Mechanism of Activation of Protein Synthesis Initiation in Mitogen-stimulated T Lymphocytes*

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The pronounced stimulation of protein synthesis in T lymphocytes in response to mitogens is partly due to increased cell size and hence ribosome number. There is also a large increase in translation rate per ribosome as a result of an increased rate of initiation. In response to mitogen, levels of both eukaryotic initiation factor (eIF)-2 and guanine nucleotide exchange factor, GEF, increase in parallel with ribosomes which is consistent with a general increase in the translational machinery but cannot explain the increase in activity per ribosome. However, as total eIF-2 accumulates, the ratio of phosphorylated eIF-2α (eIF-2(αP)) to eIF-2α decreases. Further, the levels of eIF-2(αP) and GEF in resting T lymphocytes are similar. As eIF-2(αP) inhibits GEF by effectively sequestering the exchange factor in an inactive 1:1 complex, the level of GEF available for protein synthesis initiation must be very low in resting cells. Hence, as GEF is synthesized and rises above the level of eIF-2(αP), there will be a disproportionate increase in GEF available for initiation compared with the increase in total GEF. This increase in available GEF is probably great enough to support the increase in translation rate per ribosome as well as the increase in ribosome number.

Mature T lymphocytes exist in a resting state, both in vivo and when maintained in culture. Their growth is arrested in the Go stage of the cell cycle, and they do not proliferate despite the presence of adequate nutrients in the culture medium. Consistent with their non-proliferative state, resting T lymphocytes exhibit very low rates of protein synthesis. Evidence from studies of both cells (Kay et al., 1971; Cooper et al., 1975) and cell-free systems prepared from T lymphocytes (Ahern and Kay, 1973; Ahern et al., 1974; Kay et al., 1975) has suggested that this deficit in the rate of protein synthesis is due to a block at the level of initiation. One means by which protein synthesis initiation might be regulated in T lymphocytes is through a translational inhibitor that would act to inhibit directly and specifically a step in the initiation pathway. Potent translational inhibitors have been reported in extracts of T lymphocytes from porcine blood (Kay et al., 1978) and rabbit thymus (Kruger and Cooper, 1980); in both cases, initiation appeared to be the target of the inhibition. We have examined the nature and activity of a similar translational inhibitor in extracts of T lymphocytes from rat spleen. Our results suggest the presence of this inhibitor in T lymphocyte extracts is artifactual.

When cultured in the presence of the appropriate mitogen, T lymphocytes show a dramatic increase in their rate of protein synthesis. The rise in the overall protein synthesis rate reflects an increase in the rate of translation per ribosome (Kay et al., 1971; Ahern and Kay, 1973; Kay et al., 1975) and is thought to be caused by an increase in the rate of initiation (Ahern et al., 1974; Kay et al., 1975; Cooper and Braverman, 1977). Rates of elongation were found to be similar in resting and stimulated T lymphocytes (Kay et al., 1975). It is likely that control of initiation in T lymphocytes is complex with several initiation steps being regulated. Deficiency of ribosome dissociation factor, modulation of mRNA binding initiation factors, and mRNA masking have been suggested as mechanisms of regulation of initiation. However, no progress has been made in determining how these steps are modulated. It has been shown that the cap binding protein, eIF-4E, is strikingly more phosphorylated in stimulated T cells compared with resting cells (Boal et al., 1990), but it is not known how this modification modulates the factor's activity. It has also been suggested that one or more of the initiation factors involved in the binding of Met-tRNA to the 40 S ribosomal subunit, one of the first steps in the initiation pathway, may be an important regulatory site during T lymphocyte activation (Ahern et al., 1974). In the present study, we examined the potential role of the initiation step involving the Met-tRNA binding factor, eIF-2, a known major control point in initiation in eukaryotes, in the regulation of the protein synthesis rate in T lymphocytes.

In one of the first steps of initiation, eIF-2, in the active form eIF-2-GTP, mediates the binding of Met-tRNA to the 40 S ribosomal subunit (Pain, 1986; Moldave, 1985). When a round of initiation is complete, eIF-2 is released with the concomitant hydrolysis of GTP, yielding eIF-2-GDP. This form of eIF-2 is inactive in initiation and the active form, eIF-2-GTP, must be regenerated prior to another initiation cycle. Another initiation factor, guanine nucleotide exchange factor (GEF, also known as eIF-2B, RF, or co-eIF-2) catalyzes the release of GDP from eIF-2 and the binding of GTP (Ochoa, 1983).

The activity of eIF-2 in initiation is regulated by phosphorylation, through modulation of the activity of GEF. Phosphorylated eIF-2 (eIF-2(αP)) is a potent competitive inhibitor of GEF, capable of effectively sequestering an equimolar amount of the exchange factor and preventing it from acting on eIF-2-GDP (Siekerka et al., 1984; Rowlands et al., 1988a). In this way, the level of eIF-2(αP) determines the level of catalytically active GEF. Relatively small changes in eIF-2

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† The abbreviations used are: eIF, eukaryotic initiation factor; GEF, guanine nucleotide exchange factor; eIF-2(αP), phosphorylated eIF-2; SDS, sodium dodecyl sulfate.
phosphorylation then can lead to large changes in the level of catalytically active GEF, which in turn can have a profound effect on the overall translation rate by controlling the amount of active eIF-2 available for initiation. In hemedeficient reticulococyte lysate, activation of hemeregulated inhibitor, an eIF-2-α kinase, leads to phosphorylation of only a small fraction of eIF-2 but a dramatic decrease in GEF activity and complete shutdown of protein synthesis (Pain, 1986). In Ehrlich ascites tumor cells, inhibition of protein synthesis in response to physiological stresses correlates closely with a large decrease in GEF activity resulting from a relatively small increase in eIF-2 phosphorylation (Rowlands et al., 1988b).

In this communication, we show that eIF-2 and GEF levels in rat T lymphocytes increase in parallel with ribosomes when the cells are stimulated by the mitogen concanavalin A. Further, we demonstrate that the rise in the total level of eIF-2 is accompanied by a significant decrease in the proportion of eIF-2 that is phosphorylated. Thus, total GEF increases to a greater extent than does phosphorylated eIF-2. We infer that this increase in total GEF relative to eIF-2(αP) leads to a comparatively large increase in GEF that is not inhibited by eIF-2-α(ÐeIF-2(αP) and is active in initiation, and we hypothesize that this provides at least one mechanism by which mitogen-stimulated cells increase their rate of protein synthesis per ribosome.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**Translational Inhibitor in Rat T Lymphocyte Lysate Is a Ribonuclease; Calf T Lymphocyte Lysate Lacks a Detectable Translational Inhibitor**—A number of reports suggests that the low rates of protein synthesis in resting T lymphocytes may result from the presence of potent translational inhibitors. The present study undertook the investigation of lysates of T lymphocytes from rat spleen and calf spleen for the presence of translational inhibitors, and of the potential role of such inhibitors in T lymphocyte protein synthesis regulation. Lysate was prepared from rat T lymphocytes that were purified from a mixture of splenocytes by the nylon wool technique. Addition of 7.5 μg of T lymphocyte lysate protein (1 in 100 dilution with respect to cell cytoplasma) to a reticulocyte lysate cell-free protein-synthesizing system inhibited protein synthesis completely by 15 min (Fig. 1A, Miniprint). This inhibition was almost completely overcome by the ribonuclease inhibitor, RNasin. It is unlikely that a ribonuclease, that is present at such high activity, is a physiological regulator of protein synthesis, particularly given that lysate from mitogen-stimulated cells still displays very high levels of ribonuclease, despite high rates of protein synthesis in these cells (data not shown). Further, lysate prepared from calf T lymphocytes does not contain a detectable translational inhibitor (Fig. 1B). Addition of as much as 50 μg of calf T lymphocyte lysate protein, an amount nearly 7-fold higher than that which brought about potent inhibition by the rat T lymphocyte lysate, has no significant effect on the protein synthesis rate in the reticulocyte cell-free translation system. Taken in sum, these results suggest that the potent translational inhibitor detected in T lymphocyte lysate is a ribonuclease that is unlikely to have a regulatory role in protein synthesis. Since purification of T lymphocytes by the nylon wool technique is known to result in contamination by macrophages (Julius et al., 1973), which contain high ribonuclease activity, it is possible that the ribonuclease activity observed in rat T lymphocyte lysate is due to macrophage contamination. Our measurements of nonspecific esterase activity (a marker for macrophages) indicate that lysate from rat cells is more contaminated with macrophages than lysate from calf cells, providing support for this possibility (data not shown). Alternatively, a ribonuclease may be released from T lymphocyte lysosomes during cell lysis.

**Mitogenic Activation of T Lymphocyte Protein Synthesis Is Accompanied by an Increase in Total eIF-2 and a Decrease in the Proportion of Total eIF-2 That Is Phosphorylated**—Studies of the differences between resting and mitogen-stimulated cells have suggested that changes at the level of initiation play a crucial role in the activation of protein synthesis by mitogens (Kay et al., 1971; Ahern et al., 1974; Cooper and Braverman, 1977). Particularly, evidence has been presented for possible regulation by factor(s) involved in the binding of Met-tRNA to the 40 S ribosomal subunit (Ahern et al., 1974). In the present study, the potential regulatory role of the initiation step involving the factor eIF-2 was examined.

To study the changes in protein synthesis initiation that occur when T lymphocytes are induced to proliferate by mitogens, rat T lymphocytes were cultured in the presence of the mitogen concanavalin A. Such treatment caused these cells to increase their rate of protein synthesis 10-15-fold during the first 24 h; in the experiment shown in Fig. 2 (Miniprint), a 15-fold increase was recorded. As Fig. 2 further shows, this large stimulation of the protein synthesis rate was accompanied by approximately a 4-fold increase in the total RNA content of the cells. Since ribosomal RNA comprises the great majority of cellular RNA (Henshaw et al., 1971), it can be concluded that the cells increased their ribosome level nearly 4-fold in this time period as reported by others (Cooper, 1969; Kay and Cooper, 1969). The increase in ribosome number suggests that there is a general increase in the total protein synthesis machinery in response to mitogenic activation. It can also be seen from Fig. 2 that the synthesis of new ribosomes proceeded very slowly, if at all, in the first 4 h of incubation, even though the rate of protein synthesis rose by about 50% during this time. This observation suggests that during the initial stages of activation the increase in the overall translation rate is primarily due to increased translation on existing ribosomes, as has been reported previously for human T lymphocytes (Cooper and Braverman, 1977). The nearly 4-fold higher increase in the protein synthesis rate relative to the rise in the ribosome pool during the later stages of stimulation also agrees with earlier reports of an increase in the rate of translation per ribosome.

An examination of the total eIF-2 level in T lymphocytes during the same incubation time course showed that eIF-2 was being synthesized in parallel with ribosomes in the mitogen-stimulated cells. Lysates, prepared from T cells as described under "Experimental Procedures," were dissolved in SDS sample buffer and their eIF-2 content analyzed by immunoblotting a SDS-polyacrylamide electrophoresis gel. Fig. 3A illustrates clearly the increase in eIF-2 in concanavalin A-stimulated cells. Laser densitometry revealed that the eIF-2 level in stimulated cells increased about 3.5-fold with stimulation during the same incubation period compared to unstimulated control T lymphocytes.

Portions of this paper (including "Experimental Procedures" and Figs. 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
The extent to which eIF-2 is able to support initiation is governed by the proportion of the eIF-2 pool that is phosphorylated, since eIF-2(aP) can prevent the recycling of unphosphorylated eIF-2 by sequestering GEF. We thus examined the levels of phosphorylated eIF-2α at different times of incubation. The autoradiograph of an immunoblot of an isoelectric focusing gel, Fig. 4A, shows that the proportion of total eIF-2α that is phosphorylated decreased in response to mitogenic stimulation. Laser densitometry reveals that the ratio changed from 33% phosphorylated eIF-2α at time 0 to 12.5% phosphorylated eIF-2α at 16 hours (Fig. 4B) with most of the change resulting from an increase in unphosphorylated eIF-2 as eIF-2(aP) increased only slightly. At 24 h, eIF-2 phosphorylation was markedly increased in both stimulated and control cells (data not shown); however, it is likely that this increase was caused by the depletion of nutrients from the culture medium and is not relevant to the overall proliferation response. Thus, the increase in total eIF-2 in response to mitogenic activation is accompanied by a decrease in the proportion of total eIF-2 that is phosphorylated.

**Mitogenic Activation of T Lymphocyte Protein Synthesis Is Accompanied by an Increase in Total GEF**—We examined changes in the GEF level in T lymphocytes in response to mitogenic stimulation. To measure GEF in T lymphocytes, lysates were prepared from control and concanavalin A-stimulated cells at different times of incubation and their GEF content measured using a modification of the ternary complex assay described previously (Panniers and Henshaw, 1983). Briefly, GEF was assayed by the ability of lysate to stimulate the formation of the ternary complex eIF-2·GTP·Met-tRNA from eIF-2·GDP, GTP, and Met-tRNA. Only GEF is known to stimulate this reaction, so that the increase in ternary complexes formed above background (spontaneous complex formation) is a measure of the amount of GEF present.

Similar to our finding in Ehrlich cells, there is a potent inhibitor of GEF in T lymphocyte lysates (data not shown). To overcome the effects of this inhibitor, lysates were diluted into a range known to produce a linear response. At the dilution used, the amount of added purified eIF-2·GDP (12 nM) serving as substrate for the reaction was in large excess relative to the amounts of both eIF-2 and eIF-2(aP) introduced into the reaction by the addition of small amounts of lysate. From Western blots of lymphocyte extracts (data not shown), we estimate that the concentration of eIF-2 in resting T lymphocytes is 400 nM. Cellular material was diluted at least 4000-fold to estimate GEF activity. Therefore, the maximum endogenous eIF-2 concentration in the assay was only 0.1 nM representing a negligible background. Most importantly, the amount of eIF-2(aP) in the reaction was estimated to be 0.03 nM, well below its $K_i$ for GEF (0.2 nM in Ehrlich cells (Rowlands et al. 1988b)), making any sequestration of GEF by endogenous eIF-2(aP) negligible. Therefore, the assay effectively measured the total GEF in the lysate, rather than free GEF (free being that GEF available for cycling eIF-2·GDP and not trapped by eIF-2(aP)). Reactions were linear.

**FIG. 3.** A, an autoradiogram of an immunoblot of an SDS electrophoresis gel loaded with samples (30 μl) of T lymphocyte lysate prepared from cultures incubated plus or minus concanavalin A for the indicated times. B, estimate of the relative amounts of eIF-2 by laser densitometry of the autoradiograph shown in A. □, plus concanavalin A; ■, minus concanavalin A.

**FIG. 4.** A, T lymphocytes were incubated plus or minus concanavalin A, and samples, which were removed at the indicated times, were analyzed by isoelectric focusing gel electrophoresis and immunoblotting with a monoclonal anti-eIF-2α antibody. The positions of eIF-2α (upper band) and eIF-2α(aP) (lower band) were revealed by autoradiography that detects 125I-labeled secondary antibody. B, percent eIF-2 phosphorylated (eIF-2(aP)/eIF-2α + eIF-2(aP)) was estimated by laser densitometry of the autoradiograph in A. □, plus concanavalin A; ■, minus concanavalin A.
for at least 5 min. Fig. 5 depicts ternary complexes formed (with spontaneous complexes subtracted) in the presence of an amount of cell extract known to be within a range that gives a linear response.

Using this method, a steady increase in total GEF over the 24-h incubation was measured in lysates from mitogen-stimulated cells. Fig. 5 shows that total GEF increased approximately 3-fold in treated cultures relative to untreated cultures by 24 h. This experiment has been repeated three times with only small differences in this trend. Similar to the rise in the level of eIF-2, the increase in GEF roughly parallels the increase in ribosomes. The close correlation between the increase in GEF and the increase in eIF-2 and ribosomes suggests that new GEF is synthesized as part of the general protein synthesis activation response in T lymphocytes.

The Total Level of GEF Is Near the eIF-2(αP) Level in Resting T Lymphocytes—The total GEF activity measured in the above assay is not a reflection of the amount of GEF active in initiation in T lymphocytes. The amount of GEF active in initiation depends on the level of eIF-2(αP), which, by its action as a competitive inhibitor of GEF, determines the level of GEF that can act on eIF-2-GDP. The significance of the increase in total GEF activity to the initiation rate therefore depends on the level of GEF relative to the eIF-2(αP) level in the cell. The proportion of total eIF-2 that is eIF-2(αP) in resting cells was shown above to be approximately 33% from isoelectric focusing gel electrophoresis experiments. The ratio of GEF to eIF-2 was estimated by differential elution from CM-Sephadex, as has been done previously for Ehrlich ascites tumor cells (Rowlands et al., 1988b). In cell extracts, almost all the GEF exists in a complex with eIF-2. The GEF-eIF-2 complex does not bind CM-Sephadex, whereas free eIF-2 binds strongly. This finding provides a means for the estimation of the amount of GEF relative to the amount of eIF-2. The proportion of eIF-2 in resting T lymphocytes that does not bind CM-Sephadex was found to be 23% of the total eIF-2, indicating that the ratio of GEF to eIF-2 is approximately 0.23 in these cells (data not shown). Thus, the GEF level in resting T lymphocytes is close to the eIF-2(αP) level which in turn suggests that the level of free GEF that is active in initiation in these cells is very low, which is consistent with a regulatory role for GEF and eIF-2(αP) in protein synthesis activation. The ratio of GEF:eIF-2 in stimulated cells will be similar to that found in resting cells because eIF-2 and GEF increase approximately in parallel. We calculate a GEF:eIF-2 ratio of 0.2 after 24 h of stimulation.

DISCUSSION

Previously, it has been suggested that one of the steps in polypeptide chain initiation leading to the formation of 43 S complexes (initiation complexes that consist of the 40 S ribosomal subunit, Met-tRNA, and the initiation factors eIF-2 and eIF-3) may be regulatory (Ahern et al., 1974). In this communication, the results indicate that mitogenic activation of T lymphocyte protein synthesis is accompanied by approximately a 4-fold increase in eIF-2 during the first 24 h of incubation, which parallels the increase in ribosomes. Thus, new eIF-2 is synthesized as part of the general activation of protein synthesis. Assuming that the increase in the ribosome content is a good estimate of cell growth and increase in cell size, the parallel increase in eIF-2 indicates that the concentration of eIF-2 in the cell remains relatively constant with stimulation. Estimates of cell size and cytoplasmic volume obtained for resting and mitogen-stimulated human T lymphocytes (Cooper and Braverman, 1981) also agree with the eIF-2 concentration remaining constant during cell proliferation. It appears then that eIF-2 is synthesized to keep in step with the increase in the protein synthesis machinery as the cell grows in size which suggests that the increase in eIF-2 alone is insufficient to account for the large increase in the rate of protein synthesis per ribosome that is observed in mitogen-stimulated cells.

The proportion of eIF-2 that is phosphorylated decreases in response to mitogenic stimulation. Thus, the increase in unphosphorylated eIF-2 is greater than the increase in phosphorylated eIF-2 when new eIF-2 is synthesized. New eIF-2 synthesis then provides a proportionately larger pool of unphosphorylated eIF-2 for the initiation of protein synthesis as the cell grows in size. However, the increase in unphosphorylated eIF-2 still is not sufficient to account for the observed increase in the translation rate per ribosome.

The results presented here also show that mitogenic activation of T lymphocytes is accompanied by an increase in GEF, a factor required for the cycling of eIF-2-GDP in initiation. As in the case of eIF-2, the increase in GEF parallels the increase in ribosomes though it is slightly smaller in magnitude, increasing 3-fold in 24 h. The increase does not necessarily indicate that new GEF is synthesized, since it is estimated by measurement of enzymatic activity. However, the rather close resemblance of the trend in GEF increase to the increase in eIF-2 and ribosomes makes it attractive to infer that new GEF is synthesized as part of the general activation of protein synthesis.

The parallel increase in total GEF and eIF-2 while eIF-2(αP) remains unchanged may provide a mechanism by which mitogen-stimulated cells increase their rate of protein synthesis per ribosome. In resting cells, the total level of GEF is similar to the level of eIF-2(αP). eIF-2(αP) is a potent competitive inhibitor of GEF that can trap a roughly equimolar
quantity of the exchange factor and prevent its normal catalytic activity, which means that the free GEF level in resting cells must be very low and thus probably limiting for initiation. Moreover, it means that an observed 3-fold increase in total GEF in parallel with a relatively small increase in the eIF-2(αP) level in stimulated cells will probably result in a large increase in free GEF compared with the increase in total GEF. This, in turn, may result in a pronounced increase in the rate of chain initiation and thus account for the large increase in the rate of translation per ribosome. To estimate the exact increase in free GEF, we need to know the free GEF quantity of the exchange factor and prevent its normal catalytic activity. Moreover, it means that an observed %fold increase in cells must be very low and thus probably limiting for initiation in the ratio of eIF-2:eIF2(αP) occurs over hours which correlates closely with the increase in protein synthesis as activated T lymphocytes grow in size. This close correlation argues strongly for the described mechanism of increase in active GEF being at least necessary and perhaps partly responsible for the increase in protein synthesis in T lymphocytes.

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**T Lymphocyte Protein Synthesis**

**Supplementary Material to:**
Mechanism of activation of protein synthesis initiation in mitogen stimulated T lymphocytes
by P. Jalkanen and Richard Pearse

**METHODS**

Preparation of lysis from resting T lymphocytes for assay of translational inhibitors.
T lymphocytes were purified from the spleen of adult C3H mice by the procedure of the nylon wool technique described previously (Matthew and Singh, 1985; Julius et al, 1973). Spleens were homogenized in a sterile tissue grinder with muscle mesh (250 µm) and the cells were collected in cold (7°C) RPMI 1640 medium supplemented with 10% fetal calf serum (RPMFCS). The cells were pooled, counted and the suspension was centrifuged twice for 5 minutes at 900 g at 7°C. The cells were resuspended in warm (37°C) RPMFCS to a density of 1 x 10^6 cells/ml, yielding a total volume of approximately 15 ml. The suspension was added to a 25 ml conical flask of nylon wool (2.4 g of nylon wool packed to a volume of 25 ml and subsequently preincubated for 1 hour at 37°C in RPMFCS) and was allowed to penetrate the columns. The column was incubated for 1 hour at 37°C and nonpenetrated cells were eluted by washing with 50 ml of RPMFCS (37°C). All subsequent steps were carried out at 0°C. The suspension of eluted cells was centrifuged for 5 minutes at 900 g and the cells were resuspended for 2 minutes in hypotonic buffer (20mM Tris-Cl, 150mM NaCl, pH 7.2) to lyse erythrocytes. Excess Tris (20mM Tris, 150mM NaCl, pH 7.2) was added, the suspension was centrifuged for 5 minutes at 900 g, washed once with PBS and resuspended again. The cells were then resuspended 1:1 in "Freezing Buffer" (20mM Tris-Cl, 0.1mM EDTA, 0.1 mM EGTA, 1mM DTT, 3mM ATP, 3mM MgCl₂, pH 7.4) and placed in liquid N₂. If the samples were frozen below -30°C, complete cell lysis occurs.

Assay for inhibition of protein synthesis in the rabbit reticulocyte cell-free translation system.
The cells prepared above were thawed in the presence of KCl, at a final concentration of 50mM, and the resulting cell homogenate was centrifuged for 10 minutes at 10,000 g. The supernatant was collected and allowed to sit for 10 minutes at 25°C. The reticulocyte cell-free translation system was made as described above, using the rabbit reticulocyte cell-free translation system described previously. Various amounts of lymphocyte lysate were added to protein synthesis reactions (50 or 160 µl), of which 20ml MOPS, 75mM KCl, 0.5mM MgCl₂, 1mM creatine phosphate, 10% FBS, 2mM DTT, 0.5mM ATP, 0.1mM DTT, 0.1mM EGTA, 1mM EDTA (including tyrosine), 5mM (NH₄)2SO₄, 10% rabbit reticulocyte lysate and 12 µl RNA (the pH of the reactions was 7.4). The reactions were incubated at 37°C and 10 µl aliquots were plated onto paper filters at the indicated time points. The filters were then processed to determine the amount of hot TCA precipitable radioactivity.

Concentrate Alkaline Extract of T lymphocytes in culture.
The cells purified from the tissue were processed as described above, using the rabbit reticulocyte cell-free translation system described previously. Various amounts of lymphocyte lysate were added to protein synthesis reactions (50 or 160 µl), and the reactions were incubated for 10 minutes at 37°C. At 10 minutes, the entire reaction mixture was spotted onto paper filters, and the filters were processed to determine the amount of hot TCA precipitable radioactivity.

Preparation of lysis from resting and Concentrate Alkaline Extracted T lymphocytes.
A given time point, 1ml of cell suspension was removed from each culture, 50 µl of [35S]methionine were added, and the reactions were incubated for 10 minutes at 37°C. At 10 minutes, the entire reaction mixture was spotted onto paper filters, and the filters were processed to determine the amount of hot TCA precipitable radioactivity.

**Determination of eIF-2 level in T lymphocytes.**
T lymphocyte lysates were prepared by triton lysis as described above and 30 µl of each lysate was analyzed for eIF-2 content by SDS-PAGE and immunoblotting. SDS-PAGE was performed on T lymphocyte lysate (30µl) by a modification of the procedure of Laemmli (1970) as described previously (Sorbonne et al, 1987). Gels were transferred to nitrocellulose as described by Towbin et al (1979), and eIF-2 was detected by immunoblotting using monoclonal anti-eIF-2 as detected previously (Rowlands et al, 1990). The relative amounts of eIF-2 were determined by laser densitometric scanning of autoradiographs relative to a purified eIF-2 standard.

**Purification of eIF-2a phosphorylation in T lymphocytes.**
T lymphocytes were isolated from spleens and cultured with or without concanavalin A as described above. At a given time point, 600 µl of cell suspension was removed from culture and mixed with 1ml of cold PBS. (All subsequent steps were carried out at 0°C). The suspensions were centrifuged at 900 g for 5 minutes, resuspended in 70 µl of cold RPMFCS, and were placed in a sample buffer (50mM Tris, 2% SDS, 20% glycerol, 0.1% bromphenol blue). After samples were boiled for 3 minutes, transfer to nitrocellulose and detection of eIF-2 and eIF-2a by immunoblotting was performed as described previously (Sorbonne et al, 1987) except that gels were transferred 0.4% between 30. The relative amounts of eIF-2 and eIF-2a were quantified by laser densitometric scanning of autoradiographs.

**Preparation and Purification of [35S]Met-tRNA.**
[35S]Met-Ser-tRNA (250 Ccmmoles) was prepared as described previously (Rowlands et al, 1990), except that cold cell free tRNA was used instead of rabbit liver tRNA.

Assay of GEF activity (modified ternary complex assay).
Crude extracts were used immediately after preparation for assay of GEF activity. To obtain reproducible results, extracts cannot be frozen. GEF activity was measured by a modification of the ternary complex assay as described previously (Perry and Hershey, 1983). Reactions (50 µl) containing 50mM MOPS, pH 7.4, 100mM KCl, 3mM MgCl₂, 1mM DTT, 0.1mM GTP, 3mM phosphorothioate pyruvate, 40 µg/ml pyruvate kinase, 0.1mM GTP, 10% rabbit reticulocyte lysate and 12 µl RNA were incubated at 37°C. At a given time point, 20 µl aliquots were removed, mixed with 500 µl of ice cold quench buffer (50mM MOPS, pH 7.4, 100mM KCl, 3mM MgCl₂, 0.1mM DTT and 0.1mM EDTA) to stop the reaction and the filters were washed twice with 500 µl of quench buffer, were dried and then radioactivity determined by liquid scintillation spectrophotometry. The amount of ternary complexes formed above that formed spontaneously (without the addition of extract or GEF) is an estimate of the relative amount of GEF present.

**Determination of eIF-2a levels in resting T lymphocytes.**
Lysates from resting T lymphocytes were prepared as described above, except that the cells were lysed by resuspending in Triton X lysate and addition of Triton X-100 to a final concentration of 0.1% (All subsequent steps were performed at 0°C). The lysate was resuspended in the presence of KCl, at a final concentration of 50mM, and centrifuged for 10 minutes at 10,000 g, and the supernatant was collected. 200 µl of supernatant were made 0.5M with respect to KCl by the addition of 3M KCl. The high salt supernatant was centrifuged for 60 minutes at 50,000 rpm in a Beckman 70 Ti rotor, and the resulting high-speed post-nuclear supernatant was dialyzed overnight against 11 of E₆₈ buffer (220mM MOPS, pH 7.4, 0.2mM DTT, 0.2mM EDTA, 10% glycerol and 1mM KCl) as indicated by (Suba). The ratio of GEF to eIF-2a was estimated by a modification of a method used previously for c-Ha ras transfected tumor cells (Rowlands et al, 1990). 200 µl of the dialyzed material were mixed with an equal volume of CM-Sepharose equilibrated in E₆₈, in a 1ml E₆₈ Eppendorf tube, and incubated at 0°C for 10 minutes. The suspensions were centrifuged for 30 minutes at 6000 rpm in a microfuge and the supernatant was removed (Flow through). The CM-Sepharose was washed 3 times with 1ml of E₆₈, 100 µl of E₆₈ were added, the supernatant was centrifuged as above, and the supernatant was recovered (E₆₈ wash). Both fractions were precipitated with acetone (90% at 24°C), centrifuged for 10 minutes at 12,000 rpm in a microfuge, and the precipitates were dissolved in 1x SDS-PAGE sample buffer (50mM Tris-HCl pH 6.8, 1% SDS, 10% 2-mercaptoethanol, 5% Glycerol and 0.02% bromphenol blue). The relative amounts of eIF-2 in the fractions were quantitated by SDS-PAGE and immunoblotting, as described above.
Figure 1. Protein synthesis in reticulocyte lysate was measured by 14C leucine incorporation into protein as described in materials and methods with the following additions: A. (a) none; (c) 7.5 μg rat T cell lysate protein; (B) 300 units/ml RNasin; and (a) 7.5 μg rat T cell lysate protein plus 300 units/ml RNasin.
B. (a) none; (c) 2 μg; (c) 16 μg; (a) 50 μg of rat T cell lysate protein.

Figure 2. T lymphocytes were cultured with or without concanavalin A and cell samples were removed at various times to estimate rates of protein synthesis and RNA content as described in materials and methods. The fold increase in protein synthesis (c) and RNA content (a) in concanavalin A-treated relative to untreated cells is given.