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The Heat Shock Response in HeLa Cells Is Accompanied by Elevated Expression of the c-*fos* Proto-Oncogene

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Several known inducers of the heat shock response (heat stress, arsenite, and heavy metals) were shown to cause a significant elevation of c-*fos* mRNA in HeLa cells. Heat stress resulted in a time- and temperature-dependent prolonged elevation in the level of c-*fos* mRNA, which was accompanied by increased translation of c-*fos* protein and its appearance in the nucleus. Elevated expression of c-*fos* during heat stress was paralleled by induction of hsp 70 mRNA, while levels of c-*myc* and metallothionein mRNAs declined. Treatment of HeLa cells with arsenite or heavy metals also resulted in increased levels of hsp 70, as well as c-*fos* mRNA. Although elevated expression of c-*fos* was prevented by inhibitors of RNA synthesis, analysis of relative rates of gene transcription showed that during heat stress there was a negligible change in c-*fos* transcription. Therefore, the enhanced expression of c-*fos* during the heat shock response is likely to occur primarily through posttranscriptional processes. Cycloheximide was also shown to significantly increase the c-*fos* mRNA level in HeLa cells. There results are consistent with the observation that these inducers of the heat shock response, as well as cycloheximide, repress protein synthesis and suggest that the increase in the level of c-*fos* mRNA is caused by an inhibition of protein synthesis. This supports the hypothesis that c-*fos* mRNA is preferentially stabilized under conditions which induce the heat shock response, perhaps by decreased synthesis of a short-lived protein which regulates c-*fos* mRNA turnover.

The heat shock response is characterized by the rapid induction of a small set of proteins during heat stress or following exposure to stress-inducing agents such as arsenite or toxic levels of heavy metals (for reviews, see references 5, 7, and 16). The response is regulated at a number of levels. Heat shock genes are activated transcriptionally and their mRNAs are preferentially translated over non-heat-shock mRNAs. It is known that in *Drosophila* spp., genes normally expressed at 25°C, although active at 37°C, are blocked at the level of RNA processing and that proteins normally synthesized at 25°C are translated inefficiently if at all. Cells maintained at heat shock temperatures or released from exposure to stress-inducing agents gradually return to a more normal pattern of protein synthesis with increased translation of the pre-heat-shock mRNAs and reduced heat shock protein synthesis. It is believed that heat shock proteins function at normal temperatures in early development and during the cell cycle and that they function during stress to provide thermotolerance or to protect the cell from the effects of stress-inducing agents (5, 7, 16).

Although the function of the proto-oncogene c-*fos* is unknown, it has been suggested that c-*fos* may be involved in cell differentiation (23, 35) and in the process of cell division in response to growth factors (9). The c-*fos* proto-oncogene is expressed during prenatal cell growth, development, and differentiation, after partial hepatectomy, and in monomyelocytes induced to differentiate into macrophages (for a review, see reference 34). c-*fos* is also elevated in a number of cell types stimulated by growth factors. Although c-*fos* mRNA is rapidly induced by mitogens or by differentiation-specific agents, its response to mitogens is only transient, whereas its response in differentiating cells is more sustained (9, 23, 35). In both cases, however, the c-*fos* protein is present only transiently. This contrasts with the expression of the viral (v-*fos*) counterpart, the transforming gene of the FBJ murine sarcoma virus (10). Cells constitutively expressing the v-*fos* gene have relatively high levels of v-*fos* protein and, probably for this reason, become transformed. The c-*fos* gene can also transform cells if the 3′ untranslated region is altered such that the normal protein is overproduced (18, 20). Under most conditions both the c-*fos* mRNA and the c-*fos* protein are rapidly turned over, and it appears that increased stability of either the mRNA or the protein, as well as transcriptional induction and translational controls, could affect the levels of c-*fos* protein and ultimately the cell function (34). In the process of examining the effects of heat shock on gene expression in HeLa cells, it was noted that agents which induce the heat shock response dramatically increase c-*fos* mRNA levels.

MATERIALS AND METHODS

Culture and treatment of HeLa cells. HeLa cells were cultured at 37°C at a density of 5×10^5 cells per ml in spinner flasks. Heat stress was administered by immersing the flasks in a water bath for various times at 42, 44, or 46°C as indicated in the figure legends. Other inducers of the heat shock response were added to cultures of HeLa cells as follows. Sodium arsenite was added to a final concentration of 80 μM, and the incubation was continued at 37°C for the indicated times; heavy metals (an equal mixture of cadmium chloride and zinc chloride) were added to final total concentrations of 2×10^{-4} to 2×10^{-5} M, and the incubations were continued at 37°C for the indicated times; and cycloheximide was added to final concentrations of 1 to 25 μg/ml, and the incubations were continued for 2 h at 37°C.

Northern blot analysis. Total RNA was extracted from cell pellets by using phenol-chloroform-sodium dodecyl sulfate

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(3) and precipitated with 3 M ammonium acetate to remove DNA (24). RNA (6 μg) was run in 1.5% agarose gels containing formaldehyde (14) and Northern blotted (RNA blotted) (30) to nitrocellulose in 20× SSC (1× SSC is 0.15 M NaCl, plus 0.015 M sodium citrate [pH 7.0]). Nitrocellulose filters were hybridized with ^{32}P-labeled cRNA probes and then washed under stringent conditions as described previously (2). Hybrids were detected by autoradiography. To obtain quantitative estimates of changes in mRNA levels, we analyzed autoradiographs by densitometric scanning.

Hybridization probes. The c-*fos* (mouse) and c-*myc* (mouse) clones were obtained from the American Type Culture Collection, Rockville, Md. The hsp 70 (mouse) clone was generously provided by Richard Morimoto, Northwestern University, Evanston, Ill., and the MT-I (mouse) clone was provided by Richard Palmiter, University of Washington, Seattle. The above DNAs were subcloned into SP6 vectors (Promega Biotec, Madison, Wis.) and used as templates for synthesis of high-specific-activity (2×10^9 dpm/μg) ^{32}P-labeled cRNA probes (19).

Preparation of polysomes. HeLa cells were homogenized in RSB (10 mM NaCl, 10 mM Tris hydrochloride [pH 7.4], 1.5 mM $MgCl_2$) and centrifuged at 12,000 × *g*, and the supernatant was adjusted to 0.5% (wt/vol) sodium deoxycholate and layered onto a 5 to 20% sucrose gradient over a 60% sucrose–RSB cushion. The sucrose gradient solutions were prepared in 500 mM NaCl–10 mM Tris hydrochloride (pH 7.2)–50 mM $MgCl_2$. The sucrose gradients were centrifuged at 130,000 × *g* for 3 h at 4°C, 1-ml fractions were collected, and the A_{260} of these fractions was measured (13). Polysomal fractions (fractions 1 to 6) and nonpolysomal fractions (fractions 19 to 39) were pooled. RNA was extracted and subjected to Northern blot analysis as described above.

Determination of rates of protein synthesis. HeLa cells were incubated for 0.5 h in medium containing 50 μCi of a ^{3}H-amino acid mixture (240 mCi/mg; ICN Radiochemicals, Irvine, Calif.) per ml. Aliquots (1 ml) of the labeled cells were collected by low-speed centrifugation, suspended in 1 ml of RSB, and allowed to swell at 4°C for 20 min. Cells were homogenized with a Teflon-to-glass homogenizer, and the homogenate was centrifuged at 100,000 × *g* for 0.5 h at 4°C. Aliquots (100 μl) were analyzed for trichloroacetic acid-precipitable radioactivity by collection of precipitated proteins on glass fiber filters followed by liquid scintillation counting in a toluene-based counting fluid.

Immunohistochemical detection of c-*fos*. HeLa cells were grown on gelatinized cover slips for 2 days until 50% confluent. Cells were subjected to heat shock (42°C) for various times up to 4 h and then allowed to recover at 37°C. Slides were washed in sterile phosphate-buffered saline, fixed in methanol at −20°C for 5 min, and allowed to dry. Slides were stored at −60°C or used immediately. Immunohistochemical staining with avidin-biotin-peroxidase complex (ABC) was done as described previously (1). Anti-*fos* peptide antibodies (1) were applied to cells at 4°C for 12 to 16 h at concentrations ranging from 6.6 to 0.22 μg/ml in phosphate-buffered saline containing 1% normal goat serum. To confirm these results we used two other antibodies: a second rabbit *fos* peptide antiserum (amino acid sequence 155 to 165 of mouse c-*fos*), and antiserum from a rat with a tumor raised with v-*fos*-transformed rat fibroblasts (10) (RS2 cells). Peroxidase staining was intensified by immersion in 0.5% $CuSO_4$ solution for 5 min. Slides were counterstained in fast green, dehydrated, and mounted in Permount (Fisher Scientific Co.). Photography was done on a Nikon microscope.

RNA polymerase runoff transcription assay. HeLa cells were homogenized in 0.32 M sucrose–TMKSH (10 mM Tris hydrochloride [pH 7.5], 5 mM $MgCl_2$, 25 mM KCl, 5 mM β-mercaptoethanol) containing 0.1% Triton X-100, and nuclei were pelleted by centrifugation at 1,000 × *g* for 10 min at 4°C (29). The nuclear pellet was suspended in 0.88 M sucrose–TMKSH and pelleted by centrifugation at 1,000 × *g* for 20 min. Nuclei were suspended in 2.1 M sucrose–TMKSH and pelleted at 15,000 × *g* for 30 min. The nuclei were then washed in TGEDM (10 mM Tris hydrochloride [pH 7.9], 25% glycerol, 50 mM EDTA, 10 mM dithiothreitol, 5 mM $MgCl_2$), suspended in TGEDM at 2 mg of DNA per ml, and frozen at −70°C. Runoff transcription and isolation of labeled transcripts were performed as described previously (17). Labeled transcripts (4×10^7 dpm) were hybridized at 66°C for 24 h to filter-bound RNAs complementary to c-*fos*, c-*myc*, and hsp 70 under conditions described previously (2). cRNAs were synthesized in vitro by using SP6 RNA polymerase (19), and 1 μg of each RNA was slot blotted to nitrocellulose in 20× SSC (30). Filters were baked at 80°C for 4 h before hybridization. After hybridization, filters were washed at 66°C for 1 h in 4× SSC and then at 66°C for 1 h in 2× SSC. Filters were then treated with RNases A and T_1 (17), and hybrids were detected by autoradiography. To quantitate the data, autoradiographs were analyzed by densitometric scanning.

RESULTS

c-*fos* mRNA was present at barely detectable levels in HeLa cells cultured at 37°C (Fig. 1). Shifting the incubation temperature to 42°C resulted in a slow increase in the level of c-*fos* mRNA. This increase was often detected by 1 h (Fig. 1B), reached a level approximately 15-fold greater than that of the control by 3 to 4 h, and remained elevated with continued incubation for up to 6 h at 42°C (Fig. 1A). Significantly elevated levels of c-*fos* during heat stress were consistently detected in nine experiments. The reported increase in the relative level of c-*fos* mRNA is likely to be underestimated by densitometric scanning of the autoradiograms as a result of the low level of this mRNA in control cells. The hsp 70 mRNA level was dramatically induced by heat stress (Fig. 1). Although the magnitude of the hsp 70 mRNA induction shown in Fig. 1A and B appears to be similar to that for c-*fos*, the hsp 70 autoradiogram was exposed for only 2 h compared with 24 h for c-*fos*. c-*myc* mRNA levels changed little in response to incubation at 42°C (although see Fig. 1C), while metallothionein (MT) mRNA levels decreased by 1 h (Fig. 1B).

After the incubation temperature was lowered from 42 to 37°C (recovery), c-*fos* and hsp 70 mRNA slowly returned to preshock levels (Fig. 1B). The apparent transient decline in c-*fos* mRNA in the 1-h recovery sample (Fig. 1B) was not detected in other experiments. MT mRNA levels returned to preshock values by 3 h at 37°C (Fig. 1B). The induction of both c-*fos* and hsp 70 was temperature dependent (Fig. 1C), with maximal induction occurring at 44°C. The c-*fos* and hsp 70 autoradiographs were both exposed for 5 h for a more direct comparison of their relative amounts. The level of c-*myc* mRNA, in contrast to those of c-*fos* and hsp 70, was significantly reduced by incubation at 44°C (Fig. 1C). These results show that the increase in c-*fos* mRNA levels during heat stress is time and temperature dependent.

During heat stress, c-*fos* and hsp 70 mRNAs were detected exclusively in the polysomal fraction (Fig. 2), in which the c-*fos* mRNA level was increased approximately

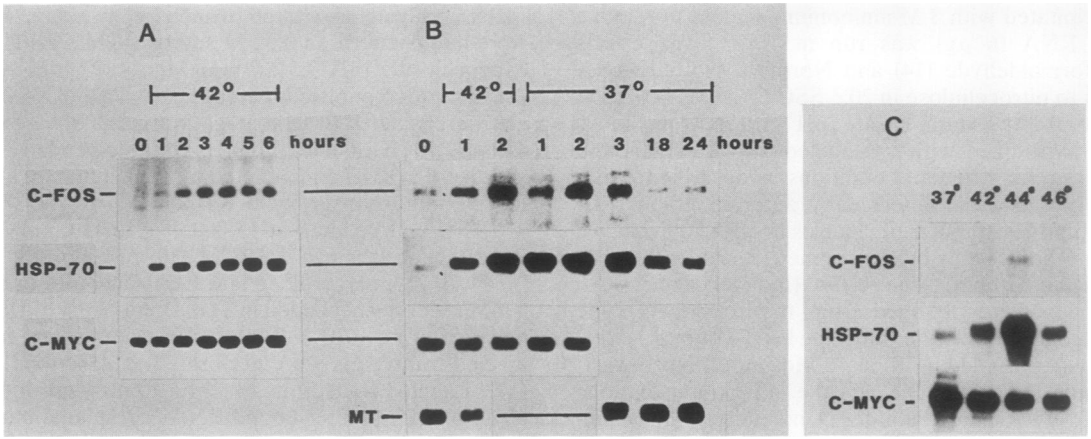

FIG. 1. Northern analysis of c-*fos*, hsp 70, c-*myc*, and MT mRNAs in HeLa cells exposed to heat stress. Cells were incubated at 42°C for up to 6 h (A) or for 2 h (B) at 42°C and then underwent a recovery period at 37°C for up to 24 h. (C) Cells were incubated at 42, 44, or 46°C for 4 h. Total RNA was subjected to formaldehyde-agarose gel electrophoresis and analyzed by Northern blot hybridization with cRNA probes.

50-fold over the level in control polysomes. Heat stress (42°C) had little effect on the distribution of c-*myc* and MT transcripts between the polysomal and nonpolysomal fractions. Some MT mRNA was also detected in the nonpolysomal fraction (Fig. 2). These results suggest that transcription, processing, and transport of c-*fos* mRNA occur during heat stress and that c-*fos* protein is synthesized at 42°C. However, the overall rate of protein synthesis during heat stress was reduced to 30% of the control rate within 0.5 h and slowly increased to 50% of the control rate by 3 h (data not shown).

HeLa cells were analyzed for the presence of c-*fos* protein by immunoperoxidase staining (Fig. 3). Control cells had low but reliably detectable levels of anti-*fos*-staining nuclear material (Fig. 3a), which could be increased by adding fresh medium (data not shown). All panels in Fig. 3 show HeLa cells stained with 6.6 μg of affinity-purified antibody per ml. The relative amounts of *fos* protein were estimated by determining the concentration of antibody at which *fos* protein was just detectable. Titration of *fos* staining by antibody dilution showed that cells cultured at 42°C for 1 and 2 h (Fig. 3b and c, respectively) contained approximately fivefold-higher concentrations of *fos* protein than did control cells. Cells incubated at 42°C for 4 h contained approximately 20-fold-higher levels (Fig. 3d). After the incubation temperature was returned to 37°C, *fos* levels fell back to fivefold higher than control levels for up to 4 h (Fig. 3f). Not all nuclei stained equally well with anti-*fos*, and c-*fos* can be rapidly lost during the recovery period (Fig. 3f). These experiments were repeated with cells in suspension and in monolayer and with three different anti-*fos* antibodies (Fig. 3e). These results established that the increased levels of c-*fos* mRNA found in HeLa cells during heat stress were accompanied by increased levels of c-*fos* protein, which in turn became associated with the nucleus.

To determine the relative contribution of gene transcrip-

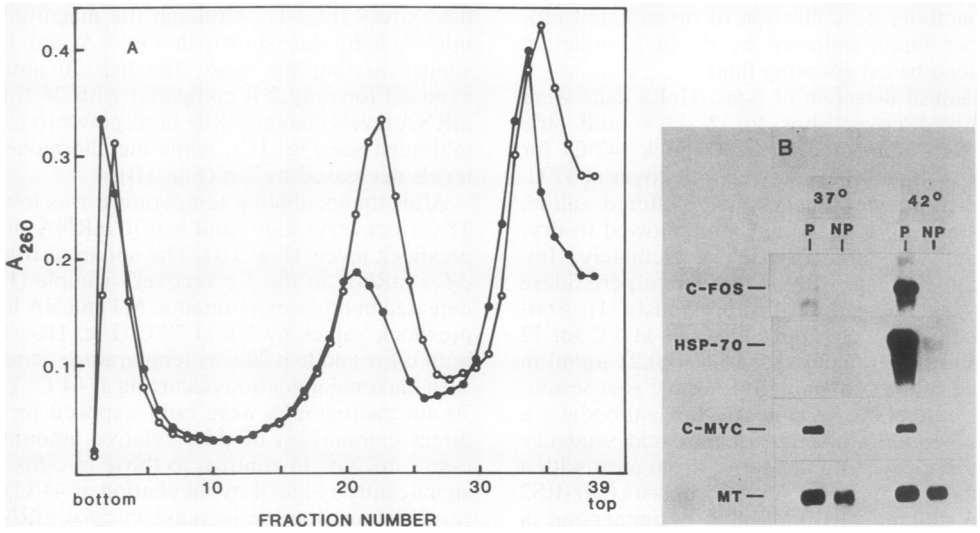

FIG. 2. Distribution of mRNAs in the polysomal and nonpolysomal fractions following heat stress of HeLa cells. (A) Cells were cultured at 37°C (●) or 42°C (○) for 4 h, and cell cytosols were fractionated into polysomal and nonpolysomal fractions by sucrose gradient centrifugation. (B) Distribution of c-*fos*, hsp 70, c-*myc*, and MT transcripts in the polysomal and nonpolysomal fractions were determined by Northern blot analysis.

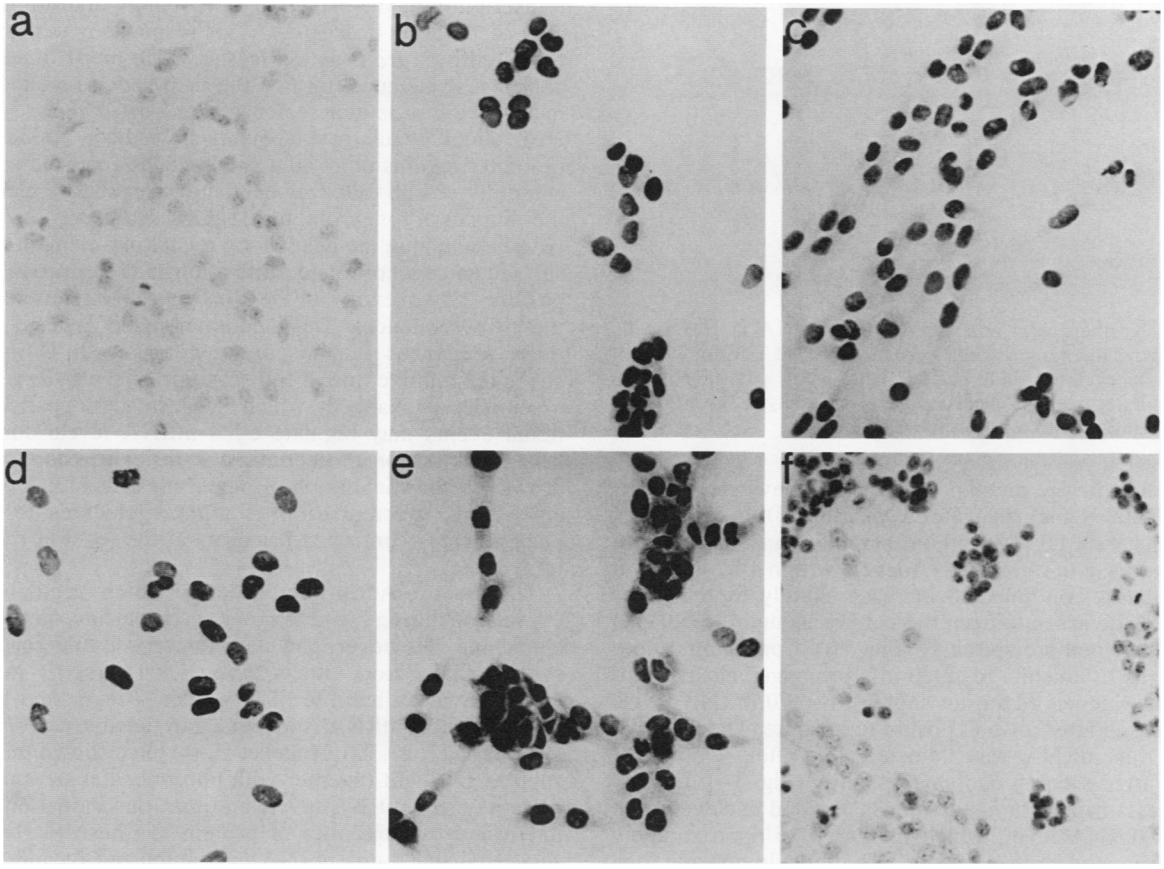

FIG. 3. Immunohistochemical staining for c-*fos* protein in heat-stressed HeLa cells. Cells were treated with anti-*fos* antibodies and stained by the avidin-biotin-peroxidase procedure. The localization of c-*fos* protein is indicated by the dark (brown)-staining nuclei. (a) Control cells; (b) 1-h heat shock (42°C); (c) 2-h heat shock; (d) 4-h heat shock; (e) 4-h heat shock, cells stained with rat anti-v-*fos* antiserum; (f) 4-h heat shock at 42°C and 1.5 h recovery at 37°C. Magnification, ×250.

tion to the induction of c-*fos*, HeLa cells were incubated at 42°C for 0.5 h and RNA polymerase runoff transcription assays were performed with isolated nuclei. Densitometric scanning of the autoradiogram in Fig. 4 showed that heat stress induced a 7.5-fold increase in the relative rate of hsp 70 gene transcription. Transcription of c-*myc* and c-*fos*, in contrast, remained essentially unchanged (1-fold and 1.6-fold respectively) (Fig. 4). Treatment of HeLa cells with actinomycin D or α-amanitin during heat stress completely prevented the increases in c-*fos* and hsp 70 mRNAs (data not shown). These results establish that continued transcription of the c-*fos* gene occurs during heat stress and suggest that posttranscriptional events are likely to contribute significantly to the enhanced expression of this gene.

Other agents known to induce the heat shock response

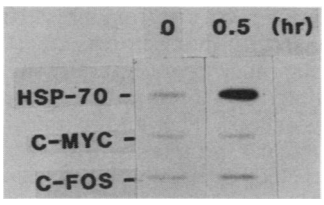

FIG. 4. Effect of heat stress on the relative rate of transcription of c-*fos*, hsp 70, and c-*myc* genes. HeLa cells were harvested after incubation at 42°C for 30 min. Nuclei were isolated, and runoff transcription assays were performed in the presence of [^{32}P]GTP. Labeled runoff transcripts were hybridized to filter-bound RNA complementary to hsp 70, c-*myc*, and c-*fos*. RNase A- and RNase T_1-resistant hybrids were detected by autoradiography.

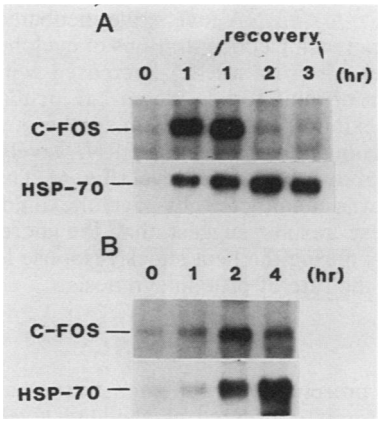

FIG. 5. Northern analysis of c-*fos* and hsp 70 mRNAs in HeLa cells exposed to arsenite or heavy metals. (A) Cells were incubated at 37°C in the presence of 80 μM sodium arsenite for 1 h, washed, and transferred to fresh medium, and the incubation was continued for 3 h (recovery). (B) Cells were incubated at 37°C with 10^{-4} M heavy metals for up to 4 h. Total RNA was subjected to formaldehyde-agarose gel electrophoresis and analyzed by Northern blot hybridization with cRNA probes.

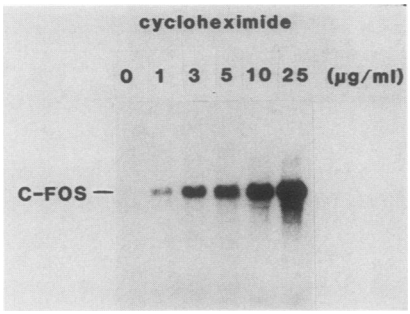

FIG. 6. Northern blot analysis of c-*fos* mRNA in HeLa cells exposed to cycloheximide. Cells were incubated at 37°C for 2 h with 0 to 25 μg of cycloheximide per ml. Total RNA was subjected to formaldehyde-agarose gel electrophoresis and analyzed by Northern blot hybridization with a c-*fos* cRNA probe.

(arsenite and heavy metals) also caused elevation of c-*fos* and hsp 70 mRNA levels in HeLa cells (Fig. 5). Incubation of HeLa cells with 80 μM sodium arsenite caused at least a 40-fold increase in c-*fos* mRNA levels within 1 h. The level of this mRNA continued to increase slightly for 1 h after removal of the arsenite from the culture medium (recovery) and then declined precipitously (Fig. 5A). Continuous exposure of cells to arsenite resulted in a prolonged elevation of c-*fos* mRNA levels (data not shown). hsp 70 mRNA levels also increased after a 1-h exposure to arsenite. The maximal level of this mRNA was detected 2 h after removal of arsenite, after which it decreased slightly (Fig. 5A). Incubation of HeLa cells with heavy metals resulted in elevation of the c-*fos* mRNA level, which increased to approximately 10-fold above the control level for 2 h of incubation and then declined. Increasing the concentration of heavy metals in the culture medium resulted in a prolonged elevation of c-*fos* mRNA (data not shown). hsp 70 mRNA levels were also increased in cells incubated with heavy metals (Fig. 5B). These results establish that a variety of agents which induce the heat shock response cause an elevation in the level of c-*fos* mRNA in HeLa cells.

One of the characteristics of the heat shock response is a reduction in the synthesis of non-heat-shock proteins (5, 7, 16). To examine the involvement of protein synthesis in the regulation of c-*fos* mRNA levels, we incubated HeLa cells for 2 h with increasing concentrations of cycloheximide (Fig. 6). The level of c-*fos* mRNA increased with increasing concentration of inhibitor. During a 2-h incubation with 25 μg of cycloheximide per ml, protein synthesis was inhibited by 95% (data not shown) and c-*fos* mRNA levels increased at least 80-fold above the control level (Fig. 6). The level of hsp 70 mRNA was unaffected by cycloheximide (data not shown). These results suggest that the increase in c-*fos* mRNA levels during the heat shock response is related to a reduction in the rate of protein synthesis.

DISCUSSION

The data presented here demonstrate that the proto-oncogene c-*fos* is expressed at elevated levels during the heat shock response in HeLa cells. This effect was obtained with several inducers (heat stress, arsenite, and heavy metals) of the heat shock response and was accompanied by the induction of hsp 70 gene expression.

The mechanisms regulating the expression of c-*fos* during heat shock differ from those regulating hsp 70 expression. As shown in this work (Fig. 4) and in previous studies (5, 7, 16), transcription of the hsp 70 gene is rapidly and dramatically increased during heat stress. This response is thought to be mediated by a *trans*-acting factor which binds to a specific consensus sequence, termed the heat shock regulatory element, in the promoter region of the hsp 70 gene (5, 7, 16). Heat shock regulatory elements have been found in the promoter regions of all heat shock genes analyzed so far. A computer search found no heat shock regulatory element in the human c-*fos* promoter region. However, sequences closely matching the heat shock regulatory element consensus sequence were found at position 1135 in intron 1 and at position 2814 in exon 4 of the c-*fos* gene (see reference 33 for the c-*fos* sequence). The functional significance, if any, of these sequences remains to be determined. During heat stress the relative rate of transcription of the c-*fos* gene was essentially unchanged, which suggests that posttranscriptional events may regulate c-*fos* mRNA levels during the heat shock response. In contrast, c-*fos* expression following growth factor stimulation of quiescent cells (12, 25, 26, 32) and during differentiation of monomyelocytes to macrophages (21) is controlled primarily at the level of transcription.

The posttranscriptional events which regulate c-*fos* expression during the heat shock response have not yet been elucidated. However, the data suggest a role for protein synthesis inhibition. Others have shown that c-*fos* mRNA is labile, having a half-life of less than 30 min, and that the half-life of this mRNA is increased in the absence of protein synthesis (11, 21, 22). Angel et al. (4) have shown that stress such as UV light together with phorbol ester treatment can increase c-*fos* mRNA levels and that this induction can be intensified by inhibitors of protein synthesis. It has been suggested that c-*fos* mRNA is destabilized by interactions between a short-lived protein and a conserved A+U-rich sequence in the 3′ untranslated region of the c-*fos* mRNA (27, 31). Inducers of the heat shock response (heat stress, arsenite, and heavy metals) have been shown in this work and in previous studies (7, 8, 15, 28) to inhibit protein synthesis. The finding that these inducers, as well as cycloheximide, cause an increase in the level of c-*fos* mRNA is consistent with the hypothesis that elevated expression of c-*fos* during the heat shock response reflects an increased stability of this mRNA, owing to the decreased synthesis of a protein which regulates the stability of c-*fos* mRNA.

The role, if any, that c-*fos* may play in the response of a cell to stress is uncertain. Bravo et al. (6) have suggested that c-*fos* is involved in the process of growth competence, and a variety of other studies have demonstrated that growth factor stimulation of quiescent cells can induce a rapid, transient expression of c-*fos* (9, 23, 34, 35). It is possible that the increased level of c-*fos* mRNA found during the heat shock response in HeLa cells facilitates the reinitiation of the cell cycle during recovery from stress. In this regard, it is conceivable that c-*fos* may serve as a heat shock protein. The classical heat shock proteins are thought to provide a protective function (5, 7, 16), but their precise physiological roles are poorly defined. It is known that hsp 70 (16) and c-*fos* (34) are nuclear proteins which are transiently expressed during the cell cycle, and it has been suggested that they may also play a role in cell differentiation. However, whether enhanced expression of c-*fos* during the heat shock response is a general phenomenon remains to be tested. Several observations suggest this possibility. We have detected induction of c-*fos* following heat stress of NR6 mouse fibroblasts (unpublished results) and following heavy metal treatment of the embryonal carcinoma cell line OC15S1 (1a).

Heat stress, arsenite, and heavy metals can also cause elevated levels of c-*fos* mRNA in 3T3, A431 (human epithelioid carcinoma), and NB2a (mouse neuroblastoma) (R. M. Gubits, Abstr. Annu. Meet. Am. Soc. Cell Biol. 1986, abstr. no. 611, p. 166a; R. Müller, personal communication) cells. On the other hand, preliminary studies suggest that c-*fos* mRNA is not significantly elevated in CV-1 (African green monkey kidney) or NB2 (rat lymphoma) cells during heat stress (unpublished results). The results reported here suggest the possibility that only cell types in which the c-*fos* gene is already transcriptionally active will demonstrate elevated levels of c-*fos* mRNA during the heat shock response.

In conclusion, the proto-oncogene c-*fos* is expressed at significantly elevated levels during the heat shock response in HeLa cells. Elevated c-*fos* mRNA levels are not due primarily to increased transcription rate, but are likely to reflect posttranscriptional events. The evidence suggests that decreased protein synthesis as a result of exposure to heat stress, arsenite, or heavy metals may be involved in the regulation of c-*fos* expression during the heat shock response, perhaps by decreasing c-*fos* mRNA turnover. These observations suggest that there may be other mRNAs which are posttranscriptionally induced during the heat shock response and that these mRNAs may have important physiological functions during the stress response.

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