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Transcriptional Activation and Subsequent Control of the Human Heat Shock Gene During Adenovirus Infection

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A cDNA copy of the major human heat shock mRNA was cloned. The clone is complementary to the mRNA encoding the major 70-kilodalton heat shock protein as shown by hybrid arrest translation. We utilized the cloned DNA to measure induction of the gene during adenovirus infection. The mRNA increases in abundance approximately 100-fold during a wild-type adenovirus infection but does not increase more than 2-fold during an infection in which there is no E1A gene function [high multiplicity of infection of an E1A (−) mutant]. Furthermore, by measuring transcription in isolated nuclei, we found that the induction was transcriptional and was mediated by the E1A gene product. The induction was not maintained, however. After a peak level was obtained, transcription returned to preinfection levels. This decline was also reflected in the cytoplasmic mRNA abundance indicating a rapid turnover of the heat shock mRNA. This rapid turnover of the heat shock mRNA appears to be induced by the viral infection since the heat shock mRNA was found to be stable when synthesized in an adenovirus-transformed cell line.

The study of gene control necessitates the use of a particular gene system that is amenable to study and that is, of course, subject to control in a defined manner. The use of viral systems to study gene regulation offers certain advantages over that of cellular systems, principally due to the availability of well-defined genomes and gene products as well as to the availability of mutants defective in functions involved in the regulatory events. Adenovirus mutants have revealed regulatory events during the lytic infection of HeLa cells. During early infection, five viral transcription units are activated through the action of a *trans*-acting viral protein, the E1A gene product (5, 15). The mechanism for the induction appears to be transcriptional (21), although there may also be post-transcriptional effects (16). Such systems of viral gene control are limited, however, with respect to the question of how representative such inducible or repressible genes may be of the eucaryotic cell in general. However, we have recently described an induction of the synthesis of a cellular protein through the action of the E1A gene product of adenovirus (22). This protein of 70 kilodaltons (kd) is also induced in HeLa cells by a heat shock and is related to the major heat shock protein of *Drosophila* sp. (for a review, see reference 2).

Our previous studies have mainly utilized measurements of protein synthesis to demonstrate an induction of the heat shock gene. Given the known mechanism of induction of the adenovirus genes, as mediated by the E1A protein, it was thus essential to clearly establish at what level the heat shock expression was being activated. To answer these questions, we proceeded to clone a cDNA copy of the human heat shock mRNA. The clone detects a major mRNA species of 2.7 kilobases (kb) in heat-shocked HeLa cells. We utilized the cDNA clone to investigate the expression of the gene during adenovirus infection. We found that the specific action of the adenovirus E1A gene indeed induces the heat shock gene at the level of transcription. Of further interest is the continued control of the expression of the gene after induction. Transcription is tightly regulated such that after the initial induction there is a decline back to preinduced levels. In addition, the stability of the mRNA appears to be tightly controlled during the viral infection.

MATERIALS AND METHODS

Cells and virus. HeLa cells and 293 cells were maintained in Spinner cultures in Joklik-modified minimal essential medium containing 5% fetal calf serum. Monolayer cultures of HeLa, 293, and human embryonic kidney (HEK) cells were maintained in Dulbecco modified Eagle medium containing 10% fetal calf serum. The growth and purification of wild-type adenovirus type 5 (Ad5) and *dl*312 have been described previously (20, 21).

Cloning of the heat shock mRNA. Initially, a cDNA library was constructed essentially as described by

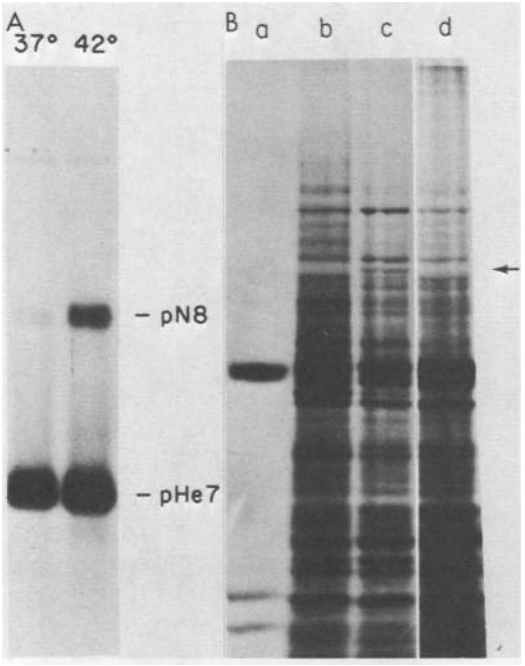

FIG. 1. (A) Northern blot analysis of mRNA from normal HeLa cells and heat-shocked HeLa cells and probed with a specific cDNA clone. Poly(A)$^+$ RNA was prepared from HeLa cells grown at 37°C or grown at 42°C for 3 h; 2 μg of each was then separated in a 1.2% agarose-formaldehyde gel. The RNA was then transferred to nitrocellulose and hybridized with a mixture of two ^{32}P-labeled cloned probes: pN8 is the heat-shock-specific clone, and pHe7 is a clone to a common HeLa mRNA. (B) Hybrid-arrest translation employing the heat shock mRNA and the heat shock cDNA clone. Poly(A)$^+$ RNA, prepared from control and heat-shocked HeLa cells, was translated in vitro in a rabbit reticulocyte lysate. In addition, a sample of the heat shock RNA was hybridized to the heat shock clone, pHSG, and then translated in vitro. Translation products were analyzed in a 10% acrylamide-sodium dodecyl sulfate gel followed by fluorography. The arrow indicates the position of the 70-kd heat shock protein. Lane a, endogenous translation; lane b, translation of a normal HeLa mRNA; lane c, translation of heat shock mRNA; lane d, translation of hybridized heat shock mRNA. Exposure for lanes a through c was for 1 day; exposure for lane d was for 4 days.

Norgard et al. (23) by using mRNA from early adenovirus-infected cells. RNA was prepared from approximately 8×10^8 HeLa cells infected with adenovirus type 2 (Ad2) (5,000 particles per cell) for 7.5 h. Approximately 100 μg of polyadenylated [poly (A)$^+$] RNA was used to prepare double-stranded cDNA and then tailed with dCMP as described previously (23). The deoxyribosylcytosine-tailed cDNA was annealed to deoxyribosylguanosine-tailed, *Pst*-digested pBR322 and used to transform *Escherichia coli* RR1. Colonies were screened by a method described previously (12) with a ^{32}P-labeled cDNA probe enriched in heat shock mRNA sequences. Poly(A)$^+$ RNA was prepared from heat-shocked HeLa cells and fractionated by sucrose gradient centrifugation, and then the fractions were translated in vitro in a reticulocyte lysate. Those gradient fractions enriched in the heat shock mRNA were then used to synthesize the ^{32}P-labeled cDNA probe. Positive colonies were picked, and plasmid DNA was prepared. This DNA was labeled by nick translation (27) and used to probe an RNA gel blot of control and heat shock mRNA. One clone (pN8) detected a 2.7-kb RNA that was induced by heat shock. This clone had an insert size of 150 nucleotides.

To obtain a clone with a larger insert size, a cDNA library was prepared by using a modification of the technique described previously (25). The RNA for cloning was from 293 cells infected with the Ad5 mutant *ts*125 for 12 h at 42°C. Under these conditions, the heat shock mRNA represents a major constituent of the poly(A)$^+$ RNA fraction. The procedures for cloning were as described, except that the linker molecule was a *Hin*dIII digest of pBR322 tailed at the *Pst* site with deoxyribosylguanosine residues. Colonies were screened with an M13 subclone of the pN8 insert. DNA was prepared from a positive colony and assayed for specific hybridization in an RNA gel blot as described above. The specific isolated clone was termed pHSG and had an insert size of 1,000 nucleotides.

Hybrid-arrest translation. Poly(A)$^+$ RNA was obtained from heat-shocked and normal HeLa cells. The heat shock RNA (10 μg) was hybridized to 0.2 μg of *Pvu*II-digested pHSG in 10 mM piperazine-*N*,*N*'-bis(2-ethanesulfonic acid), pH 6.4–400 mM NaCl–2 mM EDTA–80% formamide in a 130-μl volume. The mixture was heated to 65°C for 10 min and then incubated at 54, 52, 50, and 48°C for 1 h each. The solution was then made to 0.2 M with sodium acetate and ethanol precipitated four times. The final pellet was washed in 80% ethanol and dried. The RNA was dissolved in water and translated in vitro with a rabbit reticulocyte lysate. Products were analyzed by electrophoresis in a 10% acrylamide-sodium dodecyl sulfate gel (18) followed by fluorography.

Preparation and analysis of RNA. The procedures for the preparation of cytoplasmic RNA and the selection of poly(A)$^+$ RNA have been described previously (20). The analysis of RNA by agarose-formaldehyde gel electrophoresis has been described (7), as well as the procedures for transfer to nitrocellulose (29).

Isolated nuclei transcription assays. The procedures for transcriptional rate measurements with isolated nuclei have been described previously (7).

RESULTS

Isolation of a cDNA clone specific for the 70-kd heat shock protein mRNA. Our long-term interest in these studies was to isolate and characterize any and all cellular genes induced as a result of the action of the adenovirus E1A gene product. We initially chose to directly screen for a clone specific for the heat shock mRNA, as it was clear from our previous experiments that this particular mRNA should be induced by adenovirus (22). Such a clone was obtained, as described above, and is demonstrated in Fig. 1A employing the clone in hybridization to a North-

ern blot of mRNA from HeLa cells grown at 37°C or mRNA from HeLa cells heat shocked at 42°C. The pN8 clone detected a 2.7-kb mRNA that was increased in abundance approximately 20- to 50-fold by the heat shock. As a control, the blot was also hybridized with a HeLa cDNA clone (pHe7) that did not respond to the heat shock; clearly, the abundance of this mRNA does not change.

Our previous experiments involved the identification of the 70-kd heat shock protein as the one induced by adenovirus (22). We therefore determined, utilizing hybrid-arrest translation, whether the mRNA detected by pHSG indeed encoded the 70-kd major heat shock protein. Poly(A)$^+$ mRNA was prepared from HeLa cells grown at 37°C or heat shocked at 42°C and translated in vitro in a rabbit reticulocyte lysate. As shown in Fig. 1B, the synthesis of the 70-kd heat shock protein is the major difference between the translation products of the normal HeLa mRNA (Fig. 1B, lane b) and the heat shock mRNA (Fig. 1B, lane c). We then hybridized the heat shock mRNA with pHSG and analyzed the translation products. As shown in Fig. 1B, lane d, the translation of the 70-kd protein was abolished, with no effect on the translation of the other proteins. It therefore appears certain that pHSG is indeed a cDNA clone of the mRNA encoding the 70-kd heat shock protein.

Induction of the heat shock mRNA during adenovirus infection. With a specific probe in hand, we were able to measure directly the effect of adenovirus infection on the production of the heat shock mRNA. We compared the cytoplasmic abundance of the heat shock mRNA with that of another HeLa mRNA for which we possessed a cDNA clone. The RNAs from these infections were fractionated by agarose-formaldehyde gel electrophoresis, transferred to nitrocellulose, and then hybridized with the pHSG probe (Fig. 2A). The abundance of the heat shock mRNA increased dramatically as a result of adenovirus infection. Surprisingly, however, the level of the heat shock mRNA then fell such that by 13 h postinfection there was only 9% of the maximal amount remaining in the cells. In contrast, the concentration of the mRNA complementary to pHe7 did not change through the course of the experiment. In particular, there was no decline in the abundance at a time when the concentration of the heat shock mRNA dropped rapidly. Therefore, the control of the abundance of the heat shock mRNA is specific and not a general occurrence. In addition to the major 2.7-kb RNA species, there are reproducibly two minor RNA species of 2.3 and 1.5 kb induced by adenovirus infection, as well as by heat shock, that are detected with the pHSG probe. At the present time we do not know the relatedness of these three RNA species. They may represent transcripts of a gene family, particularly in view of the organization of the genes in *Drosophila* sp. (2), or they might also represent products of a single transcript

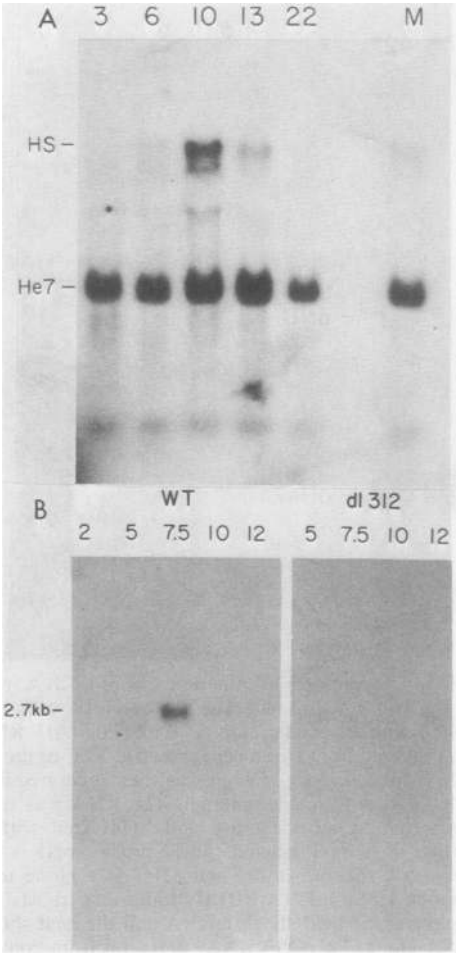

FIG. 2. (A) Specificity of the induction of the heat shock mRNA during adenovirus infection. HeLa monolayer cultures were infected with Ad5 (100 particles per cell), and samples were taken at the indicated times (in hours) to prepare cytoplasmic RNA. Equal amounts of cytoplasmic RNA (50 μg) were subjected to electrophoresis in an agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized with a mixture of ^{32}P-labeled pN8 (HS) and pHe7 (He7). Lane M, Mock-infected sample. (B) Induction of the heat shock mRNA during adenovirus infection. HeLa cells were infected with wild-type (WT) Ad5 (100 particles per cell) or with *dl*312 (10,000 particles per cell), and then samples of 5×10^7 cells were taken at the indicated times. Total cytoplasmic RNA (50 μg) was analyzed by agarose-formaldehyde gel electrophoresis. After transfer to nitrocellulose, the 2.7-kb heat shock mRNA was visualized by hybridization to ^{32}P-labeled pN8.

generated by processing. It is, however, possible that as many as three genes, related in sequence, are induced during adenovirus infection.

In a separate experiment, HeLa cells were infected with wild-type adenovirus or with the E1A mutant *dl*312, and at various times postinfection samples were removed and cytoplasmic RNA was prepared. Furthermore, the *dl*312 infection was carried out at a multiplicity that was 100-fold higher than the wild-type infection. The rationale for such a protocol is as follows. Under these conditions of a high-multiplicity *dl*312 infection there is a normal level of production (as compared with wild-type levels) of the various early viral gene products (22), and in fact, a wild-type yield of *dl*312 virions is produced (28). Presumably, there is still an inefficient level of transcription from the various early genes, but now from many more templates. Therefore, when comparing the two infections (wild-type versus high-multiplicity *dl*312), the only difference is the absence of the E1A gene products in the *dl*312 infection. One can then assess the requirement for this viral gene product in the induction of the heat shock gene. As shown in Fig. 2B, the level of the heat shock mRNA rose rapidly in the wild-type infection such that by 7.5 h postinfection there was a nearly 100-fold increase in the concentration of the mRNA. In this particular experiment, Spinner cultures of HeLa cells were employed rather than monolayers as in Fig. 2A. As a result, faster kinetics of infection were obtained. In contrast to the wild-type infection, the abundance of the heat shock RNA did not change significantly in the *dl*312-infected cells. The high multiplicity of infection does not itself prevent induction since a similar infection with wild-type virus still results in the increase in the heat shock mRNA (data not shown). There was a modest increase of approximately twofold in the abundance of the RNA, which was also seen in mock-infected cultures and is apparently due to the manipulations of the experiment (centrifugation and resuspension of the cells). Finally, assay of the pHe7 complementary mRNA in a *dl*312 infection revealed no change in abundance (data not shown).

Transcriptional induction of the heat shock gene mediated by the E1A gene product. The function of the adenovirus E1A gene product during a viral infection is to induce the expression of the five early viral transcription units (5, 15). Furthermore, the mechanism of this induction appears to reside at the transcriptional level (21), although there is evidence suggesting a post-transcriptional role as well (16). If the induction of the heat shock gene by the E1A protein was part of the same phenomenon as the induction of the viral genes, then it clearly was important to assess directly the level of induction of the gene, whether it was transcriptional or not. We therefore measured transcription directly by allowing isolated nuclei to label nascent RNA in vitro. Such an assay provides a reasonable measure of the primary transcription rates of a gene (7, 14, 19, 30). For the experiment, HeLa cells were either mock infected, infected with wild-type Ad5, or infected with the E1A deletion mutant *dl*312. Once again, the *dl*312 infection was at a 100-fold higher multiplicity than the wild-type infection. The experiment then involved preparing nuclei from the three infections at various times postinfection and labeling the nascent RNA by incubation of the nuclei in a reaction mixture containing [^{32}P]UTP. The labeled RNA was then hybridized to plasmid DNAs that were spotted on nitrocellulose, and RNase-resistant hybrids were scored by autoradiography. Transcription of the heat shock gene was barely detectable in the mock-infected cells (Fig. 3). The transcrip-

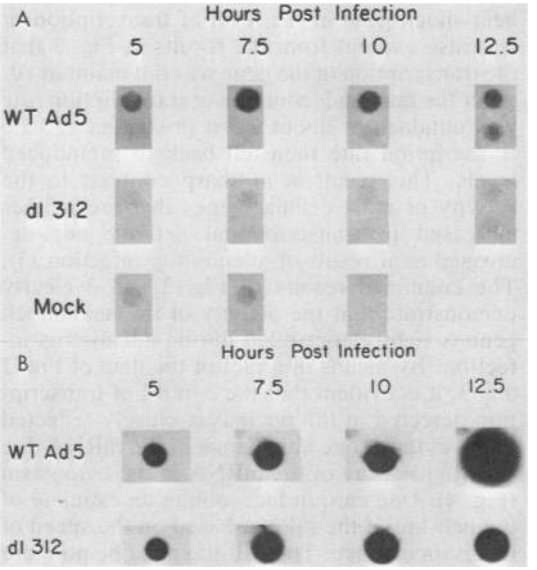

FIG. 3. Transcriptional activation and control of the heat shock gene. Samples of HeLa cells were either mock infected, infected with wild-type (WT) Ad5 (100 particles per cell), or infected with *dl*312 (10,000 particles per cell). At the indicated times postinfection, samples of 1×10^8 cells were removed, nuclei were prepared, and in vitro transcription carried out as described in the text. The labeled nuclear RNA was then hybridized to filters containing pHSG and pBR322 or to filters containing pE2 (a probe for the adenovirus E2 gene) and pBR322. After washing and treatment with RNase, the hybrids were used to expose X-ray film. (A) Hybridization to pHSG (upper dot) and pBR322 (lower dot). (B) Hybridization to pE2.

tion of the gene in the wild-type Ad5-infected cells increased approximately 20-fold by 7.5 h postinfection. Thus, it is clear that the induction of the heat shock gene as seen by mRNA analysis is largely due to an increased transcription rate of the gene. Furthermore, the results of the *dl*312 infection clearly show that this increased transcription rate of the heat shock gene in wild-type Ad5 infection is due to the action of the E1A gene product since in the absence of the gene in the *dl*312 infection there was no greater induction of the heat shock transcription than occurred in the mock infection. And, it was clear that the high-multiplicity *dl*312 infection was effective as suggested before. That is, the E2 gene was transcribed at the same rate in the *dl*312-infected cells as in the wild-type infected cells (Fig. 3B); the same was true for the other early genes (data not shown). Thus, the absence of induction of the heat shock gene in *dl*312 infection must be due to an absence of E1A function.

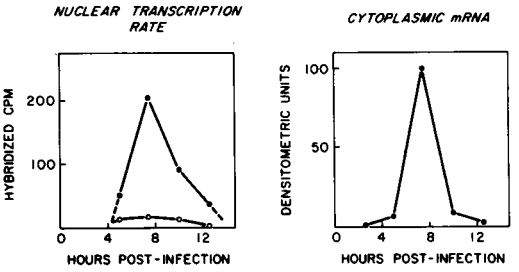

FIG. 4. Control of the expression of the heat shock gene during adenovirus infection. The data of Fig. 2 and 3 have been quantitated and plotted. For transcription rates, the hybridizations depicted in Fig. 3 were cut out and counted by scintillation counting. The hybridized counts per minute above background hybridization to pBR322 DNA was plotted for wild type (●) and *dl*312 (○). To obtain the plot for cytoplasmic mRNA abundance, the autoradiogram depicted in Fig. 2B was scanned with a densitometer, and the relative values were plotted.

Control of the expression of the heat shock gene during adenovirus infection. In addition to the observation that the E1A protein induces the heat shock gene at the level of transcription, it was also evident from the results in Fig. 3 that the transcription of the gene was not maintained. After the maximal induction of transcription rate was obtained at about 7.5 h postinfection, the transcription rate then fell back to preinduced levels. This result is in sharp contrast to the activity of most cellular genes that are neither increased in transcriptional activity nor decreased as a result of adenovirus infection (3). The combined results of Fig. 2 and 3 clearly demonstrate that the activity of the heat shock gene is tightly controlled during adenovirus infection. By means of a plot of the data of Fig. 2 and 3, it is evident that the control of transcription detected in the nucleus is closely reflected in the cytoplasmic abundance of the mRNA due to a rapid decay of the mRNA in the cytoplasm (Fig. 4). One can, in fact, obtain an estimate of the half-life of the mRNA based on the speed of its disappearance. The half-life must be no more than 40 min and likely is even shorter. This half-life, in fact, approaches that measured for the early adenovirus RNAs in a lytic infection (4, 31).

Heat shock RNA is abundant in an adenovirus-transformed cell line that expresses E1A. The adenovirus-transformed cell line 293 was created by transformation of HEK cells with fragmented Ad5 DNA (10). These cells constitutively express the E1A and E1B genes (1), and thus, one would expect a continual induction of the heat shock gene. In fact, we previously have shown that these cells were synthesizing increased levels of the protein as compared with the level synthesized in HeLa cells (22). This fact is even more dramatically illustrated by a direct measurement of the heat shock RNA levels by Northern blot (Fig. 5). RNA was prepared from 293 or HEK cells, and equal amounts were analyzed by agarose-formaldehyde gel electrophoresis. The level of the heat shock RNA in 293 cells is at least 100-fold higher than in the HEK cells, as we simply cannot detect the presence of the RNA in the HEK cells.

Control of the stability of the heat shock mRNA. In addition to defining an induction of the heat shock gene, the data of Fig. 4 clearly demonstrate that the heat shock mRNA decays with a very rapid half-life in the adenovirus-infected cells. Is this rapid decay of the heat shock mRNA an intrinsic property of the mRNA itself, or is the decay induced as a result of the viral infection? To answer this question, we measured the stability of the heat shock mRNA in the absence of a viral infection. This was done by utilizing the 293 cells where the endogenous synthesis of the mRNA is relatively high such that the metabolism of the RNA can be measured. Specifically, we wanted to avoid any circumstance such as heat shock which might conceivably alter the stability of the RNA. To determine the half-life of the mRNA in 293 cells, we utilized the assay of measuring the kinetics of labeling of the RNA to a steady state. This procedure, originally described by Greenberg (11), provides an accurate estimation of the stability of an RNA and avoids the use of inhibitors to effect a pulse-chase. The cytoplasmic stability of an RNA is reflected as the time it requires to label the RNA to a steady-state condition given a constant rate of delivery to the

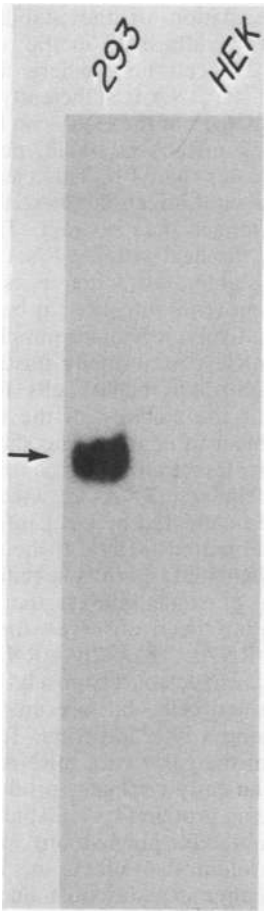

FIG. 5. Abundance of the heat shock mRNA in adenovirus-transformed 293 cells. Cytoplasmic RNA was prepared from 293 cells and from HEK cells. Fifty micrograms of each was subjected to electrophoresis, and the heat shock mRNA was visualized by hybridization after blotting to nitrocellulose.

cytoplasm. Such a measurement is shown in Fig. 6 for the heat shock mRNA synthesized in the 293 cells, as well as for an additional HeLa mRNA, the mRNA complementary to pHe7 that we have previously found to be stable in HeLa cells (3; also see Fig. 2). The heat shock mRNA accumulates linearly in the cytoplasm, as does the mRNA complementary to pHe7, throughout the period of the experiment—8 h in all. Therefore, the half-life of the heat shock mRNA must be greater than 4 to 5 h; otherwise, a steady state in labeling would have easily been reached within the time of the experiment. It would thus appear that the heat shock mRNA is not intrinsically short lived but rather that the rapid decay observed in the adenovirus-infected cells must be a property of the viral infection.

DISCUSSION

The results presented in this paper demonstrate that a functional E1A gene of adenovirus is required for the induction of the heat shock gene in HeLa cells. It is important to keep in mind that it is not merely a general adenovirus infection that induces the heat shock gene. Rather, the induction is the result of the action of a specific viral gene product. Thus, a defined inducer of the heat shock gene is identified, an inducer that can be analyzed and followed in the cell. Whether the E1A gene product alone is sufficient for induction or requires other viral or cellular components is, of course, not yet clear. This is not the only instance of induction of the heat shock protein by a virus infection. Recent reports have shown that herpes simplex virus (17), Newcastle disease virus (8), and simian virus 40 and polyomavirus (24) each induce an increased synthesis of the 70-kd heat shock protein. It is still to be determined, however, whether in these instances the induction is due to the action of a specific viral gene product.

What then is common among these genes (early adenovirus and heat shock) so as to allow coordinate induction? We have previously suggested that the E1A protein was not a direct activator of transcription in the sense of recognizing and binding to promoter sequences (9, 21). Instead, the activation of the viral genes by the protein appears to be indirect, involving an interaction with components of the host cell. This point is best illustrated by the fact that

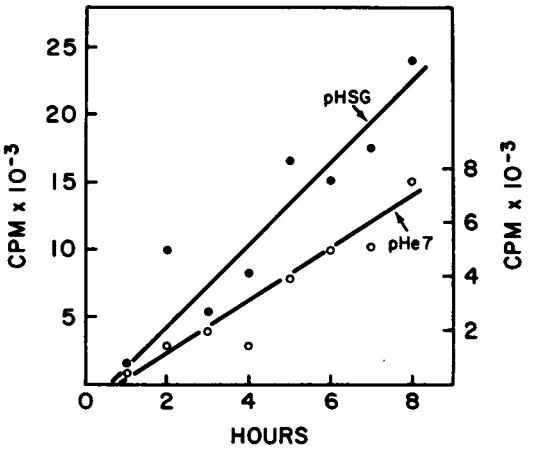

FIG. 6. Stability of the heat shock mRNA in 293 cells. A suspension culture of 293 cells was concentrated to 10^6 cells per ml. At this time [^{3}H]uridine was added to a concentration of 125 μCi/ml and a final uridine concentration of 14 μM. Samples of 5×10^7 cells were withdrawn at the indicated times, and cytoplasmic RNA was prepared. The labeled RNAs were then hybridized to filters bearing the pHSG plasmid (●) or the pHe7 plasmid (○).

there is cell-specific complementation of the E1A function; that is, certain cells will support early viral transcription in the absence of E1A function, whereas others will not (M. J. Imperiale et al., submitted for publication). Of importance is the further observation that in those cells that complement the E1A function there is a high level of heat shock gene expression in the absence of heat shock. Thus, we would suggest that there is a common target of action in the cell for both the adenovirus E1A protein and heat shock. Although we have previously suggested a negative regulation of transcription which is relieved by the action of the E1A protein, this seems unlikely in the sense of a repressor, given the observations that the control of the heat shock promoter appears to be positive (26). As an alternative, we would suggest that the negative control might just be the absence of certain cellular transcriptional factors. That is, that the action of the E1A protein might be to increase the available supply of transcriptional factors, one of which is utilized by the heat shock promoter.

The other aspect of these results that is noteworthy is the apparent tight control of the expression of the heat shock gene after induction. Shortly after the peak transcription rate is achieved, transcription of the gene drops to the same level as that before induction. This is in contrast to the majority of cellular genes that continue to be transcribed during adenovirus infection (3). In fact, the heat shock gene appears to be regulated in much the same manner as the early viral genes. The factor or factors responsible for the decline in transcription are of considerable interest. Several possibilities would explain this decline. It is possible that this may be a normal phenomenon of induction of the heat shock gene; for instance, a continued heat shock of cells does not result in a continued expression of the gene and has led to the suggestion that the heat shock gene may be autoregulated. This possibility, we feel, is unlikely given the abundance of the heat shock mRNA in 293 cells. If the protein regulated the expression of the gene, one would not expect such a high level ever to be maintained. Alternatively, the decline in transcription may be a virus-induced effect mediated by a viral gene product, or it may simply be that the level of the inducer, the E1A protein, declines during this period of infection. This latter possibility, in fact, may be the case since recent preliminary experiments measuring the concentration of E1A protein with specific antibody indicates that the concentration of the protein is maintained only transiently (L. Feldman et al., unpublished data).

Perhaps the most striking result of the control of the expression of the heat shock gene concerns the regulation of the stability of the mRNA. When synthesized in the adenovirus-transformed 293 cell line, where the rate of production of the RNA is sufficiently high such that the metabolism of the RNA can be studied, the heat shock mRNA is stable, possessing a half-life of greater than 4 h. This clearly differs from that in a viral infection where the half-life must be no longer than 40 min. This finding indicates that the heat shock mRNA is intrinsically rather stable, but when it is produced during an adenovirus infection, it becomes unstable. Alternatively, it remains possible that the heat shock mRNA is normally unstable but is somehow stabilized in the 293 cells. Regardless, it is clear that the stability of the heat shock mRNA is subject to change. The short half-life during a lytic infection is in contrast to the majority of cellular mRNAs in which stability appears to be unaffected by virus infection (Babich et al., submitted). Thus, if the stability in this case is regulated, there is specificity to the control. It is of some interest that the same phenomenon has been observed for the early adenovirus mRNAs. The early mRNAs are stable when synthesized apart from a lytic infection (i.e., transformed cells) but are unstable when produced during a lytic infection. The induced rapid decay of the early viral mRNAs is due to the action of an early viral gene product, the 72-kd DNA binding protein (4; A. Babich and J. R. Nevins, submitted for publication). Whether the 72-kd viral protein also affects the heat shock mRNA or whether an additional induced cellular protein is responsible is not clear.

Finally, the finding that the heat shock mRNA decays rapidly upon the shutdown of the transcription of the gene adds another example to the list of genes that exhibit this property. Of course, the same is true for the early adenovirus RNAs as the early phase of viral infection proceeds (4). Another clear example is the developmentally regulated mRNAs of *Dictyostelium* sp. (6, 19). These aggregation-induced RNAs are stable during induction. However, when transcription declines, the mRNAs are selectively degraded. The histone mRNAs follow the same course. The histone mRNAs are stable during S phase but become labile at other times of the cell cycle (13). Thus, the control of mRNA stability may be a common feature of inducible eucaryotic genes.

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LITERATURE CITED

1. **Aiello, L., R. Guiloyle, K. Huebner, and R. Weinmann.** 1979. Adenovirus 5 DNA sequences present and RNA sequences transcribed in transformed human embryo kidney cells (HEK-Ad5 or 293). Virology **94**:460–469.
2. **Ashburner, M., and J. J. Bonner.** 1979. The induction of gene activity in *Drosophila* by heat shock. Cell **17**:241–254.
3. **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 translocational discrimination. Mol. Cell. Biol. **3**:1212–1221.
4. **Babich, A., and J. R. Nevins.** 1981. The stability of early adenovirus mRNA is controlled by the viral 72 kd DNA binding protein. Cell **26**:371–379.
5. **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.
6. **Chung, S., S. M. Landfear, D. D. Blumberg, N. S. Cohen, and H. F. Lodish.** 1981. Synthesis and stability of developmentally regulated *Dictyostelium* mRNAs are affected by cell-cell contact and cAMP. Cell **24**:785–797.
7. **Clayton, D. F., and J. E. Darnell.** 1983. Changes in liver-specific compared to common gene transcription during primary culture of mouse hepatocytes. Mol. Cell. Biol. **3**:1552–1561.
8. **Collins, P. L., and L. E. Hightower.** 1982. Newcastle disease virus stimulates the cellular accumulation of stress (heat shock) mRNAs and proteins. J. Virol. **44**:703–707.
9. **Feldman, L. T., M. J. Imperiale, and J. R. Nevins.** 1982. Activation of early adenovirus transcription by the herpesvirus immediate early gene: evidence for a common cellular control factor. Proc. Natl. Acad. Sci. U.S.A. **79**:4952–4956.
10. **Graham, F. L., J. Smiley, W. C. Russell, and R. Nairn.** 1977. Characteristics of a human cell line transformed by DNA from human adenovirus 5. J. Gen. Virol. **36**:59–72.
11. **Greenberg, J. R.** 1972. High stability of messenger RNA in growing cultured cells. Nature (London) **240**:102–104.
12. **Grunstein, M., and D. S. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. U.S.A. **72**:3961–3965.
13. **Heintz, N., H. L. Sive, and R. G. Roeder.** 1983. Regulation of human histone gene expression: kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. Mol. Cell. Biol. **3**:539–550.
14. **Hofer, E., and J. E. Darnell.** 1981. The primary transcription unit of the mouse B-major globin gene. Cell **23**:585–593.
15. **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.
16. **Katze, M. G., H. Persson, and L. Philipson.** 1981. Control of adenovirus early gene expression: posttranscriptional control mediated by both viral and cellular gene products. Mol. Cell. Biol. **1**:807–813.
17. **Khandjian, E. W., and Turler.** 1983. Simian virus 40 and polyoma virus induce synthesis of heat shock proteins in permissive cells. Mol. Cell. Biol. **3**:1–8.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
19. **Landfear, S. M., P. Lefebvre, S. Chung, and H. F. Lodish.** 1982. Transcriptional control of gene expression during development of *Dictyostelium discoideum*. Mol. Cell. Biol. **2**:1417–1426.
20. **Nevins, J. R.** 1980. Definition and mapping of adenovirus 2 nuclear transcription. Methods Enzymol. **65**:768–785.
21. **Nevins, J. R.** 1981. Mechanism of activation of early viral transcription by the adenovirus E1A gene product. Cell **26**:213–220.
22. **Nevins, J. R.** 1982. Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A gene product. Cell **29**:913–919.
23. **Norgard, M. V., M. J. Tocci, and J. J. Monahan.** 1980. On the cloning of eukaryotic total poly(A)-RNA populations in *Escherichia coli*. J. Biol. Chem. **255**:7665–7672.
24. **Notarianni, E. L., and C. M. Preston.** 1982. Activation of cellular stress protein genes by herpes simplex virus temperature-sensitive mutants which overproduce immediate early polypeptides. Virology **123**:113–122.
25. **Okayama, H., and P. Berg.** 1982. High-efficiency cloning of full-length cDNA. Mol. Cell. Biol. **2**:161–170.
26. **Pelham, H. R. B.** 1982. A regulatory upstream promoter element in the *Drosophila* hsp 70 heat-shock gene. Cell **30**:517–528.
27. **Rigby, R. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling DNA to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
28. **Shenk, T., N. Jones, W. Colby, and D. Fowlkes.** 1979. Functional analysis of adenovirus host range mutants defective for transformation of rat embryo cells. Cold Spring Harbor Symp. Quant. Biol. **44**:367–375.
29. **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.
30. **Weber, J., W. Jelinek, and J. E. Darnell.** 1977. The definition of large viral transcription unit late in Ad2 infection of HeLa cells: mapping of nascent RNA molecules labeled in isolated nuclei. Cell **10**:611–616.
31. **Wilson, M. C., J. R. Nevins, J. M. Blanchard, H. S. Ginsberg, and J. E. Darnell.** 1979. The metabolism of mRNA from the transforming region of adenovirus type 2. Cold Spring Harbor Symp. Quant. Biol. **44**:447–455.