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Selective Induction of Human Heat Shock Gene Transcription by the Adenovirus E1A Gene Products, Including the 12S E1A Product

M. CELESTE SIMON,[1] KARL KITCHENER,[2] HUNG-TEH KAO,[1] EILEEN HICKEY,[3] LEE WEBER,[3] RICHARD VOELLMY,[4] NATHANIEL HEINTZ,[2] AND JOSEPH R. NEVINS[1]*

Laboratory of Molecular Cell Biology, Howard Hughes Medical Institute,[1] and Laboratory of Biochemistry and Molecular Biology,[2] The Rockefeller University, New York, New York 10021; Department of Biology, University of South Florida, Tampa, Florida 33620[3]; and Department of Biochemistry, University of Miami School of Medicine, Miami, Florida 33101[4]

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We have previously shown that the human 70-kilodalton heat shock protein gene (*hsp70*) is induced by the adenovirus E1A gene product and during the S-G_2 phase of the cell cycle. In this study, we investigated the effect of E1A on the expression of other human *hsp* genes. A gene encoding one form of the hsp89 protein (*hsp89α*) was activated during an adenovirus infection with kinetics similar to those of activation of *hsp70*. The induction required a functional E1A gene. However, the *hsp89* transcript was not cell cycle regulated. Genes encoding another form of hsp89 and the hsp27 protein were not induced by E1A or during the cell cycle. Further examination of *hsp70* expression revealed a greater complexity than previously seen. S1 nuclease analysis using an *hsp70* cDNA as well as a distinct *hsp70* genomic clone demonstrated three related *hsp70* transcripts; two were induced by E1A, and one was not. Both of the E1A-inducible genes were regulated during the cell cycle. All three were induced by heat shock. These results suggest common aspects of control among certain members of this family of cellular genes distinct from heat shock control. Finally, using viruses that express the individual E1A proteins, we found that the *hsp70* gene is induced by the 12S and the 13S E1A products. The efficiency of induction by the 12S product was somewhat less than that by the 13S product but only by a factor of less than 2. This is in contrast to the induction of early viral genes, for which the 13S product is considerably more efficient than the 12S product.

The adenovirus E1A gene is a regulatory gene that specifies products that both activate (2, 16, 35) and repress (3, 11, 50) transcription of unlinked genes. During a lytic viral infection, five early transcription units are stimulated by the action of the product of the 13S E1A RNA, a 289-amino-acid protein (42). The precise mechanism by which this induction occurs is not understood, but it appears to involve an increase in cellular transcription factor(s) (13, 21, 22, 57). A mechanism involving cellular transcription factors is consistent with the fact that the process is not restricted to the early viral genes. It was initially shown that a cellular gene (*hsp*70) encoding the 70-kilodalton heat shock protein is stimulated by E1A (36). Furthermore, this induction is much the same as the induction of the early viral genes in that the kinetics of activation are similar and the activation is transcriptional (19). Subsequently, it was shown that one other cellular gene, the β-tubulin gene, is induced by E1A (45). Of additional interest is the observation that the *hsp70* gene is also regulated as a function of the cell cycle (18, 56). In addition, a subset of cellular genes whose expression is growth regulated are also induced by adenovirus infection, although in this instance there is no evidence for E1A-mediated stimulation (26). These observations suggest a possible link between an E1A-like control and control of gene expression during cell growth.

Indeed, E1A expression can alter the growth properties of cells. Introduction of the E1A gene into primary cell cultures can lead to immortalization (14). In combination with E1B or other oncogenes, E1A expression can transform cells to an oncogenic state (9, 23, 43, 49). Although a cellular counterpart of the E1A gene has not been identified, there is evidence for a cellular activity that can complement E1A mutants with respect to early transcription (15). Interestingly, the presence of this activity in F9 embryonal carcinoma cells, but not in differentiated F9 cells, correlates with the oncogenic state of the cells (46) and the presence of a transcription factor that is also activated by the viral E1A (41).

Here we report a search for additional cellular genes that may be controlled by E1A. Given the striking activation of the *hsp70* gene and the fact that this gene is part of a multigene family, we determined whether any of the other *hsp* genes are affected by E1A. In fact, two other *hsp* genes were induced by E1A, and at least two others were not. In addition, we found that the 12S E1A product can induce the *hsp* genes, a result with implications for the role of transcription induction is oncogenesis.

MATERIALS AND METHODS

Cells and virus. HeLa and 293 Spinner cultures were maintained in Joklik modified minimal essential medium supplemented with 5% fetal calf serum. Heat-shocked cultures were placed in a water bath at 42°C for 2 h. Monolayer cultures of HeLa cells were grown in Dulbecco modified Eagle medium containing 10% fetal calf serum. Wild-type (WT) and mutant adenovirus type 5 (Ad5) were grown in Spinner cultures as described previously (34). Mutant viruses *dl*312 (17) and *dl*1500 and *pm*975 (31, 32) were purified from infected 293 cells; WT adenovirus was prepared from HeLa cells. The particle concentration of purified virus stocks was determined by UV absorbance (34). Infectious focus-forming units were determined by indirect immunofluorescence by using rabbit antiserum directed against Ad5 late antigens (7, 47).

* Corresponding author.

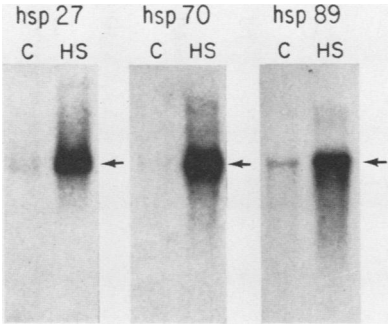

FIG. 1. Detection of specific and distinct heat shock RNAs. Shown is Northern blot analysis of mRNAs from normal (C) and heat-shocked (HS) HeLa cells probed with *hsp70* (pUR-HS), *hsp89* (pHS801), and *hsp27* (pHS208) cDNAs. RNA was isolated from suspension cultures grown at 37 or 42°C for 2 h, fractionated in a 1.2% agarose–formaldehyde gel, and transferred to nitrocellulose. Each lane contained 20 μg of total HeLa cell cytoplasmic RNA. Arrows indicate the heat-induced transcripts.

Infection of cells. For infection of suspension cultures of HeLa cells, the appropriate number of cells was collected by centrifugation at 600 × *g* for 5 min and suspended in minimal essential medium without serum at a concentration of 10^7 cells per ml. Virus was added at the appropriate multiplicity, and the suspension was continually mixed for 20 min at room temperature and then diluted to 5×10^5 cells per ml with fresh warm minimal essential medium containing 5% fetal calf serum. For infection of monolayer cultures, the medium was removed from plates with confluent growth and the cells were washed once with warm Dulbecco modified Eagle medium without serum. Virus was diluted in Dulbecco modified Eagle medium (no serum), and 0.5 ml was added to the plate (100 mm). After incubation of the plate at 37°C in a CO_2 incubator for 1 h, 10 ml of fresh complete medium was added.

RNA and transcription rate analyses. Cytoplasmic RNA was prepared by using 5% Nonidet P-40 at pH 8.6 as described previously (34). The procedure for Northern blot (RNA blot) analyses of purified RNA has been described previously (19, 48). Steady-state levels of RNA were quantitated by S1 nuclease protection assays (2). DNA probes were prepared by restriction enzyme digestion and end labeling: phosphatase treated 5′ termini were phosphorylated with [γ-^{32}P]ATP and T4 polynucleotide kinase (27), and 3′ termini were labeled by using the Klenow fragment of *Escherichia coli* DNA polymerase I (6). S1-nuclease-resistant DNAs were denatured and analyzed by 8.3 M urea–6% polyacrylamide gel electrophoresis. Adenovirus early gene E2 expression was assayed by using an [α-^{32}P]UTP uniformly labeled RNA probe (28). Hybrids were digested with RNase T_2 and analyzed on urea-polyacrylamide gels. Transcription assays of isolated nuclei were performed with virus-infected HeLa cells as described by Clayton and Darnell (4).

HeLa cell synchronization. HeLa cells grown in suspension were synchronized at the G_1-S phase boundary with a thymidine-aphidicolin block (10, 38). DNA synthesis was monitored by incorporation of [^{3}H]deoxycytidine as a function of time after release from treatment with aphidicolin.

RESULTS

Induction of *hsp89*. To assay the expression of the various *hsp* genes during an adenovirus infection of HeLa cells, we used a group of cDNA clones that detect mRNAs encoding two forms of hsp89 (α and β), hsp70, and hsp27 (12). A Northern blot analysis of RNA from control and heat-shocked HeLa cells that were probed with three of these cDNAs (*hsp89α*, *hsp*70, and *hsp27*) is shown in Fig. 1. Each cDNA detected a distinct mRNA whose abundance was substantially increased during heat treatment, including 2.95-kilobase *hsp89*, 2.6-kilobase *hsp70*, and 0.96-kilobase *hsp27* transcripts. Translation of hybrid-selected mRNAs has identified the product of each mRNA and thus the labeling as hsp89, hsp27, etc. (12, 19).

Using the cDNA clones, we determined whether any of these genes, besides *hsp70*, were stimulated in an adenovirus infection. Steady-state levels of the *hsp89α* mRNA increased in abundance in Ad5-infected HeLa cells at 7.5 h postinfection (Fig. 2), although the increase was somewhat less than that previously observed for the *hsp70* RNA. This increase appeared to be mediated by E1A, since *dl*312-infected cells did not contain elevated levels of *hsp89α* mRNA (Fig. 2A). In contrast to *hsp89α* and *hsp70* mRNAs, there appeared to be very little, if any, change in the abundance of *hsp27* mRNA. The time course of *hsp89* gene activation is shown in Fig. 2B. Once the increase in abundance occurred, the *hsp89* mRNA levels were maintained. This is in contrast to the rapid decline of *hsp70* mRNA after the peak of accumulation is reached (19). The same blot was also hybridized to the *hsp27* cDNA, and no induction was detected. On the basis of these results, we conclude that an adenovirus infection can stimulate other members of the *hsp* gene family, but not all.

E1A-mediated transcription induction. Based on our previous studies, it is clear that the activation of the viral genes by the E1A gene product is transcriptional (35). This is also true for the *hsp70* gene (19). We therefore determined whether the increase in *hsp89α* mRNA abundance was due to transcriptional activation. Nuclei were prepared from WT- or *dl*312-infected cells and incubated with [^{32}P]UTP. The *dl*312 infection was performed at a 100-fold higher multiplicity than was the WT infection to ensure equivalent

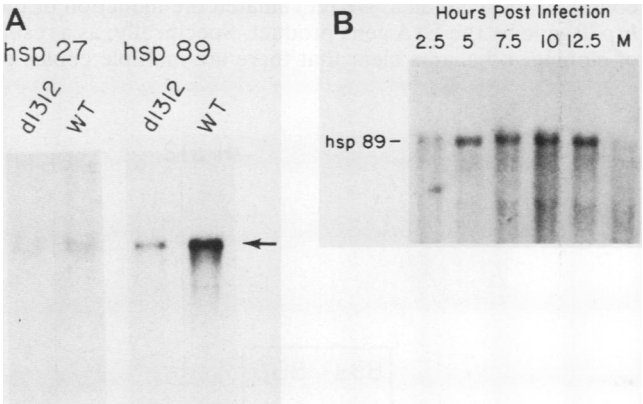

FIG. 2. Induction of *hsp89* mRNA during adenovirus infection. (A) HeLa cells were infected with 1,000 particles of WT or *dl*312 Ad5 per cell, and 5×10^7 cells of each were harvested 7.5 h later. Each lane contained 20 μg of total HeLa cell cytoplasmic RNA hybridized with ^{32}P-labeled pHS201 (*hsp27*) or ^{32}P-labeled pHS801 (*hsp89α*). The arrow indicates the induced *hsp89* transcript. (B) HeLa cells were infected with Ad5, and 5×10^7 cells was harvested at the indicated times after infection and compared with mock-infected cells (M). RNA samples (20 μg) were fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and probed with pHS801.

expression of early viral proteins other than E1A (36). The labeled RNA was then hybridized to filters containing *hsp70* and *hsp89* cDNAs. The increase in transcription of the *hsp70* gene was about 15-fold (Fig. 3). Transcription of the *hsp89α* gene was also increased by E1A, also approximately 15-fold. Thus, the increase in the abundance of the *hsp89α* mRNA (Fig. 2) was due to an increase in the transcription of the gene. Also shown in Fig. 3 is an assay for a distinct heat shock gene (*hsp89β*) encoding a different 89-kilodalton protein. cDNA clones of *hsp89α* and *hsp89β* do not cross hybridize. Clearly, the transcription of this gene was not affected by the adenovirus E1A gene product. On the basis of these results and those shown in Fig. 2, we conclude that at least two *hsp* genes (*hsp89β* and *hsp27*) are not induced by the E1A gene and at least two *hsp* genes are induced (*hsp89α* and *hsp70*).

Lack of cell cycle control of *hsp89*. Our previous experiments demonstrated that the *hsp70* gene, as detected with our cDNA clone, is regulated in growing HeLa cells in a cell-cycle-dependent fashion (18). Maximal *hsp70* expression was observed during the late S-early G_2 phase of the cell cycle (18). Similar results have been obtained after serum stimulation (56) and synchronization by selective mitotic detachment (29) of HeLa cells, although the peak of induction was somewhat earlier. Because the *hsp89α* and *hsp70* genes are both induced by E1A, we determined whether the *hsp89α* gene is also regulated during the cell cycle. RNA was prepared from HeLa cells synchronized by aphidicolin-thymidine block, fractionated in an agarose-formaldehyde gel, transferred to nitrocellulose, and probed with the *hsp89α* cDNA. The level of *hsp89α* RNA did not fluctuate as a function of release into the cell cycle (Fig. 4). These RNA samples did exhibit elevated levels of *hsp70* mRNA 13 h after release, at the S-G_2 boundary (data not shown). We conclude that unlike *hsp70*, which responds to cell cycle regulation and E1A control, *hsp89α* is apparently affected only by E1A (in addition to heat shock). In addition, the level of the *hsp27* transcript did not fluctuate as a function of the cell cycle (data not shown).

Multiple *hsp70* genes and control by E1A. During the course of these studies, we reevaluated the induction of the *hsp70* gene by the E1A gene product. Specifically, as a result of cloning, it became clear that there are multiple copies of

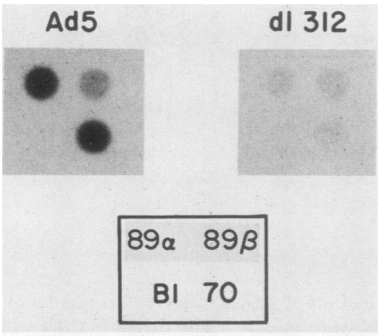

FIG. 3. E1A-dependent transcriptional activation of *hsp70* and *hsp89* during an adenovirus infection. HeLa cells were infected with WT Ad5 (100 particles per cell) or *dl*312 (10,000 particles per cell). Seven hours after infection, samples (10^8 cells) were harvested and nuclei were isolated. Transcription was measured as described in the text. The labeled nuclear RNA was hybridized to filters bearing 5 μg of pUR-HS (*hsp70*), pHS801 (*hsp89α*), pHS811 (*hsp89β*), and pBR322. The filters are labeled as follows: *hsp89α*, 89α; *hsp89β*, 89β; *hsp70*, 70; plasmid control, B1.

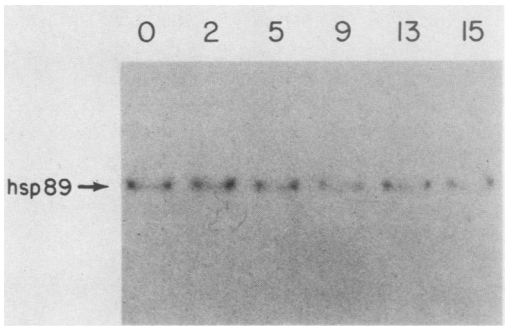

FIG. 4. Analysis of *hsp89* RNA levels during the cell cycle. HeLa cells were synchronized by a thymidine-aphidicolin block and then released by suspending the cells in fresh medium. At the indicated times (hours) after release, 5×10^7 cells were harvested and cytoplasmic RNA was prepared. RNA samples (20 μg) were then analyzed by Northern blotting; pHS801 (*hsp89*) was used for hybridization.

the *hsp70* gene in human cells, as is the case in *Drosophila* and yeast cells (5), and we questioned whether each of these genes was subject to E1A control. Two distinct genomic clones encoding hsp70 have been isolated (51, 53; B. J. Benecke, personal communication). One of the genomic clones (53) appears to be the gene encoding the transcript homologous to our cDNA, as judged by sequence analysis (H.-T. Kao, unpublished data). Furthermore, two *hsp70* mRNAs were revealed by S1 nuclease analysis with our *hsp70* cDNA clone (Fig. 5A). The cDNA was 3′ end labeled at an *Ava*I site and hybridized to control and heat shock RNA (left panel). A 410-nucleotide band represented full protection of the insert, including a portion of the poly(A) sequence, whereas the 325-nucleotide band apparently represented protection up to the poly(A) segment. We surmise that there was nicking by S1 at the poly(A) tail to give the 325-nucleotide band, particularly since the ratio of the intensity of the 410-nucleotide band to that of the 325-nucleotide band was variable. In addition to these apparent fully protected fragments, an 80-nucleotide band was also observed. The ratio of the amount of this product to that of the larger S1 product did not vary with the conditions of S1 treatment. Interestingly, the position of the end of the protection was close to the distance from the *Ava*I site to the end of the coding region of the transcript. It would thus appear that the cDNA detected two *hsp70* transcripts that diverge in sequence near the 3′ untranslated sequence. It is unlikely that the transcript giving rise to the 80-nucleotide S1 product is formed by alternative splicing or by use of an alternate poly(A) site since there is not a splice site consensus at the point of divergence or a poly(A) site signal. Rather, given the reproducibility of the results with S1, the position of cleavage with respect to the coding sequence, and the observation of multiple copies of *hsp70* genes detected with this clone (53; H.-T. Kao, unpublished data), it appears that this RNA is the product of a related but distinct gene. Each of these transcripts was induced by E1A (Fig. 5A, right panel). Furthermore, each of these transcripts was regulated during the cell cycle (Fig. 5A, center panel), as was previously shown for the total *hsp70* RNA population detected by the cDNA in a Northern blot analysis (18).

In addition to the two *hsp70* transcripts detected with the cDNA, there is at least one additional *hsp70* transcript. The gene isolated by Voellmy et al. (51) is distinct from the gene isolated by Wu et al. (53), since sequences at the 5′ ends of

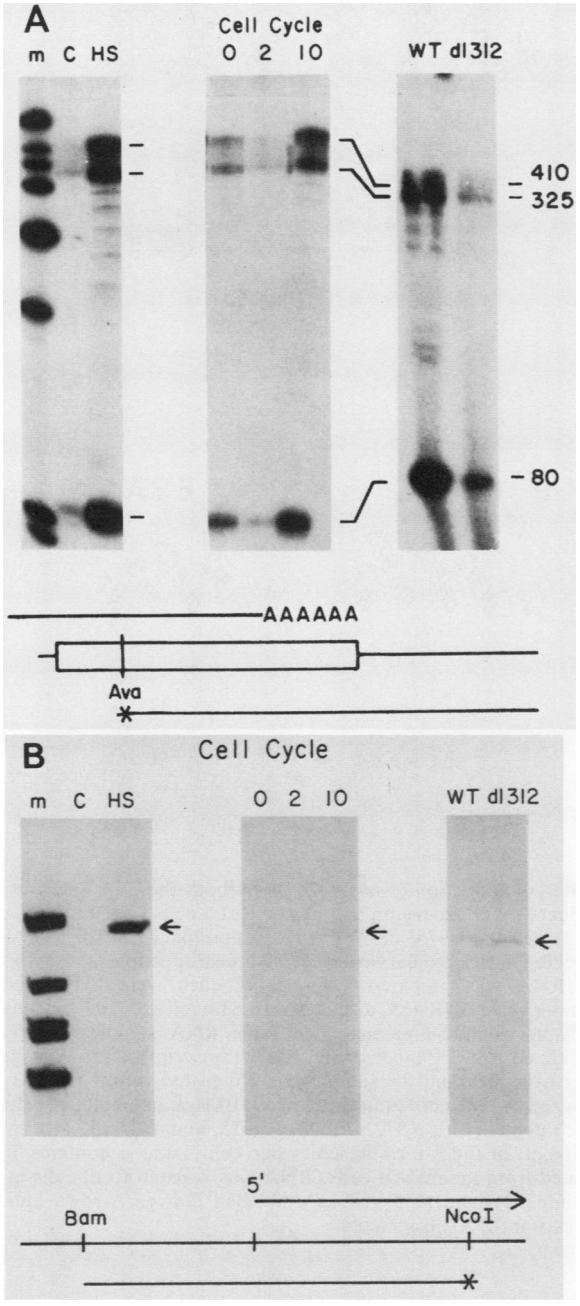

FIG. 5. Three distinct transcripts revealed by S1 nuclease analysis with *hsp70* DNA probes. (A) The *hsp70* cDNA probe pUR-HS was 3' end labeled at an *Ava*I site within the insert (bottom panel). Depicted in the left panel is hybridization of the labeled DNA to 10-μg samples of HeLa cell RNA from normal control cultures (C) and heat-shocked cells (HS). Depicted in the middle panel is hybridization of the probe to RNA from cells released for the indicated times from a thymidine-aphidicolin block. Depicted in the right panel is hybridization of the probe to RNA prepared from cells infected for 7 h with *dl*312 or WT Ad5. The positions of S1-nuclease-resistant DNA fragments of 410, 325, and 80 nucleotides are indicated. (B) An *hsp70* genomic clone (51) was 5' end labeled at an *Nco*I site which is 491 nucleotides from the RNA 5' end (bottom panel). The labeled DNA was hybridized to 10 μg of the same RNA samples used in panel A. The arrows indicate a 491-nucleotide protected fragment.

the two genes do not match. We used the genomic clone of Voellmy et al. (51) to assay for *hsp70* RNA in adenovirus-infected cells (Fig. 5B). A 5'-end-labeled probe for S1 nuclease analysis was prepared to allow distinction between this *hsp70* transcript and related transcripts. The probe clearly detected a heat-inducible mRNA (left panel). However, when the probe was hybridized to RNA from a WT or *dl*312 adenovirus infection, no induction was observed (right panel). Futhermore, analysis of cell cycle RNA samples (middle panel) revealed barely detectable levels of *hsp70* transcripts and no increase in expression. Thus, we conclude that there are at least three functional *hsp70* genes in HeLa cells, two of which are induced by E1A and during late-S-phase cell growth and one of which is unaffected.

***hsp* gene induction by individual E1A products.** The E1A gene actually encodes three distinct products by virtue of the production of three differentially spliced mRNAs (40). The product of the smallest RNA (9S) is probably not important for transcriptional control, since it is only detected late in a productive infection (44) well after the time when transcription of viral genes, as well as the *hsp* genes, is activated. Furthermore, adenovirus early genes E2 and E3 are not transcribed by mutant viruses producing only the 9S E1A mRNA or when cotransfected with the E1A 9S cDNA (33, 58). The two larger mRNAs (13S and 12S) are spliced in the same reading frame and produce proteins with identical N termini and C termini. They differ by the presence of an additional 46 amino acids in the 13S product. Extensive analyses of mutants have demonstrated that the larger E1A protein (289 amino acids), the product of the 13S RNA, is mainly responsible for transcriptional activation of early viral genes, although the smaller protein (243 amino acids) does possess some activity (8, 24, 25, 31, 33, 52). To determine the E1A requirements for activation of transcription of an endogenous chromosomal gene, in this instance an *hsp70* gene, we measured *hsp70* RNA levels in cells infected with the Ad5 mutants *pm*975 ($13S^+$ $12S^-$), *dl*1500 ($13S^-$ $12S^+$), and *dl*312 ($13S^-$ $12S^-$). As shown in two experiments (Fig. 5), the *hsp70* mRNA was increased ~20-fold in WT-infected cells compared with *dl*312-infected cells. In addition, a similar stimulation of *hsp70* gene expression was observed in *pm*975-infected cells. Thus, as is true for the early viral genes, the 13S product alone appears to be as functional in transcriptional stimulation as the combination of 13S and 12S products. Strikingly, *dl*1500 (12S only) also induced the expression of *hsp70* mRNA. The data obtained from four separate experiments with the Ad5 mutants *pm*975, *dl*1500, and *dl*312 are presented in Table 1. The induction by *dl*1500 was often somewhat less than that by the WT or *pm*975 but was clearly and reproducibly above that by *dl*312. Furthermore, results with a 3'-end-labeled probe indicated that transcription of both of the E1A-responsive *hsp70* genes (Fig. 5) was increased in Ad5 *dl*1500-infected cells (data not shown). *hsp89*α mRNA levels were also increased in cells infected with Ad5 *dl*1500 (data not shown). These results are in contrast to those for the induction of early viral genes: previous experiments have shown that the 12S product was much less efficient. This was shown for the E2A gene by using RNA from the same experiment. The induction of E2A in either a WT or *pm*975 infection was on average 50 times greater than that in a *dl*1500 infection (Fig. 6). As also seen by others (52), we reproducibly observed an increase in E2 RNA in a *dl*1500 ($12S^+$) infection over that in a *dl*312 infection, indicating that the 12S product retains some inducing activity, even for the viral genes. However, the clear result from these experiments was the differential

TABLE 1. Induction of *hsp70* RNA and E2A RNA by infection with WT and E1A mutant viruses

Expt	Virus	RNA level (fold)[a]	
		hsp70	E2A
1	*dl*312	1	1
	*dl*1500	9	2
	*pm*975	12	250
	WT	20	380
2	*dl*312	1	1
	*dl*1500	8	10
	*pm*975	17	145
	WT	28	125
3	*dl*312	1	1
	*dl*1500	20	13
	*pm*975	17	370
	WT	17	418
4	*dl*312	1	1
	*dl*1500	13	8
	*pm*975	16	320
	WT	27	280

[a] RNA levels were determined by densitometric scanning of films of S1 nuclease analyses. Experiments 1 and 3 are depicted in panels A and B of Fig. 6.

induction of the *hsp*70 gene, since the 12S product was nearly as efficient as the 13S product.

DISCUSSION

As documented here and elsewhere (19, 45), the adenovirus E1A gene can stimulate transcription of cellular chromosomal genes. Although several of the E1A-induced genes are also induced by heat shock, the E1A response is not just another form of "stress," since not all of the heat shock genes are induced by E1A. We identified at least three members of the *hsp* gene family, an *hsp89* gene (*hsp89*β), an *hsp70* gene, and an *hsp27* gene, that were heat inducible but did not respond to an adenovirus infection. Thus, the two activation mechanisms, E1A and heat shock, appear to be quite distinct. This is further suggested by the observation that heat shock cannot replace E1A in the activation of viral genes (M. J. Imperiale and J. R. Nevins, unpublished data). Heat shock promoters are complex, containing several distinct genetic elements and sites for transcription factor interaction. The human *hsp70* gene responds to a variety of stimuli, including serum, heavy metals, and polyomavirus large tumor antigen, and different elements of the promoter have been shown to be responsible (20, 55). Heat shock induction involves the activation of a particular factor that interacts with the conserved control element (37). Based on all of these results, it seems most likely that different factors are involved in E1A control; this was borne out by our experiments with the same *hsp70* gene. A heat shock control element (30, 39) is found at position −110 relative to the transcription initiation site of the human *hsp70* gene, and plasmids that contain this sequence are both E1A and heat shock inducible. Deletion of these sequences, leaving only 77 nucleotides of upstream sequence, eliminates the heat shock response but leaves the E1A inducibility intact (M. C. Simon et al., in preparation).

A major impetus for the study of cellular transcription control by the E1A gene product is the fact that the E1A gene has profound effects on cellular growth regulation. E1A

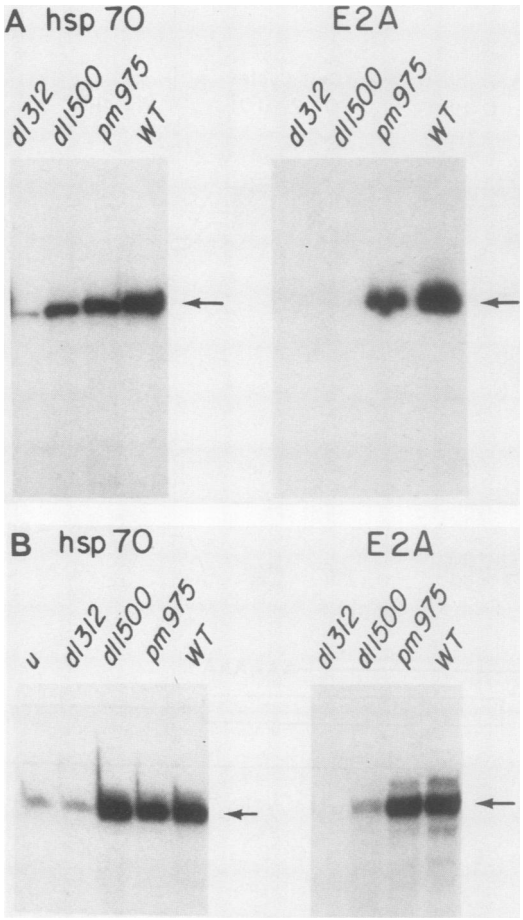

FIG. 6. Induction of *hsp70* RNA by both the E1A 13S and 12S products. (A) Suspension cultures of HeLa cells were infected with *dl*312, *dl*1500, *pm*975, and WT Ad5 at a multiplicity of 1,000 particles per cell. Cells were harvested at 7.5 h postinfection, and cytoplasmic RNA was prepared. Samples of each were hybridized to 5′-end-labeled pUR-HS, digested with S1 nuclease, and analyzed in a 6% acrylamide–urea gel. The same RNA samples were also hybridized to a ^{32}P-labeled SP6 RNA transcript of the E2A gene. After hybridization, the samples were digested with T_2 RNase and analyzed in a 6% acrylamide–urea gel. (B) HeLa monolayer cultures were infected with *dl*312, *dl*1500, *pm*975, and WT Ad5 at a multiplicity of 20 focus-forming units per cell. Lane u contains RNA isolated from uninfected cells. RNA was isolated from cells at 7 h postinfection and analyzed as described above. Arrows indicate S1-resistant DNA fragments.

together with E1B or a number of other viral and cellular oncogenes can transform cells to an oncogenic state (9, 43, 49). E1A alone can generate immortalized cell lines from primary cultures (14). It might be anticipated that the transcriptional regulatory activity of E1A with respect to cellular genes might be part of this process. For instance, a role for E1A-mediated transcriptional repression in adenovirus transformation has been suggested (25, 33). The E1A proteins can repress the activity of the simian virus 40, polyomavirus, or Ad2 E1A enhancers in transfection experiments (3, 50) and the transcription of endogenous immunoglobulin genes (11). Several transformation-defective E1A mutants have been identified that are positive for induction of the E2, E3, and E4 mRNAs, as well as the human β-globin gene cloned into the adenovirus genome (25, 33). However,

these transformation-defective mutants failed to repress a gene linked to the simian virus 40 enhancer, as well as endogenous cellular genes. Consequently, viral transformation and transcription repression appear to be linked.

The analyses of the transformation-defective mutants described above indicate that a function other than normal transactivation must be involved in transformation. However, these studies do not rule out the involvement of transactivation. More to the point are various studies that show that the 12S product can effect immortalization and at least partial transformation in the absence of the 13S product. Since the 13S product is necessary for normal viral transcription activation, it has been suggested that the E1A transcription-inducing activity is not required for transformation (25, 31). However, the results presented in this report must alter these arguments since it was found that the *hsp70* gene can be induced by the 12S E1A gene product. In some experiments, the level of induction was not as great as with the 13S product but was certainly well above that in the complete absence of E1A (*dl*312). Perhaps this is the reason for the apparent discrepancy between our findings and a previous report that suggested no stimulation of the *hsp70* gene by the 12S E1A product (54). In the experiments of Wu et al. (54), the induction of the *hsp70* gene by WT virus was only seven- to eightfold above that by *dl*312. Thus, stimulation that was 30% of that of the WT would be only two- to threefold above background and could easily be overlooked. In our experiments, the stimulation of *hsp70* by WT virus was 20- to 30-fold greater than that in a *dl*312 infection. Under these conditions, the 12S-mediated stimulation was clearly evident. It is also evident based on the results presented in Fig. 5 and Table 1 that the 12S product can also effect an inefficient stimulation of E2 transcription. In most experiments, there was a clear increase of E2 RNA in a *dl*1500 infection compared with that in a *dl*312 infection. This is consistent with other studies that demonstrated an induction of viral transcription by the 12S E1A product (8, 24, 52). We conclude that the 12S E1A gene product does possess transcription-inducing activity but that the efficiency of this activity depends on the target gene. The *hsp70* gene may not be the only example of E1A-12S-mediated stimulation since it was recently shown that there is an increase in synthesis of the proliferating cell nuclear antigen in either 12S or 13S virus infection of primary baby rat kidney cells (59). Of potential interest is the fact that these two genes induced by the 12S E1A mutant (if indeed proliferating cell nuclear antigen gene induction is transcriptional) are also induced by an addition of serum to starved cultures. Possibly the induction of these genes is related to the E1A function responsible for stimulation of DNA synthesis in quiescent cells, which also localizes to the 12S E1A product.

Obviously, these findings have potential significance for the role of E1A in oncogenesis. It could be imagined that E1A is responsible for the activation or repression or both of certain key cellular genes, the products of which allow for continued cell proliferation. Perhaps the best suggestion of such a role is the relationship between the E1A-like transcriptional control in F9 embryonal carcinoma cells and the oncogenic state of these cells. F9 cells are tumorigenic and exhibit an E1A activity, whereas F9 cells differentiated with retinoic acid and cyclic AMP are not tumorigenic and have lost the transcriptional regulatory activity (15, 46). Unfortunately, we still do not know whether the activation or repression of any of the cellular genes affected by E1A is important for the process of immortalization or transformation. These arguments are based only on correlations and cannot be viewed as conclusive. Ultimately, it will be necessary to show that the expression of one of the E1A-induced cellular genes is essential for the transformed state.

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