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# Induced Heat Shock mRNAs Escape the Nucleocytoplasmic Transport Block in Adenovirus-Infected HeLa Cells

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**Under conditions in which cytoplasmic accumulation of HeLa cell mRNAs has been blocked by adenovirus infection, *hsp70* family mRNAs are transported from the nucleus to the cytoplasm at near normal efficiency subsequent to heat shock. Heat shock does not reverse the general virus-induced block to host cell mRNA transport. The heat shock mRNAs are translated within the cytoplasm of the infected cell but at substantially reduced efficiency compared with that of uninfected cells. Thus, the *hsp70* family of mRNAs can escape the transport block but not the translational block instituted late after adenovirus infection. The β-tubulin gene family is induced by the viral E1A gene after infection, and its mRNAs also accumulate in the cytoplasmic compartment. Given these two examples, it seems likely that the process of transcriptional induction allows the resulting mRNA to escape the viral block of transport.**

When procaryotic or eucaryotic cells are subjected to stress such as severely elevated temperature, they respond by rapidly synthesizing a small number of proteins, the so-called heat shock or stress proteins (reviewed in references 1, 9, 30, 39, and 44). One group of heat shock proteins related in amino acid sequence is termed the HSP70-like family. Cross-hybridization analysis suggests that this family contains at least 10 members, although these estimates include at least one pseudogene (17, 36, 47). DNAs coding four members of this family have been cloned from mammalian cells, and their products have been termed (39) HSP70, which is not detected in unstressed cells (32, 50); HSC70, which is constitutively synthesized and weakly induced upon stress (38, 47); GRP78, a constitutive but inducible component of the endoplasmic reticulum (28, 37); and HSX70, which is constitutively synthesized but further induced by stress (53), the adenovirus E1A protein (25, 54), or serum (55). We used the preceding designations in this report in the absence of an accepted uniform terminology for these genes.

We used the heat-inducible HSP70 family as a test system to explore the fate of an induced cellular transcription unit late after adenovirus infection. Cellular gene expression is shut off at two levels within adenovirus-infected cells. Host cell mRNAs continue to be transcribed and processed in the nucleus but fail to appear in the cytoplasm (2, 6). This effect is mediated by a protein complex that includes two adenovirus-coded products, the E1B-55K and E4-34K polypeptides (4, 16, 31, 41, 42, 51). The second level of shutoff occurs in the cytoplasm of the infected cell, where host cell mRNAs are displaced from active polysomes (e.g., see reference 40).

When the *hsp70* gene family is induced in adenovirus-infected HeLa cells, heat shock mRNAs accumulate within the cytoplasm at nearly the same efficiency as in uninfected cells. The *hsp70* mRNAs are poorly translated, however, within the infected-cell cytoplasm. The mRNA from a second induced gene (β-tubulin transcription is induced by E1A; 48) also reaches the cytoplasm of adenovirus-infected cells. At least in some cases, the process of transcriptional induction allows the resulting mRNA to escape the normal adenovirus-induced inhibition of host cell mRNA transport.

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## MATERIALS AND METHODS

**Viruses and cells.** H5*dl*309 served as the wild-type adenovirus type 5 (Ad5) in these studies. This virus was generated from H5*wt*300, which is a plaque-purified derivative of a virus stock obtained from H. Ginsberg. H5*dl*309 was selected as a variant that contains only one *Xba*I cleavage site, located at 3.8 map units; it is phenotypically wild type (24).

HeLa suspension cells (American Type Culture Collection) were maintained in medium supplemented with 5% calf serum.

**Heat shock conditions.** Uninfected or infected HeLa suspension cell cultures (about 70 ml) in glass vessels were subjected to heat shock by incubation in a circulating water bath equilibrated at the appropriate temperature.

**RNA preparation and analysis.** Total cytoplasmic RNA was isolated by lysing cells in hypotonic buffer containing Nonidet P-40, pelleting the nuclei, and extracting the supernatant (18). The nuclei were suspended in 7 M guanidinium thiocyanate, and total nuclear RNA was isolated by pelleting through CsCl (8). Steady-state RNAs were analyzed by ribonuclease protection (35) with $^{32}$P-labeled probe RNAs derived from DNA segments cloned into vectors containing promoters for bacteriophage SP6 or T7. The *hsx70*-specific probe was derived from a 424-base-pair subclone corresponding to the 5′ end of the mRNA coded by an *hsx70* genomic clone (53), and the *hsp70*-specific probe was derived from a 375-base-pair subclone corresponding to the 5′ end of the mRNA coded by an *hsp70* genomic clone (50). Ad5-specific clones were E1A/E1B (0 to 5.1 map units) and a tripartite leader sequence (leader 2) (16.7 to 21.5 map units with several hundred base pairs deleted from the leader 1-leader 2 intron [152 and 80 base pairs of intron sequence remain adjacent to the 5′ and 3′ splice sites, respectively]).

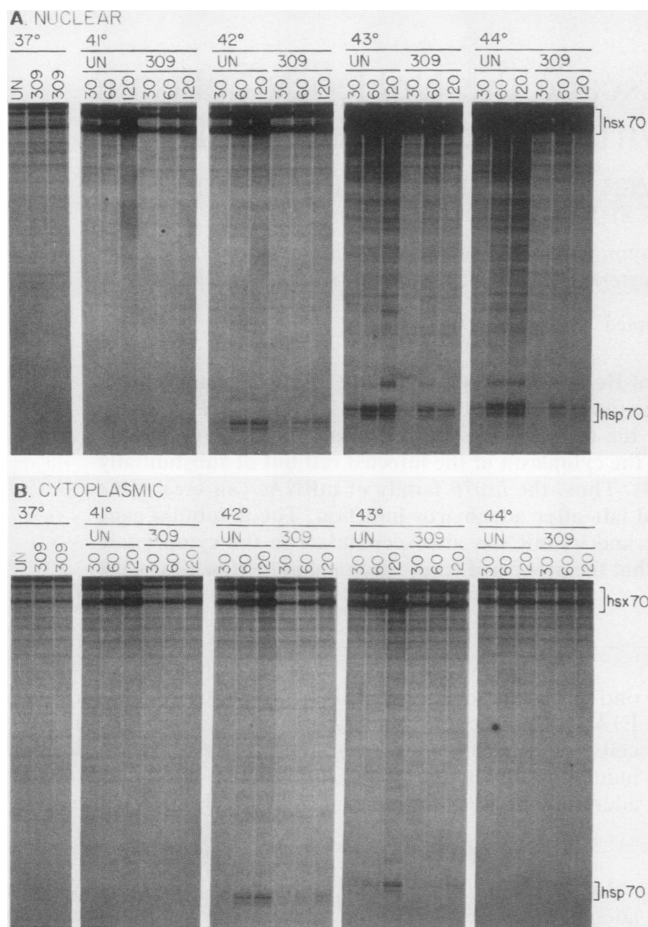


FIG. 1. Temperature dependence of *hsx70* mRNA induction in uninfected cells and cells at 20 h after infection with Ad5. Uninfected and Ad5-infected (multiplicity of infection, 25 PFU per cell) HeLa cells were shifted from 37°C to the heat shock temperatures indicated. After 30, 60, or 120 min at the elevated temperature, nuclear (A) and cytoplasmic (B) RNAs were prepared and analyzed by RNase protection with $^{32}$P-labeled *hsx70*-specific probe RNA. UN, Uninfected; 309, infected with *dl*309. Bands corresponding to *hsx70*- and *hsp70*-specific RNAs are labeled.

Transcription rates were measured in isolated nuclei essentially as described by Hofer and Darnell (22) and Groudine et al. (14). Nuclei prepared from infected cells were incubated for 15 min at 30°C in the presence of [$^{32}$P]UTP (750 μCi/ml, 410 Ci/mmol), and nuclear RNA was isolated, degraded by treatment with 0.2 N NaOH for 10 min at 0°C, and hybridized to single-stranded or denatured double-stranded DNA probes bound to nitrocellulose filters (4.7 μg of probe DNA per spot) by the method of McKnight and Palmiter (34). After one round of hybridization, a second DNA-containing filter was added to the hybridization mixture to ensure quantitative results.

Cytoplasmic accumulation of mRNA was measured by previously described procedures (2, 3, 13). In brief, infected cells were labeled with [$^{3}$H]uridine (200 μCi/ml, 50 Ci/mmol) in the presence of added unlabeled uridine (14 μM). Approximately $10^7$ infected cells were harvested at each interval, and cytoplasmic RNA was prepared (8) and then hybridized to DNA probes as described for nuclear RNAs.

Plasmids used to generate DNA probes contained the following inserts: a human *hsx70* genomic clone (53); human β-tubulin cDNA (27); the human β-actin gene segment (15); the human histone H4 gene (45); Ad5 E1A (0 to 4.5 map units); Ad5 E1B (5.6 to 7.9 map units); Ad5 L1 (31.5 to 37.3 map units); Ad5 L3 (53 to 58.5 map units).

**Protein analysis.** HeLa suspension cells were metabolically labeled with [$^{35}$S]methionine (50 μCi/ml, 1,100 Ci/mmol) for 30 min either at a heat shock temperature or immediately after shifting from the elevated temperature to 37°C. Preparation of total cellular extracts, immunoprecipitation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were performed as described previously (43). Immunoprecipitation of 70-kilodalton (kDa) heat shock polypeptides was done with an antiserum (a gift of P. Hinds and A. Levine) prepared by immunization with a synthetic oligopeptide corresponding to a region of homology among *HSP70*, *HSX70*, and *HSC70* (21).

## RESULTS

**Heat shock mRNAs accumulate in the cytoplasm of Ad5-infected HeLa cells.** The temperature dependence of induction of *hsx70* and *hsp70* mRNAs was examined in HeLa cells. Total nuclear or cytoplasmic RNA was prepared after cultures were kept for 30, 60, or 120 min at various elevated temperatures, and *hsx70-hsp70*-specific RNAs were assayed by RNase protection by using a probe derived from the 5′ end of an *hsx70* gene (53). To monitor the effect of adenovirus infection on the metabolism of heat shock mRNAs, uninfected cultures were compared with cells infected 20 h earlier with wild-type Ad5. Both nuclear and cytoplasmic compartments contained a low, constitutive level of *hsx70* RNA at 37°C, and the level was not perturbed by infection (Fig. 1). An *hsx70*-specific doublet was consistently observed in RNase protection assays, presumably representing either two 5′ start sites or RNA products of divergent *hsx70* genes (53). Both *hsx70*-specific bands were rapidly induced in the nucleus upon heat shock at 41 to 44°C (Fig. 1A). Steady-state levels of *hsx70* RNAs were consistently reduced by a factor of two to three within infected cell nuclei as compared with uninfected cell nuclei after heat shock, and this reduction appears to be the direct consequence of an approximately twofold reduction in transcription rate (Table 1). In infected cell nuclei, *hsx70* RNA levels dropped somewhat (less than twofold) as the period of heat shock was extended from 60 to 120 min. The steady-state level of *hsx70*-specific RNA rapidly increased in the cytoplasmic compartment following heat shock at all of the temperatures tested (Fig. 1B). Cytoplasmic levels were

TABLE 1. Relative *hsx70-hsp70* transcription rates in HeLa cells subjected to 42°C heat shock

| Heat shock period (min) | Relative transcription rate (fold)[a] | |
|---|---|---|
| | Uninfected cells | Infected cells |
| 0 | 1.0 | 1.1 |
| 30 | 5.7 | 3.0 |
| 60 | 6.8 | 3.3 |
| 90 | 3.7 | 2.2 |
| 120 | 2.5 | 1.6 |

[a] Transcription rates were determined by nuclear run-on analysis either in uninfected cells or late after infection (20 h) at 25 PFU per cell. Cells were either maintained at 37°C or shifted to 42°C for the periods indicated. Rates are expressed relative to the value observed for the transcription unit in uninfected cells at 37°C (45 cpm above a background of 30 cpm, which was subtracted from all determinations). Virus-infected samples did not exhibit induced *hsx70-hsp70* transcription in the absence of heat shock. This was expected, since EIA-mediated induction is transient and occurs during the early phase of infection (25).

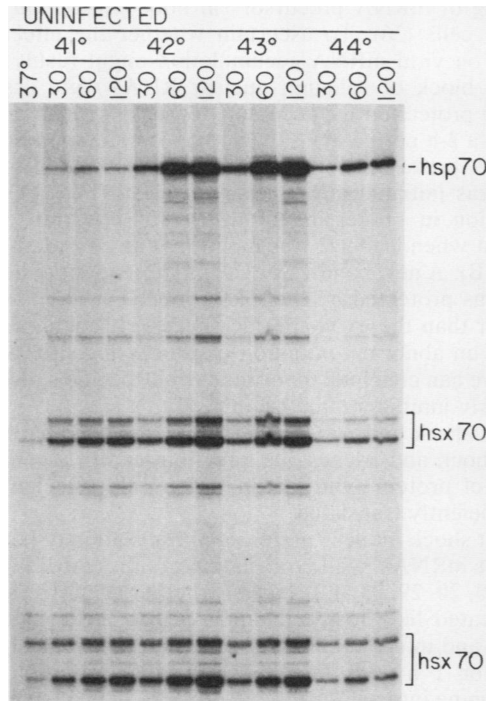


FIG. 2. Temperature dependence of *hsp70* mRNA induction in uninfected cells. The same uninfected HeLa cell RNA preparations described in the legend to Fig. 1 were analyzed by RNase protection with $^{32}P$-labeled *hsp70*-specific probe RNA. Bands corresponding to *hsp70*- and *hsx70*-specific RNAs are labeled.

reduced at the highest temperature, 44°C. The fraction of RNA which was heat shock specific in the cytoplasm was markedly lower than in the nucleus, presumably because of the preferential loss of small RNAs in the procedure used for isolation of nuclear RNA.

A second set of smaller bands was detected after heat shock (Fig. 1A and B). Their pattern of expression differed from the *hsx70*-specific products in that they were present only in cells exposed to elevated-heat treatments. This pattern was appropriate for *hsp70* RNA expression (e.g., references 39 and 50). To confirm this possibility, a probe corresponding to the 5'-terminal region of the *hsp70* mRNA was used to reanalyze the uninfected cell RNA samples (Fig. 2). RNA homologous to the probe accumulated with kinetics identical to that observed for the small bands, and small pairs of doublets were evident that corresponded to *hsx70* RNAs. Thus, both heat shock-specific probes can detect *hsx70*- and *hsp70*-specific RNAs. In each case, the hybridization of probe with heterologous RNA leads to the production of small bands in the RNase protection assay because of cleavage at mismatches in the heteroduplex formed between the probe and RNA.

We conclude that the precise nature of the heat shock response varies over the 41 to 44°C range. Further, adenovirus infection has only a modest effect on the response, principally at the level of transcriptional initiation. Cytoplasmic accumulation of heat shock-specific RNAs is not prevented by the generalized virus-induced shutdown of host cell mRNA accumulation.

**Heat shock reduces cytoplasmic accumulation of some Ad5 mRNAs.** Heat shock at 42°C was chosen to evaluate effects on viral mRNA metabolism. At this temperature, both *hsx70* and *hsp70* RNAs were induced (Fig. 1 and 2). No effect of

TABLE 2. Relative adenovirus transcription rates in HeLa cells subjected to 42°C heat shock

| Transcription unit | cpm ($10^3$)/1,000-nucleotide probe[a] | | |
|---|---|---|---|
| | 37°C | 42°C | 42°C/37°C |
| EIA | 0.95 | 0.86 | 0.9 |
| L1 | 15.1 | 16.7 | 1.1 |
| L3 | 19.9 | 27.8 | 1.4 |

[a] Transcription rates were determined by nuclear run-on analysis at 20 h after infection at 25 PFU per cell. Cells were either maintained at 37°C or shifted to 42°C for 30, 60, 90, or 120 min. The time at 42°C had very little effect on transcription rate, so heat shock values are the average for all determinations at the elevated temperature.

heat shock on steady-state levels of viral mRNAs was observed at 20 h after infection (data not shown). This is not surprising, since the half-life of early mRNAs has increased by this time (52) and substantial quantities of stable late mRNAs have accumulated. Clearly, no dramatic change in viral mRNA half-life occurred in response to heat shock, and changes in rates of accumulation were too small to impact on overall mRNA pool sizes during the 2-h heat shock period. Further, heat shock had no significant impact on the rates of E1A, L1, and L3 transcription at 20 h after infection (Table 2).

To probe posttranscriptional effects of heat shock, uninfected and infected (20 h) HeLa cell cultures were brought to 42°C and labeled continuously with [$^3$H]uridine for the next 2 h. The rate of appearance of labeled RNA in the cytoplasmic compartment was monitored by hybridization to specific probe DNAs. This experiment can provide information on relative transport rates and RNA stability (2, 3, 13). After increase of the temperature to 42°C, *hsx70-hsp70* RNAs were strongly induced and accumulated rapidly in uninfected cells and to only a slightly reduced extent in infected cells (Fig. 3A). Transcription rates of the *hsx70-hsp70* genes began to drop after 60 min (Table 1). Therefore, it is not clear whether the plateau at 90 min reflects the half-life of *hsx70-hsp70* RNAs or the reduction in transcription rates. The heat shock-specific RNAs accumulated at a time after infection when other cellular mRNAs (actin [Fig. 3C] and histone H4 [Fig. 3D]) failed to exit the nucleus.

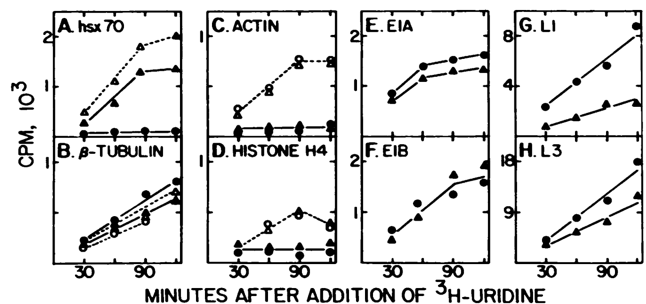


FIG. 3. Kinetics of mRNA accumulation in the cytoplasm of uninfected cells and cells at 20 h after infection with Ad5. Uninfected and Ad5-infected (multiplicity of infection, 25 PFU per cell) HeLa cells were shifted from 37 to 42°C, and [$^3$H]uridine was simultaneously added to initiate the accumulation time course, which took place entirely at the elevated temperature. Portions of the cultures were harvested at the indicated intervals, and RNAs were prepared and hybridized to the indicated single-stranded probe DNAs. Symbols: ○, uninfected cells at 37°C, △, uninfected cells at 42°C; ●, infected cells at 37°C; ▲, infected cells at 42°C.

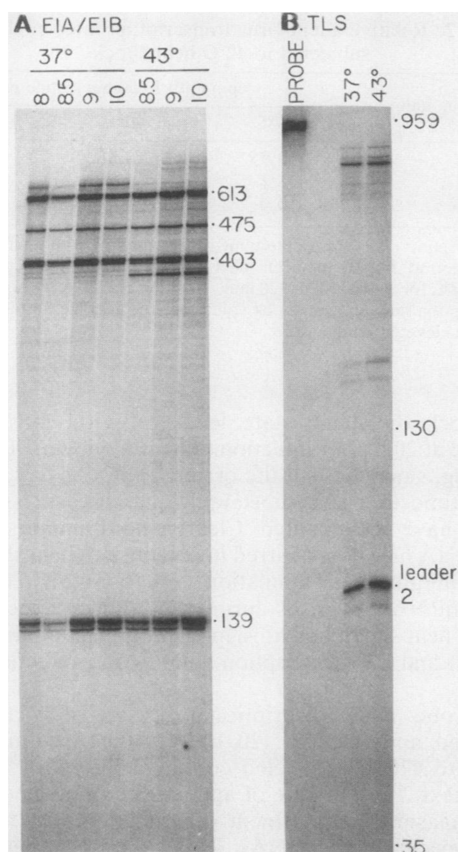


FIG. 4. Effect of elevated temperature on steady-state nuclear RNAs coded by Ad5. HeLa cells were infected at a multiplicity of infection of 25 PFU/ml and subjected to heat shock at 43°C for 30, 60, or 120 min, beginning at 8 h after infection (A), or for 120 min, beginning at 20 h after infection (B). Nuclear RNAs were prepared and analyzed by RNase protection with either E1A-E1B-specific (A) or tripartite leader sequence (TLS) leader 2-specific (B) $^{32}$P-labeled probe RNA. The sizes of protected fragments are indicated in nucleotides. (A) Fragments of 613, 475, and 403 nucleotides correspond to E1A-specific exons, and the 139-nucleotide fragment corresponds to the 5' portion of the 5' exon of E1B. (B) The indicated sizes correspond to marker fragments, and the leader 2-specific band is designated.

Accumulation of early viral mRNAs (E1A [Fig. 3E] and E1B [Fig. 3F]) was not significantly affected by the increased temperature. However, late viral mRNAs (L1 [Fig. 3G] and L3 [Fig. 3H]) accumulated at substantially reduced rates subsequent to heat shock. Of the two late classes of mRNA tested, the L1 family was more severely inhibited than the L3 family. The half-lives of these two mRNAs were monitored by a chase in the presence of actinomycin D and found to be unaltered (data not shown). Finally, β-tubulin mRNAs, encoded by a gene family whose transcription is induced by adenovirus infection (48), accumulated in the cytoplasm of late virus-infected cells (Fig. 3B).

We conclude that a 42°C heat shock at 20 h after Ad5 infection partially inhibits cytoplasmic accumulation of at least some late viral mRNAs but not the early mRNAs tested. The normal virus-induced block to host cell mRNA accumulation exerts only a modest effect on both heat shock mRNAs and a second induced transcription unit, β-tubulin.

**Heat-shock at 43°C does not inhibit splicing of viral mRNAs.** Severe heat shock treatments have been shown to inhibit splicing of mRNA precursors in both *Drosophila* (56) and mouse cells (26). To ascertain whether the effect of heat shock on viral mRNA accumulation might result from the partial block of splicing, nuclear RNAs were assayed by RNase protection for accumulation of unspliced precursors during a 2-h period at 43°C. No bands corresponding in size to E1A precursors were observed when the temperature shift was initiated at 8 h after infection (Fig. 4A), and no inhibition in processing of the major late transcript was evident when leader 2 was monitored at 20 h after infection (Fig. 4B). A new band was evident subsequent to heat shock that was protected by the E1A probe (Fig. 4A), but it was smaller than the exon-specific species. It could result from use of an abnormal initiation or processing site. Nevertheless, we can conclude that adenovirus-specific splicing is not seriously inhibited after 2 h at 43°C.

**Heat shock and Ad5 translation are not compatible.** Both heat shock and adenovirus infection lead to a general inhibition of protein synthesis in which only selected mRNAs are efficiently translated.

Heat shock induces preferential translation of heat shock-specific mRNAs, while translation of other mRNAs is inhibited (10, 20, 29, 33, 49). To test whether adenovirus mRNAs are treated like host cell mRNAs after heat shock, uninfected and infected cultures were maintained at 41, 42, and 43°C for 1 h and then tested for incorporation of [$^{35}$S] methionine into acid-insoluble product. The infected culture was assayed at 20 h after infection with Ad5, when the preponderance of translation is virus specific (see Fig. 5). Total incorporation was inhibited to similar extents in infected and uninfected HeLa cells (Table 3). Thus, viral translation is inhibited by heat shock.

Late after adenovirus infection, host cell mRNAs are displaced from polysomes and host-specific translation is dramatically inhibited. To determine whether heat shock mRNAs escape this inhibition, HeLa cells were subjected to heat shock at various temperatures for 1 h, beginning at 20 or 30 h after infection with Ad5, and then labeled with [$^{35}$S]methionine for 30 min. Comparison of polypeptides labeled in uninfected cells with those synthesized in infected cells demonstrated that cellular translation was markedly inhibited by 20 h (Fig. 5A) and almost completely inhibited by 30 h after infection (Fig. 5B). Heat shock-specific proteins were readily detected in uninfected cells after incubation at 41 to 43°C and labeling either at the heat shock temperature or upon shift down to 37°C (Fig. 5A). Heat shock proteins were produced to a lesser extent in

TABLE 3. Translational efficiencies in HeLa cells subjected to heat shock

| Temperature (°C) | Relative translation efficiency (fold)[a] | |
|---|---|---|
| | Uninfected cells | Infected cells |
| 37 | 1.00 | 1.00 |
| 41 | 0.60 | 0.70 |
| 42 | 0.43 | 0.47 |
| 43 | 0.30 | 0.35 |

[a] Uninfected cells or cells at 20 h after infection at 25 PFU per cell were incubated at the indicated temperatures for 60 min and then metabolically labeled with [$^{35}$S]methionine for an additional 30 min at the same temperature. Incorporation was normalized to that observed in either uninfected or infected cells maintained at 37°C. Incorporation in infected cells at 37°C was 59% of that observed in uninfected cells. In infected and uninfected cells labeled at 37°C after heat shock for 1 h at the various temperatures, efficiency of incorporation relative to that of cells maintained at 37°C was increased to approximately 60 to 70%.

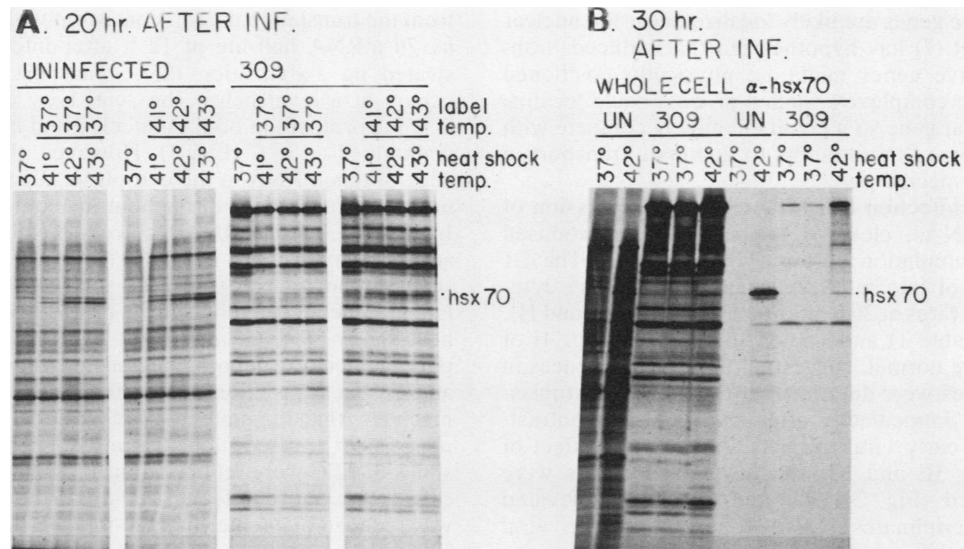


FIG. 5. Temperature dependence of protein synthesis in uninfected and Ad5-infected cells. Uninfected HeLa cell cultures and cultures infected at a multiplicity of 25 PFU per cell were subjected to heat shock at the indicated temperatures (temp.) for 60 min, beginning at 20 h after infection (A), or 120 min, beginning at 30 h after infection (B). Cells were labeled with [$^{35}$S]methionine for 30 min at either the indicated temperature (A) or the elevated temperature (B). UN, Uninfected; 309, Ad5 infected; α-*hsx70*, whole-cell extract immunoprecipitated with antibody to 70-kDa heat shock proteins. *hsx70*-specific bands are designated.

infected cells and were most readily observed when cultures were radioactively labeled at the heat shock temperature (Fig. 5A). Immunoprecipitation with an antibody specific for the *HSP70*, *HSX70*, and *HSC70* polypeptides (21) confirmed the identity of the heat shock-specific product, although it was not possible to distinguish precisely which members of the 70-kDa family of heat shock proteins are represented. Nevertheless, this experiment demonstrated that synthesis of heat shock proteins was reduced by a factor of about 20 at 30 h after Ad5 infection as compared with uninfected cells (Fig. 5B). Since *hsx70-hsp70* mRNA levels were reduced only two- to three-fold (Fig. 1; data not shown), it is clear that viral infection inhibited translation of heat shock mRNAs after induction of their accumulation by elevated temperature.

## DISCUSSION

Adenovirus infection does not block cytoplasmic accumulation of all cellular mRNAs as previously assumed. Exceptions to the generalized block include the *hsx70-hsp70* mRNAs after induction by heat treatment (Fig. 1 and 3) and the β-tubulin mRNA family (Fig. 3), which is transiently activated early after infection by the adenovirus E1A product. We have also observed cytoplasmic accumulation of beta interferon mRNA upon induction in adenovirus-infected cells (unpublished data). Thus, there are at least three instances in which mRNAs are able to bypass the adenovirus block of host cell mRNA transport from nucleus to cytoplasm. In each case, the gene is induced subsequent to infection, suggesting that the induction process somehow overcomes the viral block.

The experiments reported here were all performed in HeLa cells. The effect of adenovirus infection on *hsx70* expression has also been examined in 293 cells, where the gene is constitutively expressed at elevated levels (25). Cytoplasmic accumulation of *hsx70* mRNA is substantially, but not completely, inhibited late after Ad5 infection, and its accumulation is partially restored by heat shock (M. Moore, Ph.D. thesis, Princeton University, Princeton, N.J., 1986; U. C. Yang and S. J. Flint, personal communication).

The mechanism underlying the viral block of host cell mRNA transport is not yet understood. Thus, it is difficult to speculate on the mechanism by which induced genes can escape the block. It seems likely that more than mass action is involved since the beta interferon gene, once induced, is less active in terms of transcription rate than is the actin gene, whose mRNAs fail to reach the cytoplasm.

Two general models could explain the block of transport of cellular mRNAs in adenovirus-infected cells. The first model postulates a signal in the sequence or structure of viral mRNAs. This would be recognized by the virus-coded E1B-55K–E4-34K protein complex, required for host shutoff (5, 41, 51), enabling it to distinguish virus from host mRNAs. However, nonadenovirus mRNAs expressed from genes on the viral chromosome accumulate in the cytoplasm late after infection (e.g., preproinsulin I [12], α-globin [19], and herpes simplex virus thymidine kinase [Moore and Shenk, submitted for publication]). As a result, it seems unlikely that a discrimination signal is built into viral mRNAs. The second model proposes that the viral chromosome is localized within the nucleus so that it can dominate transport of mRNAs to the cytoplasm at its local pore or that host transcription units are displaced from sites at which efficient transport can occur. This localization model fits a variety of observations. (i) It predicts transport of nonviral mRNAs coded by genes on the viral chromosome. (ii) It is known that host cell shutoff cannot occur if viral DNA replication is inhibited (reviewed in reference 11). This is also predicted by the model since it could be necessary for the viral chromosome to colonize and dominate most, if not all, nuclear pores to achieve host cell shutoff. (iii) The localization model can readily accommodate continued transport of induced genes. Hutchinson and Weintraub (23) demonstrated that DNaseI-sensitive domains in chromosomes are preferentially localized at the nuclear periphery and along channels communicating with the periphery. Since active or potentially active genes exhibit enhanced sensitivity to DNaseI digestion, it

follows that active genes are likely localized near the nuclear periphery. Blobel (7) has hypothesized that induced, transcriptionally active genes might be physically positioned near nuclear pore complexes. Such a process could localize an induced cellular gene so it could effectively compete with a viral chromosome that attempts to dominate transport in the vicinity of a specific pore.

Whereas viral infection had little effect on expression of *hsx70-hsp70* mRNAs, elevated temperature compromised cytoplasmic accumulation of some viral mRNAs. The L1 and L3 families of late mRNAs accumulated in the cytoplasm at reduced rates at 20 h after infection (Fig. 3G and H). Transcription (Table 1) and nuclear processing (Fig. 4) of late mRNAs were normal, suggesting that the differences in accumulation rates were due to either transport or cytoplasmic stabilization immediately after transport. In contrast, accumulation of early viral mRNAs showed little effect of heat shock (Fig. 3E and F), and host cell mRNAs were largely unaffected (Fig. 3B, C, and D). Thus, elevated temperature discriminates between early and late viral mRNAs (at least those tested) and treats early viral species like host cell mRNAs. It is interesting that the E1B-55K–E4-34K protein complex, which is responsible for both inhibition of host-specific mRNA transport and enhancement of virus-specific transport, also discriminates between early and late viral mRNAs. Transport of early mRNAs is not dependent on the complex, while late mRNA transport is inhibited in its absence (41). Further, accumulation of the L1 family of late mRNAs is more strongly dependent on the complex than is the L3 family (41), paralleling the somewhat greater heat sensitivity of L1 than L3 mRNA accumulation. These observations suggest that function of the E1B-55K–E4-34K protein complex is at least partially impaired by elevated temperature. Generalized shutoff of host mRNA transport was not reversed by heat shock (Fig. 3C and D). Thus, either the generalized shutoff is irreversible or the complex remains at least partially functional at 42°C.

Whereas the mechanisms underlying cytoplasmic accumulation of viral and heat shock mRNAs appear reasonably compatible, their translational strategies are not. Elevated temperature leads to a generalized reduction in translation of non-heat shock mRNAs (10, 20, 33, 46), and it caused a similar reduction late after infection (Table 3), when the preponderance of protein synthesis was virus specific. Elevated temperature clearly inhibited viral translation. Similarly, although cytoplasmic levels of heat shock mRNAs were reduced only severalfold in virus-infected as compared with uninfected cells, the level of *hsx70*-specific translation was reduced by a factor of 20 at 30 h after infection (Fig. 5B). Adenovirus infection clearly inhibited heat shock protein synthesis.

In cells maintained at 37°C, a 70-kDa heat shock-specific protein was synthesized at a low but discernible level (Fig. 5). Since we detected *hsx70*, but not *hsp70*, mRNA at 37°C, it seems very likely that the heat shock protein detected is *hsx70*. The rates of *hsx70* mRNA translation were very similar in uninfected cells and cells at 20 or 30 h after infection. In contrast, actin translation was dramatically reduced by 20 h after infection relative to that of uninfected cells. The fraction of cytoplasmic RNA which is *hsx70* mRNA was nearly identical in uninfected cells and cells at 20 h after infection (Fig. 1B). Thus, continued *hsx70* mRNA translation is not due to a large excess of this RNA in infected cells. *hsx70* mRNA appears to escape the inhibition of host mRNA translation before induction by heat shock. This implies that *hsx70* RNA is not stabilized by removal from the translational pool, consistent with measurements of *hsx70* mRNA half-life at 12 h after infection that demonstrated no stabilization (25). Transport of *hsx70* mRNA occurs at a level below the sensitivity of the continuous-labeling protocol in both uninfected and infected HeLa cells maintained at 37°C (Fig. 3). However, the continued presence of this mRNA in the cytoplasm suggests that it continues to be transported throughout the course of the infection. In contrast, actin mRNA was very inefficiently transported and translated by 20 h after infection. These observations suggest that mRNA transport and translation are coupled late in infected cells, but this is probably not the case. After heat shock at 42°C, *hsx70* and *hsp70* mRNAs were transported efficiently in both infected and uninfected cells (Fig. 1 and 3), but translation in infected cells was increased very much less than in uninfected cells (Fig. 5). The rates of *hsx70* plus *hsp70* transcription and mRNA transport upon heat shock at 42°C were reduced approximately 2-fold in infected cells relative to uninfected cells, but the rate of translation was reduced by greater than 20-fold. In contrast, in cells not subjected to heat shock, the rates of mRNA transport and translation were strongly correlated. Therefore, coupling of mRNA transport and translation is unlikely to be direct in infected cells.

Why, then, might the transcripts of certain cellular genes continue to be transported and translated during the late phase of infection? It is possible that the virus requires a continuing supply of certain host gene products, and it could activate these genes through the E1A proteins. Both *hsx70* and the β-tubulin gene are transcriptionally activated by E1A, and transcripts of both genes appear to be transported late in infected cells. Thus, it is possible that E1A transcriptional activation also leads to activation of mRNA transport and translation. This leads to the prediction that β-tubulin mRNA will be efficiently translated, while *hsp70* mRNA induced by heat shock will be inefficiently translated late in infected cells.

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