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Cell stress and translational inhibitors transiently increase the abundance of mammalian SINE transcripts

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ABSTRACT

The abundance of *Alu* RNA is transiently increased by heat shock in human cell lines. This effect is specific to *Alu* repeats among Pol III transcribed genes, since the abundance of 7SL, 7SK, 5S and U6 RNAs is essentially unaffected by heat shock. The rapid induction of *Alu* expression precedes the heat shock induction of mRNAs for the ubiquitin and HSP 70 heat shock genes. Heat shock mimetics also transiently induce *Alu* expression indicating that increased *Alu* expression is a general cell-stress response. Cycloheximide treatment rapidly and transiently increases the abundance of *Alu* RNA. Again, compared with other genes transcribed by Pol III, this increase is specific to *Alu*. However, as distinguished from the cell stress response, cycloheximide does not induce expression of HSP 70 and ubiquitin mRNAs. Puromycin also increases *Alu* expression, suggesting that this response is generally caused by translational inhibition. The response of mammalian SINEs to cell stress and translational inhibition is not limited to SINEs which are *Alu* homologues. Heat shock and cycloheximide each transiently induce Pol III directed expression of B1 and B2 RNAs in mouse cells and C-element RNA in rabbit cells. Together, these three species exemplify the known SINE composition of placental mammals, suggesting that mammalian SINEs are similarly regulated and may serve a common function.

INTRODUCTION

The nearly one million *Alu* repeats interspersed throughout human DNA share a 282 nt consensus which includes an internal promoter for RNA polymerase III (Pol III) (1,2). *Alu*s are generated by retrotransposition through an RNA intermediate, and the 5′ end of most *Alu* repeats corresponds precisely to the Pol III transcriptional start site, showing that these promoters function *in vivo* (1,2). However, despite the abundance of potential templates, corresponding Pol III directed *Alu* transcripts are expressed at a very low level in cultured cells (3,4). The abundance of *Alu* elements, and the paucity of corresponding transcripts, are each curious.

All mammalian DNAs contain short, highly repetitive, retrotransposed sequences, usually called SINEs (1,2,5), which can be assigned to either of two large superfamilies of sequences. *Alu* repeats are ancestrally related to 7SL RNA, as are rodent B1 repeats. Rodent DNAs contain a second SINE family, B2 repeats, ancestrally derived from tRNA sequences. Here we refer to *Alu*, B1 and other elements which are related to 7SL RNA as being *Alu*-like, and to mammalian SINEs which are ancestrally derived from tRNA precursors as being B2-like. There are no detectable B2-like elements in DNAs of higher primates, while in most mammalian orders, SINEs are exclusively B2-like elements. For example, rabbit DNA contains B2-like C elements, but does not contain *Alu*-like SINEs (6). With respect to SINE composition, mammals belong to one of three groups: those containing exclusively *Alu*-like elements, e.g. human; those containing exclusively B2-like elements, e.g. rabbit; and those containing both types, e.g. mouse (1,2). Either mammalian SINEs do not have a common function, or that function must be satisfied by nonhomologous sequences in different mammalian orders. However, each of these two superfamilies is ancestrally derived from a sequence having a translational role and an internal Pol III promoter.

Because of their ubiquitous interspersion within Pol II directed transcription units, SINEs are abundantly expressed in short-lived nuclear transcripts (1,2). Here, we consider only Pol III directed SINE transcripts, as these are more likely to have a defined function. In HeLa cells, these cytoplasmic transcripts co-sediment (11S) with SRP (4). The 282 nt consensus *Alu* sequence is a dimeric tandem repeat which is followed by an A rich region resembling a poly A tail. Consequently, full length *Alu* transcripts have an average length of 400 nt and fractionate as poly A plus RNA. Shorter (118 nt) cytoplasmic transcripts, called sc*Alu* RNA, correspond to the left monomer of the dimeric *Alu* consensus sequence and fractionate as poly A minus RNA (7). *Alu* RNA and sc*Alu* RNA in HeLa cells are present in the range of 100–1000 copies/cell (4). The steady-state expression of rodent B1 RNA is also low in cultured cells, and there is evidence for a processed scB1 RNA (7,8). Relative to *Alu* and B1 repeats,

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rodent B2 repeats are more abundantly expressed in cultured cells (1,2,9).

Results from *in vitro* transcription studies suggest that the expression of *Alu* RNA is down-regulated at multiple levels (10–13). For example, *Alu* repeats are heavily methylated in cultured cells and somatic human tissues (14). Methylation represses *Alu* transcription *in vitro* by a factor which specifically binds methylated DNAs (11,12), and treating HeLa cells with 5-azacytidine to demethylate *Alu*s stimulates their transcriptional expression (4). Subsequently, we have found that demethylated *Alu* templates transiently transfected into HeLa cells are inactive, whereas the identical templates are abundantly expressed in 293 cells (unpublished). These results indicate that demethylation is not sufficient for *Alu* expression, and that some other effect of 5-azacytidine induces *Alu* expression in HeLa cells. Chronic exposure of cells to 5-azacytidine is toxic, causing us to speculate that *Alu* expression may respond to cell stress. One aspect of the heat shock response, as well as other cell stress treatments, is altered transcriptional expression (15). While B2 repeats are not necessarily an ideal model for *Alu* repeats, heat shock greatly increases B2 expression (16,17).

Infection of cells with adenovirus, HIV and HSV induces *Alu* expression (18–20). A combination of at least four adenovirus gene products is required to induce *Alu* expression, indicating the complexity of this response (18). Among many other effects, viral infection selectively inhibits translational expression of certain host mRNAs. This effect is reminiscent of the cell stress response which selectively inhibits the translational expression of non-heat shock mRNAs (21). Cycloheximide treatment stimulates *Alu* expression in an adenovirus-transformed human cell line, 293 cells, again indicating a possible connection between *Alu* expression and the translational state of the cell (18).

For these reasons, we investigated whether SINE expression might be increased by cell stress and translational inhibition. Here, we show that expression of both *Alu*-like and B2-like SINE RNAs is transiently induced by cell stress and translational inhibition, suggesting a unifying role for all mammalian SINEs.

MATERIALS AND METHODS

Cell culture

HeLa cells and mouse NIH 3T3 cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) containing 5% calf serum and 5% fetal bovine serum; 293 cells (human transformed primary embryonal kidney cells, ATCC) were grown in α-minimal medium supplemented with 10% new-born calf serum. RAB-9 cells (rabbit skin fibroblast, ATCC) were grown in Eagle's Basal Medium with Earle's BSS, 5% calf serum and 5% fetal bovine serum. All cells were maintained in 5% CO_2 at 37°C. A variety of heat shock conditions were explored in preliminary experiments with results similar to those reported here. For heat shock experiments reported here, cells were heated in 175 cm^2 flasks containing 30 ml of medium in a 45°C water bath. The heating time was 30 min for HeLa, 293 and RAB-9 cells, and 12 min for NIH 3T3 cells (16). After heat shock, cells were incubated at 37°C for the time indicated prior to RNA extraction. For cycloheximide treatment experiments, cells were grown in the presence of 100 μg/ml of cycloheximide (18,22) for the time indicated prior to RNA extraction. Similar experiments were performed on other treatments of cells: puromycin (100 μg/ml) (22), α-amanitin (4 μg/ml), arsenite (50 μM) and ethanol (1%) (23).

RNA preparation and RNP isolation

Cytoplasmic and nuclear RNAs from cells were prepared and separated into poly(A)$^+$ and poly(A)$^-$ RNAs as described previously (4). Similarly, cytoplasmic RNPs were fractionated by sucrose gradient centrifugation as previously described (4).

Oligodeoxyribonucleotides

The following custom-synthesized oligonucleotides were used for primer extension and Northern blot analysis:

Table 1.

Name	Sequence 5′→3′	Position	Size of extension product
Alu 21mer (4)	GCGATCTCGGCTCACTGCAAG	238–218	238 (240)
P-S *Alu* (18)	TTAGTAGAGAC/$_G$GGGGTTTCACCATG	120–96	120
Alu #71 (24)	GGTTTCACCGTGTTAGCCA	89–107	107
7SL (25)	ATGCCGAACTTAGTGCGG	129–112	129
5S (26)	AAAGCCTACAGCACCCGGTATT	120–99	120
7SK (27)	CGGGGATGGTCGTCCTCTTC	160–141	160
U6 (28)	TATGGAACGCTTCACGAATTTGC	102–80	102
β-Actin (29)	GCCTGGGGCGCCCCACGAT	118–100 (coding)	190
HSP70 (30)	CGCGAGAAGAGCTCGGTCCTT	150–130 (non-coding)	150
Ubiquitin (31)	CCACCTCAAGGGTGATGGTCTT	52–31 (coding)	148
Mouse B1 (32)	CTGGCTGTCCTGGAACTCACT	110–90	110
Mouse B2 (33)	TACACTGTAGCTGTCTTCAGACA	155–133	155
Rab-1 (6)	TCYRYTGGTTCACTCCCCAAAT	258–238	258
Rab-2 (6)	GGYCCCAAGNACTTGGGCCAT	159–139	159

Primer extension assays

Primer extension assays were performed with slight modifications of our previous methods (4). Primers labeled by T4 polynucleotide kinase with [γ-^{32}P]ATP were ethanol precipitated. RNA in 10 µl of annealing buffer (10 µM Tris–HCl, pH 7.5, 1 µM EDTA, 0.3 M KCl) and 5–10 pmol (~10^5 c.p.m.) of a primer were incubated at 95°C for 2 min and then allowed to anneal at 56, 46 (7SL only) or 64°C (P–S *Alu* only) for ≥1 h. Following addition of 40 µl of extension buffer (10 mM Tris–HCl pH 8.6, 5 mM $MgCl_2$, 5 mM DTT, 1 mM dNTP and 1 U Inhibit-ACE), the reaction mixture was incubated with 20 U AMV reverse transcriptase (Life Science Inc.) for ≥3 h at 42°C. Subsequent analysis of the primer extension products was performed as previously described (4). The resulting gel was exposed to X-ray film, optionally using an intensifying screen at –70°C. Quantitation of the extension products was performed with the Fujix BAS 1000 PhosphorImager.

Northern blot analysis

For Northern blots from agarose gels, RNA was separated on 1.5% agarose containing 7% formaldehyde and 1 × MOPS (Sigma) (34). After soaking in transfer buffer to remove formaldehyde, the gel was transferred to Hybond-N$^+$ membrane (Amersham) overnight and the membrane was dried at 80°C. Blotting from 5% polyacrylamide gels (40:1 acrylamide:bis) was performed as previously described (4). The membrane was incubated with prehybrization buffer (50 µM phosphate buffer, pH 6.8, 5 × SSC, 10 × Denhardt's solution, 0.5% SDS and 100 µg/ml yeast tRNA) at 44°C for 6 h, and then was hybridized with oligoprobe #71 (10 ng/ml) (24) in hybridization buffer (25 mM phosphate buffer, pH 6.8, 5 × SSC, 10 × Denhardt's solution, 0.5% SDS) at 44°C for 20 h. The membrane was washed twice with 5 × SSC, 0.1% SDS at room temperature and once at the hybridization temperature for 10 min. Autoradiography and PhosphorImager analysis was performed as described above.

RESULTS

Cell stress specifically induces *Alu* expression in human cell lines

Primer extension by reverse transcriptase provides a sensitive assay to detect Pol III directed *Alu* transcripts in HeLa cells against the background of *Alu*s present in Pol II transcripts (4). The 240 nt primer extension product predicted for full length Pol III directed *Alu* transcripts when using *Alu* 21mer oligonucleotide is observed in 293 cells (Fig. 1A). The assignment of this product to Pol III directed transcripts has previously been verified by the corresponding cDNA sequences (4). Primers mapping elsewhere in the *Alu* consensus sequence reconfirm this assignment in the present study (data not shown). Additionally, a 350 nt primer extension product, previously assigned to a HeLa mRNA containing an *Alu* repeat, is observed in 293 cells (Fig. 1A).

Heat shock of 293 cells induces a transient increase in *Alu* RNA level as demonstrated by the abundance of the 240 nt primer extension product (Fig. 1A). In this particular experiment, there is a 4-fold induction of *Alu* RNA after a 2 h recovery from heat shock. Heat shock at lower temperatures (42°C) also transiently induces *Alu* expression (data not shown).

Whereas *Alu* expression is transiently induced by heat shock, the abundance of 7SL RNA, 7SK RNA, 5S RNA and U6 RNA is essentially unchanged by heat shock during the time period of the *Alu* response (Fig. 1B). Among Pol III transcripts tested, the increase due to heat shock is specific to *Alu* repeats. The constant level of these other Pol III transcripts incidentally provides an internal control, demonstrating that the amount of RNA being examined in primer extension assays is constant. In each of the experiments reported below (specifically heat shock of mouse and rabbit cells and cycloheximide treatment of human, mouse and rabbit cells), this same study of other Pol III directed transcription units was routinely performed with virtually identical results. Accordingly, we do not show additional examples of the experiment depicted in Figure 1B.

Actin mRNA, which is not heat shock inducible (17), shows a slight decrease after a long recovery from heat shock (Fig. 1C). HSP 70 mRNA and ubiquitin mRNA are also subject to heat shock (17,31). Transient increases in the levels of the predicted primer extension products for HSP 70 and ubiquitin mRNAs reach a maximum after 4 h of recovery from heat shock (Fig. 1C). Under these experimental conditions, the heat shock response of *Alu* transcripts is faster than the response of known heat shock mRNAs. However, the kinetics and magnitude of the heat shock response depend critically on the dose and severity of the treatment so that under other conditions, the relative kinetics of *Alu* and HSP RNAs may be different (17).

Like basal *Alu* transcripts in HeLa cells (4), heat shock induced *Alu* transcripts fractionate as cytoplasmic, poly(A)$^+$ RNAs and cytoplasmic fractions containing these transcripts co-sediment with SRP at 11S (not shown). By Northern blot analysis, using a hybridization probe positioned in the right *Alu* monomer, the size range of the induced *Alu* transcripts centers on 300–500 nt (Fig. 2A). A 6-fold increase of this transcript level is observed in heat shocked 293 cells in agreement with the results from the primer extension assay described above. The basal level of full length *Alu* transcripts is much lower in HeLa cells (4; Fig. 2A). Otherwise, the results obtained for heat shocking HeLa cells are similar to those for 293 cells (Fig. 2A; data not shown). The length heterogeneity of *Alu* transcripts, which is more apparent in high resolution acrylamide gels (Fig. 2B), indicates that many loci must be expressed. We have not yet investigated the relative activity of different *Alu* loci.

Probes positioned in the left *Alu* monomer hybridize to both full length and 118 nt sc*Alu* RNA. The abundance of sc*Alu* RNA is not significantly increased (~150% of the control) by heat shock (Fig. 2B). The differential response of *Alu* RNA and sc*Alu* RNA to heat shock is considered in the Discussion.

Ethanol and arsenite elicit the cell stress response similar to heat shock (35). Treatment of cells with either ethanol or sodium arsenite induces a transient increase in *Alu* expression (data not shown) indicating that *Alu* induction is a component of the general cell stress response.

Translational inhibition induces *Alu* expression in human cell lines

Treatment of HeLa cells with cycloheximide transiently induces *Alu* expression (Fig. 3). Induction occurs within 20 min of exposure to cycloheximide (Fig. 3) resulting in a 20-fold increase after 3 h (Fig. 3). While the effects of translational inhibition are likely to be extremely complex, the rapidity of this response

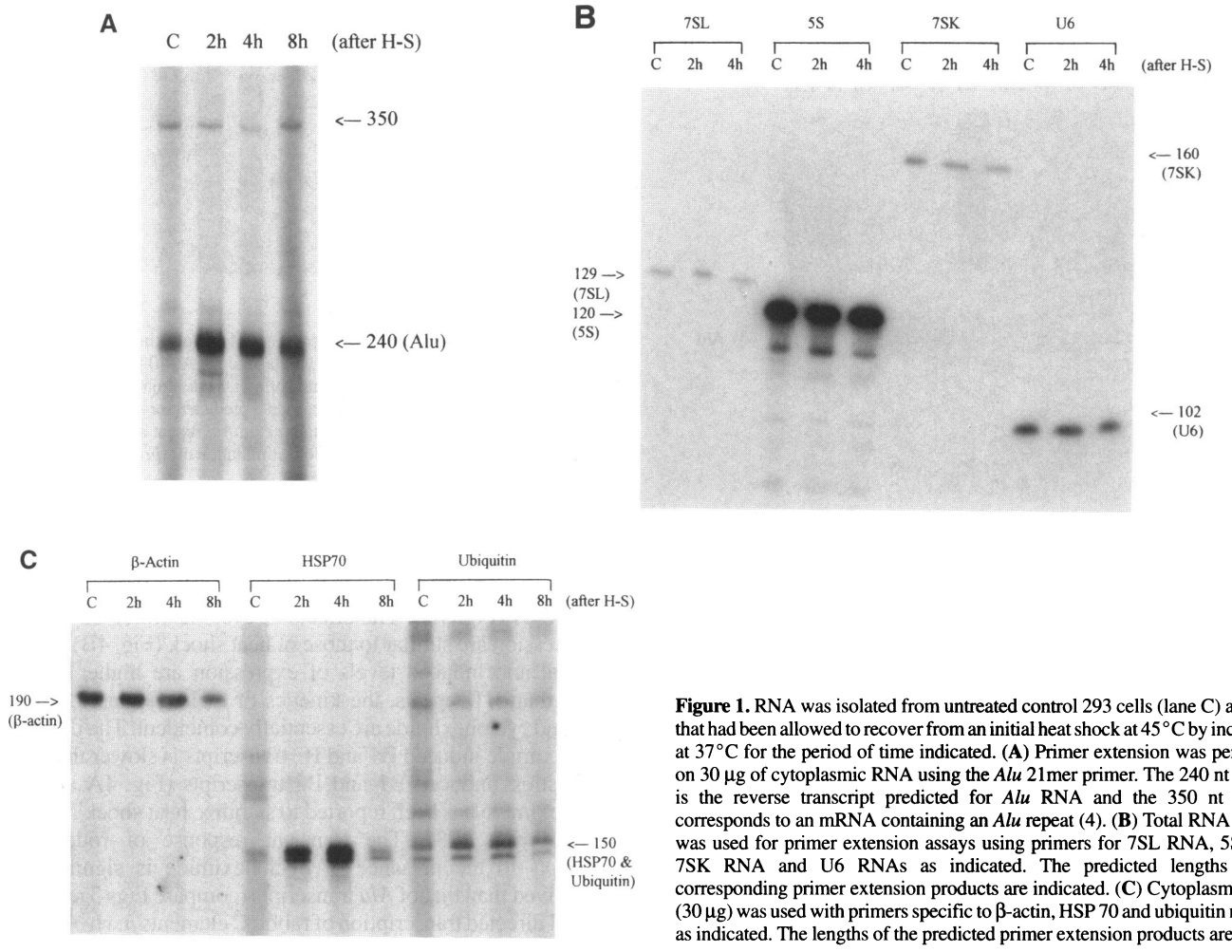


Figure 1. RNA was isolated from untreated control 293 cells (lane C) and cells that had been allowed to recover from an initial heat shock at 45°C by incubation at 37°C for the period of time indicated. (**A**) Primer extension was performed on 30 μg of cytoplasmic RNA using the *Alu* 21mer primer. The 240 nt product is the reverse transcript predicted for *Alu* RNA and the 350 nt product corresponds to an mRNA containing an *Alu* repeat (4). (**B**) Total RNA (10 μg) was used for primer extension assays using primers for 7SL RNA, 5SrRNA, 7SK RNA and U6 RNAs as indicated. The predicted lengths of the corresponding primer extension products are indicated. (**C**) Cytoplasmic RNA (30 μg) was used with primers specific to β-actin, HSP 70 and ubiquitin mRNAs as indicated. The lengths of the predicted primer extension products are shown.

suggests that *Alu* expression is tightly coupled to the translational state of the cell. As assayed by Southern blot hybridization and primer extension, 293 cells also show a transient response when treated with cycloheximide (Fig. 2A; data not shown). These results confirm and extend Panning and Smiley's initial observations that cycloheximide stimulates *Alu* expression in 293 cells and that the level of stimulation observed is variable (18). The effect of cycloheximide is not limited to adenovirus-transformed cells and the response is transitory.

A 20-fold increase in the steady state concentration of *Alu* RNA converts *Alu* transcripts from the barely detectable (100–1000 copies) in uninduced HeLa cells into a relatively abundant RNA (Figs 2A and 3). These estimates are confirmed by comparing the intensities of primer extension products for 7SL RNA and for cycloheximide induced *Alu* RNA enriched in an 11S cytoplasmic fraction by sucrose gradient sedimentation. On a cell equivalent basis, *Alu* transcripts in cycloheximide stimulated HeLa cells approach 5% of the level of an abundant transcript 7SL RNA.

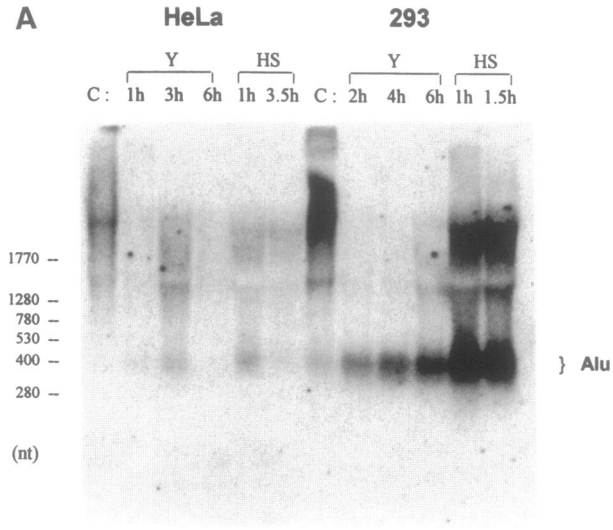
Like the heat shock induced transcripts, the cycloheximide induced *Alu* transcripts are cytoplasmic, poly(A)$^+$ RNAs in HeLa and 293 cells have an average size of ~400 nt (Fig. 2A) and the subcellular fraction containing *Alu* RNA co-sediments with SRP. Relative to full length transcripts, the abundance of 118 nt sc*Alu* RNA is not stimulated by cycloheximide (Fig. 2B).

In contrast to the previous results for heat shocked cells, the 350 nt primer extension product shows a transient decrease upon cycloheximide treatment (Fig. 3). To account for this response, cycloheximide might decrease either its rate of transcriptional expression or its lifetime.

The effect of cycloheximide, like that of heat shock, is specific to *Alu* among Pol III directed transcripts, as no increase in the abundance of 7SL RNA, 7SK RNA, 5S RNA or U6 RNA is observed, as assayed by primer extension (not shown). In agreement with previous publications, and in contrast to heat shock (22,36,37; Fig. 1C), cycloheximide does not induce the expression of HSP 70 mRNA or ubiquitin mRNA (data not shown). *Alu* expression, while part of the cell stress response, can be thus induced independently of the transcriptional expression of other heat shock genes.

Puromycin, which blocks protein synthesis at a different point than cycloheximide, also transiently induces *Alu* expression (not shown). We conclude that translational inhibition rapidly induces transient expression of *Alu* RNA and that this induction is independent of novel protein synthesis.

The effect of α-amanitin on the cycloheximide induction of *Alu* RNA was examined as a further test of whether the induced *Alu* transcripts are Pol III directed. In quantities sufficient to inhibit Pol II, but not Pol III directed transcription, α-amanitin alone actually stimulates *Alu* expression and α-amanitin and cycloheximide in combination have an additive effect (data not shown). We also notice that continuous exposure of cells to cycloheximide for 1 day (Fig. 3) causes a second increase in *Alu* expression.



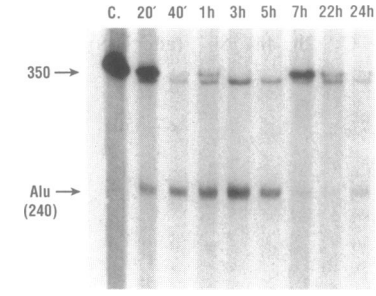


Figure 3. HeLa cells were grown in the presence of cycloheximide (100 µg/ml) for the indicated periods of time, and cytoplasmic RNA (40 µg) was assayed by primer extension using the *Alu* 21mer primer. C designates control cells. Positions of the 240 nt primer extension product predicted for *Alu* RNA and the 350 nt product for a HeLa mRNA containing an *Alu* repeat are indicated. By PhosphorImager analysis, there is a 16-fold stimulation of *Alu* expression at the peak response.

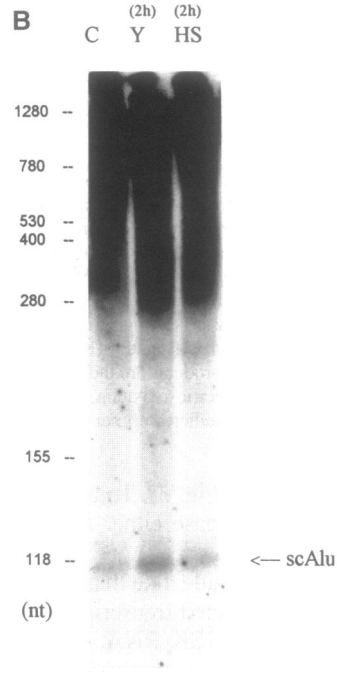


Figure 2. Northern blot analysis of cycloheximide and heat shock induced *Alu* transcripts. (**A**) Cytoplasmic RNA from HeLa and 293 cells, as indicated, was separated by agarose gel electrophoresis and hybridized with *Alu* 21mer. C designates untreated control cells, HS designates heat shock, Y designates cycloheximide treatment, and the number of hours following recovery from heat shock or cycloheximide addition are indicated. The amounts of RNA employed were 110 µg for all 293 cell samples, 70 µg for cycloheximide treated HeLa cells and 100 µg for heat shocked HeLa cells. The approximate size range of full length *Alu*s is indicated. (**B**) Cytoplasmic RNA (75 µg) from 293 cells was separated on an acrylamide gel and, after blotting, hybridized with *Alu* #71 oligonucleotide. Lane designations are identical to those in (A). To detect sc*Alu*, the autoradiogram of full length transcripts is intentionally overexposed.

Heat shock and cycloheximide induce non-primate SINE expression

Heat shock of mouse cells induces a rapid transient increase in the abundance of B1 repeat RNA, in exact analogy to its effect on *Alu* expression in human cells (Fig. 4A). Confirming previous findings (16,17,23), heat shock also increases the level of B2 repeat RNA (Fig. 4A). The effects of cycloheximide on B1 and B2 expression are similar to those of heat shock (Fig. 4B). While the basal and induced levels of expression are higher for B2 repeats than B1 repeats, the kinetics of their responses to heat shock and cycloheximide are essentially coincident. The decay of cycloheximide induced B1 and B2 transcripts is slower than that of cell stress induced B1 and B2 transcripts (Fig. 4A and B). Cycloheximide has been reported to stabilize heat shock induced B2 transcripts (23). The transient response of rodent B2 transcripts to the presence of cycloheximide is significantly longer-lived than that of *Alu* transcripts (compare Figs 3 and 4B).

Pol III directed transcription of rabbit C elements *in vivo* has not yet been demonstrated. Three primer extension products are observed in rabbit cells when using the primer Rab-1 (Fig. 5A and B). The principal product, 258 nt, corresponds to the product length predicted for Pol III directed transcripts from the rabbit C element consensus sequence (6). Individual C elements exhibit insertions with respect to this consensus sequence (6), thereby accounting for the minor primer extension products. The assignment of these RNAs as being Pol III directed transcripts was verified by using a second oligonucleotide, Rab-2, positioned elsewhere in the rabbit C element consensus sequence (not shown). We therefore conclude that members of the different subfamilies of rabbit C elements are expressed *in vivo* by Pol III.

Heat shock and cycloheximide treatment of rabbit cells induce a transient increase in the abundance of Pol III directed C element transcripts (Fig. 5A and B). The kinetics of both the heat shock and cycloheximide responses resemble those observed in rodent cells for B1 and B2 transcripts (Figs 4 and 5). In both species, the decay of the cycloheximide response back to the basal level exceeds 1 day, and is significantly slower than that observed for human *Alu* transcripts.

DISCUSSION

Translational inhibition and cell stress are interrelated

Expression of human *Alu* and rodent B1 transcripts exhibits a classic heat shock response; the effects of ethanol and sodium arsenite show that *Alu* expression is generally subject to the cell

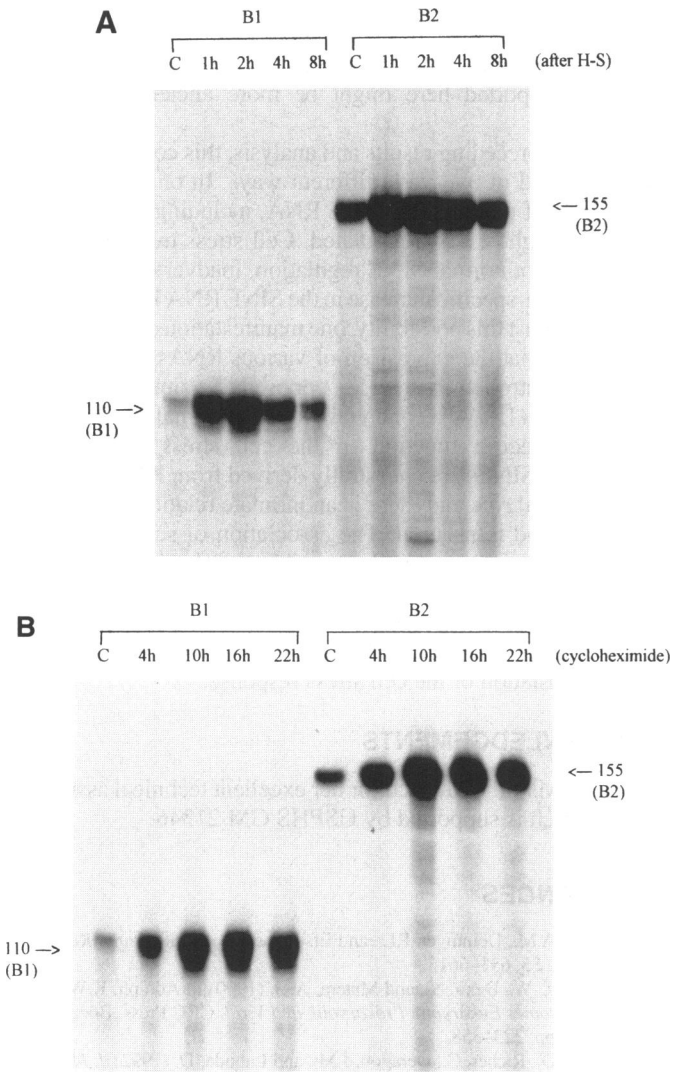


Figure 4. Cytoplasmic RNA (10 μg) from mouse 3T3 cells was examined by primer extension using oligonucleotide primers for B1 and B2 transcripts. The predicted sizes of the primer extension products are indicated by arrows and C designates control cells. (**A**) Cells were heat shocked and allowed to recover for the indicated period of time by incubation at 37°C. (**B**) Cells were grown in the presence of cycloheximide for the indicated period of time.

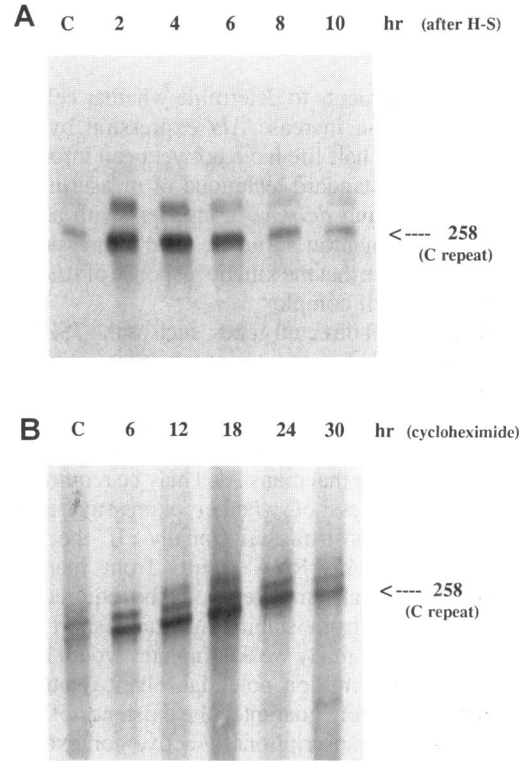


Figure 5. Cytoplasmic RNA (20 μg) from rabbit (RAB-9) cells was examined by primer extension using oligonucleotide primer Rab-1. The predicted length of the primer extension product for Pol III transcripts of rabbit C elements is indicated by an arrow. Minor bands are discussed in the text. Lane C designates control cells. (**A**) Cells were heat shocked and allowed to recover for the indicated period of time by incubation at 37°C. (**B**) Cells were grown in the presence of cycloheximide for the indicated period of time.

stress response. In addition to cell stress, cycloheximide and puromycin rapidly induce expression of human *Alu* and rodent B1 sequences. An important issue is whether *Alu* induction by cell stress and translational inhibition follows a common pathway or represents two unrelated phenomena.

There is an intimate connection between cell stress and translational inhibition such that either might be viewed as a cause or an effect of the other. Denatured proteins induce the cell stress response, and one early event in this response is inhibition of translation of non-heat shock mRNAs (21,35). Presumably, translational inhibition under cell stress conditions prevents the further accumulation of misfolded protein products. Translation in the presence of inhibitors, such as cycloheximide and puromycin, is expected to produce truncated and misfolded nascent polypeptides. Cells, treated with cycloheximide or puromycin, modify HSP 27 and become tolerant to a subsequent heat shock at temperatures <42°C, but do not increase the abundance of heat shock mRNAs (22,36,37). In this sense, exposing cells to translational inhibitors invokes a partial cell stress response. In summary, while the increased expression of *Alu*-like elements is part of the normal cell stress response, their induction is also triggered by translational inhibition, which might be regarded as either a downstream event in the general cell stress regulatory pathway or as an inducer of a partial cell stress response.

Alu expression is inducible by a sufficient concentration of α-amanitin to inhibit Pol II directed mRNA transcription as well as by two translational inhibitors. The rapidity of *Alu* induction as well as its independence from both mRNA and protein synthesis suggest that it results from modifying the activity of an existing factor. HSP 27, modified by treatment of cells with cycloheximide, is one possibility (36–38).

Either transcriptional or post-transcriptional activation might require many *Alu*s

Alu expression might be increased by either transcriptional activation or increased life time of the transcripts. The effects of heat shock and cycloheximide are specific to *Alu* transcripts in that there is no change in the abundance of 7SL RNA, 7SK RNA, 5S RNA and U6 RNA, each of which is a Pol III directed transcript. However, these four RNAs are already abundant and

long lived; a significant increase in either their transcription or their half life might not perceptibly increase their steady state concentrations.

Preliminary experiments to determine whether cell stress and translational inhibition increase *Alu* expression by increasing transcription or RNA half life have not yet been informative. As just one example, a standard technique of measuring RNA life times involves blocking *de novo* transcripts with actinomycin. However, since α-amanitin stimulates *Alu* expression, there is every reason to believe that the kinetic response of *Alu* expression to actinomycin is itself complex.

Conventional Pol III directed genes, such as the 7SL RNA gene having 5′ enhancer sequences, are much more active templates than *Alu* repeats (39,40; unpublished results this laboratory). The increase in *Alu* RNA expression can be dramatic, approaching 5% of the abundance of 7SL RNA. Because *Alu*s are inherently weak templates, we speculate that many *Alu*s may be required to mount this response, regardless of whether expression is increased transcriptionally or post-transcriptionally. If the cell stress induced increase in *Alu* RNA results from increasing *Alu* transcription, many weak templates would be required. If instead, this response results from stabilizing many short-lived transcripts, the products of many weak templates would be required. The existence of one million potential, albeit relatively weak, templates may effectively guarantee the existence of a sufficient number of *Alu*s in a transcriptionally active context in any cell type to mount the required response. This reasoning tacitly assumes that *Alu* RNA may have a role in the cell stress response, an assumption discussed in the conclusion.

We also observe that treatment with α-amanitin and chronic long term exposure to either cycloheximide (~1 day) or 5-azacytidine (~1 week) causes an increase of *Alu* RNA in human cells (4; this study). Each of these treatments might indirectly stress cells or alter translational expression. Regardless of which of these interpretations are correct, translational inhibition by cycloheximide (Fig. 3) more immediately causes *Alu* induction.

While cell stress and translational inhibition increase the abundance of *Alu* RNA, these treatments have little or no effect on the expression of sc*Alu* RNA. Similarly, 5-azacytidine stimulates the expression of *Alu* RNA without affecting the abundance of sc*Alu* RNA (4). These findings do not contradict the proposal that sc*Alu* RNA results from processing *Alu* transcripts, but merely introduce a further requirement that this processing is limited by some factor other than the abundance of the primary transcripts.

Stress induced expression of mammalian SINEs suggests a common function

An assumption underlying this study is that understanding the regulated expression of *Alu*-like elements should provide insight into their presumed function. A major difficulty with this proposal is that most mammals do not have SINEs which are homologous to *Alu* repeats. Rodent B2 repeats and rabbit C repeats belong to the superfamily of mammalian SINEs derived from tRNAs (Introduction). Like that of human *Alu* and mouse B1 repeats, the expression of mouse B2 and rabbit C repeats is also transiently induced by heat shock and translational inhibition. The three species examined here are likely to be representative of all placental mammals which have either only *Alu*-like repeats, only B2-like repeats, or both. Therefore, we confidently expect that cell stress and translational inhibition would increase the level of SINE RNA in all mammals. B2-like SINEs have been identified in plants, insects and vertebrates, suggesting that the responses reported here might be more anciently conserved (1,2,41,42).

Based on preceding results and analysis, this conclusion might be interpreted in two very different ways. In one view, normal expression of mammalian SINE RNA, including especially *Alu* repeats, is tightly down regulated. Cell stress treatments might simply disrupt normal SINE regulation, inadvertently causing a transient, non-specific increase in the SINE RNA level. However, arguing against this possibility, one manifestation of the cell stress response is that the expression of various RNAs is subjected to precisely controlled changes, as opposed to a simple breakdown in regulation (15). A second view is that mammalian SINE RNA serves a specific function in the cell stress response. All mammalian SINEs are ancestrally derived from RNAs that have a translational role and there is an intimate relationship between cell stress and translation. The association of sc*Alu* RNA with SRP proteins underscores the possible translational role of this transcript (43). For these reasons, the induction of SINE expression by cell stress and translational inhibition causes us to speculate that divergent SINE transcripts may have a common role in translation or the cell stress response.

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