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Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins

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ABSTRACT **The immunosuppressant rapamycin (RAP) has been demonstrated to specifically inhibit the activity of p70 S6 kinase ($p70^{s6k}$) and subsequent phosphorylation of ribosomal S6 protein in mammalian cells. Addition of RAP to proliferating lymphoid cells resulted in inhibition of protein synthesis before any changes in the rate of cell proliferation. When the cellular composition of proteins was examined by gel electrophoresis, RAP dramatically inhibited synthesis of selective proteins, particularly elongation factor 2 (eEF-2). The inhibition of eEF-2 synthesis by RAP was at the translational level. Further, RAP inhibited the polysomal association of mRNAs encoding not only eEF-2 but also elongation factor 1-α and ribosomal proteins without affecting mRNA translation of any of a number of nonribosomal proteins. Since levels of activity of $p70^{s6k}$ are correlated with the rate of biosynthesis of eEF-2, $p70^{s6k}$ might be involved in coordinate translational regulation of ribosomal protein mRNAs in higher eukaryotes, which have a conserved sequence at their 5′ end. Specific inhibition of ribosomal protein synthesis likely explains the differential antiproliferative effect of RAP on proliferating and mitogen-activated quiescent cells.**

Rapamycin (RAP), a macrolide with potent immunosuppressive activity, is effective in prolonging survival of organ allografts and preventing the onset of autoimmune disease in animals (1). RAP inhibits the proliferation of lymphocytes and other types of mammalian cells. Cell cycle progression is arrested in early G_1 phase of the cycle or the G_1 phase is significantly prolonged when the drug is added to quiescent cells stimulated with mitogens (2–4). Recent studies have demonstrated that RAP specifically inhibits p70 S6 kinase ($p70^{s6k}$) activity with little or no alteration in several other early signal transduction events involved in mitogen-induced activation of cells (2, 4–8). In mammalian cells, activated $p70^{s6k}$ phosphorylates ribosomal S6 protein (9, 10), and inhibition of $p70^{s6k}$ by RAP is associated with inhibition of ribosomal S6 phosphorylation *in vivo* (2). Furthermore, microinjection of quiescent rat fibroblasts with polyclonal antibodies against $p70^{s6k}$ abolished serum-induced entry into S phase of the cell cycle (11). These studies suggest that inhibition of $p70^{s6k}$ may be responsible for the inability of activated resting cells to traverse through the G_1 phase of the cell cycle.

However, in contrast to results obtained with resting cells, RAP did not immediately affect cell cycle progression when the drug was added to cycling cells, despite inactivation of $p70^{s6k}$ activity (4). Cells continued to proliferate normally for at least two or three additional cycles in the presence of RAP and in the absence of detectable activity of $p70^{s6k}$. These data indicated that $p70^{s6k}$ activity and S6 phosphorylation may not be essential for proliferation once cells have entered the cell cycle. Here, we demonstrate that RAP selectively inhibits the translation of mRNAs encoding ribosomal proteins and elongation factors.

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MATERIALS AND METHODS

Cell Culture. The human B-lymphoblastoid cell line Ramos [American Type Culture Collection (ATCC) CRL 1596] was maintained in RPMI 1640 medium (GIBCO, NY) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (HyClone), 2 mM L-glutamine (GIBCO), 2-mercaptoethanol (0.05 mM), 100 units of penicillin per ml, and 100 μg of streptomycin per ml (GIBCO). RAP and FK506 were obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute, and Fujisawa Pharmaceutical (Osaka), respectively, and dissolved in ethanol to give 1–10 mg/ml stock solutions.

[^{3}H]Thymidine and ^{3}H-Labeled Amino Acid (^{3}H-Amino Acid) Incorporation. DNA synthesis was evaluated by incorporation of [^{3}H]thymidine into cells as described (3). General protein synthesis was evaluated by incorporation of ^{3}H-amino acid mixture (Amersham) into cells. After labeling cells with 5 μCi (1 Ci = 37 GBq) of the ^{3}H-amino acid mixture per ml, cells were washed twice with ice-cold phosphate-buffered saline (PBS) and then lysed in PBS containing 1% SDS followed by addition of 7% trichloroacetic acid/1% pyrophosphate. The precipitates were loaded on GF/A filters and washed extensively with 7% trichloroacetic acid/1% pyrophosphate. Radioactivity was measured by scintillation counting.

Kinase Assay of $p70^{s6k}$. Specific activity of $p70^{s6k}$ was determined by ^{32}P incorporation into S6 peptide in the immune complex as described (4).

[^{35}S]Methionine Incorporation into Proteins and Immunoprecipitation. Cells were pretreated with the drugs for 30 min in methionine-free medium, and then 200 μCi of [^{35}S]methionine per ml (ICN) was added to the cultures for 2 hr. Cells were lysed in RIPA buffer, and lysates were separated by an SDS/10% polyacrylamide gel. Gels were fixed, treated with Chemiamplifier solution, and dried. Radiolabeled species were visualized by autoradiography. The radioactivity of each lane was also scanned by PhosphorImager and analyzed by ImageQuant analysis (Molecular Dynamics). For immunoprecipitation, the [^{35}S]methionine-labeled protein samples were incubated at 4°C for 1 hr either with rabbit polyclonal antibodies against elongation factor 2 (eEF-2; EF-2 Ab 118) (12) or with a mouse monoclonal antibody against hsp90 (clone 9D2, StressGen, Biotechnologies, Sidney, Canada).

Abbreviations: RAP, rapamycin; $p70^{s6k}$, p70 S6 kinase; eEF-2, elongation factor 2; eEF-1α, elongation factor 1α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mRNP, mRNA–ribonucleoprotein complex; PCNA, proliferating cell nuclear antigen; DHFR, dihydrofolate reductase.

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Immune complexes were absorbed to protein G/protein A-coupled beads (Oncogene Science). Precipitated proteins were separated and visualized as described above.

Northern Blot Analysis. Northern blot analysis was performed as described (4). Probes used here were hamster eEF-2 cDNA (*Sma* I–*Bgl* II fragment of pHEW 1) (13) and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (4).

Analysis of mRNA in Polysomal and Nonpolysomal Fractions. Fractionation of polysomes and translationally inactive mRNA–ribonucleoprotein complexes (mRNPs) was performed as described (14). Briefly, after washing cells ($2–5 \times 10^7$) once with PBS, cells were suspended for 10 min at 0°C in 1 ml of a buffer containing 10 mM NaCl, 10 mM Tris·HCl (pH 7.4), 15 mM $MgCl_2$, 1.2% Triton X-100, 1.2% deoxycholate, and a ribonuclease inhibitor, RNasin at 200 units/ml (Promega). Nuclei were pelleted by centrifugation for 2.5 min at 10,000 × *g*. The postnuclear supernatant (300 μl) was layered over 11 ml of a 0.5–1.5 M sucrose gradient with a 1-ml cushion of 1.5 M sucrose. The sucrose solutions contained 2 mM Tris·HCl (pH 7.4), 25 mM NaCl, 5 mM $MgCl_2$, and 100 μg of heparin per ml. The gradients were centrifuged at 105,000 × *g* for 3 hr at 4°C in a Beckman SW28 rotor. Twelve fractions of 1 ml each were collected, and RNA was extracted as described (15). The RNA from each fraction was then applied to nylon membranes using a slot blot apparatus. Membranes were hybridized with radiolabeled probes of hamster eEF-2 cDNA, human GAPDH cDNA, human eEF-1α cDNA (ATCC no. 37854), human ribosomal protein S4 cDNA (ATCC no. 65108), human ribosomal protein S24 cDNA (ATCC no. 65452), human ribosomal protein S3 cDNA probe (ATCC no. 65824), human ribosomal protein S14 cDNA probe (ATCC no. 59246), human c-*myc* cDNA (4), mouse dihydrofolate reductase (DHFR) cDNA (ATCC no. 37295), human cdc-2 cDNA (provided by S. Elledge) (16), human cyclin A cDNA (provided by T. Hunter) (17), and human proliferating cell nuclear antigen (PCNA) cDNA (ATCC no. 61054). Probes for human ribosomal S6 and S17 were made by PCR amplification using specific primers and human cDNA obtained by mitogen-activated T cells. The specific primers used here were as follows: for S6, 5′-TTCAAGATGAAGCTGAACATCTCC (5′ sense, cDNA sequences across exons 1 and 2), 5′-GTCCAGGAATATCCTTCTCTCCTT (3′ antisense, exons 3 and 4) (18); for S17, 5′-AACATGGGCCGCGTTCGCACCAAA (5′ sense, exons 1 and 2), 5′-CCAAGGCTGAGACCTCAGGAACAT (3′ antisense, exons 3 and 4) (19). The resultant PCR products were 376 bp (S6) and 277 bp (S17). Membranes were then washed under high stringency and specific mRNA was visualized by autoradiography.

RESULTS

RAP Inhibits Protein Synthesis but not Cell Proliferation or DNA Synthesis of Cycling Cells Despite the Inactivation of $p70^{s6k}$. Exponentially proliferating cells continue to cycle in an apparently normal manner for a period of time in the presence of RAP (4). Proliferating Ramos cells were treated with RAP (10 ng/ml) at a dose sufficient to achieve complete inhibition of $p70^{s6k}$ in this and other cells (2, 4–8). As shown in Fig. 1*A*, increases in cell number were not affected by RAP during the first 3 days of culture. The growth rate gradually decreased in the presence of RAP over 72 hr. DNA synthesis was not affected by RAP in the first 24 hr after addition of the drug (Fig. 1*B*), and the profile for DNA content, determined by flow cytometry of propidium iodide-stained cells, was also similar in the initial 24 hr (data not shown). Although cell proliferation continued in the RAP-treated cells for two additional cycles (up to 72 hr), protein synthesis was rapidly inhibited (Fig. 1*C*). During the first 3 hr of culture after addition of the drug, RAP inhibited overall protein synthesis

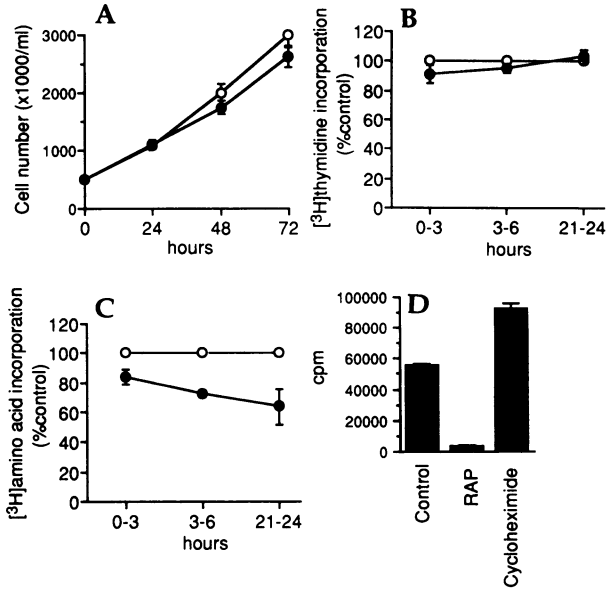

FIG. 1. (*A*) Effects of RAP on cell proliferation. Ramos cells (5×10^5 per ml) were cultured in the presence of RAP (10 ng/ml, ●) or vehicle (0.1% ethanol, ○) for the indicated times. Cell counts were evaluated using a Coulter Counter. (*B*) DNA synthesis. After addition of RAP or control vehicle, thymidine incorporation was measured after a 3-hr pulse at the indicated times with 5 μCi of [^{3}H]thymidine per ml (6.7 Ci/mmol) by scintillation counting. The results are shown as % control at each time point. (*C*) Protein synthesis. General protein synthesis was measured by incorporation of ^{3}H-amino acid into cells (see text). Cells were pulse-labeled for 3 hr at the indicated times with 5 μCi of the ^{3}H-amino acid mixture per ml. (*D*) $p70^{s6k}$ activity. Cells were treated with vehicle (control), RAP (10 ng/ml), or cycloheximide (50 ng/ml) for 30 min. Specific activity of $p70^{s6k}$ was determined by ^{32}P incorporation into S6 peptide in the immune complex as described (4).

by about 15%, as measured by incorporation of ^{3}H-amino acids. Protein synthesis gradually decreased at later time points, reaching 40% lower levels than in control cultures after 24 hr. Perhaps as a consequence of continuing cell proliferation with partial inhibition of protein synthesis, cell size progressively decreased within 12–24 hr in RAP-treated samples (mean diameter of cells after 24 hr: control cells, 9.8 μm vs. RAP-treated cells, 8.8 μm, estimated by Coulter Counter). In these same cells, we confirmed that RAP inhibited $p70^{s6k}$ activity completely within 30 min (Fig. 1*D*), and inhibition was sustained throughout the entire time course of the experiments (4). In parallel to the inhibition of $p70^{s6k}$ activity, *de novo* phosphorylation of ribosomal S6 protein *in vivo* was inhibited within 30 min of addition of RAP (data not shown).

RAP Inhibits Protein Synthesis in a Selective Manner. To further characterize the inhibition of protein synthesis induced by RAP, we examined protein synthesis by electrophoretic separation of proteins following [^{35}S]methionine labeling of Ramos cells. Cells were pretreated with RAP for 30 min in methionine-free medium, and then [^{35}S]methionine was added to the cultures for 2 hr. The cells were harvested, and the lysates were separated by SDS/10% polyacrylamide gel. Fig. 2*A* demonstrates scanning data of relative radioactivity of each lane in the gel using the PhosphorImager. The intensity of only a few bands was dramatically altered following addition of RAP. One of the most significant decreases induced by the drug was observed in a protein with an apparent molecular mass of 97 kDa. As a comparison, we added a low concentration (50 ng/ml) of cycloheximide, an inhibitor of peptidyltransferase, to the cultures. This concentration of cycloheximide inhibited overall protein synthe-

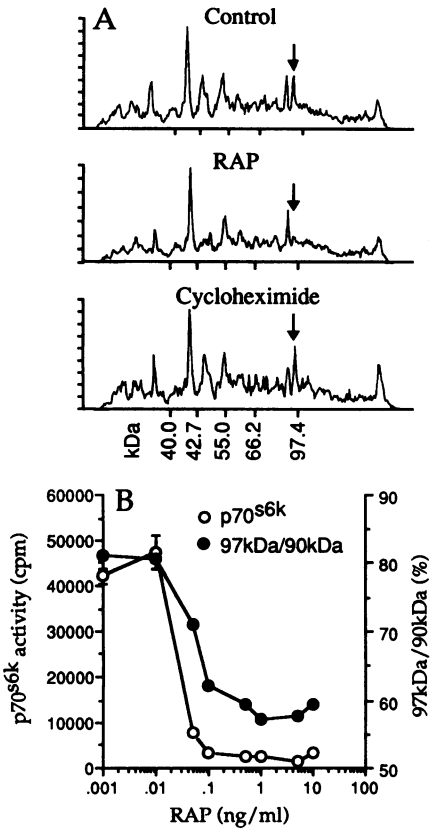

FIG. 2. RAP specifically inhibits [35S]methionine incorporation of a 97-kDa band. (A) Cells were pretreated with vehicle (control), RAP (10 ng/ml), or cycloheximide (50 ng/ml) for 30 min in methionine-free medium, and then 200 μCi of [35S]methionine per ml was added to the cultures for 2 hr. Cells were lysed in RIPA buffer, and lysates were separated by SDS/10% polyacrylamide gel. Gels were fixed in methanol/acetate solution, treated with Chemiamplifier solution, and dried. The radioactivity of each lane was then scanned by the PhosphorImager using ImageQuant analysis. An arrow indicates the 97-kDa band. (B) Cells were incubated with various concentrations of RAP (0.01–10 ng/ml) and labeled with [35S]methionine for 2 hr as described above. The ratio of the peak value (%) of the 97-kDa band to that of the 90-kDa band was determined using PhosphorImager. Cells were also treated with the same concentration of RAP for 2 hr and the activity of p70s6k was measured.

sis by about 15%, similar to the overall decreases induced by RAP. Under these conditions, [35S]methionine incorporation was decreased more generally than observed with RAP. Moreover, the relative incorporation of [35S]methionine into the 97-kDa band was augmented by cycloheximide (Fig. 2A, cycloheximide). This concentration of cycloheximide also increased both p70s6k activity and the rate of S6 phosphorylation, as has been demonstrated previously (10) (Fig. 1D).

To investigate the relationship between the specific inhibition of the 97-kDa protein and p70s6k activity by RAP, we examined the dose-dependent effect of the drug on these events. As an estimation of specific inhibition of the 97-kDa band, we compared the ratio (%) of the peak value of the 97-kDa band by PhosphorImager to that of the 90-kDa band, which was the most prominent peak seen adjacent to the 97-kDa band (see Fig. 2A, control). As shown in Fig. 2B, the dose–response to RAP for the specific inhibition of incorporation of [35S]methionine into the 97-kDa band was in parallel to that of the inhibition of p70s6k activity.

Identification of the 97-kDa Protein as eEF-2. To identify this 97-kDa protein, cell lysates radiolabeled with [35S]methionine were separated using two-dimensional gel electrophoresis (isoelectric focusing method). RAP significantly and selectively decreased the level of a series of proteins with an approximate size of 97 kDa and with an apparent isoelectric point in the range of 6.5–6.8 (data not shown). Based on a comparison with a comprehensive two-dimensional gel protein data base (20), these proteins were located in a region in which eEF-2 migrated. The identity of these proteins was confirmed by immunoblotting using specific antibodies directed against eEF-2 (data not shown). To confirm the decrease in [35S]methionine incorporation into eEF-2, we performed immunoprecipitation of cell lysates using the same antibodies. [35S]Methionine incorporation into eEF-2 was dramatically inhibited by RAP (Fig. 3). Moreover, the mobility of eEF-2 was identical to that of the 97-kDa protein, which RAP inhibited in the one-dimensional gel. As a control, [35S]methionine incorporation into a 90-kDa heat shock protein (hsp90) was examined by immunoprecipitation of the same lysates with specific antibody against hsp90. In contrast to eEF-2, [35S]methionine incorporation into hsp90 was not significantly affected by RAP (Fig. 3). Synthesis of other proteins similarly examined, including β-actin, β-tubulin, PCNA, cyclin A, and transferrin receptor, also was not affected by RAP (data not shown). The inhibition of eEF-2 synthesis was dependent on RAP binding to FK506 binding proteins since addition of an excess of FK506 reversed the inhibitory effect and was accompanied by an increase in p70s6k activity (data not shown).

Inhibition of eEF-2 Synthesis by RAP Is Due to a Decrease in Translation of eEF-2 mRNA. To determine whether the apparent decrease in [35S]methionine incorporation into eEF-2 induced by the drug was due to a decrease in synthesis or an increase in the rate of degradation of the protein, continuously growing Ramos cells were first labeled with [35S]methionine for 2 hr, washed three times, and resuspended in medium for another 1, 3, or 16 hr in the presence or absence of RAP. As shown in Fig. 4A, RAP did not affect the relative amount of [35S]methionine present in the immunoprecipitated eEF-2 at any of these time points. These data indicate that RAP did not affect the degradation of eEF-2 but that its effects were at the level of synthesis of the protein. In addition, the data confirm that eEF-2 has a long half-life, as previously demonstrated in other systems (21). In addition, Ramos cells treated with RAP for 2 hr had similar or slightly higher levels of eEF-2 mRNA than control cells (Fig. 4B). These results suggest that the inhibition of eEF-2 protein synthesis by RAP was due to a decrease in the translation of eEF-2 mRNA.

To analyze more directly whether or not RAP inhibited synthesis of eEF-2 at the level of translation, polysomal

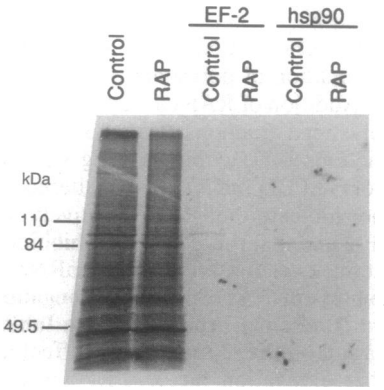

FIG. 3. Immunoprecipitation of eEF-2. The [35S]methionine-labeled samples were prepared as described in the legend to Fig. 2A. The lysates were immunoprecipitated with rabbit polyclonal antibody against eEF-2 or with mouse monoclonal antibody against hsp90. Precipitated proteins were separated and visualized. Whole cell lysates were also separated in the same gel (the two left lanes).

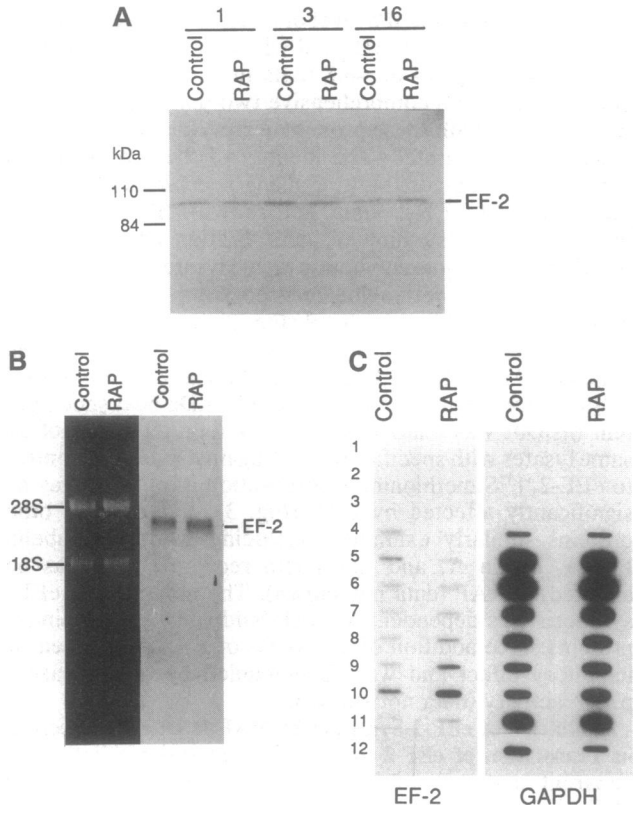

FIG. 4. (*A*) Degradation of eEF-2 protein. Untreated Ramos cells were first labeled with [^{35}S]methionine for 2 hr as described in the legend to Fig. 2*A*, washed with regular medium three times, and resuspended in regular medium in the presence or absence of RAP (10 ng/ml). After the indicated times (hours), radiolabeled eEF-2 protein was immunoprecipitated and analyzed. (*B*) mRNA analysis of eEF-2. Ramos cells were incubated for 2 hr with the control vehicle or RAP (10 ng/ml), and total RNA (5 μg) was separated and blotted onto nylon membrane. The membrane was probed with radiolabeled eEF-2 cDNA, and eEF-2 mRNA (about 3 kb) was visualized by autoradiography. As a control for total RNA amount, 28S and 18S rRNA, visualized by ethidium bromide staining, are also shown. (*C*) RAP-induced shift of eEF-2 mRNA from polysomes to mRNP fraction. Ramos cells (1×10^7) were cultured with RAP (10 ng/ml) or vehicle for 2 hr, and fractionation of polysomes and mRNPs was performed using sucrose gradient (0.5–1.5 M) centrifugation (see text). Twelve fractions of 1 ml each were collected, and RNA was extracted. Half of the RNA from each fraction was then applied to duplicate nylon membranes using a slot blot apparatus. Membranes were hybridized with a radiolabeled eEF-2 cDNA probe or a GAPDH cDNA probe and visualized by autoradiography.

association of eEF-2 mRNA was analyzed using sucrose gradient fractionation of polysomes and translationally inactive mRNPs. Addition of RAP caused a shift in eEF-2 mRNA from the polysomal fractions (fractions 4–7) to the mRNP fractions (fractions 9–11) within 2 hr (Fig. 4*C*). In contrast, the distribution of GAPDH mRNA was unaltered by RAP. These results further indicate that RAP selectively inhibited translation of eEF-2 and that this selective inhibition of translation may occur at the level of recruitment of mRNA to polysomes.

Translation of mRNAs Encoding Elongation Factor 1-α (eEF-1α) and Ribosomal Proteins Is Also Inhibited by RAP. Since translation of eEF-2 mRNA was affected by RAP, we tested another elongation factor, eEF-1α, and other ribosomal proteins where mRNA translation is apparently coordinately regulated by mitogenic activation (14, 22–25). As a control, we also examined the translation of mRNAs encoding various nonribosomal proteins, including immunoglobulin μ chain, PCNA, DHFR, p34^{cdc2}, p33^{cdk2}, cyclin A, cyclin E, and c-*myc*. As demonstrated in Fig. 5, addition of RAP to cycling Ramos cells caused a shift in mRNAs encoding not only eEF-2 but also eEF-1α, S3, S6, S14, and S24 from the polysomal fractions to the mRNP fractions within 2 hr, suggesting that the translation of these mRNAs was also inhibited by RAP. Similar shifts in the mRNAs encoding S4 and S17 were observed in the presence of RAP (data not shown). In contrast, no shift was observed in any unrelated mRNA, including those encoding GAPDH, PCNA, c-*myc*, DHFR, p34^{cdc2}, or cyclin A (Fig. 5). mRNAs encoding immunoglobulin μ chain, p33^{cdk2}, or cyclin E were also not affected by RAP (data not shown). The selective inhibition of synthesis of ribosomal proteins was also confirmed by metabolically labeling Ramos cells with [^{35}S]methionine for 2 hr and subsequent separation of ribosomes (data not shown). These results indicate that translation of mRNAs encoding ribosomal proteins including elongation factors was selectively inhibited by addition of RAP.

DISCUSSION

We have demonstrated that the immunosuppressive agent rapamycin inhibits the translation of mRNAs encoding elongation factors and ribosomal proteins in cultures of continuously proliferating human B-lymphoblastoid cells. Inhibition appeared to be selective as the translation of mRNAs encoding various other nonribosomal proteins was not inhibited. In addition, synthesis of most protein species was not affected by the drug in [^{35}S]methionine-labeling experiments except for the elongation factors and ribosomal proteins. Interestingly, the mRNAs for which translation was inhibited by RAP had a common sequence of cytidine–pyrimidine stretches (CYYYYYY) at their 5′ terminus. This sequence is conserved in all of the mRNAs encoding ribosomal proteins examined so far in higher eukaryotes (see refs. 26 and 27 for recent studies in the field and references therein) and is also detected in mRNAs encoding elongation factors in mammalian cells (28, 29). To the best of our knowledge, this conserved sequence at the 5′ end of mRNA has not been observed in other mRNAs reported encoding nonribosomal proteins in mammalian cells, including those studied here. In addition, most of the mRNAs encoding ribosomal proteins and elongation factors have a (G+C)-rich region following the cytidine–pyrimidine stretch sequence. These common structures suggest that such mRNAs may be uniquely regulated. In fact, mRNAs encoding ribosomal proteins and elongation factors exist in large amounts but are translated poorly in resting cells (14, 22–25). Following activation of the cells by mitogens, these mRNAs are shifted to the polysomal fraction within 30 min and synthesis of the proteins begins prior to the general increase in protein synthesis (30). The timing is compatible with that of p70^{s6k} activation (4, 8) and S6 phosphorylation (31). This polysomal shift was also inhibited by addition of RAP, without altering the translational status of nonribosomal protein mRNA in mitogen-activated primary T-cell activation (unpublished data). RAP did not alter the proliferative status of cycling cells, at least within the first 24–72 hr, but did induce the shift of ribosomal protein and elongation factor mRNAs from polysomal to nonpolysomal fractions. This shift of specific mRNAs is therefore not likely the consequence of changes in proliferative status. Since RAP selectively inhibited p70^{s6k} activity, which is responsible for phosphorylation of ribosomal S6 protein *in vivo*, one hypothesis is that p70^{s6k} activity may facilitate the translation of these selected mRNAs. In fact, the activity of p70^{s6k} and the rate of eEF-2 synthesis were correlated in the various experiments described above. Furthermore, S6 occupies a position in which it may modulate the interaction of ribosomes with mRNA and may be involved directly in the initiation process (31). It is possible that phosphorylation of S6, the consequence of p70^{s6k} activation, is involved in the

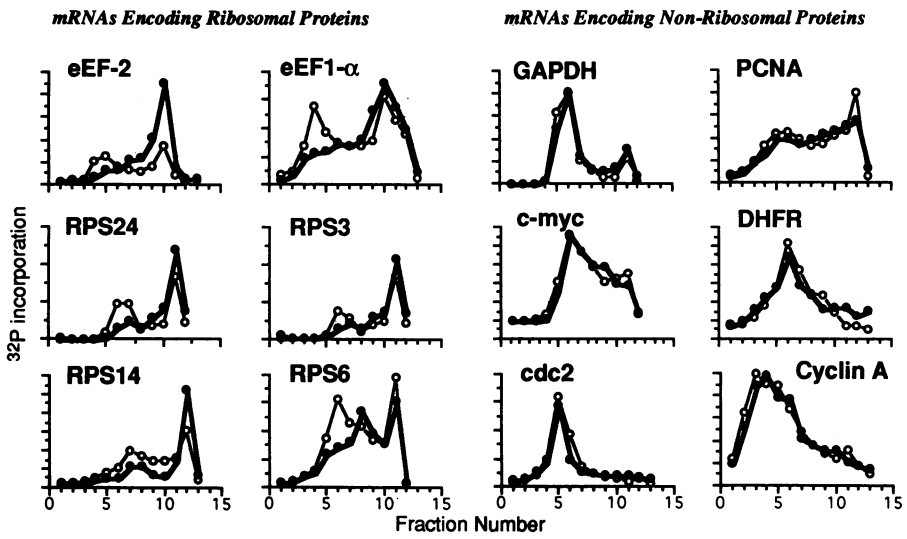

FIG. 5. RAP induces a shift of eEF-1α and ribosomal protein mRNAs from polysomes to mRNP fraction. Ramos cells (1×10^7) were cultured with RAP (10 ng/ml, ●) or vehicle (○) for 2 hr, and fractionation of polysomes and mRNPs and slot blot hybridization of mRNA of each fraction were performed as described in the legend to Fig. 4*C*. Membranes were hybridized with radiolabeled cDNA probe for eEF-2, eEF-1α, ribosomal proteins S24, S3, S14, and S6 (RPS24, RPS3, RPS14, and RPS6), GAPDH, PCNA, c-*myc*, DHFR, cdc2, or cyclin A. The radioactivity of slot blots was scanned using the PhosphorImager and the relative count in each fraction was determined by ImageQuant analysis.

coordinate regulation of translation of mRNAs encoding ribosomal proteins and elongation factors that have the conserved sequence at their 5′ terminus. The data presented here are in contrast to the results of microinjection of anti-$p70^{s6k}$ antibody, which inhibited overall protein synthesis, including that of c-*fos* (11), and this discrepancy needs further evaluation.

The need for a rapid increase in synthesis of ribosomal proteins and elongation factors in mitogen-activated cells coupled with the long half-life of their proteins in continuously proliferating cells likely explains the differential sensitivity of resting cells and proliferating cells to RAP (3, 4). Resting cells, which have relatively fewer ribosomal units (32), will require *de novo* synthesis of ribosomal proteins and elongation factors in order to accelerate the rate of protein synthesis, in preparation for cell division. On the other hand, growth of cycling cells, which already have significant amounts of ribosomal units, may continue through the cell cycle for a period of time, even when the synthesis of ribosomal proteins and elongation factors is rapidly and markedly decreased by RAP. The potent antiproliferative activity of RAP, which is distinct from that exerted by cyclosporin A or FK506, may thus reside in the inhibition of synthesis of ribosomal proteins and elongation factors.

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