The EMBO Journal vol.9 no.12 pp.3831–3837, 1990

Increased eIF-2α expression in mitogen-activated primary T lymphocytes

Roger B.Cohen, Thomas R.Boal and Brian Safer

Laboratory of Molecular Hematology, Section on Protein and RNA Biosynthesis, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA

Communicated by G.Blobel

G_0 human T cells synthesize protein at low rates and contain very low levels of eIF-2α mRNA. eIF-2α plays a pivotal role in the earliest regulated steps of translation initiation. We examined eIF-2α gene expression in normal human T cells stimulated with PHA. Nuclear run-on assays indicate low rates of eIF-2α gene transcription in G_0 cells and these change 2-fold with PHA treatment. Actinomycin D chase experiments show that the $t_{1/2}$ of eIF-2α mRNA is similar in G_0 and PHA-treated T cells. Analysis of nuclear RNA with probes specific for eIF-2α intron sequences shows that increased eIF-2α expression after PHA treatment is largely due to intranuclear stabilization of the primary transcript. The increase in eIF-2α mRNA does not require new protein synthesis. Hence, expression of this gene appears to be a part of the primary response program of T cells when they are exposed to mitogen.

Key words: eIF-2α/mRNA/stabilization/T cell/translation

Introduction

Resting (G_0) human peripheral blood T cells are metabolically quiescent. DNA synthesis is undetectable and rates of RNA and protein synthesis are low (reviewed in Hume and Weidemann, 1980; Ashman, 1984). When T cells are activated with mitogenic lectins such as phytohemagglutinin (PHA), a complex cascade of biochemical events occurs that eventually results in cell proliferation and the acquisition of immunologic competence (reviewed in Crabtree, 1988). A key event in this process is an early and rapid increase in protein synthesis. Conversion of single ribosomes to polyribosomes and increased translational activity are evident within the first few hours of mitogenic stimulation (Wettenhall and London, 1974; Cooper and Braverman, 1977). The rate of protein synthesis continues to increase during the first 48–72 h of lymphocyte activation, ultimately achieving levels which are >10 times that of the initial rate (Ahern and Kay, 1975; Udey and Parker, 1981). The high levels of protein synthesis which occur early in lymphocyte activation appear to be at least one of the pivotal events in the activation process since partial inhibition of translation delays and depresses T cell activation (Varesio and Holden, 1980).

The basis of the low rates of protein synthesis in the resting T cell remains unexplained. Many hypotheses have been proposed, including the presence of inhibitors of translation initiation (Kay *et al.*, 1978; Ferrer *et al.*, 1980), lack of met-tRNA$_i$ (Cooper and Braverman, 1981), and inaccessibility of cellular messages to the translational machinery (Wettenhall *et al.*, 1976). The idea of quantitative or qualitative deficiencies in translation initiation factors has also been pursued by a number of laboratories (Ahern *et al.*, 1974; Wettenhall and Slobbe, 1979; Resche *et al.*, 1980). Kay *et al.* (1979) have shown *in vitro* that the depressed translational activity of cytoplasmic lysates made from resting lymphocytes could be rescued with exogenous eIF-2. Regulation of protein synthesis via modulation of the level of eIF-2, or one of its individual subunits α, β or γ, is a logical hypothesis. eIF-2 catalyzes the binding of met-tRNA$_i$ to the $43S_N$ ribosomal subunit by the formation of a ternary complex with GTP. This is the first regulated step of protein synthesis initiation (Jagus *et al.*, 1981). We have recently obtained genomic clones for the α subunit of eIF-2 (Hümbelin *et al.*, 1989). We therefore decided to re-examine the role of altered eIF-2 expression during mitogenic activation of G_0 T cells.

The eIF-2α gene is >30 kb in size and contains four large introns (Hümbelin *et al.*, 1989). On the basis of the function which it encodes it may be considered as a member of the housekeeping gene family. In this report we show that the accumulation of eIF-2α mRNA differs drastically between G_0 and actively growing cells in contrast to eIF-2α gene transcription which varies only modestly. We show that PHA alters the rate of degradation of eIF-2α primary transcripts in the nucleus and that this leads to the accumulation of large quantities of cytoplasmic eIF-2α mRNA. This intranuclear mechanism occurs without any accompanying changes in eIF-2α mRNA cytoplasmic half-life and does not depend on *de novo* protein synthesis. Mechanisms based on stabilization of nuclear precursors may underlie the regulation of a variety of housekeeping genes including those encoding translation factors.

Results

PHA causes a dramatic increase in eIF-2α mRNA expression

Following mitogenic stimulation, quiescent (G_0) T lymphocytes execute a programmed sequence of events which leads to DNA synthesis and cell division within 24–30 h. Recently considerable attention has been focused on the transcriptional regulation of growth regulatory genes such as c-*myc* and c-*fos* during this process (reviewed in Crabtree, 1988). Mitogen binding is also followed by a general increase in protein synthesis and this rapid acquisition of translational competence is a fundamental part of the cellular response to mitogens, particularly during the first 24 h of pre-replicative phase. This portion of the mitogenic response occurs early and we and others have detected significant increases in protein synthetic rates within 15 min (Wettenhall and London, 1974; T.Boal, unpublished data).

© Oxford University Press

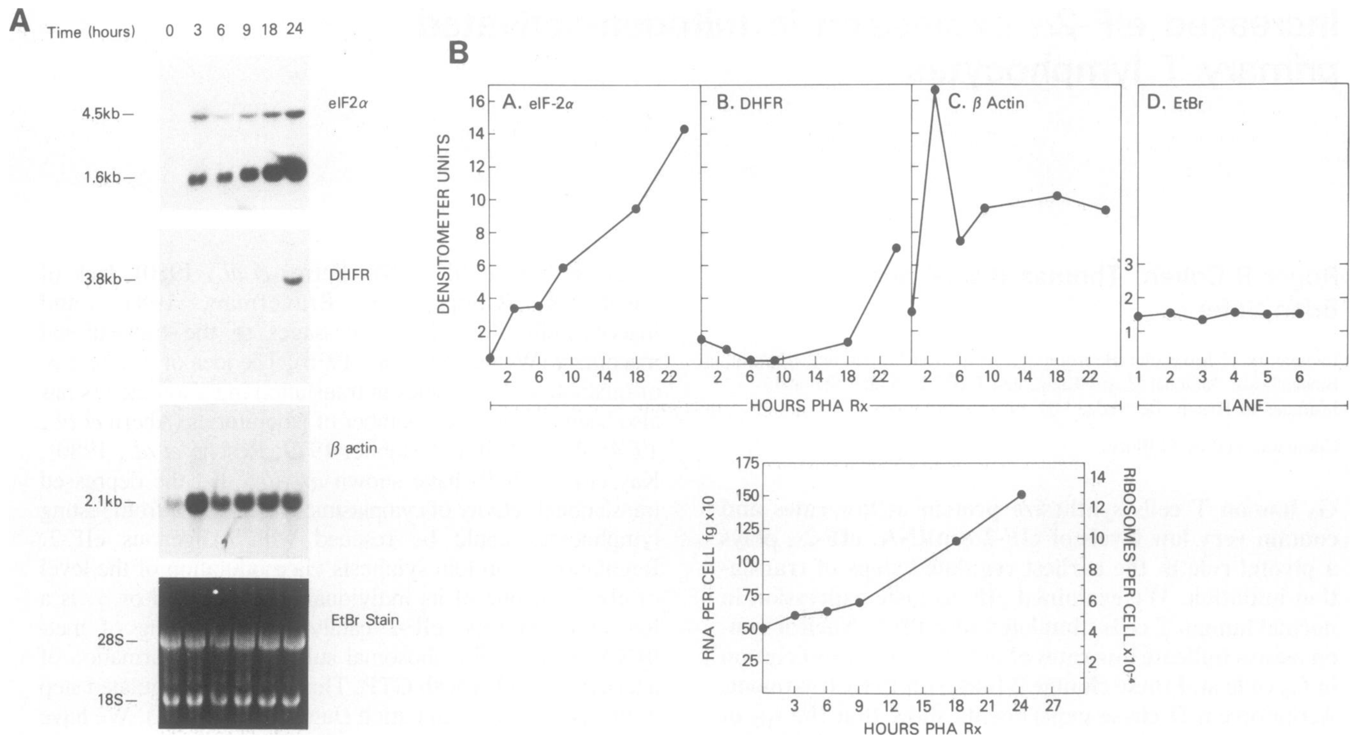

Fig. 1. (**A**) Kinetics of eIF-2α mRNA accumulation after treatment of T cells with PHA. G_0 T cells (time 0) were treated with 5 μg/ml PHA. Total cellular RNA was isolated from the cells at various times after PHA addition. RNA (10 μg/lane) was fractionated on agarose–formaldehyde gels, blotted onto Nytran membranes and sequentially hybridized with eIF-2α, DHFR and β actin cDNAs which had been radiolabeled with ^{32}P by random priming to a specific activity of 2×10^9 c.p.m./μg. The positions of 28S and 18S rRNAs are indicated. A photograph of ethidium bromide stained RNA is shown. For sequential hybridization blots were stripped with 50% formamide/6 × SSPE for 60 min at 65°C. Autoradiographic exposures of 12 h are shown for eIF-2α and DHFR. The exposure time for the β actin blot was 15 min. (**B**) Autoradiogram and a negative of the ethidium stain photograph in (A) were scanned by soft laser densitometry and the results displayed.

We have recently cloned and characterized the promoter region and first two introns of the eukaryotic translation initiation factor 2 α subunit (eIF-2α) (Hümbelin *et al.*, 1989). This protein is one of three subunits of initiation factor eIF-2, which catalyzes the binding of initiator met-$tRNA_i$ to native $43S_N$ ribosomal subunits (Jagus *et al.*, 1981). Since this is the first regulated step of protein synthesis initiation, we decided to examine expression of this gene during T cell activation by PHA.

Peripheral blood lymphocytes were purified by continuous countercurrent flow elutriation (Carter *et al.*, 1987) and were further fractionated using nylon wool columns in order to remove adherent cells (mostly B cells and macrophages). Preparations of T cells which resulted were >95% pure by FACS analysis (data not shown). To determine whether eIF-2α mRNA levels are regulated during T cell activation, we isolated total cellular RNA from G_0 T cells and T cells stimulated with PHA for the indicated times and performed a Northern blot analysis. Figure 1A illustrates the autoradiograms obtained when Northern blots are sequentially probed with cDNAs for eIF-2α, dihydrofolate reductase and β actin. Quantitation of this data by densitometry is presented in Figure 1B. The data shown are representative of six separate experiments with cells from six individuals. Equal amounts of total RNA (10 μg per lane) were analyzed at each time point. At time zero, the levels of eIF-2α mRNA are quite low and in this particular experiment are detectable only after overexposure of the autoradiogram. Following the addition of PHA, eIF-2α mRNA levels increase linearly for the first 24 h. During this period the pool of eIF-2α mRNA expands >50-fold, eventually reaching the high level which appears to be characteristic of other actively dividing cells (i.e. HeLa, K562 and MELC, data not shown). We note here the presence of two bands of 1.6 and 4.5 kb on the Northern blot. The major 1.6 kb species has been described previously in HeLa cell RNA (Ernst *et al.*, 1987). We have detected the second minor 4.5 kb band in a variety of cell types with cDNA or oligonucleotide probes. This minor species always varies in parallel with the major 1.6 kb band. Since there is only one copy of the eIF-2α gene we believe that this species results from use of an alternative upstream transcription start site or a downstream polyadenylation site. It does not represent nuclear precursor RNA since the band is also seen when cytoplasmic poly$(A)^+$ RNA is analyzed (data not shown). The precise origin of this second band was not explored further in these experiments.

In contrast to eIF-2α, DHFR mRNA levels, represented by a 3.8 kb species (Morandi *et al.*, 1982), remain low for most of the pre-replicative phase. By 24 h, when DNA replication begins, DHFR mRNA levels rise, consistent with the role of this protein in *de novo* thymidylate and purine biosynthesis. As others have found, β actin mRNA is easily detectable in G_0 cells (Degen *et al.*, 1983), and increases during the first day. The apparent transient increase in β actin mRNA expression is an artifact due to gel loading. The ethidium bromide stain of the RNA preparations which were blotted in this analysis is shown in order to document that approximately equal amounts of undegraded RNA were studied at each time point. The lower panel in Figure 1B shows the average increase in total RNA over this 24 h period (~3-fold). It is clear, therefore, that the large increase

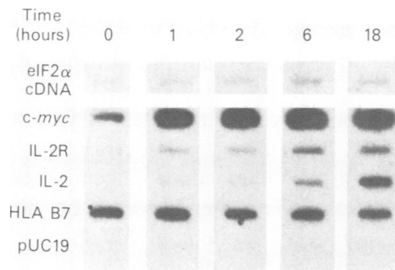

Fig. 2. Nuclear run-on assays of PHA-treated T cells. Purified T cells (10^8 cells per time point) were treated with PHA for the indicated times and nuclei were prepared. Equal amounts (c.p.m.) of radiolabeled RNA from the run-on reactions were hybridized to nitrocellulose filters that contained DNA probes (5 μg per slot) for eIF-2α cDNA, c-*myc*, IL-2 receptor cDNA, IL-2 cDNA, HLA B7 cDNA and pUC19. After washing and RNase digestion filters were exposed to Kodak XAR film for 14 days at −70°C. Fold increases in eIF-2α hybridization were estimated by soft laser densitometry. Parts per million (p.p.m.) calculations were made by cutting out individual slots, counting them by liquid scintillation and subtracting background hybridization to pUC19.

in the eIF-2α mRNA pool (at a minimum >50-fold) reflects a process which is distinct from the general overall increase in total RNA. Furthermore, the kinetics of increase in eIF-2α mRNA differ from those of another gene encoding a housekeeping function, i.e. DHFR.

Transcription of the eIF-2α gene does increase slightly with PHA treatment

In order to determine the mechanism(s) responsible for the 50- to 100-fold increase in eIF-2α mRNA, nuclear run-on transcription assays were performed (Hofer and Darnell, 1981). This assay measures the density of RNA polymerase II molecules on the eIF-2α transcription unit and thus reflects the transcriptional activity of the gene. Nuclei isolated from T cells at the indicated times following PHA stimulation were pulse-labeled with [α-^{32}P]GTP. Following purification radiolabeled RNA was then hybridized to a panel of cDNAs. The results of such an analysis are shown in Figure 2. Incorporation of radiolabel per cell increased 3- to 4-fold during the first 18−24 h, in agreement with the increase in total RNA over this period (Figure 1B). To adjust for this increase equal numbers of counts ($\sim 6 \times 10^6$ per filter) were hybridized to the five filters shown. Consistent with the results of others, we observed that transcription of genes such as c-*myc*, IL-2 and IL-2 receptor, increases significantly during the course of the experiment (Krönke *et al.*, 1985; Crabtree, 1988; Zipfel *et al.*, 1989). In contrast, the hybridization signal over the eIF-2α cDNA clone increases only 2-fold as measured by densitometry. We note that the signal for eIF-2α transcripts is reproducibly very low at all times during the experiment and that this may be characteristic of certain housekeeping genes including DHFR (Leys *et al.*, 1984; R.Cohen and B.Safer, unpublished observations). We also did not detect a more prominent increase in eIF-2α transcription rates when very early time points (5 min, 15 min and 30 min) following PHA treatment were analyzed (data not shown).

Role of cytoplasmic transcript stability

Since the >50-fold increase in eIF-2α expression greatly exceeds the modest increase in transcription rate, we considered whether an alteration of the eIF-2α mRNA half-

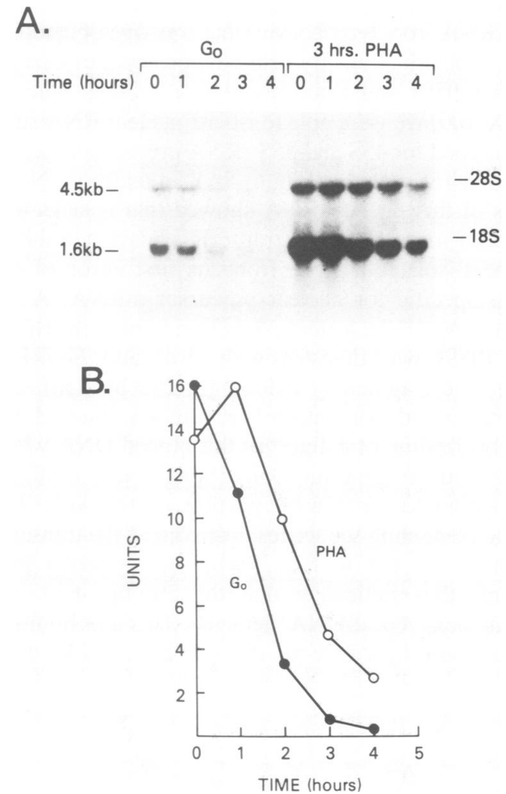

Fig. 3. Half-life of mature eIF-2α mRNA in G_0 and PHA-treated T cells. A Northern blot was prepared from total cellular RNA (10 μg per lane) from G_0 or PHA-treated (3 h) T cells exposed for 0−4 h to actinomycin D (5 μg/ml). **Upper panel**: autoradiogram of blot hybridized to eIF-2α cDNA. **Lower panel**: autoradiogram was scanned by soft laser densitometry (BioMed) and the results displayed graphically. Closed circles show data from G_0 cells. Open circles show data from PHA-treated cells. Data from G_0 cells were generated from an overexposed autoradiogram and the results are normalized to those from PHA-treated cells.

life could explain the large increase observed in eIF-2α mRNA levels. Resting or PHA-treated (3 h) T lymphocytes were incubated with actinomycin D (5 μg/ml) in order to block new transcription. At 60 min intervals, total cellular RNA was prepared and the level of eIF-2α mRNA was quantitated by Northern analysis (Figure 3). In both quiescent and PHA-treated cells, we note a decrease in the major 1.6 kb and minor 4.5 kb eIF-2α mRNA species (panel A) over time. Panel B shows a $t_{1/2}$ of $\sim$60−75 min for eIF-2α in either untreated or treated cells. The increased expression of eIF-2α mRNA in PHA-stimulated lymphocytes, therefore, cannot be explained adequately on the basis of increased transcription or decreased cytoplasmic mRNA degradation.

PHA causes an increase in eIF-2α nuclear transcript stability

A number of recent investigations have described post-transcriptional control mechanisms through which cellular mRNA levels are modulated by changes in nuclear precursor or hnRNA stability. Our data showing that neither increased transcription nor increased cytoplasmic stability can account completely for the large increase in eIF-2α mRNA suggested that post-transcriptional processes in the nucleus might be altered by PHA treatment of lymphocytes. To address this

mechanism we first developed a procedure for isolating nuclear RNA from lymphocytes that was free of cytoplasmic mRNA contamination. By using the protocol of Chang and Clayton (1989) and three successive washes with 0.5% Triton X-100, we were able to isolate nuclear RNA substantially enriched for 45S and 35S rRNA precursors and devoid of 28S and 18S rRNAs (Figure 5A). In addition, Northern analysis of this nuclear RNA showed that it lacks the 1.6 and 4.5 kb mature eIF-2α transcripts (data not shown).

We next isolated a probe from the first intron of eIF-2α that was specific for nuclear precursor mRNA. A 3.3 kb *Sst*I−*Eco*RV eIF-2α genomic subclone in pUC18 (Hümbelin *et al.*, 1989) was digested with *Ava*I and *Eco*RI. This releases five fragments, including a 299 bp *Ava*I−*Eco*RI fragment derived from intron I (Figure 4A, intron I probe). Blot hybridization of a digest of this cloned DNA with nick translated human genomic DNA (Song *et al.*, 1988) shows hybridization to an 816 bp portion of the *Sst*I−*Eco*RV subclone containing sequences upstream of the transcription start sites, but not to the 299 bp *Ava*I−*Eco*RI intron I fragment, thus indicating that the 299 bp *Ava*I−*Eco*RI fragment lacks repeat DNA elements. This 299 bp fragment also does not hybridize to the eIF-2α cDNA (data not shown). Finally, this 299 bp fragment hybridizes to a single band of the correct size when used to probe restriction digests of human genomic DNA (data not shown). As shown in Figure 4B, the 299 bp *Ava*I−*Eco*RI intron I probe hybridizes only to sequences in nuclear RNA but not to total cytoplasmic and cytoplasmic poly(A)$^+$ RNA. The eIF-2α cDNA probe, on the other hand, detects sequences in all three RNA fractions. Together, these observations indicate that the eIF-2α intron I probe is highly specific for eIF-2α primary transcripts containing intron I.

Nuclear and total RNA was then prepared from either G_0 or PHA-treated (3 h) T cells (Figure 5A). We have attempted repeatedly without success to detect primary transcripts of eIF-2α as discrete bands by Northern blotting or ribonuclease protection assays using intron probes. What we do see after stimulation is a fine higher molecular weight smear of hybridization which we believe corresponds to primary transcripts being processed and degraded (data not shown). We feel that this failure is due mainly to the large precursor size (>30 kb) as well as to the rapid degradation of processed introns. We therefore analyzed nuclear RNA by dot blots. Equal amounts of total and nuclear RNA were immobilized on nylon membranes, covalently cross-linked to the membranes with ultraviolet light and probed with either cDNA or intron I fragments. In agreement with the previous Northern analysis data (Figure 1A), G_0 lymphocytes contain undetectable levels of precursor or mature eIF-2α mRNA in nuclear and cytoplasmic fractions (Figure 5B). The inability to detect nuclear eIF-2α precursor

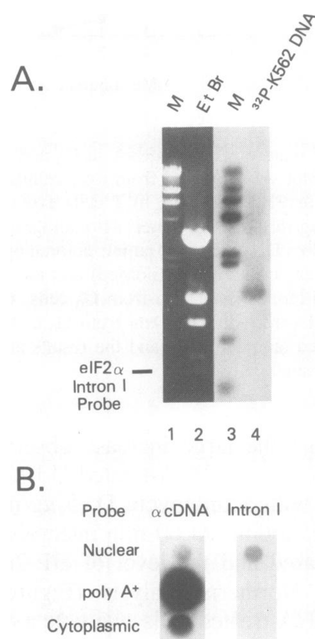

Fig. 4. Intron probe is specific for nuclear RNA precursor. (**A**) A plasmid containing a 3.3 kb *Sst*I−*Eco*RV fragment of the eIF-2α gene (Hümbelin *et al.*, 1989) was digested with *Ava*I and *Eco*RI. This releases five fragments of 3.5, 1.3, 0.82, 0.45 and 0.3 kbp. After agarose gel electrophoresis the fragments were visualized with ethidium bromide staining. Lane 1 shows a *Hin*dIII digest of λ DNA and lane 2 shows the *Ava*I−*Eco*RI digest of eIF-2α DNA. The 299 bp *Ava*I−*Eco*RI intron I fragment is indicated. The gel was blotted onto a Nytran membrane and hybridized with 200 ng of sheared human DNA (from K562 cells) which had been radiolabeled with ^{32}P by random priming to a specific activity of 4×10^8 c.p.m./μg. Lane 3 shows ^{32}P-labeled λ *Hin*dIII markers. Lane 4 shows an overnight exposure of eIF-2α DNA after hybridization to radiolabeled human DNA.
(**B**) Nuclear cytoplasmic and cytoplasmic poly(A)$^+$ RNA was prepared from K562 cells as described in Materials and methods. RNAs were dotted onto Nytran membranes (5 μg per dot) as described (Costanzi and Gillespie, 1984). RNAs were covalently cross-linked to the membranes with UV light and hybridized with eIF-2α cDNA and intron I probes which had been radiolabeled with ^{32}P by random priming to specific activity of 2×10^9 c.p.m./μg.

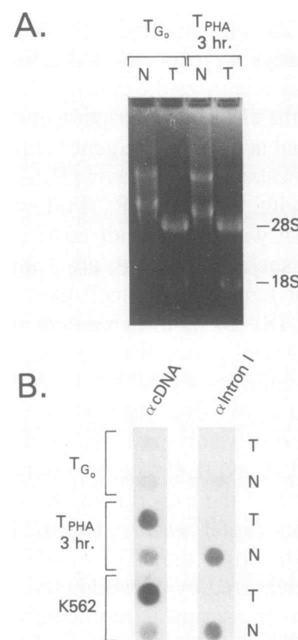

Fig. 5. PHA treatment of T cells causes stabilization of eIF-2α precursor mRNA in the nucleus. Nuclear (N) and total cellular (T) RNA were prepared as described (Chirgwin *et al.*, 1977; Chang and Clayton, 1989). RNAs were visualized by agarose−formaldehyde gel electrophoresis (5 μg/lane) and ethidium bromide staining (**A**). 5 μg aliquots of total and nuclear RNA from G_0 and PHA-treated (3 h) T cells were dotted onto Nytran membranes as described (Costanzi and Gillespie, 1987). After cross-linking with UV light, membranes were hybridized with radiolabeled eIF-2α or intron I probes. For purposes of comparison, total and nuclear RNA was prepared from K562 cells in logarithmic phase growth. An overnight autoradiographic exposure is shown.

mRNA contrasts with the clear evidence of low but detectable levels of eIF-2α gene transcription by nuclear run-on analysis (Figure 3). Following 3 h of PHA treatment, however, we detect a large increase in eIF-2α mRNA in total and nuclear RNA using the cDNA probe. Particularly striking is the large increase in nuclear eIF-2α precursor mRNA which we now detect with the intron I probe. We also note the reversal of the ratio of nuclear to total RNA detected by the eIF-2α cDNA probe under different conditions. In the G_0 cell (Figure 5B, T_{G0}) the ratio favors nuclear RNA whereas in the PHA-treated cell (Figure 5B, $T_{PHA3\ hr}$) the ratio favors total RNA. These data imply that nuclear−cytoplasmic transport of eIF-2α mRNA may also increase after PHA treatment. We believe that detection of eIF-2α precursor requires the 15 to 25-fold enrichment achieved by nuclear RNA preparation because precursors for such housekeeping genes are often synthesized at low rates (Figure 2) and are processed and transported at rapid rates. In summary, PHA treatment transforms the resting T cell into an actively growing cell such as K562, in which eIF-2α precursor and product are also easily detectable (Figure 5B, lowest set of dots).

These results indicate that one of the principal mechanisms underlying the lower levels of eIF-2α transcripts in G_0 T lymphocytes is their post-transcriptional degradation either before or during the processing of pre-mRNA to mRNA. Following PHA activation of T cells, most eIF-2α transcripts are rapidly converted to mature mRNA instead of being destroyed. Thus, growth regulation of eIF-2α mRNA expression is controlled at two levels. To a minor degree gene expression is regulated by an increase in *de novo* transcription. The major mechanism, however, appears to be regulation of primary transcript stability in the nucleus.

PHA-stimulated eIF-2α expression is independent of protein synthesis

Whether newly made protein is required for the induction of eIF-2α mRNA was explored by the use of the protein synthesis inhibitor cycloheximide. We treated quiescent T cells with PHA in the presence of 10 μg/ml cycloheximide. Treatment with the protein synthesis inhibitor alone for 6 h caused a 2-fold increase in eIF-2α mRNA levels (Figure 6). Treatment with cycloheximide and PHA led to an increase in eIF-2α mRNA that was greater than the increase seen with PHA alone (Figure 6). Cycloheximide does not significantly affect the level of β_2-microglobulin mRNA (Figure 6, bottom panel). The results demonstrate that the PHA mediated increase in eIF-2α mRNA is not sensitive to protein synthesis inhibitors. The effects of PHA are potentiated by cycloheximide but this effect is additive rather than synergistic. The insensitivity of eIF-2α regulation to protein synthesis inhibitors places the eIF-2α gene in the broad category of other primary or immediate genetic response genes in T cell activation (Zipfel *et al.*, 1989).

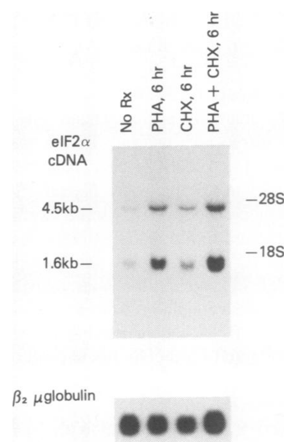

Fig. 6. Induction of eIF-2α gene expression does not depend upon protein synthesis. Peripheral blood T cells were treated for 6 h with PHA (5 μg/ml), cycloheximide (CHX, 10 μg/ml) or both CHX and PHA. Total RNA was isolated, fractionated by gel electrophoresis (10 μg/lane), blotted onto Nytran membranes and hybridized with the eIF-2α cDNA. The locations of the 28S and 18S rRNA markers are shown. The blot was also stripped and re-hybridized with a synthetic 30mer specific for the coding strand within the first exon of the human β_2-microgloublin gene (Suggs *et al.*, 1981) in order to confirm the presence of equal amounts of RNA in each lane.

Discussion

G_0 human T cells synthesize low levels of protein and express virtually no eIF-2α mRNA. Activation by mitogenic lectins such as PHA results in a rapid rise in eIF-2α mRNA levels and these levels continue to rise for the next 24 h. Increased eIF-2α mRNA levels are paralleled by increases in eIF-2α protein and the acquisition of translational competence (Boal,T.R., Cohen,R.B., Montine,K.S., Sonenberg,N. and Safer,B., manuscript in preparation). Activation of eIF-2α gene expression does not require new protein synthesis and the rapid and dramatic increase in eIF-2α mRNA is very disproportionate to the modest 2-fold increase in transcription initiation. Modulation of the rate of eIF-2α RNA degradation in the cytoplasm also does not appear to be responsible for increased eIF-2α expression following PHA treatment. Rather, our data indicate that the most important mechanism which accounts for the increase in eIF-2α mRNA is intranuclear stabilization of the primary transcript. Given that basal transcription rates change only slightly when compared to those of stimulated T cells, the low levels of eIF-2α mRNA in G_0 cells must be the result of almost complete degradation of eIF-2α hnRNA in the nucleus.

We wish to note that the autoradiographic signals corresponding to the eIF-2α transcripts in nuclear run-on experiments are very low. Indeed, they are present at only ~1 p.p.m. and are visible only after very long autoradiographic (>7 day) exposures in contrast to differentiation specific genes such as c-*myc* and IL-2. We and others have observed similar low rates of transcription or polymerase density on housekeeping genes such as tubulin (Cohen and Sheffery, 1985) and DHFR. The frequency of transcription initiation for DHFR, for example, has been estimated as one transcript per gene per hour (Leys *et al.*, 1984). Thus, low invariant rates of transcription for housekeeping genes may be a general feature and post-transcriptional mechanisms may figure more prominently in their regulation (Carneiro and Schibler, 1984).

Another mechanism which could account for some of the data would be a transcriptional elongation block or premature transcription termination as has been reported for c-*myc* (Bentley and Groudine, 1986) and HIV (Kao *et al.*, 1987). We feel that such a mechanism is unlikely, though, since the intron I probe lies very close to the transcriptional start site.

Regulation of cytoplasmic mRNA levels by modulation of intranuclear precursor half-life has now been demonstrated for a number of genes including malic dehydrogenase (Song *et al.*, 1988), DHFR (Leys *et al.*, 1984), class I MHC (Vaessen *et al.*, 1987), myeloperoxidase (Tobler *et al.*, 1988) and acid-1 glycoprotein (Vannice *et al.*, 1984). In the case of *Xenopus* ribosomal protein genes, a specific post-transcriptional regulation mediated through regulation of processing efficiency and stability of intranuclear transcripts has been proposed (Amaldi *et al.*, 1989). Morris and co-workers, in studies on the regulation of ornithine decarboxylase in lymphocyte activation (Morris *et al.*, 1988; White *et al.*, 1987), have commented explicitly on some of the obvious characteristics which distinguish genes regulated at the transcriptional level from those regulated at the post-transcriptional level. They have proposed two broad regulatory classes, with genes encoding housekeeping functions such as DHFR, ornithine decarboxylase and now eIF-2α, belonging to the post-transcriptional class. Growth regulatory genes such as c-*myc*, on the other hand, belong to the class controlled primarily by changes in transcription rate. Indeed, it is tempting to speculate, as Leys *et al.* (1984) and Carneiro and Schibler (1984) have, that housekeeping genes do, in fact, constitute a separate regulatory class. Genes in this class may be regulated more by negative than positive mechanisms in the following sense. In general, genes in this class tend to be constitutively transcribed at rates which seem to vary little among different cell types and physiological conditions. In the case of eIF-2α, for example, the gene product (i.e. primary transcript) apparently is destroyed in the nucleus when it is not needed, an outcome that may be facilitated in general by the relatively large size of housekeeping gene primary transcripts (eIF-2α is >30 kb). When the metabolic needs of the cell change, the primary gene product is stabilized long enough to become spliced and exported into the cytoplasm. Clearly, as the case of eIF-2α amply demonstrates, changes in efficiency of nuclear processing can effect dramatic fluctuations in message levels with minimal alterations in transcription rate.

RNA accumulation after mitogen addition is a general phenomenon in that total cellular RNA rises 2- to 3-fold after 24 h (Figure 1B; Ashman, 1984; Ahern and Kay, 1975). Changes in eIF-2α mRNA pool size are significantly out of proportion to this overall increase, however, which implies a specific regulatory role for this message increase. We are not proposing that eIF-2α mRNA plays a role in the 'commitment' process of T cell activation in which transcriptionally regulated genes such as IL-2 and its receptor figure so prominently. Instead, we imagine that genes such as eIF-2α play a supporting, but nevertheless critical role. The resistance of eIF-2α gene expression to cycloheximide also indicates a role as a so-called primary response gene (Zipfel *et al.*, 1989).

A number of workers have shown that in resting lymphocytes only a small fraction of hnRNA gets to the cytoplasm (Kraft and Shortman, 1970; Berger and Cooper, 1984; Green, 1977), results that are consistent with what we observe for eIF-2α. They predicted that increased processing and nuclear export would account for much of the increase in cytoplasmic mRNA which is observed after lectin treatment. To our knowledge, eIF-2α represents the first RNA species for which precisely such a mechanism has been clearly demonstrated in the lymphocyte system.

It is also interesting in this regard to compare the G_0 state in resting T cells with the G_0 state achieved in serum-starved mouse fibroblasts. Efficiency of poly(A)$^+$ RNA transfer from nucleus to cytoplasm also decreases when fibroblasts are deprived of serum and increases when cells are re-fed (Johnson *et al.*, 1975). Pardee and co-workers (Gudas *et al.*, 1988) have shown that thymidine kinase mRNA is an example of such a regulated gene product. Another example of a gene that is regulated at the nuclear RNA level during mitogenic activation is 24p3, which is induced when quiescent monkey kidney cells are mitogenically activated by expression of SV40 T antigen (Hraba-Renevey *et al.*, 1989). We have observed, in striking contrast to the case of the G_0 T cell, that there is little change in steady-state eIF-2α mRNA levels when exponentially growing fibroblasts are compared with serum-starved and re-fed fibroblasts (B.Safer and T.Boal, unpublished results).

The exact mechanism by which eIF-2α precursor degradation ceases when T cells are treated with PHA remains a matter for future investigation. A number of mechanisms are plausible including the inhibition by PHA of general or gene specific RNases (Kraft and Shortman, 1970; Green, 1977), the modification of factors which promote general or gene specific splicing and nuclear export, and the induction of factors that specifically stabilize certain hnRNAs.

Materials and methods

T cell preparation and culture

Human peripheral blood mononuclear cells from normal volunteers were fractionated by continuous counterflow elutriation coupled with density gradient sedimentation (Carter *et al.*, 1987). The lymphocyte enriched fraction was then plated in plastic dishes containing RPMI 1640 medium (Biofluids) supplemented with 5% fetal bovine serum at a density of 3×10^6 cells/ml. Following overnight incubation at 37°C, non-adherent T and B cells (depleted of macrophages) were harvested and passed over nylon wool columns (Henry, 1980) to enrich further for T cells. The resulting mixtures of T cells are $>95\%$ pure by FACS analysis using anti-Leu-4 antibodies (Becton-Dickinson) to identify T cells. Cells were activated by the addition of PHA (5 μg/ml, Sigma) and PMA (100 ng/ml, Pharmacia) (Melton *et al.*, 1987), or PHA alone.

Preparation and analysis of RNA

RNA was prepared by guanidine isothiocyanate lysis (Chirgwin *et al.*, 1977) and CsCl gradient ultracentrifugation. RNAs were electrophoretically separated on agarose–formaldehyde gels (Ausubel *et al.*, 1987), stained with ethidium bromide and blotted onto Nytran (Schleicher and Schuell) membranes. RNA was covalently cross-linked to the filters with UV light (Fotodyne) and hybridized to radiolabeled DNA inserts labeled to specific activities of $2-3 \times 10^9$ c.p.m./μg by random priming (Feinberg and Vogelstein, 1983). Hybridization buffers were prepared according to Church and Gilbert (1984) and wash conditions were as previously described (Ausubel *et al.*, 1987). Poly(A)$^+$ RNA was isolated as described (Aviv and Leder, 1972) using type III oligo(dT) resin purchased from Collaborative Research.

Transcription in isolated nuclei

Nuclei were prepared and pulse-labeled with [α-^{32}P]GTP according to Hofer and Darnell (1981) except that radiolabeled RNA fragments were separated from unincorporated radiolabel by G-50 Sephadex chromatography. Hybridization to cDNA clones immobilized as slots on nitrocellulose membranes was performed as previously described (Cohen and Sheffery, 1985).

Probe identity and preparation

The following individuals kindly provided DNA probes: c-*myc*, Dr K.Kelly; Il-2 and IL-2 receptor cDNAs, Dr Richard Klausner; eIF-2α cDNA, Dr J.Hershey (Ernst *et al.*, 1987); dihydrofolate reductase (DHFR) cDNA, Dr A.Nienhuis; β actin cDNA, Dr J.Quinn. HLA B7 probe is from ATCC.

Nuclear RNA preparation

The procedure described by Chang and Clayton (1989) was used to prepare nuclei. Vanadium ribonucleoside complexes were purchased from BRL. Following three washes with 0.5% Triton X-100 nuclei were lysed with guanidine isothiocyanate and nuclear RNA isolated by CsCl ultracentrifugation (Chirgwin *et al.*, 1977).

Acknowledgements

The authors gratefully acknowledge the assistance of Mr Charles Carter of the Clinical Center Blood Bank who provided the peripheral blood lymphocytes and Ms Monica Yu who performed the FACS analyses.

References

Ahern,T. and Kay,J.E (1975) *Exp. Cell Res.*, **92**, 513–515.
Ahern,T., Sampson,J. and Kay,J.E. (1974) *Nature*, **248**, 519–521.
Amaldi,F., Bozzoni,I., Beccari,E. and Pierandrei-Amaldi,P. (1989) *Trends Biochem. Sci.*, **14**, 175–178.
Ashman,R. (1984) In Paul,W.E. (ed), *Fundamental Immunology*. Raven Press, New York.
Ausubel,F.M., Brent,R., Kingston,R.E., Moore,O.D., Smith,J.A., Seidman,J.G. and Struhl,K. (1987) *Current Protocols in Molecular Biology*. John Wiley, New York.
Aviv,H. and Leder,P. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 1408–1412.
Bentley,D.L. and Groudine,M. (1986) *Nature*, **321**, 702–706.
Berger,S. and Cooper,H.L. (1978) *Biochim. Biophys. Acta*, **517**, 84–98.
Carneiro,M. and Schibler,U. (1984) *J. Mol. Biol.*, **178**, 869–880.
Carter,C.S., Leitman,S.F., Cullis,H., Muhl,L.M., Nason-Burchenal,K., Rosenberg,S. and Klein,H.G. (1987) *Transfusion*, **27**, 362–365.
Chang,D.D. and Clayton,D.A. (1989) *Cell*, **56**, 131–139.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W. (1977) *Biochemistry*, **18**, 5294–5299.
Church,G.M .and Gilbert,W. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1991–1995.
Cohen,R.B. and Sheffery,M. (1985) *J. Mol. Biol.*, **182**, 109–129.
Cooper,H.L. and Braverman,R. (1977) *J. Cell Physiol.*, **93**, 213–225.
Cooper,H.L. and Braverman,R. (1981) *J. Biol. Chem.*, **256**, 7461–7467.
Costanzi,C. and Gillespie,D. (1987) *Methods Enzymol.*, **152**, 585–587.
Crabtree,G.R. (1988) *Science*, **243**, 355–361.
Degen,J.L., Neubauer,M.G., Friezner-Degen,S.J.F., Seyfried,C.E. and Morris,D.R. (1983) *J. Biol. Chem.*, **258**, 12153–12162.
Ernst,H., Duncan,R. and Hershey,J.W.B. (1987) *J. Biol. Chem.*, **262**, 1206–1212.
Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.*, **132**, 6–13.
Ferrer,M., Burrone,O.R. and Algranati,I.D. (1980) *FEBS Lett.*, **121**, 203–206.
Green,R.C. (1977) *Exp. Cell Res.*, **110**, 215–223.
Gudas,J.M., Knight,G.B. and Pardee,A.B. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 4705–4709.
Henry,C. (1980) In Mishell,B.B. and Shiigi,S.M. (eds), *Selected Methods in Cellular Immunology*. W.H.Freeman, San Francisco, pp. 182–185.
Hofer,E. and Darnell,J. (1981) *cell*, **23**, 585–593.
Hraba-Renevey,S., Turler,H., Kress,M., Salomon,C. and Weil,R. (1989) *Oncogene*, **4**, 601–608.
Hümbelin,M., Safer,B., Chirorini,J.A., Hershey,J.W.B. and Cohen,R.B. (1989) *Gene*, **81**, 315–324.
Hume,D.A. and Weidemann,M.J. (1980) *Mitogenic Lymphocyte Transformation: a General Model for the Control of Mammalian Cell Proliferation and Differentiation*. Elsevier/North Holland, Amsterdam.
Jagus,R., Anderson,W.F. and Safer,B. (1981) *Prog. Nucl. Acid Res. Mol. Biol.*, **25**, 127–185.
Jagus,R. and Kay,J.E. (1979) *Eur. J. Biochem.*, **100**, 503–510.
Johnson,L.F., Williams,J.G., Abelson,H.T., Green,H. and Penman,S. (1975) *Cell*, **4**, 69–75.
Kao,S.-Y., Calman,A.F., Luciw,P.A. and Peterlin,B.M. (1987) *Nature*, **330**, 489–493.
Kay,J.E., Benzie,C.R., Dicker,P. and Lindahl-Kiessling,K. (1978) *FEBS Lett.*, **91**, 40–44.
Kay,J.E., Wallace,D.M., Benzie,C.R. and Jagus,R. (1979) In Quastel,M.R. (ed.), *Cell Biology and Immunology of Leukocyte Function*. Academic Press, NY, pp. 107–115.
Kraft,N. and Shortman,K. (1970) *Biochim. Biophys. Acta*, **217**, 164–175.
Krönke,M., Leonard,W.J., Depper,J.M. and Greene,W.C. (1985) *J. Exp. Med.*, **161**, 1593–1598.
Leys,E.J., Crouse,G.F. and Kellems,R.E. (1984) *J. Cell Biol.*, **99**, 180–187.
Melton,L.B., Lakkis,F.G., Deloria,D., Williams,J.W. and Strom,T.B. (1987) *Transplantation Proceedings*, **19**, 331–332.
Morandi,C., Master,J.H., Motte,N. and Attardi,G. (1982) *J. Mol. Biol.*, **156**, 583–607.
Morris,D.R., Allen,M.L., Rabinovitch,P.S., Kuepfer,C.A. and White,M.W. (1988) *Biochemistry*, **27**, 8689–8693.
Resch,K., Wood,T. and Cooper,H.L. (1980) *FEBS Lett.*, **117**, 284–288.
Song,M.-K.H., Dozin,B., Grieco,D., Rall,J.E. and Nikodem,V.M. (1988) *J. Biol. Chem.*, **263**, 17970–17974.
Suggs,S.V., Wallace,R.B., Hirose,T., Kawashima,E.H. and Itakura,K. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6613–6617.
Tobler,A., Miller,C.W., Johnson,K.R., Selsted,M.E., Rovera,G. and Koeffler,H.P. (1988) *J. Cell Physiol.*, **186**, 215–225.
Udey,M.C. and Parker,C.W. (1981) *J. Immunol.*, **126**, 1106–1113.
Vaessen,R.T.M.J., Houweling,A. and van der Eb,A. (1987) *Science*, **235**, 1486–1488.
Vannice,J.L., Taylor,J.M. and Rigold,G.R. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 4241–4245.
Varesio,L. and Holden,H.T. (1980) *J. Immunol.*, **124**, 2288–2294.
Wettenhall,R.E.H. and London,D.R. (1974) *Biochim. Biophys. Acta*, **349**, 214–225.
Wettenhall,R.E.H. and Slobbe,A. (1979) *Biochim. Biophys. Acta*, **563**, 400–412.
Wettenhall,R.E.H., Slobbe,A. and Higgins,T.J.V. (1976)*Biochim. Biophys. Acta*, **432**, 312–322.
White,M.W., Oberhauser,A.K., Kuepfer,C.A. and Morris,D.R. (1987) *Mol. Cell. Biol.*, **7**, 3004–3007.
Zipfel,P.F., Irving,S.G., Kelly,K. and Siebenlist,U. (1989) *Mol. Cell. Biol.*, **9**, 1041–1048.

Received on June 8, 1990; revised on August 8, 1990