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HeLa Cell β-Tubulin Gene Transcription Is Stimulated by Adenovirus 5 in Parallel with Viral Early Genes by an E1a-Dependent Mechanism

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We report that the rate of transcription of cellular β-tubulin genes increases during the early phase of adenovirus infection of HeLa cells, with kinetics very similar to those routinely found for viral genes. This activation depends upon adenovirus early region E1a, which encodes products that activate early virus transcription. To compare the responses of viral and cellular genes to E1a, we infected HeLa cells with *dl*312, a transcriptionally inactive deletion mutant that lacks a functional E1a gene. We then superinfected the cells with a helper virus, *dl*327, which encodes active E1a products, and measured changes in the rates of transcription of various cell and viral genes. Early region E3 of *dl*312 was activated 0 to 6 h postinfection and then repressed at 8 h postinfection, thus reproducing the two-step kinetics characteristic of a wild-type infection. Synthesis of β-tubulin nuclear RNA was also transiently induced two- to six-fold, rising and falling in a manner similar to E3 transcription. An increase in helper virus multiplicity gave an increase in β-tubulin stimulation, but *dl*312 alone, even at a high multiplicity of infection, gave no induction, confirming the requirement for E1a. β-Actin nuclear RNA was actively synthesized before infection, but it was not further stimulated by the virus. Cellular β-globin gene transcription was not stimulated by the virus, although transcription of a transfected β-globin plasmid was induced by the virus or from a cotransfected E1a expression plasmid. We conclude that adenovirus 5 can stimulate β-tubulin gene transcription. We discuss the significance for the viral life cycle of viral stimulation of cell genes and consider possible mechanisms in the light of the results obtained with β-actin and β-tubulin.

Adenovirus provides a particularly useful model for studying transcriptional control in animal cells because the viral genes subject to control are well defined, and mutants involved in the regulatory events are available. Furthermore, transcription is by the host RNA polymerase II, and thus control of the transcription of viral genes may be informative about the mechanisms that control cellular genes.

Proteins encoded by the adenovirus E1a transcription unit play a crucial role in activating transcription from viral early promoters (4, 24, 34, 35, 38, 39). E1a yields 12S and 13S early mRNAs which encode 51- and 48-kilodalton (kd) peptides, respectively (14, 33, 42). Under normal conditions of infection, mutants such as *dl*312, which fail to express any E1a peptides, or HR1, which expresses the 48-kd peptide and an amber-terminated fragment of the 51-kd peptide, also do not transcribe the adenovirus early regions E1b, E2, E3, or E4 (25, 42). Additional mutants which are defective in some manner in E1a peptide expression also show that the 51-kd peptide is the major E1a product required for transcription activation (14, 33, 42, 45).

E1a is expressed from the earliest stages of infection. DNA sequences upstream from the E1a promoter exhibit properties of transcriptional enhancers and are proposed to turn on E1a transcription through a *cis*-acting mechanism upon entry of viral DNA into the cell (22, 23, 50). E1a proteins then activate other early transcription units through a *trans* mechanism. These include E1b, E2, E3, E4, and the promoter proximal segment of the major late transcription unit (35, 37, 44). From 0 to 4 h postinfection, the transcription rates of these early regions rise and reach a peak. From 4 to 6 h postinfection, these transcription units are repressed, and the rate of viral transcription declines (35, 44). Although the mechanism causing transcription decline is not known, a temperature-sensitive mutant of the 72,000-molecular-weight DNA-binding protein (DBP) encoded in region E2a fails to repress region E4, suggesting that the E4 promoter is negatively controlled by the DBP (2, 5, 36). Negative regulation of E4 by DBP has also been demonstrated in vitro (21).

Several independent lines of evidence suggest that E1a not only activates transcription of viral genes, but also has a profound effect on cellular growth mechanisms. The E1a gene products are sufficient to immortalize primary rodent cells (17), and in conjunction with E1b, E1a gene products can transform these cells. One adenovirus-transformed cell line, 293 cells, which are human embryonic kidney cells transformed by fragments of adenovirus 5 (Ad5) DNA (18), expresses E1a constitutively and may be used to propagate viral E1a mutants.

Several further lines of experimentation also suggest that adenovirus early functions can influence the expression of cellular genes. Schrier et al. (43) have recently shown that the oncogenic Ad12 E1a gene products, in contrast to the nononcogenic Ad5 E1a, turn off expression of cellular class I major histocompatibility antigens in Ad12-transformed baby rat kidney cells. During Ad5 infection expression of histone genes is also repressed (12). In contrast, the thymidine kinase (27, 28, 40, 46) dihydrofolate reductase (53) and the 72- to 74-kd heat shock protein genes (26) are stimulated

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during early infection. In addition, transfection experiments with the cloned cellular β-globin gene suggest that cellular promoters, present on plasmids, may be recognized by the E1a transcription activation mechanism (19, 49).

We also asked whether adenoviral early functions can effect the transcription of cellular genes at their normal loci. We show that transcription of cellular β-tubulin nuclear RNA is stimulated by a viral early function with kinetics that parallel transcription of the viral early genes themselves. We contrast this result with the effect of the virus on other cellular genes in chromosomal and plasmid-transfected form. We propose that the mechanism for viral activation of β-tubulin transcription may be directly relevant to cell gene control during viral infection.

MATERIALS AND METHODS

Cells and viruses. HeLa cells were maintained in spinner culture in Joklik modified minimal essential medium containing 5% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). HeLa monolayer cells were grown in Dulbecco modified Eagle medium containing 10% fetal calf serum. Viruses *dl*327 and *dl*312 were provided by T. Shenk. Viral stocks were prepared in spinner cultures of 293 cells. The viruses were used as lysates and were prepared as previously described (13).

DNA procedures. DNA probes for Ad5 and cellular transcripts were prepared from recombinant DNA plasmids. Clone pA6 (0 to 4.5 map units [m.u.] Ad2 in pBr322) was obtained from M. Wilson, and a single-stranded M-13 clone of Ad2 DNA (89.7 to 93 m.u.) from A. Berk was the probe for E4. The E3-specific probe, pXbaD, was prepared by cloning *Xba*I fragment D of Ad5 (78.5 to 84.3 m.u.) into the *Xba*I site of pUC12. The cDNA clones for β-actin (pHf βA-1, in PCDV-1) and β-tubulin (Dβ-1, in pUC12) were obtained from L. Kedes and N. Cowan, respectively. β-Globin sequences were detected with a pBR322 clone corresponding to the unique human 5.2-kilobase genomic *Bgl*II fragment (pH β-6). This clone was obtained from T. Maniatis. Growth of bacteria and preparation and handling of recombinant plasmid DNAs were in accordance with the National Institutes of Health guidelines for recombinant DNA research.

Pulse-labeling. For superinfection experiments, HeLa cells were concentrated by centrifugation to 3×10^7/ml in serum-free medium and infected with 200 particles of *dl*312 per cell. After a 30-min absorbtion at 37°C, cells were diluted to 4×10^5 cells per ml in prewarmed medium containing 10 mM hydroxyurea and 5% fetal calf serum. *dl*312-infected HeLa cells were superinfected with *dl*327 at 2,000 particles per cell at 2 h postinfection under the conditions described above. At 2 h postinfection or at 2, 4, 6, and 8 h postsuperinfection, portions containing 2×10^8 cells were withdrawn and concentrated to 5×10^6 cells per ml in prewarmed medium containing 10 mM hydroxyurea, 5% fetal calf serum, and 160 μCi of [^{3}H]uridine per ml (30 Ci/mmol; New England Nuclear Corp., Boston, Mass.). After incubation at 37°C with agitation for 30 min, cells were lysed with Nonidet P-40, and nuclear RNAs were extracted as described below.

RNA extraction and analysis. A culture sample containing 2×10^8 cells was washed twice by suspension in 20 ml of ice-cold phosphate-buffered saline and centrifugation. Cells were then suspended in 20 ml of ice-cold Iso-Hi pH buffer (0.14 M NaCl, 0.01 M Tris [pH 7.4], 0.0015 M $MgCl_2$), and 2 ml of 5% Nonidet P-40 in IsoHi pH was added with mixing. Cells were allowed to lyse by incubation on ice for 10 min, and then the nuclei were pelleted by centrifugation. The nuclei were washed once in 10 ml of cold Iso-Hi pH. The nuclei were treated with 400 μg of iodoacetate-treated DNAse I in 2 ml of HSB buffer (0.5 NaCl, 0.01M Tris [pH 7.4], 0.05M $MgCl_2$, 0.002M $CaCl_2$) plus 0.001 M $CaCl_2$ at 37°C for 5 min. The digested nuclei were then adjusted to 0.2% sodium dodecyl sulfate and 0.01 M EDTA, treated with 50 μg of proteinase K for 30 min at 37°C, and finally diluted to a volume of 6 ml with 0.05 M sodium acetate (pH 5.1)–0.01 M EDTA–0.2% sodium dodecyl sulfate. An equal volume of phenol saturated with 0.05 M sodium acetate (pH 5.1) was added, and the fractions were incubated at 65°C for 5 min, followed by centrifugation and extraction of the aqueous phase with equal volumes of 50% phenol–50% $CHCl_3$ and finally with an equal volume of $CHCl_3$.

Preparation of DNA filters and RNA hybridization was done as previously described by Fraser and Ziff (13).

DNA transfections. Subconfluent cultures of HeLa cells were transfected with form I plasmid DNA by the calcium phosphate coprecipitation technique (16). Approximately 10^6 cells were plated onto 10-cm^2 tissue culture dishes 20 h before the addition of the precipitate and refed fresh medium 6 h before addition of the precipitate. While the solutions were vortexed, 0.5 ml of the DNA-$CaCl_2$ mixture (20 μg of DNA, 124 mM $CaCl_2$, 10 mM Tris [pH 7.6]) was added dropwise to 0.5 ml of transfection cocktail (50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES], 280 mM NaCl, and 1.5 mM Na_2HPO_4 adjusted to pH 7.13). After 30 min, the precipitates were applied to the cells and incubated for 13 h at 37°C. The medium was then removed, and the cells were washed twice in phosphate-buffered saline and then incubated at 37°C in 10 ml of medium containing 10 mM hydroxyurea. These cells were cotransfected with DNA containing the human β-globin gene (pH β-6; 10 μg) and an E1a-containing plasmid (dIIIG; Ad5, 0 to 7.8 m.u. in pBR322; 10 μg). The cells which were to be infected with virus were only transfected with pH β-6 (20 μg). After these cells were washed with phosphate-buffered saline, the pH β-6-transfected cells were incubated at 37°C for 1 h in the presence (*dl*327 infected) or absence (mock infected) of 2,000 particles per cell of *dl*327 diluted in 1 ml of medium without serum. After 60 min, 9 ml of medium containing 10 mM hydroxyurea was added to the cells and incubated at 37°C. At the times indicated (see Fig. 7), total cytoplasmic RNA was purified by the procedure described by Favaloro et al. (11).

RNA blot analysis. For analysis of total cytoplasmic RNA, 5 μg of RNA was applied to sheets of nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.) by using a 96-well dot manifold. The blots were hybridized as described by Thomas (48). Specific RNAs were detected with gel-purified, nick-translated, ^{32}P-labeled fragments (plasmid-vector sequences excised) of the E1a and β-globin genes (32).

RESULTS

Adenovirus mutant *dl*312 promoters activated by helper virus superinfection. To compare the effects of adenovirus early functions on the transcription of viral and cellular genes, we devised a viral system for E1a complementation. In this system, one mutant virus which lacks a functional E1a gene relies upon a second helper virus, provided through superinfection, for E1a products that activate early transcription. The genome which provides the activating E1a products is thus separated from the target viral early genes. We compared the responses of viral early genes, chromosomal genes, and a transfected cellular gene to the transcription activation program of the helper. The system utilizes two deletion mutants of Ad5 (Fig. 1). One, *dl*312, lacks the

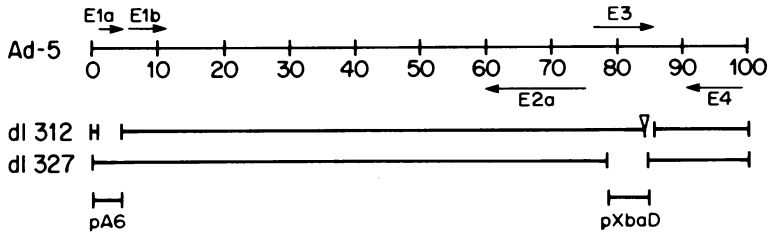

FIG. 1. Diagramatic representation of helper and mutant adenoviral genomes. The Ad5 wild-type genome is indicated by the solid line and is divided into 100 m.u. The arrows indicate the location and direction of transcription of regions transcribed during early infection. The genomes of the mutant adenoviruses, *dl*312 and *dl*327, are similar to Ad5 except for deletions of viral DNA or insertion of human DNA as indicated. Transcriptional activity of early region E3 in *dl*312 was monitored with a probe, pXbaD (78.5 to 84.3 m.u.), that corresponds to the deletion in *dl*327. Similarly, transcriptional activity of E1a in *dl*327 was monitored with a probe, pA6 (0 to 4.5 m.u.), that corresponds to the deletion in *dl*312. Symbols: —| and |—, deletion; ▽, insertion.

E1a promoter and most of the E1a coding region and fails to transcribe its early regions under normal conditions of infection of HeLa cells (24, 34). The other, *dl*327, has an intact E1a region but bears a deletion in region E3 (T. Shenk, personal communication). Because E3 is nonessential for viral growth in tissue culture, *dl*327 displays wild-type transcription kinetics (R. Stein, unpublished data). The scheme for our experiments was to infect cells with *dl*312 and then to activate the *dl*312 early genes by superinfection with *dl*327. Because E1a is partially deleted from *dl*312, an E1a-specific probe, pA6 (Fig. 1), could be used to monitor E1a expression from the helper virus *dl*327. Similarly, because most of E3 is deleted from *dl*327, a probe specific for E3, pXbaD, could be used to monitor the response of this early region in *dl*312 to products encoded by the helper virus. In control infections with *dl*312 or *dl*327 alone, these probes yielded viral-specific transcription from the E3 and E1a regions (data not shown).

To test the ability of the helper to complement the target virus, HeLa cells were infected with *dl*312 and then superinfected with *dl*327. E3 transcription from *dl*312 was stimulated 15- to 25-fold above mock superinfections by *dl*327 (data not shown). Furthermore, E1a transcripts (presumed to provide the activating function) were readily detected from the helper. From these results it is clear that superinfection by the helper virus indeed turns on target virus region E3 transcription. We next determined the kinetics of activation

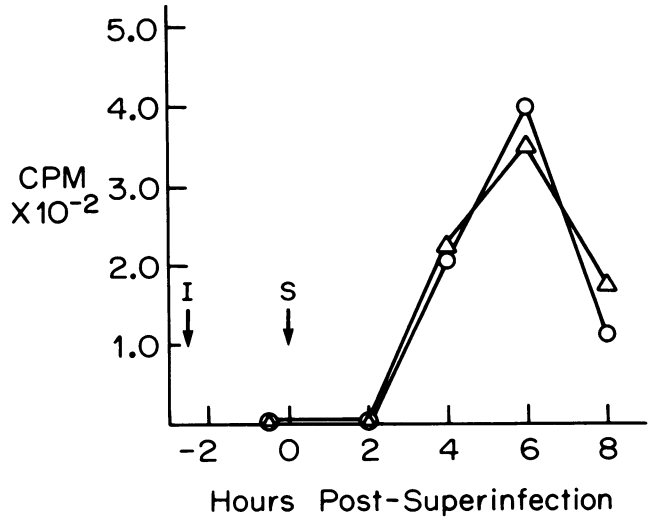

FIG. 2. Kinetics of E3 and E1a transcription in cells infected with *dl*312 and superinfected with *dl*327. HeLa cells were infected at 200 particles per cell with *dl*312 (at time I), followed by superinfection at 200 particles per cell with *dl*327 (at time S). Cells were incubated at 37°C in medium containing 10 mM hydroxyurea. At the times indicated, 2×10^8 cells were removed and labeled for 30 min with [^{3}H]uridine (160 μCi/ml). Labeled nuclear RNA was extracted and hybridized to filters with plasmids containing the following adenovirus DNA inserts: E1a (pA6; 0 to 4.5 m.u.) and E3 (pXbaD; 78.5 to 84.3 m.u.) Symbols: △, E1a; ○, E3.

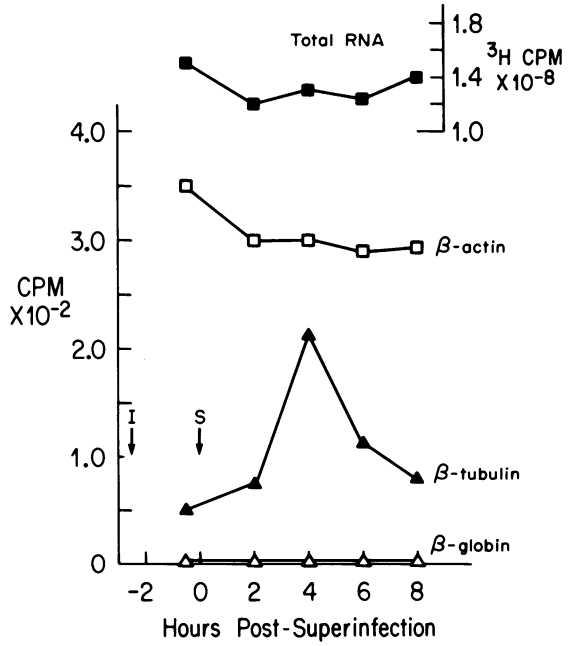

FIG. 3. Effect of *dl*327 superinfection on kinetics of cellular transcription. The results for viral transcription in the same experiment are shown in Fig. 2. Total [^{3}H]uridine incorporation into nuclear RNA was determined by trichloroacetic acid precipitation. Labeled nuclear RNA was hybridized to the following cellular-specific probes: β-tubulin (cDNA; Dβ-1), β-actin (cDNA; pHf βA-1), and β-globin (genomic; pH β-6). Nuclear RNA labeled with [^{3}H]uridine in the experiment shown in Fig. 2 was analyzed by hybridization to probes for cellular genes. Probes are described in the text. Total [^{3}H]uridine incorporation (■) was determined by trichloroacetic acid precipitation.

of E3 by helper virus and compared this with the previously established course of activation of E3 in a wild-type infection. In Fig. 2, viral early-region transcription rates were measured by [^{3}H]uridine pulse-labeling of *dl*312-infected cells for 30 min just before helper superinfection and at 2, 4, 6, and 8 h after *dl*327 helper virus superinfection. In this and subsequent kinetic experiments, the culture medium contained hydroxyurea (except as noted) to inhibit viral DNA replication, thus blocking late transcription (47). E1a transcription increased in the interval of 0 to 6 h post-superinfection and then declined at 8 h. E3 transcription was activated with similar kinetics. This biphasic profile of activation and repression is the same as observed in wild-type infections (37, 44). From these results we conclude that the temporal regulation of early adenovirus transcription can be imposed upon *dl*312 by factors acting in *trans* supplied by *dl*327.

Cellular gene transcription responds specifically to *dl*327 helper virus superinfection. We next asked whether the E1a-dependent mechanism which activated *dl*312 early transcription also influenced the rate of transcription of cellular genes. Further, if cellular gene transcription was activated, how was this related kinetically to viral gene activation? To answer these questions, we determined the rates of transcription of cellular genes for β-tubulin, β-actin, and β-globin in *dl*327-superinfected cells during the early transcription stage. Pulse-labeled nuclear RNA from the experiment shown in Fig. 2 was assayed by filter hybridization to human β-tubulin and β-actin cDNA probes. Both β-tubulin and β-actin genes are present as multigene families (7). In the case of the β-tubulin probe, Dβ-1 (20), at least three expressed gene transcripts were detected in HeLa cell RNA-DNA hybrids (see Discussion). β-Globin transcription was determined with a human probe corresponding to a unique 5.2-kilobase genomic *Bgl*II fragment containing the entire gene plus unique flanking sequences. The overall level of β-tubulin transcription rose rapidly after *dl*327 superinfection, such that by 4 h post-superinfection there was a nearly fourfold increase (Fig. 3). This peak was reached somewhat earlier than viral E3 transcription, which was maximal at 6 h in the same experiment (Fig. 2). β-Tubulin transcription subsequently declined and by 8 h post-superinfection approached the preinduced levels. The stimulation of β-tubulin transcription was not simply the result of an elevation in the overall rate of RNA synthesis in the helper virus-superinfected cells. The total trichloroacetic acid-precipitable [^{3}H]uridine nuclear RNA determined at each time point and shown in Fig. 3 was essentially invariant over the 8-h post-superinfection period. This argued against the possibility that β-tubulin activation was the result of a generalized effect on transcription rate. The result is in agreement with the previous finding that most cellular genes are not affected transcriptionally by adenovirus early infection (1).

Although the overall transcription rate did not change, transcription rates for many individual genes, in addition to the β-tubulin rate, may have been nonspecifically stimulated. To test of this possibility, we measured transcription of cellular β-actin nuclear RNA. β-Actin nuclear RNA was transcribed at high levels before helper virus superinfection (Fig. 3). However, the rate did not change either during the 0- to 6-h interval when the viral and β-tubulin rates increased or at 8 h when these rates declined.

We next asked whether a gene not normally expressed in HeLa cells, the β-globin gene, was activated by the helper virus. As expected, no β-globin transcripts were detected in HeLa nuclear RNA before superinfection (Fig. 3). However, no stimulation of β-globin transcription was found during the 0- to 6-h period when helper virus activates both β-tubulin and viral transcription.

From these results we conclude that an adenovirus early function is capable of stimulating the transcription of cellular β-tubulin nuclear RNA in parallel with viral early genes. The stimulation is selective in that (i) it is not reflected in the overall rate of RNA synthesis, (ii) the high level of transcription of β-actin nuclear RNA found in uninfected cells is not

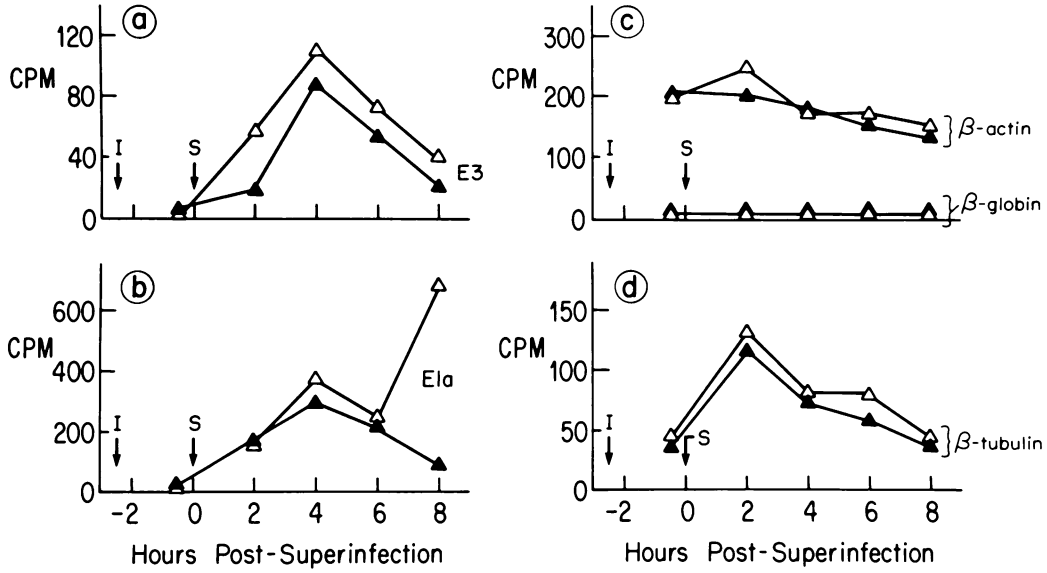

FIG. 4. Induction of cellular β-tubulin transcription by *dl*327 superinfection is not dependent on hydroxyurea. Cell were infected with *dl*312 at time I at 200 particles per cell. The infected culture was divided equally and incubated in the presence (▲) or absence (△) of hydroxyurea. After superinfection at time S with *dl*327 at 2,000 particles per cell, the cells were incubated in medium of the same composition used during infection. At the indicated times, 2×10^8 cells were removed and labeled with [^{3}H]uridine. Labeled nuclear RNA was extracted and hybridized to filters with probes for E3 (a), E1a (b), β-actin and β-globin (c), and β-tubulin (d). Details of the labeling procedure and probes are given in the text.

further stimulated during the viral early period, and (iii) β-globin fails to be transcribed despite the simultaneous activation of β-tubulin transcripts in the same cells.

β-Tubulin stimulation during *dl*327 superinfection is independent of hydroxyurea treatment. DNA replication and late viral transcription were blocked in the previous experiments by maintaining the infected cells with the drug hydroxyurea. To exclude the possibility that changes in β-tubulin transcription were a response to the DNA replication block caused by hydroxyurea, we analyzed nuclear transcripts in cells infected with *dl*312 and then superinfected with *dl*327,

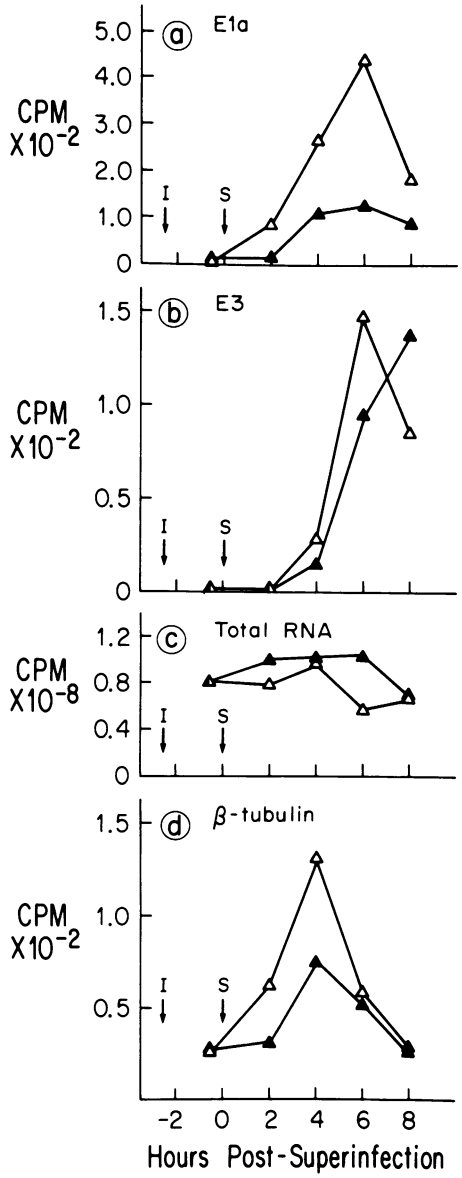

FIG. 5. The effect of E1a multiplicity on E3 and cellular β-tubulin transcription. HeLa cells were infected with *dl*312 at time I as described in the legend to Fig. 2. The *dl*312-infected culture was divided in two. After time S, half was superinfected with *dl*327 at 200 particles per cell (▲), and the other half was superinfected with *dl*327 at 2,000 particles per cell (△). [^{3}H]uridine-labeled nuclear RNA was extracted from cells labeled at the specified times and hybridized to filters with plasmids containing probes for E1a (a), E3 (b), and β-tubulin (d). In (c), total ^{3}H-labeled nuclear RNA was determined by trichloroacetic acid precipitation.

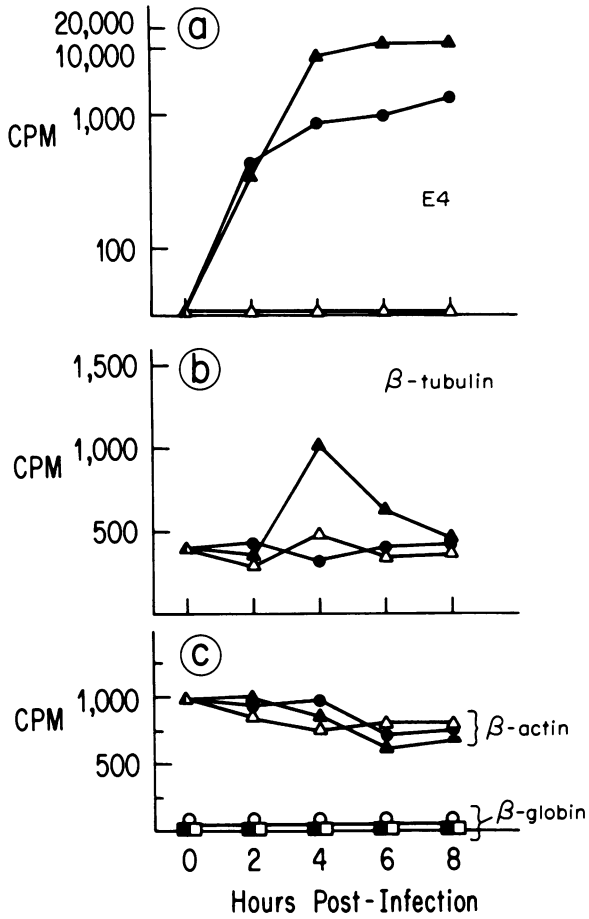

FIG. 6. Transcription of cellular β-tubulin is not induced by *dl*312, even at very high multiplicities of infection. Cells were infected with either *dl*312 at 10,000 particles per cell (●) or *dl*327 at 2,000 particles per cell (▲) or were mock infected (△). At the indicated times, 2.5 × 10^8 cells were removed and labeled with [^{3}H]uridine (300 μCi/ml) for 30 min at 37°C. Nuclear RNA was extracted and hybridized to probes for E4 (a), β-tubulin (b), and β-actin and β-globin (c). For β-globin the symbols are: ■, *dl*312; □, *dl*327; and ○, mock infected.

both in the presence and absence of hydroxyurea. The transcription of E3, β-tubulin, β-actin, and β-globin follow the same course in the presence or absence of the drug (Fig. 4a, c, and d). The transcription of E1a is slightly altered when hydroxyurea is omitted from the culture (Fig. 4b). Since the only difference was an elevation in E1a rate at the 8-h time point in the unblocked culture, most probably this increase resulted from the onset of late transcription, which elevates E1a expression (37, 44). When probes for late-specific viral regions were hybridized to these nuclear RNA samples, late transcripts were only detected in the untreated cultures at 8 h postsuperinfection (R. Stein, unpublished data), supporting this interpretation. We conclude that hydroxyurea inhibition of DNA synthesis does not account for the induction of β-tubulin transcription.

Level of induction of β-tubulin dependent on helper virus multiplicity. If an early viral product such as E1a is responsible for β-tubulin activation, then changing the multiplicity of *dl*327 helper virus, which changes the multiplicity of the E1a gene, might be expected to change either the rate or the level

of β-tubulin induction. To determine the effect of helper multiplicity, cells were infected with *dl*312 at 200 particles per cell and incubated for 2 h. The culture was then divided equally and superinfected with *dl*327 at 200 or 2,000 particles per cell. The ratio of E1a gene to target E3 gene and to cellular β-tubulin genes was therefore 10-fold higher in the second superinfected culture than in the first. Cells were pulse-labeled with [^{3}H]uridine just before superinfection and at 2, 4, 6, and 8 h post-superinfection. Nuclear RNA was isolated and hybridized to filters for E1a, E3, and β-tubulin. The results are shown in Fig. 5. A 10-fold increase of helper virus multiplicity increased the maximal level of transcription from the E1a gene of the helper virus four to fivefold (Fig. 5a). The rate of transcription from region E3 in *dl*312 was increased to a lesser extent by the change in multiplicity of *dl*327 superinfection (Fig. 5b). However, E3 transcription reached a maximal rate more rapidly at the higher *dl*327 multiplicity.

Helper multiplicity had little effect on the total cellular transcription rate (Fig. 5c), consistent with the result shown in Fig. 3. However, the maximal rate of transcription of cellular β-tubulin nuclear RNA did rise as a consequence of increasing *dl*327 multiplicity (Fig. 5d). At both low and high *dl*327 multiplicities, β-tubulin transcription reached a maximum rate at 4 h postinfection. However, this maximal rate was only twofold greater than the zero time rate when the viral multiplicity was 200 particles per cell. It was sixfold greater than the zero time rate when infection was with 2,000 particles per cell. Higher virus multiplicity gave greater stimulation.

Adenoviral induction of β-tubulin transcription requires a functional E1a gene. The increased stimulation of β-tubulin transcription at higher helper virus multiplicity suggests that the E1a gene is responsible for viral induction of β-tubulin transcription in HeLa cells. To establish the requirement for E1a more firmly, and to exclude the possibility that β-tubulin induction was the consequence of a viral factor unrelated to E1a (such as saturation of receptors on the cell membrane by virus), cells were infected with a very high multiplicity of *dl*312, 10,000 particles per cell. This is fivefold higher than the multiplicity of helper virus used in the experiment shown in Fig. 2 and 3 and 50-fold higher than the multiplicity of *dl*312 in that experiment. As controls, other cultures were mock infected or infected with *dl*327 at the normal multiplicity of 2,000 particles per cell. Portions of these cultures were pulse-labeled with [^{3}H]uridine at several times postinfection, and nuclear RNAs were assayed as before. The results of this experiment are given in Fig. 6. We measured the time course of expression of early region E4 (Fig. 6a). As expected, in the very-high-multiplicity *dl*312 infection, the level of E4 transcripts were greatly reduced relative to the *dl*327 infection. Nevins (34) reported that viral genes can bypass the requirement for E1a at these high multiplicities, albeit inefficiently. Our finding of a 10-fold reduction in signal from *dl*312 relative to *dl*327, despite a fivefold higher multiplicity, is consistent with this report. β-Tubulin transcription was transiently induced by *dl*327 ca. 2.5-fold above a mock-infected control and reached a peak at 4 h postinfection, at the same time as E4 reached its maximal rate (Fig. 6b). However, even with 10,000 particles of *dl*312 per cell, β-tubulin transcription was unchanged from the rate in a mock-infected culture. β-Globin and β-actin transcription (Fig. 6c) were unaffected by very-high-multiplicity *dl*312 infection and by *dl*327 (as previously seen in Fig. 3 and 4). We conclude that *dl*312 alone is unable to activate β-tubulin transcription, even at very high multiplicities of infection.

Adenoviral early infection induces transcription from a transfected β-globin gene. In the experiments described

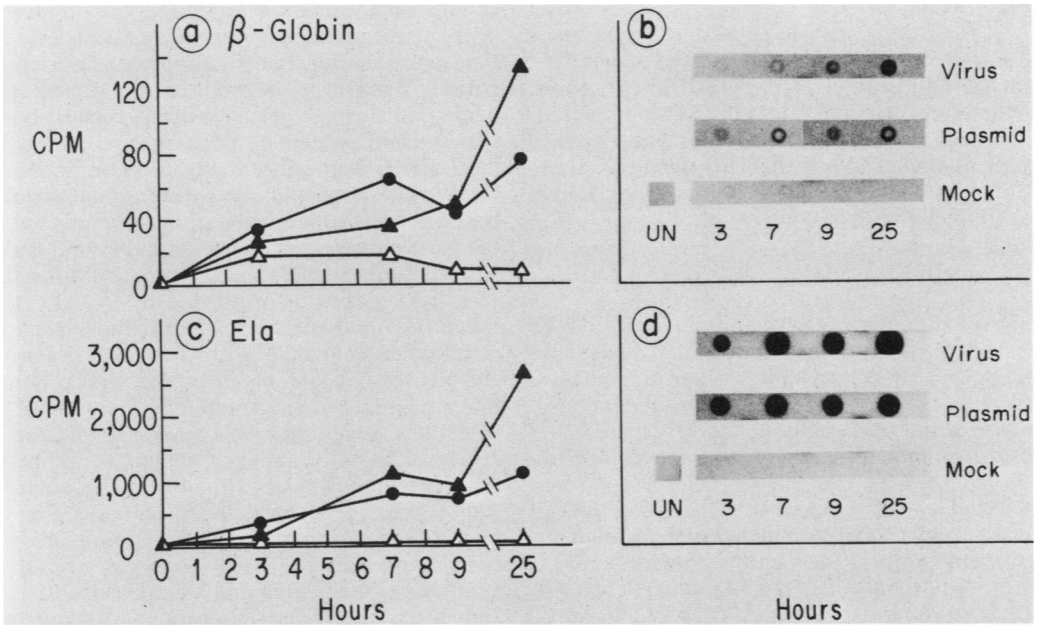

FIG. 7. Both *dl*327 and E1a expression plasmids induce transcription from a transfected β-globin plasmid. Subconfluent HeLa cells were transfected with a β-globin plasmid or cotransfected with a β-globin and an E1a expression plasmid by using the calcium phosphate technique. After 13 h, the calcium phosphate precipitates were removed, and the β-globin transfected cells were either mock infected or *dl*327 infected for 1 h at 37°C. The cells were then incubated in medium supplemented with 10 mM hydroxyurea for the indicated times. Total cytoplasmic RNA was isolated, and 5 μg was applied to a dot blot and probed for β-globin in (a) and (b) and E1a transcripts in (c) and (d). Panels b and d are autoradiograms of the dot hybridization pattern of RNA isolated at various times postinfection. Panels a and c are plots of the results from (b) and (d), respectively, obtained by cutting out dot hybrids of virus- (▲), plasmid- (●), and mock- (△) treated cells and scintillation counting.

above, transcription of the cellular β-globin gene was not activated by the virus. In contrast, in studies of a plasmid clone of the β-globin gene by Green et al. (19), transcription of the β-globin plasmid was stimulated by adenovirus infection and by cotransfection into HeLa cells together with an E1a expression plasmid (1). We wished to confirm that under our conditions of infection, the plasmid form of the gene would be activated by the virus, although we had found that the chromosomal form was not activated. If the plasmid form was activated, we also wished to determine whether the virus-stimulated increase in transcription of the β-globin plasmid paralleled the course of expression of E1a, a feature which was characteristic of the induction of chromosomal β-tubulin transcription by *dl*327, described above. We transfected HeLa cells with the β-globin plasmid and 13 h later infected them with *dl*327. The infected cells were incubated in the presence of hydroxyurea to inhibit late viral transcription and to increase the amount of early viral products. Because in this experiment the globin DNA was transfected, we determined β-globin gene expression by measuring the levels of β-globin cytoplasmic RNA rather than nuclear transcripts, because nuclear transcription rates of transiently expressed genes determined by pulse-labeling have not been well characterized and might not faithfully reflect the rates of initiation at the β-globin promoter. β-Globin and E1a expression were determined by dot blot analysis of cytoplasmic RNA isolated 3, 6, 9, and 25 h postinfection. As controls, cells transfected with the β-globin gene were either mock infected or cotransfected with an E1a plasmid. The results of this experiment are shown in Fig. 7. Figure 7a and c give the time courses for β-globin and E1a cytoplasmic RNA, respectively, obtained by quantitating the dot blots. Figure 7b and d are autoradiographic assays of these same dot-blots. β-Globin RNA transcription from the transfected gene was stimulated by viral infection, as well as by the E1a expression plasmid (Fig. 7a and b). E1a cytoplasmic RNA was encoded by the virus and the expression plasmid (Fig. 7c and d). Levels of cytoplasmic β-globin RNA increased in parallel with the amount of E1a transcript encoded during viral infection and by the cotransfected E1a gene. These results show that the virus can activate transcription from a transfected β-globin gene and suggest that the level of this induction increases with higher levels of expression of E1a, as was the case with cellular β-tubulin.

DISCUSSION

The transcription of adenovirus early genes is activated by protein products from the adenovirus E1a region. The mechanism is *trans* acting and affects multiple viral promoters (4, 24, 33, 34, 38, 39). The activation program follows characteristic kinetics, in which the transcription rate first increases from 0 to 4 h postinfection and then decreases from 4 to 6 h postinfection (37, 44).

Viral effects on cellular transcription. In the present work, we analyzed the action of E1a by complementing an adenovirus E1a deletion mutant, *dl*312, with a superinfecting helper virus, *dl*327, which encodes E1a. We compared the effect of the helper virus on the transcription of an early viral gene, E3, with the effect upon cellular genes. We show that superinfection imposes a wild-type early-transcription pattern on the *dl*312 E3 promoter. We also show that levels of HeLa β-tubulin nuclear RNA are reproducibly stimulated two- to sixfold by the helper virus during the early stage of infection. The results are notable in two respects. First, β-tubulin responds with kinetics which parallel the activation and subsequent repression of the transcription of the viral early gene, E3. Also, β-tubulin activation is dependent upon the transcription activator E1a, because it does not take place during infections by the E1a deletion mutant *dl*312 alone, even at high multiplicities of infection. Also, the level of β-tubulin induction increases when the level of E1a expression is raised through increased multiplicities of the helper virus. These data suggest that changes in levels of pulse-labeled cellular β-tubulin nuclear RNA we observe are regulated at the level of transcription and that activation is by a mechanism analogous to that which turns on the viral genes. We cannot exclude the possibility that changes in nuclear stability also contribute, although we believe that this is unlikely to be a major factor.

In humans, there are 15 to 20 members of the beta-tubulin gene family (7). Many of these are pseudogenes (29, 51, 52). The cDNA probe used in our studies, Dβ-1 (20), corresponds to a β-tubulin gene whose transcripts yield two β-tubulin mRNAs of 2.6 and 1.8 kilobases, which differ in their 3′ untranslated sequences. A second β-tubulin gene yields a single 2.6-kilobase mRNA (20), and at least one additional β-tubulin gene is expressed at high levels in HeLa cells (30). Although these genes diverged in their 3′ untranslated sequences, they share high homology in the coding regions represented in our probe. Thus, our experiments do not distinguish among the three β-tubulin transcription units expressed in HeLa cells in response to viral early signals. Our probe also shares homology with β-tubulin pseudogenes. However, 9 of 10 human β-tubulin pseudogenes analyzed thus far are intronless and are derived from reintegrated DNA copies of mRNA (30). It is unlikely that the intronless pseudogenes have promoters, reducing the probability that they contribute transcripts detected in our experiments.

It is apparent from the present studies that different cellular genes are affected by the virus in different ways. β-Actin sequences are transcribed at a high rate before infection, and this rate is neither elevated during the activation period, a result also reported by Babich et al. (1), nor decreased during the subsequent repression period. Note that as with β-tubulin, RNA in our β-actin hybrids could have arisen from more than one gene in the β-actin family (7). We also show that the chromosomal β-globin gene is not transcribed at a detectable rate in uninfected cells and that the gene remains inactive during the viral early period.

The results suggest the existence of at least three classes of cellular genes distinguished by the effect of viral early functions on their transcription: those that respond transcriptionally in parallel with viral genes, those that are active and not further stimulated by the viral program, and those that are inactive and refractile to viral signals. A fourth class of cellular genes may be represented by the class I major histocompatibility antigens, which are repressed transcriptionally by the E1a gene from the oncogenic Ad12 strain (43). The cellular genes which are activated by viral infection must contribute only a small fraction to the total transcriptional pool, since in our work the overall rate of RNA synthesis is unaffected and since Babich et al. (1) have also shown that the transcription rates of many specific cellular genes are insensitive to adenoviral infection.

Role of E1a in activating β-tubulin transcription. We showed in Fig. 6 that 10,000 particles of *dl*312 per cell are incapable of activating β-tubulin transcription. We conclude that E1a is required either directly or indirectly for β-tubulin activation. With indirect mechanisms, E1a might only serve to activate a second viral or cellular gene which encodes the

relevant product. Nevins (34) reported that during high-multiplicity infections of HeLa cells by *dl*312, early transcription units bypass their requirement for E1a and are activated with kinetics similar to those of a wild-type infection at 200 particles per cell. Consistent with this report, in the experiment shown in Fig. 6, the levels of early nuclear RNA produced during the *dl*312 infection are only a fraction of that found during the wild-type control infection at 2,000 particles per cell. At this low level of early gene expression, β-tubulin was not induced in the absence of E1a. This suggests that E1a itself, not just a product induced by E1a, is responsible for activation of β-tubulin transcription. However, the results shown in Fig. 5 suggest that gene dosage is important in stimulating β-tubulin. Thus, we cannot exclude the possibility that higher levels of an early viral factor(s) other than that obtained in the experiment shown in Fig. 6 would induce β-tubulin, even in the absence of E1a. If, however, other viral factors are the active agents, it is apparent that they depend upon E1a for their expression.

Comparison with the heat shock gene. Kao and Nevins (26) recently reported that adenovirus infection of HeLa cells transiently induces transcription of the cellular gene for the 72- to 74-kd heat shock protein. As reported here for β-tubulin, virus-induced transcription of the cellular heat shock gene is dependent upon expression of the E1a gene product. Thus, the heat shock gene also belongs to the class of genes which responds in parallel with viral genes. The case of the heat shock gene is, however, potentially more complex because a wide variety of "stress stimuli" can induce its transcription (31).

Transcription from the 72- to 74-kd heat shock protein gene is induced ca. 50-fold during adenovirus infection (26). In contrast, β-tubulin was only stimulated two- to sixfold. This difference probably reflects the difference in the basal transcription rate of these genes in the cell before infection. The 72- to 74-kd heat shock protein gene is transcribed very poorly in the absence of induction, whereas β-tubulin genes are actively transcribed, and their mRNAs account for ca. 1 to 2% of the total cellular mRNA pool (8).

Differential response of chromosomal and transfected β-globin genes. Recently Triesman et al. (49) showed that the human β-globin gene in a plasmid clone is transcribed when transfected into 293 cells, a line which expresses adenovirus early-region products from integrated viral sequences. The β-globin plasmid was also activated when cotransfected into HeLa cells, together with E1a expression plasmids. These results suggest that E1a can activate a cellular gene introduced to cells as naked DNA in a transient expression assay.

The results presented here for the chromosomal β-globin gene are in contrast to the specific finding by Green et al. (19) that a transfected β-globin plasmid is activated in nonerythroid cells by E1a products. In our study, viral early products failed to activate this same gene when it was in its normal chromosomal location in HeLa cells. Taken together, these data suggest that a *cis*-acting mechanism blocks E1a activation of the transcription of the chromosomal globin gene in HeLa cells and that this block is not imposed upon the β-globin plasmid. In the present report, adenovirus infection did activate transcription of a transfected β-globin plasmid, a finding also reported by Green et al. (19). In our experiments, the level was comparable to that induced by an E1a expression plasmid. We also measured the time course of the activation and found that it closely paralleled the kinetics of E1a levels encoded by the virus or the expression vector. The difference in the responses of plasmid and chromosomal genes to activating signals most likely reflects differences in the structures of the two forms of the β-globin gene. Because the plasmid enters as naked DNA, it is more likely to be accessible to nucleoplasmic transcription factors, such as E1a, than is the chromosomal gene, which is in a DNase I-insensitive conformation in nonerythroid cells (10).

Regulation of β-tubulin transcription. The present work demonstrates that β-tubulin may be regulated transcriptionally by an E1a-dependent mechanism. The mechanisms which normally regulate β-tubulin expression in the uninfected cell are not well established. One factor which determines cytoplasmic β-tubulin mRNA levels is the size of the pool of monomer β-tubulin protein (3). Drugs which increase the monomer pool decrease the level of β-tubulin mRNA (8). At least one component of the regulation is at the post-transcriptional level, since no changes in β-tubulin transcription are detected when the monomer pool increases (6).

The features of a promoter which are required for viral early signals to activate transcription are not yet established. However, some evidence is provided by our comparison of the cellular and plasmid forms of the β-globin gene. As discussed above, the β-globin results suggest that accessibility of the promoter to transcription factors (and not just the primary sequence) is one requirement. Perhaps the cellular β-tubulin promoter(s) more closely resembles β-globin in its plasmid form than in its cellular form and has a conformation which permits access to the viral products. An open conformation is unlikely to be the only factor, however, which determines responsiveness. If this were sufficient, many and perhaps all transcribed genes in HeLa cells would be expected to be activated, and this was not observed, either in the present study or in the report by Babich et al. (1). It is possible that the viral signals which activate and then repress β-tubulin in fact provide an analog of the cellular mechanism which normally regulates β-tubulin transcription. Alternatively, the viral factors could bypass these controls, but in a gene-specific way. It remains to be determined whether the changes we see in β-tubulin transcription are also reflected in changes in mRNA levels.

Transcription repression. Induction of β-tubulin and of heat shock transcription (26) by the virus both show two-step kinetics, with the transcription rates rising and then falling. Biphasic induction is also seen for viral early genes, and similar kinetics have been reported for certain cellular genes responsive to specific induction, such as the interferon gene when it is induced by double-stranded RNA (41). For viral promoters, the activation phase depends upon E1a products. The repressive phase of viral early gene regulation has, for region E4, been ascribed to an action of the DBP (2, 5, 36). Although a mechanism for repressing viral transcription in which DBP recognizes viral promoters is easily imagined, it is more difficult to accept direct DBP action on a cell gene, such as a β-tubulin gene(s), which is repressed in our studies at 4 to 6 h post-superinfection with helper virus. For sake of consistency, the mechanism of repression must also account for the fact that one cell gene type, β-tubulin, shows repression, but another, β-actin, is unaffected. One plausible explanation is that the repression results from a reversal of the activation mechanism, such as a decline in the levels of E1a products or cellular factors required for E1a action. This decline could be a result of cellular agents, such as proteases, or could result from other agents, such as DBP, interacting with and blocking a factor crucial to the activation mechanism. With this model, β-actin transcription is not repressed as E1a levels fall because β-actin transcription is not dependent upon E1a.

Why β-tubulin. Why do viral signals activate β-tubulin transcription? We suggested that β-tubulin transcription activation is a direct consequence of the viral mechanism for activating viral promoters. This could occur in two ways. One possibility is that β-tubulin is circumstantially activated by a mechanism that is virus specific and which has no direct cellular analog. With this alternative, one or more of the β-tubulin genes may have conformations at their promoters which fortuitously resemble an adenovirus early promoter. Thus, viral factors would be able to activate such cell genes. A second possibility is that the virus utilizes a mechanism for early gene activation which is analogous to a cellular mechanism for activation of β-tubulin transcription. Other cellular genes, such as the gene for the 72- to 74-kd heat shock protein might respond to the viral mechanism as well, although the evidence from Babich et al. (1) discussed above argues against a generalized transcription activation effect. Although it is well established that many cellular genes are transcriptionally regulated (9), the role of transcriptional regulation for the β-tubulin gene family is not yet clear. Mimicking a cellular mechanism could have two benefits for the virus. Viral genes might make maximal use of preexisting cellular machinery. Second, the mechanism might coordinately induce a class of cellular functions, and some of these could be of direct benefit to the virus. Others might be circumstantially induced. With this model, the viral factor(s) responsible for activation might correspond to a cell factor(s) which is limiting before infection and which regulates the proposed class of cell genes. To test this model, it will be of interest to identify additional cellular genes subject to viral regulation and to determine whether these genes share control mechanisms in the uninfected cell.

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LITERATURE CITED

1. **Babich, A., L. T. Feldman, J. R. Nevins, J. E. Darnell, Jr., and C. Weinberger.** 1983. Effect of adenovirus on metabolism of specific host mRNAs: transport control and specific translation discrimination. Mol. Cell. Biol. **3**:1212–1221.
2. **Babich, A., and J. R. Nevins.** 1981. The stability of early adenovirus mRNA is controlled by the viral 72Kd DNA-binding protein. Cell **26**:371–379.
3. **Ben-Ze'ev, A., S. R. Farmer, and S. Penman.** 1979. Mechanisms of regulating tubulin synthesis in cultured mammalian cells. Cell **17**:319–325.
4. **Berk, A. J., F. Lee, T. Harrison, J. Williams, and P. A. Sharp.** 1979. Pre-early adenovirus 5 gene product regulates synthesis of early viral messenger RNAs. Cell **17**:935–944.
5. **Carter, T. H., and R. A. Blanton.** 1978. Possible role of the 72,000-dalton DNA-binding protein in regulation of adenovirus type 5 early gene expression. J. Virol. **25**:664–674.
6. **Cleveland, D. W., and J. C. Havercroft.** 1983. Is apparent autoregulatory control of tubulin synthesis nontranscriptionally regulated? J. Cell. Biol. **97**:919–924.
7. **Cleveland, D. W., M. A. Lopata, R. J. McDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner.** 1980. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. Cell **20**:95–105.
8. **Cleveland, D. W., M. A. Lopata. P. Sherline, and M. W. Kirschner.** 1981. Unpolymerized tubulin modulates the level of tubulin mRNAs. Cell **25**:537–546.
9. **Derman, E., K. Kranter, L. Walling, C. Weinberger, M. Ray, and J. Darnell, Jr.** 1981. Transcriptional control of liver-specific mRNAs. Cell **23**:731–739.
10. **Emerson, B. M., and G. Felsenfeld.** 1984. Specific factor conferring nuclease hypersensitivity at the 5′ end of the chicken adult β-globin gene. Proc. Natl. Acad. Sci. U.S.A. **81**:95–99.
11. **Favaloro, J., R. Treisman, and R. Kamen.** 1980. Transcriptional maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease S1 gel mapping. Methods Enzymol. **65**:718–749.
12. **Flint, S. J., M. A. Plumb, U.-C. Yang, G. S. Stein, and J. L. Stein.** 1984. Effect of adenovirus infection on expression of human histone genes. Mol. Cell. Biol. **4**:1363–1371.
13. **Fraser, N., and E. Ziff.** 1978. RNA structures near poly(A) of adenovirus-2 late messenger RNAs. J. Mol. Biol. **124**:27–51.
14. **Gaynor, R. B., and A. J. Berk.** 1983. *Cis*-acting induction of adenovirus transcription. Cell **33**:683–693.
15. **Gaynor, R. B., A. Tsukamoto, C. Montell, and A. J. Berk.** 1982. Enhanced expression of adenovirus transforming proteins. J. Virol. **44**:276–285.
16. **Graham, F., and A. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–457.
17. **Graham, F. L., P. J. Abrahams, C. Mulder, H. L. Heyrecker, S. O. Warnaar, F. A. J. de Vries, W. Fiers, and A. J. van der Eb.** 1974. Studies on the in vitro transformation by DNA and DNA fragments of human adenovirus and simian virus 40. Cold Spring Harbor Symp. Quant. Biol. **39**:637–650.
18. **Graham, J. L., J. Smiley, W. C. Russell, and R. Nairn.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. **36**:59–72.
19. **Green, M. R., R. Treisman, and T. Maniatis.** 1983. Transcriptional activation of cloned human B-globin genes by viral immediate early-gene products. Cell **35**:137–148.
20. **Hall, J. L., L. Dudley, P. R. Dobner, S. A. Lewis, and N. J. Cowan.** 1983. Identification of two human β-tubulin isotypes. Mol. Cell. Biol. **3**:854–862.
21. **Handa, H., R. E. Kingston, and P. A. Sharp.** 1983. Inhibition of adenovirus early region IV transcription in vitro by a purified viral DNA binding protein. Nature (London) **302**:545–547.
22. **Hearing, P., and T. Shenk.** 1983. The adenovirus type 5 E1a transcription control region contains a duplicated enhancer element. Cell **33**:695–703.
23. **Imperiale, M. J., L. T. Feldman, and J. R. Nevin.** 1983. Activation of gene expression in DNA transfections by adenovirus and herpesvirus regulatory genes acting in *trans* and by a *cis*-acting adenovirus enhancer element. Cell **35**:127–136.
24. **Jones, N., and T. Shenk.** 1979. An adenovirus type 5 early gene function regulates expression of other early viral genes. Proc. Natl. Acad. Sci. U.S.A. **76**:3665–3669.
25. **Jones, N., and T. Shenk.** 1979. Isolation of adenovirus-2 type 5 host range deletion mutants defective for transformation of rat embryo cells. Cell **17**:683–689.
26. **Kao, H.-T., and J. R. Nevins.** 1983. Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. Mol. Cell. Biol. **3**:2058–2065.
27. **Kit, S., D. R. Dubbs, R. A. deTorres, and J. L. Melnick.** 1965. Enhanced thymidine kinase activity following infection of green monkey kidney cells by simian adenovirus, simian papovavirus and an adenovirus-SV40 hybrid. Virology **27**:453–457.
28. **Ledinko, N.** 1967. Stimulation of DNA synthesis and thymidine kinase activity in human embryonic kidney cells infected by adenovirus 2 or 12. Cancer Res. **12**:1459.
29. **Lemischka, I., and P. A. Sharp.** 1982. The sequence of an expressed rat α-tubulin gene and a pseudogene with an inserted repetitive element. Nature (London) **300**:330–335.

30. **Lee, M. G.-S., S. A. Lewis, C. D. Wilde, and N. J. Cowan.** 1983. Evolutionary history of a multigene family: an expressed human β-tubulin gene and three processed pseudogenes. Cell **33**:477–487.
31. **Li, G. C., D. C. Schrieve, and W. Zena.** 1982. Correlation between synthesis of heat-shock proteins and development of tolerance to heat and to adriamycin in Chinese hamster fibroblasts: heat shock and other inducers, p. 395–404. *In* M. J. Schlesinger, M. Ashburner, and A. Tissieres (ed.), Heat shock: from bacteria to man. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Maniatis, T., A. Jeffrey, and D. C. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage lambda. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
33. **Montell, C., E. F. Fisher, M. H. Caruthers, and A. J. Berk.** 1982. Resolving the function of overlapping viral genes by site-specific mutagenesis at a mRNA splice site. Nature (London) **295**:380–384.
34. **Nevins, J. R.** 1981. Mechanism of activation of early viral transcription by the adenovirus E1a gene product. Cell **26**:213–220.
35. **Nevins, J. R., H. S. Ginsberg, J.-M. Blanchard, M. C. Wilson, and J. E. Darnell, Jr.** 1979. Regulation of the primary expression of the early adenovirus transcription units. J. Virol. **32**:727–733.
36. **Nevins, J. R., and J. J. Jensen-Winkler.** 1980. Regulation of early adenovirus transcription: a protein product of early region 2 specifically represses region 4 transcription. Proc. Natl. Acad. Sci. U.S.A. **77**:1893–1897.
37. **Nevins, J. R., and M. C. Wilson.** 1980. Expression of the adenovirus-2 major late transcription unit during early infection: regulation at the level of transcription termination and RNA processing. Nature (London) **290**:113–118.
38. **Osborne, T. F., R. B. Gaynor, and A. J. Berk.** 1982. The T-A-T-A homology and the mRNA 5′ untranslated sequence are not required for expression of essential adenovirus E1a functions. Cell **29**:139–148.
39. **Perricaudet, M., G. Akusjarvi, A. Virtanen, and U. Petterson.** 1979. Structure of two spliced mRNAs from the transforming region of human subgroup C adenoviruses. Nature (London) **281**:694–696.
40. **Postel, E. H., and A. J. Levine.** 1975. Studies on the regulation of deoxypyrimidine kinases in normal, SV40 transformed and SV40 and adenovirus-infected mouse cells in cultures. Virology **63**:404–420.
41. **Raj, N. B. K., and P. M. Pitha.** 1981. Analysis of interferon mRNA in human fibroblast cells induced to produce interferon. Proc. Natl. Acad. Sci. U.S.A. **78**:7426–7430.
42. **Riccardi, R., R. Jones, C. Cepko, P. Sharp, and B. Roberts.** 1981. Expression of early adenovirus genes requires a viral encoded acidic polypeptide. Proc. Natl. Acad. Sci. U.S.A. **78**:6121–6125.
43. **Schrier, P. I., R. Bernards, R. T. M. J. Vaessen, A. Houweling, and A. J. van der Eb.** 1983. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed cells. Nature (London) **305**:771–775.
44. **Shaw, A. R., and E. B. Ziff.** 1980. Transcripts from the adenovirus-2 major late promoter yield a single family of 3′ co-terminal mRNAs and five late families. Cell **22**:905–916.
45. **Solnick, D., and M. A. Anderson.** 1982. Transformation-deficient adenovirus mutant defective in expression of region 1A but not region 1B. J. Virol. **42**:106–113.
46. **Takahashi, M., S. Veda, and T. Ogina.** 1966. Enhancement of the thymidine kinase activity of human embryonic kidney cells by infection with human adenovirus types 5 and 12. Virology **30**:741–743.
47. **Thomas, G. P., and M. B. Mathews.** 1980. DNA replication and the early to late transition in adenovirus infection. Cell **22**:523–533.
48. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
49. **Treisman, R., M. R. Green, and T. Maniatis.** 1983. Cis- and trans-activation of globin transcription in transient assays. Proc. Natl. Acad. Sci. U.S.A. **80**:7428–7432.
50. **Weeks, D. L., and N. C. Jones.** 1983. E1A control of gene expression is mediated by sequences 5′ to the transcriptional starts of the early viral genes. Mol. Cell. Biol. **3**:1222–1234.
51. **Wilde, C. D., C. E. Crowther, and N. J. Cowan.** 1982. Diverse mechanisms is the generation of human β-tubulin pseudogenes. Science **217**:549–552.
52. **Wilde, C. D., C. E. Crowther, T. P. Cripe, M. Gwo-Shu Lee, and N. J. Cowan.** 1982. Evidence that a human β-tubulin pseudogene is derived from its corresponding mRNA. Nature (London) **292**:83–84.
53. **Yoder, S. S., B. L. Robberson, E. J. Leys, A. G. Hook, M. Al-Ubaidi, C.-Y. Yeung, R. E. Kellems, and S. M. Berget.** 1983. Control of cellular gene expression during adenovirus infection: induction and shut-off of dihydrofolate reductase gene expression by adenovirus type 2. Mol. Cell. Biol. **3**:819–828.