min to remove unconverted [$^{14}$C]xanthine. The plates were chromatographed in 0.9 M guanidine hydrochloride (ultrapure; Schwartz/Mann), sprayed with En$^3$Hance (New England Nuclear Corp.), and exposed to X-ray film. XGPT converts xanthine to XMP. A phosphatase present in the cell extracts catalyzes the conversion of xanthine monophosphate to xanthosine (8). For quantitation, XMP and xanthosine were cut out and counted in a liquid scintillation counter.

## RESULTS

**Repression of endogenous insulin gene expression by Ad5 infection.** HIT T-15 cells, a clonal pancreatic beta cell line established by SV40 transformation of Syrian hamster endocrine cells (44), express significant levels of insulin mRNA (see below), although they produce insulin protein at only 2 to 5% of the rate of in vivo endocrine cells (44). To analyze the effects of E1a proteins on insulin expression, infected HIT T-15 cells with Ad5 mutant *dl*327 (an early region E3 deletion mutant which expresses a wild-type transcription program) at a high multiplicity of infection. Cytoplasmic RNA was extracted, and nuclei were prepared at 6 and 16 h postinfection (hpi). The steady-state mRNA levels for insulin, beta-actin, and E1a were quantitated by Northern analysis with specific DNA probes. Beta-actin mRNA levels served as an internal control, as its mRNA levels are invariant during adenoviral infection (1, 46). The levels of insulin mRNA relative to beta-actin mRNA decreased significantly in virus-infected cells at 6 and 16 hpi (Fig. 1a). E1a mRNA was expressed in these cells when insulin mRNA disappeared (Fig. 1b). Insulin mRNA levels were repressed by 4 hpi (Fig. 1c). Quantitation of these results showed that the levels of insulin mRNA were reduced more than 80% in virus-infected cells at both 6 and 16 hpi relative to mock-infected controls.

To determine if the specific decrease in insulin mRNA levels after *dl*327 infection was due to an inhibition of insulin transcription, we measured the rate of insulin transcription in vitro with nuclei isolated 6 and 16 hpi. Transcription of the insulin gene was reduced significantly in infected cells at both times (Fig. 2 and Table 1). In contrast, transcription of the cellular housekeeping genes, beta-actin and beta-tubulin, remained relatively constant (Table 1). We have previously reported (46) that beta-tubulin transcription is stimulated approximately sixfold by Ad5 E1a expression. This stimulation occurs during the early stage of infection (<6 hpi) and prior to the time postinfection that beta-tubulin was assayed in HIT T-15 cells in Fig. 2. Adenoviral gene transcription was measured from the E1a, E2, and E4 promoters. The rate of transcription of these genes increased between 6 and 16

TABLE 1. Effect of viral infection on insulin, beta-actin, and beta-tubulin transcription rates[a]

| Probe | Level of transcription in expt: 1 | | | | 2 (6 hpi) | |
|---|---|---|---|---|---|---|
| | 6 hpi | | 16 hpi | | | |
| | Mock infection | *dl*327 infection | Mock infection | *dl*327 infection | Mock infection | *dl*327 infection |
| Insulin | 1.00 | 0.31 | 1.0 | 0.17 | 1.00 | 0.33 |
| Beta-actin | 1.00 | 0.91 | 1.0 | 0.88 | 1.00 | 1.00 |
| Beta-tubulin | 1.00 | 0.70 | 1.0 | 1.51 | 1.00 | 1.03 |

[a] The relative levels of insulin, beta-actin, and beta-tubulin transcription were normalized within each experiment to the mock-infected-cell transcription rate for each gene. In experiment 1, which corresponds to the experiment described in Fig. 2, the ratio of relative insulin/beta-action/beta-tubulin transcription rates at 6 hpi in mock-infected cells was 1.00:0.33:1.57, and at 16 hpi it was 1.00:0.97:0.43, as quantitated by densitometric scanning of the autoradiogram. Experiment 2 was performed as experiment 1, and the ratio was 1.00:0.18:1.75.

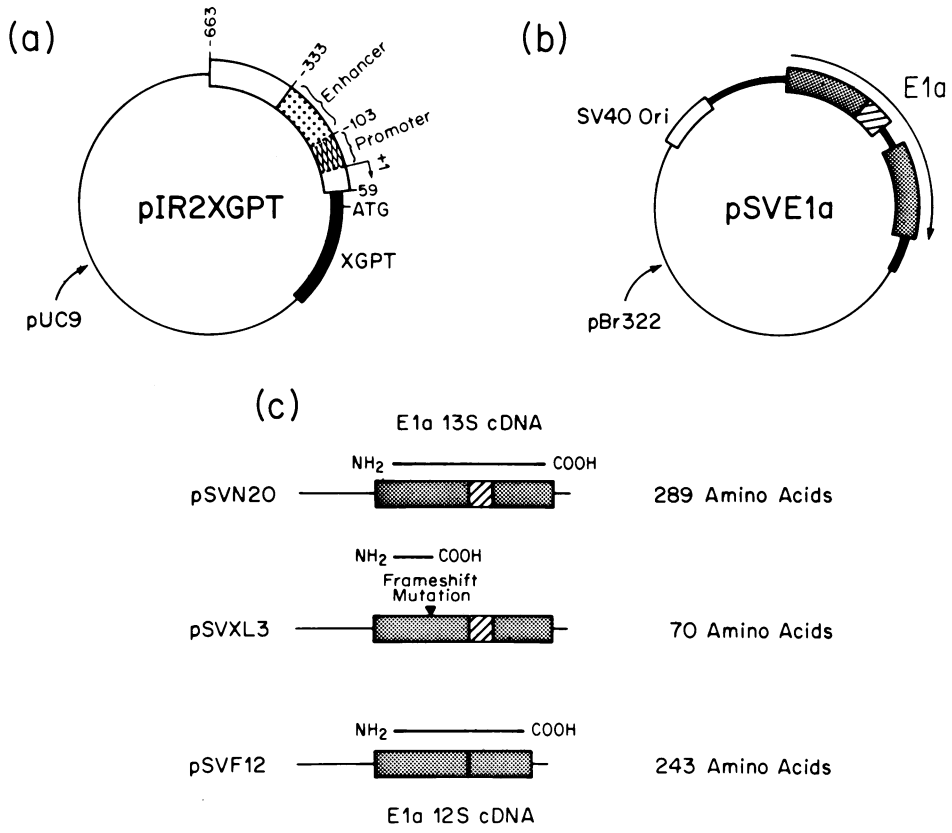


FIG. 3. Structures of recombinant expression plasmids. (a) pIR2XGPT (5.15 kilobases [kb]) consists of coding sequences of the *Escherichia coli* XGPT gene fused to sequences derived from the 5'-flanking region of the rat insulin II gene. Recombinant pIR2XGPT contains rat insulin sequences from −663 to +59 bp relative to the transcription start site (13). Open boxes denote insulin 5'-flanking DNA, the thick solid line refers to the XGPT gene, and the thin line represents pUC9 vector sequences. The insulin transcriptional enhancer and promoter are shown (10, 55). (b) pSVE1a (4.6 kb) is a clone of nucleotides 1 to 1,834 of Ad5 DNA inserted between the *Eco*RI and *Pst*I sites of pSVod, a vector derived from pML2 and containing the SV40 replication origin (Ori) but no enhancer sequences (45). The 12S mRNA sequences are denoted by spotted boxes and are also present in the 13S mRNA. The hatched box denotes sequences unique to the 13S mRNA. The thick solid lines refer to sequences flanking the E1a gene and the E1a intron, the thin line represents pBR322 sequences, and the open box represents SV40 origin sequences. (c) pSVN20 (4.5 kb) is a derivative of pSVE1a in which the Ad5 E1a genomic intron sequences are replaced by the cDNA of the 13S E1a mRNA (45). pSVF12 (4.4 kb) was constructed with the cDNA of the 12S E1a mRNA (45). pSVXL3 (4.5 kb) is a deletion or substitution mutant derived from pSVN20 by insertion of an *Xho*I linker in the 13S cDNA-coding sequences (45). In pSVXL3, the linker shifts the reading frame to encode a 70-amino-acid truncated polypeptide. The predicted sizes of the plasmid-encoded E1a gene products are shown.

hpi, as expected (Fig. 2). However, insulin gene transcription was lower at 16 hpi than at 6 hpi (Table 1). These results parallel the analysis of cytoplasmic mRNAs shown in Fig. 1 and suggest that insulin gene transcription is inhibited by an Ad5 early gene product.

We attempted to identify the viral product(s) responsible for insulin gene repression by infecting cells with recombinant viruses which selectively express either the 289- or the 243-amino-acid E1a protein. Since early viral gene expression is activated very inefficiently by the 243-amino-acid E1a protein (35, 58), infecting HIT T-15 cells with a virus expressing only the 243-amino-acid E1a protein could help to establish the dependence of insulin gene repression on the 243-amino-acid E1a protein versus other viral proteins. We found that insulin gene expression was repressed in cells infected with viruses expressing exclusively the 243-amino-acid E1a protein (*dl*347) or the 289-amino-acid (*dl*348) E1a protein (R. Stein, unpublished observations). However, at the multiplicity of infection used in these experiments (2,000 particles per cell), early adenoviral promoters E2a, E3, and E4 were active during *dl*347 infection. Although the levels of expression were low compared with that in the wild-type infection, their activity prevented us from unequivocally concluding that E1a gene products were the repressors of insulin gene expression.

**E1a repression of insulin promoter activity in transient assays.** To establish the role of E1a in insulin gene repression, we determined the effect of plasmid-encoded E1a gene products upon the expression of pIR2XGPT (Fig. 3), a plasmid containing 5'-flanking DNA sequences from the rat insulin II gene linked to the coding region of XGPT, by using a transient expression assay. The 5'-flanking region includes the insulin gene promoter and enhancer, two DNA elements that stimulate insulin gene transcription from recombinant plasmids in cultured pancreatic beta cells but not in fibroblasts (10). The rat insulin I sequences required for pancreatic beta cell-specific expression lie between residues −333 and +1 (bp) relative to the site of initiation (10, 55). The transcription control elements found within this region are the insulin promoter (−103 to +1 bp) and enhancer (−333 to

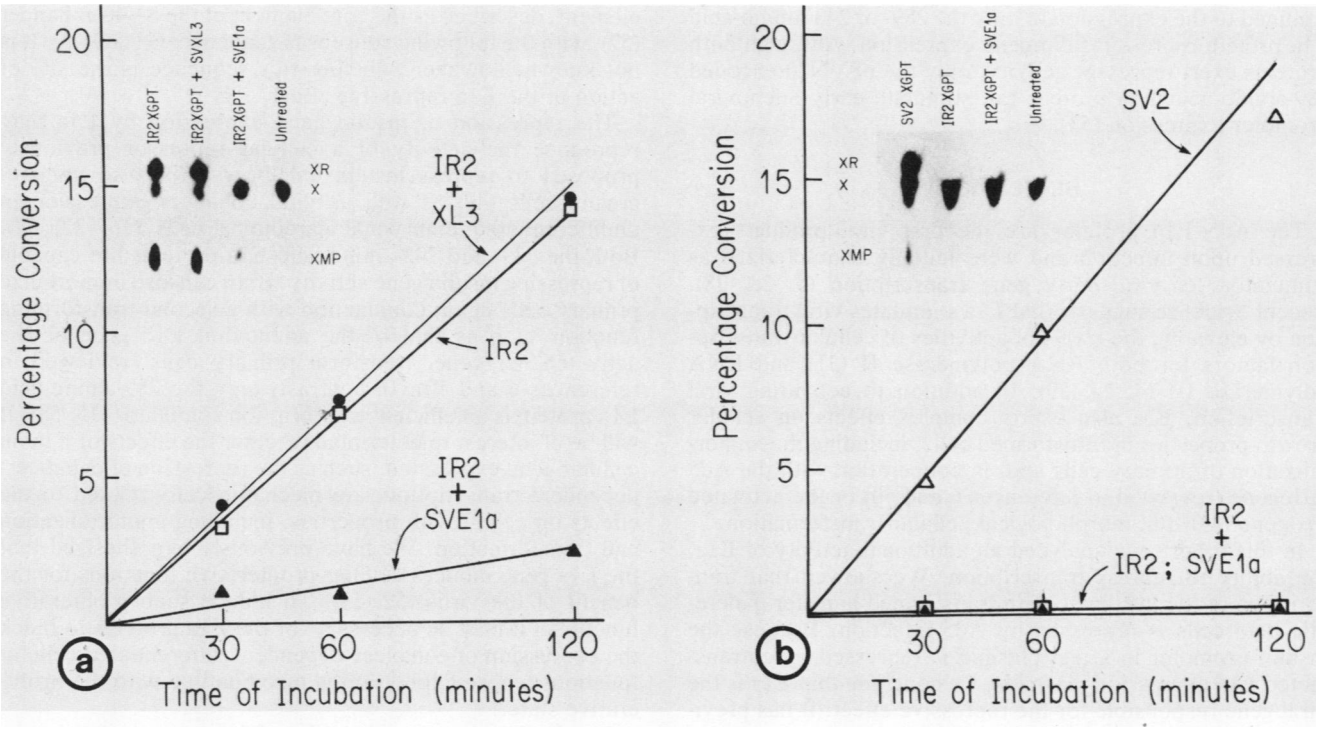


FIG. 4. Effect of vector-encoded E1a gene products on the activity of the insulin promoter. XGPT activity directed by insulin gene 5'-flanking sequences in plasmid pIR2XGPT was measured in the presence and absence of vectors expressing E1a proteins. A calcium phosphate coprecipitate containing 10 μg of pIR2XGPT and either 10 μg of plasmids encoding E1a proteins or 10 μg of pBR322 as indicated were added to HIT T-15 cells (a) and HeLa cells (b) at 30% confluence in 100-mm dishes (53), and XGPT assays were performed as described in Materials and Methods. Results are expressed as the percent conversion of [$^{14}$C]xanthine to [$^{14}$C]XMP and [$^{14}$C]xanthosine. The insets show typical autoradiograms of the XGPT assays. The spots correspond to xanthosine (XR), XMP, and unreacted xanthine (X) not extracted in the methanol step. As a control, 10 μg of pSV2XGPT containing the SV40 promoter and enhancer sequences linked to XGPT-coding sequences (37) and 10 μg of pBR322 were transfected. The transfected plasmids were as follows: □, pIR2XGPT + pBR322; ●, pIR2XGTP + pSVXL3; ▲, pIR2XGPT + pSVE1a; △, pSV2XGPT + pBR322. Abbreviations: XL3 and SVXL3, pSVXL3; IR2 and IR2XGTP, pIR2XGPT; SVE1a, pSVE1a; SV2, pSV2XGPT.

−103 bp) elements (10). The rat insulin II gene sequences found in pIR2XGPT lie between −663 and +58 bp. pIR2XGPT construction was described previously (13). The start of insulin gene transcription was mapped at +1 bp (13). pIR2XGPT was transfected into HIT T-15 cells or HeLa cells, either alone or with the following E1a expression plasmids (Fig. 3): pSVN20 (a plasmid expressing only the 289-amino-acid E1a protein), pSVF12 (a plasmid expressing only the 243-amino-acid E1a protein), pSVXL3 (a plasmid expressing a truncated 70-amino-acid E1a protein), or pSVE1a (a wild-type plasmid expressing both the 243- and 289-amino-acid proteins) (45). Insulin promoter activity was assayed from cellular extracts prepared 40 h after transfection by measuring XGPT enzymatic activity. As expected from previous studies examining the tissue specificity of the insulin promoter (10, 13, 55), pIR2XGPT was active in HIT T-15 cells but inactive in HeLa cells (Fig. 4). When pIR2XGPT was cotransfected with pSVE1a, XGPT enzymatic activity dramatically decreased in HIT T-15 cells (Fig. 4a). This decrease was not due to promoter competition between the pIR2XGPT and pSVE1a promoters, since the XGPT activity from pIR2XGPT was unaffected by the E1a frameshift mutant plasmid pSVXL3 (Fig. 4). These results confirm that the E1a proteins directly or indirectly block insulin gene expression and indicate that repression is exerted within the 5'-flanking DNA sequences which contain the insulin gene enhancer and promoter transcription control elements.

Cotransfection of pIR2XGPT with pSVE1a (Fig. 4b) did not stimulate insulin promoter activity in HeLa cells, although pSVE1a stimulates viral early promoters in HeLa cells in similar transient assays (53). This failure suggests that the insulin promoter is unresponsive to E1a in HeLa cells, since plasmid pSV2XGPT, which has a constitutively active promoter, expressed XGPT activity in HeLa cells (Fig. 4b).

XGPT activity from pIR2XGPT was reduced by plasmids encoding exclusively the 243 (pSVF12)- or 289 (pSVN20)-amino-acid E1a protein (Table 2). This result is in agreement with the finding noted above that recombinant adenoviruses

TABLE 2. Repression of XGPT activity from pIR2XGPT by E1a expression vectors in HIT T-15 cells[a]

| E1a plasmid | Predicted E1a *peptides* (no. of amino acids) | Relative XGPT enzymatic activity in expt: | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| None | | 1.00 | 1.00 | 1.00 |
| pSVE1a | 289 and 243aa | 0.18 | 0.15 | 0.23 |
| pSVXL3 | 70; truncated peptide | 1.10 | 0.96 | 1.15 |
| pSVN20 | 289 | ND | 0.16 | 0.18 |
| pSVF12 | 243 | ND | 0.25 | 0.20 |

[a] HIT T-15 cells were transfected, and XGPT enzymatic activity was measured under the conditions described in Materials and Methods. In a given experiment, the XGPT enzymatic activity from E1a-transfected cells was normalized that from control cells not transfected with E1a. ND, Not done.

confined to the expression of only the 289- or 243-amino-acid E1a protein repress insulin gene expression. Although both proteins exert repressor activity, only the pSVN20-encoded 289-amino-acid E1a protein can stimulate early adenoviral promoter expression (53).

## DISCUSSION

The Ad5 E1a proteins are the first viral products expressed upon infection and were initially characterized as stimulators of viral early gene transcription (5, 28, 38). Recent evidence suggests that E1a stimulates viral transcription by elevating the levels of activities of cellular transcription factors for both RNA polymerase II (31) and RNA polymerase III (4, 24, 59). In addition to activating viral transcription, E1a also exerts complex effects on cellular growth properties in transformed cells, including the immortalization of primary cells and, in cooperation with the Ad5 E1b gene (reviewed in references 6 and 50) or the activated *ras* gene (43), full morphological cellular transformation.

In this report, we analyzed an additional activity of E1a, the ability to repress transcription. We showed that transcription of the insulin gene in transformed hamster pancreatic beta cells is repressed by Ad5 infection. Because the insulin promoter in a test plasmid is repressed by cotransfected E1a expression plasmids, we conclude that E1a is the viral gene responsible for the repressive effect. It has previously been shown that E1a products can repress transcription from enhancer-dependent genes, including chimeric plasmids containing viral enhancers and cellular promoters (7) and viral promoters linked to their homologous viral transcription units, such as the SV40 early promoter (53) and the polyomavirus early and late promoters (51). In addition to the insulin gene studied here, another chromosomally located, tissue-specific gene, the immunoglobulin heavy-chain gene in B lymphocytes, is repressed by E1a (21). In the case of heavy-chain gene repression, E1a acts upon the heavy-chain enhancer.

The fact that a rat insulin II promoter plasmid containing residues −663 to +58 of the insulin gene is repressed by E1a strongly suggests that the repression mechanism acts within the 5′-flanking region of the gene. The nuclear runoff assay of infected cells demonstrated that repression occurs at the transcriptional level. As with E1a repression of the SV40 enhancer (7, 53), vectors for either the 289- or the 243-amino-acid E1a protein repress the insulin gene. In contrast, only the 289-amino-acid protein is an efficient transcription stimulator (15, 58), suggesting that the mechanism of transcription activation by E1a requires an E1a protein domain not necessary for the transcription repression mechanism.

The organization of regulatory sequences in the rat insulin I gene 5′-flanking region (which is closely related to that of the rat insulin II gene studied here) is complex. A region between nucleotides −103 and −333 exerts enhancer activity, and a second region between nucleotides −113 and +1 lacks enhancer activity but functions in beta cells as a promoter when linked to a constitutive murine leukemia virus enhancer (10). Specific sequences in the insulin gene promoter and enhancer also bind cellular proteins (41). The SV40 enhancer (23) and the polyomavirus transcription control region (22) also contain multiple promoter and enhancer elements. A comparison of the sequences of the regulatory regions of SV40 (60), polyomavirus (49), and the immunoglobulin heavy-chain enhancer (2), all of which are repressed by E1a (7, 21, 51), with the sequences of the rat insulin I and II genes (10, 55) reveals that they share a DNA element, described as the core element of the SV40 enhancer (57), with the following consensus sequence: $GTGG^{TTT}_{AAA}$. It is not known, however, whether this sequence is the site of action of the E1a repressive effect.

The repression of insulin gene expression by E1a may reproduce the activity of a cellular repressor previously proposed to repress insulin enhancer activity in nonpancreatic cells (40) as well as viral enhancer expression in undifferentiated embryonal carcinoma cells (16, 32, 33). Both the 289- and 243-amino-acid E1a proteins are capable of repressing insulin gene activity. Both can also immortalize primary cells or, in conjunction with a second transforming function such as that of the adenoviral E1b gene or the activated *ras* gene, transform primary cells (reviewed in references 6 and 50). In contrast, only the 289-amino-acid E1a protein is an efficient transcription stimulator (15, 58). It will be of interest to determine whether the effects of E1a on cellular gene expression, such as the repression of enhancer-dependent transcription, are mechanistically related to the effects on cell growth properties, including immortalization and transformation. We have previously hypothesized that the E1a gene induces cellular proliferative functions for the benefit of the virus (52, 53). To induce such proliferative functions, it may be necessary for the E1a proteins to block the expression of enhancer-dependent differentiated cellular functions whose expression is incompatible with the proliferative state.

The finding that the adenovirus E1a proteins can repress insulin transcription raises the possibility that viral transcription regulatory proteins with repressor functions may have the capacity to disrupt tissue-specific cellular gene expression in infections of normal host animals. If so, degenerative diseases which result from the impaired expression of tissue-specific functions could result from the repression of tissue-specific gene expression by mechanisms analogous to those described here for E1a and the insulin gene. Indeed, both herpes simplex virus type 1 and cytomegalovirus, another herpesvirus, have been isolated from the pancreas of patients suffering from insulin-dependent diabetes mellitus and have been suggested as pathogenic factors for the disease (27, 36, 56). The herpes simplex virus type 1 and cytomegalovirus immediate early proteins share certain properties with E1a in transcription assays (14, 17, 25, 26, 30, 47, 48). It is not yet known whether herpes simplex virus type 1 or cytomegalovirus plays a role in insulin-dependent diabetes mellitus or whether this form of the disease can result from the viral impairment of insulin gene expression. However, a basis for the induction of degenerative disease states through the repression of gene activity by viral regulatory proteins should be considered.

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