MOLECULAR AND CELLULAR BIOLOGY, Sept. 1984, p. 1754–1760
0270-7306/84/091754-07$02.00/0

Vol. 4, No. 9

Changes in Levels of Actin and Tubulin mRNAs upon the Lectin Activation of Lymphocytes

ERIC McCAIRNS,* DALE FAHEY, GEORGE E. O. MUSCAT, MARYANNE MURRAY, AND PETER B. ROWE
Children's Medical Research Foundation, University of Sydney, Camperdown, New South Wales 2050, Australia

Received 18 January 1984/Accepted 8 May 1984

The expression of β-actin, γ-actin, α-tubulin, and β-tubulin mRNA during the lectin activation of human peripheral blood lymphocytes was examined with specific cDNA clones. The resting lymphocyte has a low level of both α- and β-tubulin mRNAs, and these increase 10-fold after 72 h of lectin stimulation in which maximum cell transformation is achieved. Although there is a slight increase in tubulin mRNA during the first 6 h, most of the increase occurs between 6 and 24 h as the cells start to increase their RNA content and progress from G_0 into G_1. Both β- and γ-actin mRNAs are more abundant than the tubulin mRNAs in resting cells, with β-actin mRNA being the major species. Upon activation, β-actin mRNA increases threefold, whereas γ-actin mRNA increases almost sixfold. Both β- and γ-actin mRNA are elevated 2.5-fold as early as 6 h, the γ-actin mRNA level then increasing more than β-actin between 6 and 24 h, resulting in the reduced β-actin/γ-actin mRNA ratio. The lectin-stimulated lymphocyte has a similar β-actin/γ-actin mRNA ratio as that of the human leukemic T-lymphoblast cell line CCRF-CEM. These increases are over and above the general increase in polyadenylated RNA content upon lectin activation. On returning to a noncycling state, the levels of these cytoskeletal mRNAs decrease. There were two β-tubulin mRNAs present in lymphocyte cytoplasm, one of 1.8 kilobases and one of 2.8 kilobases in length. The nongrowing lymphocytes had relatively lower levels of the larger sized mRNA. Upon stimulation, the relative level of the larger sized mRNA was increased, and at 72 h the cells had approximately equal levels of both mRNAs as did the leukemic lymphoblasts.

When the growth of quiescent, serum-deprived mouse fibroblasts is initiated by the addition of serum, there is a stimulation of actin synthesis which is specific for the passage from G_0 through G_1 into S (27, 28). Upon suspension of anchorage-dependent mouse fibroblasts, the resultant growth arrest is associated with decreased actin synthesis, although the level of actin mRNA remains the same, whereas upon reattachment, the recovery is associated with an increase in actin synthesis and in actin mRNA levels (8).

Agents, such as colchicine, that disrupt microtubules and increase the level of tubulin monomer have been found to lead to an inhibition of tubulin mRNA production which, coupled with the short half-lives of both α- and β-tubulin mRNAs, quickly leads to a shutdown of tubulin synthesis (1, 4).

As the cytoskeleton is made up of microtubules and actin microfilaments in association with intermediate filaments, the above studies suggest a close relationship between cell morphology, expression of cytoskeletal protein genes, and growth control. The normal resting lymphocyte is in a quiescent (G_0) state and can be stimulated to enter the cell cycle by polyclonal mitogens such as the lectin phytohemagglutinin. Kecskemethy and Schäfer (15) have shown that actin is a major product in quiescent bovine lymph node lymphocytes; upon lectin stimulation, the level of actin increases fivefold, and tubulin now appears in significant quantities. These changes may reflect alterations in the relative amounts of the cytoplasmic mRNAs coding for these proteins or altered rates of translation of specific mRNAs. Alterations in efficiency of translation of mRNA from resting and growing cells have been demonstrated in mouse fibroblasts (7, 8), bovine lymphocytes (15), and human lymphocytes (22). To determine what changes occur in the mRNA levels for the proteins actin and tubulin upon lectin activation of human lymphocytes, we have made use of cloned cDNA probes for β- and γ-actin and α- and β-tubulin mRNAs.

The results presented here indicate that the resting lymphocyte has low levels of α- and β-tubulin mRNAs, which both increase dramatically upon stimulation. There is also an increase in β- and γ-actin mRNA levels upon stimulation but to a lesser extent than that seen with the tubulin mRNAs. The actin mRNAs are already more abundant than the tubulin mRNAs in the resting cell; upon stimulation, there is a relatively larger increase in the level of the mRNA for γ-actin than for β-actin, resulting in an altered β-actin/γ-actin mRNA ratio. On returning to a noncycling state, the levels of actin and tubulin mRNAs decrease. There were two β-tubulin mRNAs whose relative proportions varied in the growing and nongrowing lymphocytes.

MATERIALS AND METHODS

Cloned cDNA probes. Human fibroblast β- and γ-actin and α- and β-tubulin clones were kindly provided by Peter Gunning, Phyllis Ponte, and Larry Kedes. The following information was communicated regarding the clones. Clone pHFβA-3'UT is a subclone of the 3' untranslated region of the human β-actin cDNA clone pHFβA-1 (10). This subclone hybridizes only to β-actin mRNA (26). Clone pHFγA-3'UT is a subclone of the 3' untranslated region of the human γ-actin cDNA clone pHFγA-1 (10). This subclone hybridizes only to γ-actin mRNA (26). Clone pHFβT-1 is a virtually full-length cDNA clone specific for β-tubulin and was isolated from a library constructed by Okayama and Berg (25). This clone has the same sequence as the β-tubulin cDNA clone (Dβ-1) described by Hall et al. (12). Clone pHFαT-1 is a virtually full-length cDNA clone specific for α-tubulin and was isolated from the library constructed by Okayama and

* Corresponding author.

Berg (25). This clone has the same restriction analysis as the α-tubulin cDNA clone (Kα1) described by Cowan et al. (5) and was identified by its hybridization to the α-tubulin clone described by Cleveland et al. (3).

Cell culture and RNA isolation. Human peripheral blood lymphocytes were isolated, cultured, and transformed with phytohemagglutinin as described previously (22, 23). After culture for 3 days at 2×10^6 cells per ml, the medium was changed to complete medium minus phytohemagglutinin, and culturing continued until day 7 (from the initial addition of lectin). Resting cells were cultured for 16 to 24 h in the absence of phytohemagglutinin to allow them to recover from the isolation procedure. Viability and transformation were assessed as described previously (23). Nonviable cells were removed as described by Hurrell et al. (13). The human leukemic T-lymphoblast cell line CCRF-CEM was maintained in log phase of growth as described previously (16).

The isolation of cytoplasmic RNA and the purification of polyadenylated RNA from the total cytoplasmic RNA were carried out as described previously (22).

Flow cytometry. All flow cytofluorometric analyses were performed with an Ortho Instruments ICP22 flow cytometer. Cellular DNA content was measured by fluorescence after ethidium bromide-mithramycin staining as described by Taylor (31). Briefly, cells in complete medium were permeabilized with Triton and stained with an ethidium bromide-mithramycin solution. As each stained cell flowed past a light source, it was excited at 360 to 460 nm; the resulting fluorescence was measured at wavelengths greater than 550 nm.

Cellular DNA and RNA were measured simultaneously by their different fluorescence after the two-step acridine orange staining procedure described by Traganos et al. (33). Briefly, cells were made permeable to stain by treatment with Triton at low pH. Cells were then stained with a buffered acridine orange-EDTA solution. As each stained cell flowed past the light source, it was excited at 440 to 490 nm. The resulting green fluorescence due to DNA was measured between 520 and 550 nm, whereas the red fluorescence due to RNA was measured at wavelengths greater than 620 nm.

Blot analysis of lymphocyte mRNA. Polyadenylated RNA samples were denatured and run on 1% (wt/vol) agarose-formaldehyde gels as described by Lehrach et al. (18) with the gel buffer system described by Goldberg (9). After electrophoresis, RNA was transferred for 16 h to Pall Biodyne A nylon membranes by the method of Thomas (32). RNA samples were also directly dotted onto the nylon membranes by the method of Thomas (32) after formaldehyde denaturation as described by Lehrach et al. (18). After transfers, filters were baked at 80°C for 4 h. Plasmid DNA was isolated by the procedure of Ish-Horowicz and Burke (14) and labeled with deoxycytidine 5'-[α-^{32}P]triphosphate by the nick translation reaction (21) with the Amersham nick translation kit. Hybridization of nick-translated DNA to filter-bound RNA was carried out for 64 h by the method of Wahl et al. (34). The final stringency of washing was 0.5×SSC (1×SSC is 0.15 M NaCl plus 15 mM sodium citrate, pH 7.0) at 65°C. After hybridizations, filters were exposed to X-ray film at −80°C with intensifying screens.

RESULTS

Flow cytofluorometric analysis of lymphocyte stimulation. In the unstimulated resting lymphocyte, virtually all of the cells have the diploid DNA content (Fig. 1A), although DNA analysis alone cannot distinguish between G_0 and G_1 cells. There is essentially no change in DNA profile after 6 or 24 h of lectin activation (Fig. 1B and C), as no significant number of cells have started to enter S. After 3 days of stimulation with phytohemagglutinin, a substantial proportion of the cells either have doubled their DNA content and are considered to be in G_2 or M or have a DNA content between the diploid and tetraploid level and are, by definition, in S (Fig. 1D). After 7 days in culture (lectin-free medium from day 3), most of the cells have returned to a noncycling state and have the diploid DNA content (Fig. 1E). The DNA profile of exponentially growing T-lymphoblasts (CEM cells) is included for comparative purposes (Fig. 1F).

Further analysis by simultaneous DNA and RNA staining shows that the resting, G_0, lymphocyte is characterized by the diploid DNA content and low RNA content (Fig. 2A). There is essentially no change in this profile after 6 h of stimulation (Fig. 2B). However, after 24 h of lectin activation, a substantial proportion of the cells have increased their RNA content and are considered to be in G_1, although as yet there are virtually no cells with an increased DNA content (Fig. 2C). After 3 days of stimulation, there is a large increase in RNA content in cells still having the diploid DNA content representing cells in G_1. Cells with increasing DNA content are in S, whereas the cells with the tetraploid DNA content are in G_2 + M (Fig. 2D). Most of the cells entering S do so with a higher RNA content, but a small proportion appear to be cycling with a reduced RNA content (Fig. 2D). A higher RNA content is not an absolute prerequisite for a cell to enter S (6). There is also a small proportion of dead cells characterized by lower DNA and RNA content (Fig. 2D). By day 7, the cells have stopped cycling and have all returned to the diploid DNA level and lower RNA content (Fig. 2E), although the cells still have, on average, a higher RNA content than the original G_0 resting cells. The dead cells were removed from this particular analysis before flow cytometry. The noncycling day 7 cells could be restimulated with a second addition of phytohemagglutinin and tended to reach maximum transformation in 48 h this time rather than 72 h as before. The RNA-DNA analysis of exponentially growing T-lymphoblasts (CEM cells) is included for comparative purposes (Fig. 2F). Clearly, as the CEM cells double their DNA content as they move from G_1 through S to G_2 + M, there is, on average, an increase in RNA content although over a much narrower range than that seen with the stimulated lymphocytes (Fig. 2D and F).

Changes in polyadenylated RNA content upon lymphocyte activation. It is possible by flow cytometry to analyze the RNA and DNA content of each cell in a culture and thus obtain a much clearer picture of how these parameters vary than with a single average determination of the whole population. Nonetheless, the RNA content per cell is basically reflecting the change in rRNA and gives little information on how mRNA is changing. We have therefore determined the change in polyadenylated RNA content with lymphocyte stimulation, and the results are summarized in Table 1. The yield of total cytoplasmic RNA increased between 6 and 24 h, reaching a maximum at 72 h. The yield then decreased as the cells dropped out of transformation. The polyadenylated RNA began to form a bigger proportion of the total cytoplasmic RNA between 6 and 24 h. Between 24 and 72 h, the polyadenylated RNA remained a constant proportion of the total, and this proportion also began to decrease as the cells dropped out of transformation. Overall then, the yield of cytoplasmic polyadenylated RNA doubled

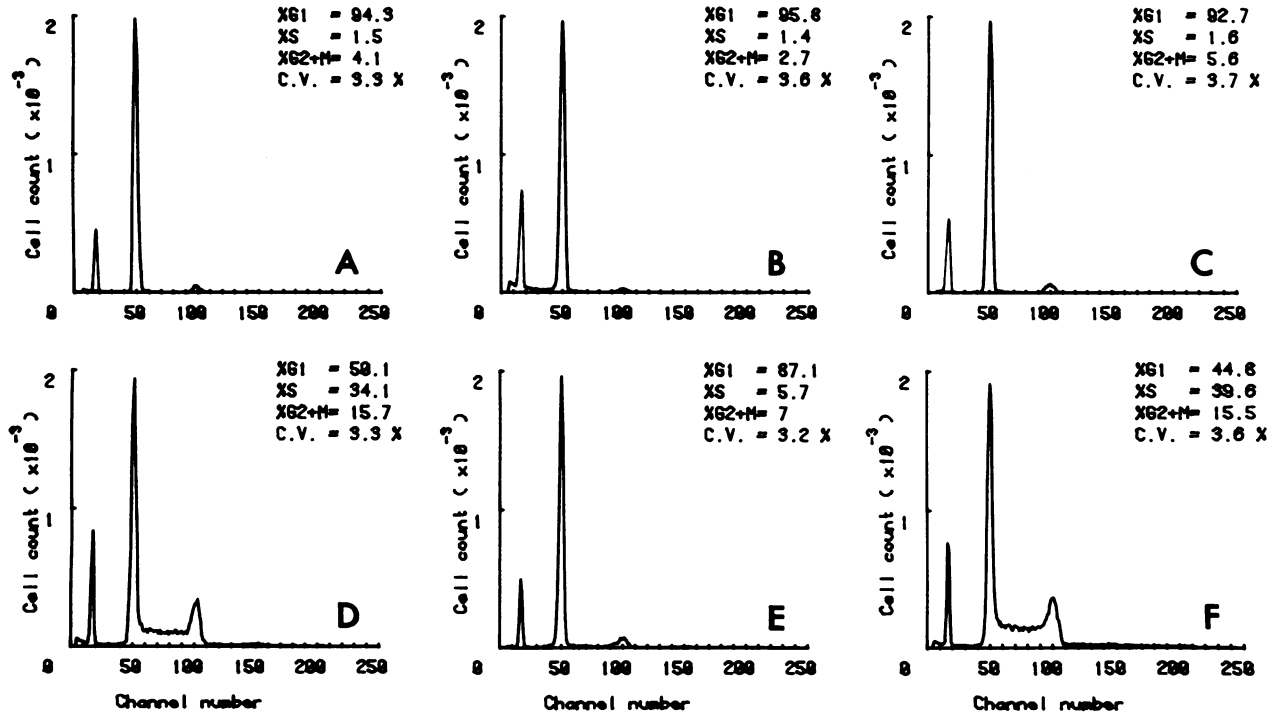

FIG. 1. Changes in the DNA distribution of lymphocytes with time of exposure to phytohemagglutinin. Cells at (A) 0 h; (B) 6 h; (C) 24 h; (D) 72 h and (E) 168 h; (F) DNA distribution of exponentially growing human leukemic T-lymphoblasts (CEM cells). Channel number corresponds to relative fluorescence intensity, which is proportional to DNA content. Ordinate scale represents number of cells. Peak between channels 10 and 20 is a chick erythrocyte internal DNA standard. Insets show computer derived histogram analyses. C.V., Coefficient of variation.

by 24 h, went up sixfold by 72 h, and although decreasing, was still at least twofold higher than the level in the resting cell on day 7 (Table 1). Exponentially growing T-lymphoblasts (CEM cells) have, on average, a lower total cytoplasmic RNA content than the fully stimulated (72 h) lymphocyte, but as the polyadenylated RNA forms a bigger proportion of the total, the actual yields of cytoplasmic polyadenylated RNA are very similar (Table 1).

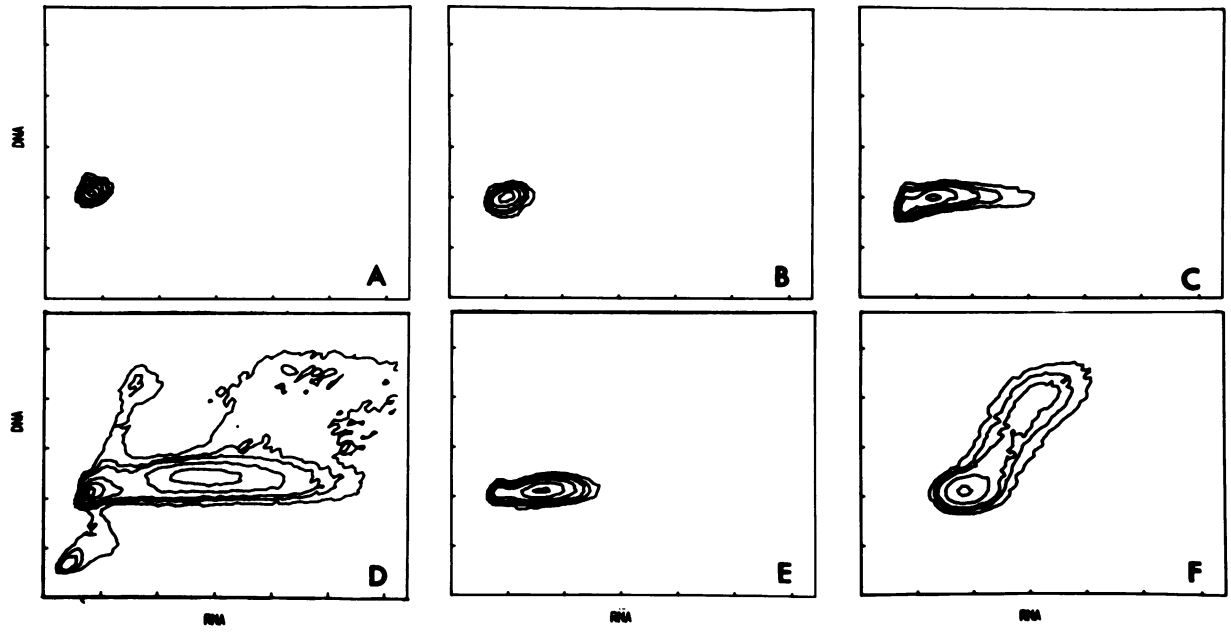

FIG. 2. Progression of lymphocytes through the cell cycle with time of exposure to phytohemagglutinin. Cells at (A) 0 h; (B) 6 h; (C) 24 h; (D) 72 h; and (E) 168 h. (F) RNA-DNA distribution of exponentially growing human leukemic T-lymphoblasts (CEM cells). Ordinate and abscissa scales are in arbitrary units which are proportional to DNA content and RNA content, respectively. Computer-drawn contour plots were generated by using six contour levels of 50, 100, 150, 300, 600, and 1,000 cells.

TABLE 1. Changes in polyadenylated RNA content with the lectin activation of lymphocytes[a]

Hours	Total cytoplasmic RNA (μg/10⁶ cells)	Poly(A)⁺ RNA (% of total)	Poly(A)⁺ RNA (ng/10⁶ cells)
0	1.0	0.9	9
6	1.0	1.0	10
24	1.5	1.7	25
72	3.5	1.7	60
168	2.0	1.2	24
CEM	2.5	2.6	65

[a] Total cytoplasmic RNA and the polyadenylated [poly(A)⁺] RNA fraction were isolated as described previously (22). Hours refers to time after lectin addition. CEM refers to exponentially growing human leukemic T-lymphoblasts (CCRF-CEM cells). Data are averages of seven determinations.

TABLE 2. Changes in actin and tubulin mRNA levels with the lectin activation of lymphocytes[a]

Hours	Relative mRNA levels			
	β-Actin	γ-Actin	α-Tubulin	β-Tubulin
0	1.0	1.0	1.0	1.0
6	2.4	2.6	2.5	2.5
24	2.8	5.0	10.6	9.5
72	3.0	5.5	11.6	11.1
168	1.8	3.8	3.9	5.7
CEM	1.3	3.3	16.3	14.1

[a] Data are from the actual counts hybridized in the type of experiment depicted in Fig. 3, and are averages of seven such determinations. The results are expressed as fold increases over the level in the resting cell (0 h), which has been set arbitrarily at 1 for each of the specific mRNAs and are related to a constant amount of polyadenylated RNA. Hours refers to time after lectin addition. CEM refers to exponentially growing human leukemic T-lymphoblasts (CCRF-CEM cells).

Changes in actin and tubulin mRNA levels upon lymphocyte activation. Changes in the levels of the polyadenylated mRNAs for actin and tubulin as the cells moved from the resting to the stimulated and back to a noncycling state were determined by a dot blot hybridization technique as illustrated in Fig. 3. The results are summarized in Table 2. The mRNA for β-actin was increased ca. 2.5-fold at 6 h and ca. 3-fold at 24 h; this was maintained up to 72 h. The mRNA for γ-actin also increased ca. 2.5-fold at 6 h but then began to rise more than β-actin mRNA, so that by 24 h it had doubled again, reaching an almost 6-fold rise by 72 h. So overall there is an approximately twofold change in the β-actin/γ-actin mRNA ratio on moving from a resting to a stimulated lymphocyte. The relative levels of β- and γ-actin mRNA in the resting cell were checked by dotting specific plasmid DNA onto the filters and hybridizing to end-labeled mRNA. This confirmed that β-actin mRNA was more abundant than γ-actin mRNA in the resting cell. Upon stimulation then, the relatively larger increase in γ-actin mRNA level has the effect of bringing the levels of β- and γ-actin mRNA much closer together. Exponentially growing T-lymphoblasts (CEM cells) have lower levels of β- and γ-actin mRNAs than the fully stimulated (72 h) lymphocytes but have a similar β-actin/γ-actin mRNA ratio. Although the resting lymphocyte has easily detectable levels of β- and γ-actin mRNAs, there is very little α- or β-tubulin mRNA. The level of both α- and β-tubulin mRNA has increased almost 2.5-fold by 6 h, but both have reached approximately 10 times their level in the resting cell by 24 h, and this level is maintained up to 72 h. Exponentially growing T-lymphoblasts (CEM cells) have a higher level of α- and β-tubulin mRNA than fully stimulated (72 h) lymphocytes. All of the above increases are expressed relative to a constant amount of polyadenylated RNA.

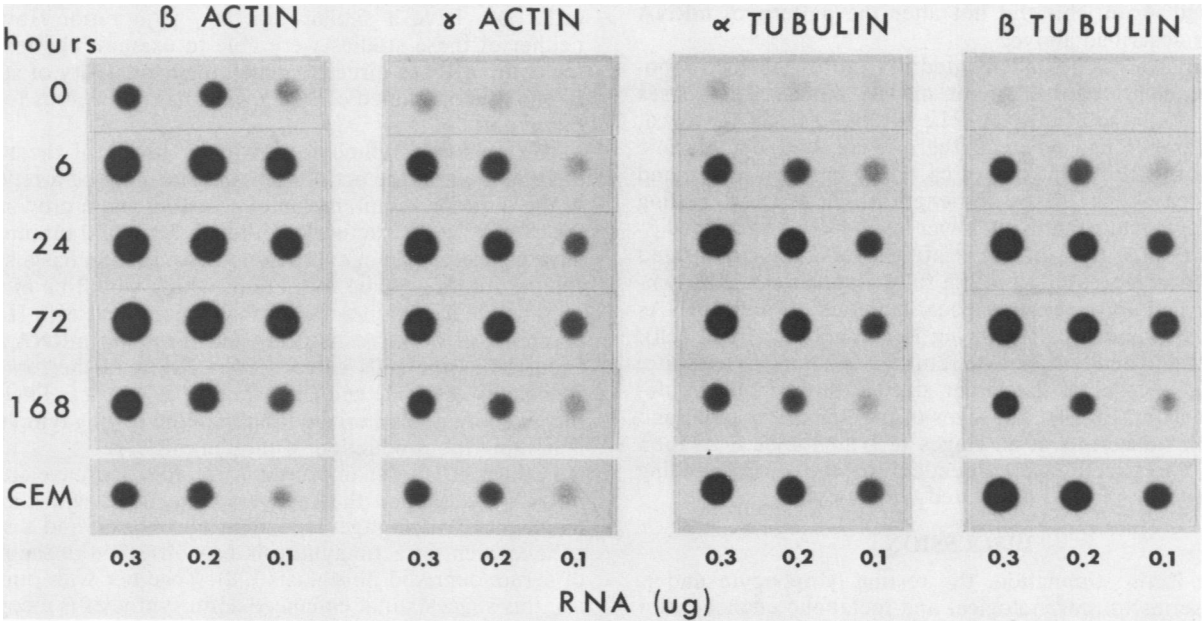

FIG. 3. Dot blot analysis of lymphocyte polyadenylated RNA. Increasing amounts of polyadenylated RNA isolated from the cells at 0, 6, 24, 72 and 168 h were dotted onto filters as shown. Samples of polyadenylated RNA from exponentially growing human leukemic T-lymphoblasts (CEM cells) are also included. Filters were hybridized with 2×10^7 cpm of nick-translated β-actin, γ-actin, α-tubulin, or β-tubulin probe as indicated. After exposure, the dots were cut out and the counts per minute hybridized were determined by liquid scintillation spectrometry.

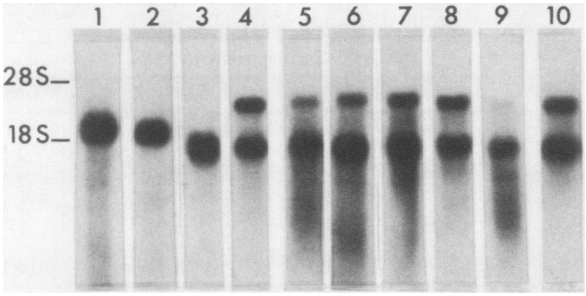

FIG. 4. Blot analysis of lymphocyte polyadenylated RNA. Lanes 1 to 4 contained 5 μg of polyadenylated RNA from stimulated lymphocytes (72 h). After gel electrophoresis and transfer to filters, these were hybridized with 2×10^7 cpm of nick-translated probes for β-actin (lane 1), γ-actin (lane 2), α-tubulin (lane 3), or β-tubulin (lane 4). Lanes 5 to 9 contained 5 μg of polyadenylated RNA from lymphocytes after 0, 6, 24, 72, and 168 h, respectively (times of lectin activation). Lane 10 contained 5 μg of polyadenylated RNA from exponentially growing human leukemic T-lymphoblasts (CEM cells). These were hybridized with 2×10^7 cpm of nick-translated β-tubulin probe as above. Exposures were for various lengths of time to show the transcript sizes.

However, the amount of polyadenylated RNA increases upon lectin stimulation so that the actual increase of each specific mRNA is much greater than this when expressed on a per-cell basis. As the cells moved back from the stimulated to a noncycling state, the level of each of the specific mRNAs started to decrease again but at day 7 were still higher than the corresponding levels in the original resting cells (Table 2). There was extremely little actin or tubulin mRNA sequence present in the nonpolyadenylated fraction of cytoplasmic RNA from resting lymphocytes. Although there was an increase in hybridizable material with all four specific probes at 24 h, which persisted until 72 h and then decreased again, this did not alter the pattern of mRNA changes described above.

Blot analysis of polyadenylated RNA after gel electrophoresis demonstrated that β-actin mRNA was ca. 2,000 bases long as was γ-actin mRNA. The α-tubulin mRNA was ca. 1,800 bases long, whereas there were two cytoplasmic mRNAs for β-tubulin, one of ca. 1,800 bases in length and one of ca. 2,800 bases in length (Fig. 4). The resting lymphocyte had a relatively lower level of the larger sized β-tubulin mRNA. By 24 h, the relative level of the larger sized species was increased, and the fully stimulated (72 h) lymphocyte had approximately equal amounts of both mRNAs as did the exponentially growing T-lymphoblast (CEM cell). As the stimulated lymphocyte returned to a noncycling state, the relative level of the larger sized β-tubulin mRNA decreased again (Fig. 4). The sizes of the β-actin, γ-actin, and α-tubulin transcripts at all stages of lymphocyte activation and in CEM cells were identical to the corresponding transcript sizes in the stimulated lymphocyte.

DISCUSSION

Upon lectin stimulation, the resting lymphocyte undergoes a series of morphological and metabolic changes as it enters the division cycle. This blast transformation, however, does not lead to a continuously dividing cell; the proliferative response rapidly dies away, and the cells return to a noncycling state. As Ling and Holt have discussed (19), the cells probably return to a prestimulated state, as their response to a second mitogenic stimulus is quicker and the noncycling cells described in this report clearly have an RNA profile distinct from that of a G_0 resting cell. It is, therefore, interesting to examine changes in gene expression as the lymphocyte moves into and out of the cell cycle. There is no change in RNA synthesis until ca. 8 h after lectin addition and DNA starts to increase at about 24 h, with the cells being maximally transformed at 72 h. By day 7, the cells have stopped cycling. This, then, points to critical areas of the proliferative response which should be investigated. Any changes in mRNA content that occur at less than 8 h can be defined as early changes preceding major transcriptional increases. Changes in mRNA that occur between 8 and 24 h could be due to new transcription but are occurring as the cells enter G_1 and precede DNA replication or S phase. Beyond 24 h, the cells move into S and G_2 + M, with virtually all of the cells having a blast-like appearance at 72 h. By day 7, all of the cells have the appearance of a small lymphocyte but have properties which distinguish them from a G_0 lymphocyte.

Although all four cytoskeletal mRNAs examined in this report are elevated 2.5-fold as early as 6 h, this accounts for most of the increase seen with β-actin in the stimulated cell, whereas γ-actin increases a further 2-fold as the cells enter G_1. Similarly, both α- and β-tubulin increase a further fourfold as the cells enter G_1. There is very little further increase in the level of any of these mRNAs as the cells move out of G_1 and into S and G_2 + M. There is, then, an altered β-actin/γ-actin mRNA ratio in lymphocytes which have entered the cell cycle compared with the resting cell. It is interesting, then, that an exponentially growing leukemic T-lymphoblast (CEM cell) has a similar β-actin/γ-actin mRNA ratio to the growing T-lymphocyte. Leavitt et al. (17) have shown that T-lymphocytes stimulated with T-cell growth factor synthesize more β-actin than γ-actin, whereas leukemic T-cells (Molt-4) synthesize equal amounts of β- and γ-actin. In contrast to this, Stark et al. (30) suggest that normal T-lymphocytes and chronic lymphocytic leukemic T-cells both have a similar β-actin/γ-actin ratio. However, neither of these studies were able to examine the levels of the actin mRNAs directly, which the availability of specific β- and γ-actin cloned cDNA probes has allowed us to do in this report.

The resting lymphocyte has lower levels of the tubulin mRNAs than of the actin mRNAs. This may be a reflection of the differing requirements of a resting and a growing cell for cytoskeletal structures. Although actin and tubulin both have a general role in cytoarchitecture, tubulin has a special role in mitotic spindle formation, which would be of major importance to a cycling rather than a quiescent cell. However, most of the increase in α- and β-tubulin mRNA levels occurred as the cells entered G_1. In view of the reports of Ben-Ze'ev et al. (1) and Cleveland et al. (4), it is likely that the level of unpolymerized tubulin in the resting lymphocyte is limiting the production of tubulin mRNA.

Farmer et al. (8) observed a twofold increase in actin mRNA levels upon the recovery associated with the reattachment of anchorage-dependent fibroblasts, and a similar enhancement of actin synthesis was observed in the rescue of serum-deprived fibroblasts (28). Together with our studies, this suggests that enhanced actin synthesis is a common feature in the progression from quiescence to growth. To this we can add the different response shown by β- and γ-actin mRNA upon stimulating cells to enter the cell cycle, and this may be related to possible functional differences of the actin isotypes. The increased levels of actin and tubulin mRNAs

upon lymphocyte stimulation are indicative of the relationship between growth and cytoskeletal protein gene expression. The resting lymphocyte is a fully differentiated cell which can be stimulated to divide in response to a foreign molecule. It is interesting, then, that the large changes in tubulin mRNA levels and the smaller changes in actin mRNA levels which are seen on lymphocyte activation are of similar magnitude to the decreases found in actin and tubulin mRNA levels with the morphological differentiation of adipocytes (29) and with rat brain development (2). As the stimulated lymphocyte returns to a noncycling state, this is also coupled with a reduction in actin and tubulin mRNA levels. The changes in mRNA levels which are described in this report may be due to alterations in transcription, processing, or stability of specific mRNAs, and the relative importance of these at the various times of activation is being investigated.

The presence of two mRNAs for β-tubulin in lymphocyte cytoplasm is interesting, and a similar situation has been described in CHO cells (4) and in HeLa cells (12). Indeed, it has been reported that there are four functional β-tubulin genes in chickens, at least two of which are expressed in any given chicken cell line examined (20). As there are numerous human β-tubulin pseudogenes, the number of functionally expressed genes has been a subject of investigation (11, 12). Gwo-Shu Lee et al. (11) have demonstrated that one expressed human β-tubulin gene could give rise to both a 1.8 and a 2.6-kilobase mRNA species as a consequence of alternative polyadenylation sites. However, the larger sized β-tubulin mRNA described by Hall et al. (12) was shown to be a mixture of two comigrating species transcribed from two different genes. So in addition to the generation of multiple mRNAs by readthrough of a site where polyadenylation can occur, there exists in human cells two distinct β-tubulin isotypes (12). Furthermore, it has been shown that a new β-tubulin mRNA is expressed with rat brain development (2), and brain and erythrocytes from chickens have been shown to contain distinct β-tubulin variants (24). We are in the process of obtaining suitable probes to determine to what extent the 2.8-kilobase β-tubulin mRNA is due to alternate pathways of processing the transcript of one gene or to the activity of a second gene (11, 12) and to what extent this may change upon lectin activation. Nonetheless, it is still interesting that the level of the larger sized β-tubulin mRNA is relatively enhanced upon mitogenic stimulation and that the stimulated lymphocyte and exponentially growing leukemic T-lymphoblasts have approximately equal levels of both mRNAs. The fact that the relative elevation in the level of the larger sized β-tubulin mRNA occurs late in the course of blast transformation may point to a relationship between this and mitosis. Gwo-Shu Lee et al. (11) have shown that HeLa, myeloma, hepatoma, and neuroblastoma cell lines all have approximately equal proportions of both β-tubulin mRNAs. As the level of the larger sized β-tubulin mRNA in lymphocytes is relatively enhanced upon mitogenic stimulation, it will be interesting to see whether this is related to a specialized requirement for microtubule assembly and function in the growing lymphocyte.

ACKNOWLEDGMENTS

We thank Peter Gunning, Phyllis Ponte, and Larry Kedes (Stanford University) for providing us with the cloned cDNA probes, Gerry Wake (Sydney University) for the use of his Cl containment facilities, Ian Taylor (Ludwig Institute for Cancer Research) for performing the flow cytofluorometric analyses, and Dorit Sauer and Annette Kalaizis for expert technical assistance.

These studies were supported by grants from the National Health and Medical Research Council of Australia and The University of Sydney Cancer Research Fund.

LITERATURE CITED

1. **Ben-Ze'ev, A., S. R. Farmer, and S. Penman.** 1979. Mechanisms of regulating tubulin synthesis in cultured mammalian cells. Cell **17**:319–325.
2. **Bond, J. F., and S. R. Farmer.** 1983. Regulation of tubulin and actin mRNA production in rat brain: expression of a new β-tubulin mRNA with development. Mol. Cell. Biol. **3**:1333–1342.
3. **Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner.** 1980. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. Cell **20**:95–105.
4. **Cleveland, D. W., M. A. Lopata, P. Sherline, and M. W. Kirschner.** 1981. Unpolymerized tubulin modulates the level of tubulin mRNAs. Cell **25**:537–546.
5. **Cowan, N. J., P. R. Dobner, E. V. Fuchs, and D. W. Cleveland.** 1983. Expression of human α-tubulin genes: interspecies conservation of 3′ untranslated regions. Mol. Cell. Biol. **3**:1738–1745.
6. **Darzynkiewicz, Z., F. Traganos, T. Sharpless, and M. R. Melamed.** 1976. Lymphocyte stimulation: a rapid multiparameter analysis. Proc. Natl. Acad. Sci. U.S.A. **73**:2881–2884.
7. **Farmer, S. R., A. Ben-Ze'ev, B.-J. Benecke, and S. Penman.** 1978. Altered translatability of messenger RNA from suspended anchorage-dependent fibroblasts: reversal upon cell attachment to a surface. Cell **15**:627–637.
8. **Farmer, S. R., K. M. Wan, A. Ben-Ze'ev, and S. Penman.** 1983. Regulation of actin mRNA levels and translation responds to changes in cell configuration. Mol. Cell. Biol. **3**:182–189.
9. **Goldberg, D. A.** 1980. Isolation and partial characterization of the Drosophila alcohol dehydrogenase gene. Proc. Natl. Acad. Sci. U.S.A. **77**:5794–5798.
10. **Gunning, P., P. Ponte, H. Okayama, J. Engel, H. Blau, and L. Kedes.** 1983. Isolation and characterization of full-length cDNA clones for human α-, β-, and γ-actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. Mol. Cell. Biol. **3**:787–795.
11. **Gwo-Shu Lee, M., S. A. Lewis, C. D. Wilde, and N. J. Cowan.** 1983. Evolutionary history of a multigene family: an expressed human β-tubulin gene and three processed pseudogenes. Cell **33**:477–487.
12. **Hall, J. L., L. Dudley, P. R. Dobner, S. A. Lewis, and N. J. Cowan.** 1983. Identification of two human β-tubulin isotypes. Mol. Cell. Biol. **3**:854–862.
13. **Hurrell, J. G. R., P. H. Black, E. Haber, and V. R. Zurawski, Jr.** 1980. A rapid and efficient procedure for the removal of nonviable cells from suspension culture of lymphoid cell lines. Aust. J. Exp. Biol. Med. Sci. **58**:373–376.
14. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9**:2989–2998.
15. **Kecskemethy, N., and K. P. Schäfer.** 1982. Lectin-induced changes among polyadenylated and non-polyadenylated mRNA in lymphocytes: mRNAs for actin, tubulin and calmodulin respond differently. Eur. J. Biochem. **126**:573–582.
16. **Kefford, R. F., R. M. Fox, E. McCairns, D. Fahey, G. E. O. Muscat, and P. B. Rowe.** 1983. Terminal incorporation of 2′-deoxyadenosine into polyadenylate segments of polyadenylated RNA in G_1-phase-arrested human T-lymphoblasts. Cancer Res. **43**:2252–2257.
17. **Leavitt, J., A. Leavitt, and A. M. Attallah.** 1980. Dissimilar modes of expression of β- and γ-actin in normal and leukemic human T lymphocytes. J. Biol. Chem. **255**:4984–4987.
18. **Lehrach, H., D. Diamond, J. M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16**:4743–4751.
19. **Ling, N. R., and P. J. L. Holt.** 1967. The activation and reactivation of peripheral lymphocytes in culture. J. Cell Sci. **2**:57–70.

20. **Lopata, M. A., J. C. Havercroft, L. T. Chow, and D. W. Cleveland.** 1983. Four unique genes required for β tubulin expression in vertebrates. Cell **32**:713–724.
21. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage λ. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
22. **McCairns, E., D. Fahey, G. E. O. Muscat, and P. B. Rowe.** 1983. The effects of lectin transformation on cytoplasmic polyadenylated RNA from human lymphocytes. Mol. Cell. Biochem. **56**:165–175.
23. **McCairns, E., D. Fahey, D. Sauer, and P. B. Rowe.** 1983. De novo purine synthesis in human lymphocytes: partial co-purification of the enzymes and some properties of the pathway. J. Biol. Chem. **258**:1851–1856.
24. **Murphy, D. B., and K. T. Wallis.** 1983. Brain and erythrocyte microtubules from chicken contain different β-tubulin polypeptides. J. Biol. Chem. **258**:7870–7875.
25. **Okayama, H., and P. Berg.** 1983. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. Mol. Cell. Biol. **3**:280–289.
26. **Ponte, P., P. Gunning, H. Blau, and L. Kedes.** 1983. Human actin genes are single copy for α-skeletal and α-cardiac actin but multicopy for β- and γ- cytoskeletal genes : 3′ untranslated regions are isotype specific but are conserved in evolution. Mol. Cell. Biol. **3**:1783–1791.
27. **Riddle, V. G. H., R. Dubrow, and A. B. Pardee.** 1979. Changes in the synthesis of actin and other cell proteins after stimulation of serum-arrested cells. Proc. Natl. Acad. Sci. U.S.A. **76**:1298–1302.
28. **Riddle, V. G. H., and A. B. Pardee.** 1980. Quiescent cells but not cycling cells exhibit enhanced actin synthesis before they synthesize DNA. J. Cell. Physiol. **103**:11–15.
29. **Spiegelman, B. M., and S. R. Farmer.** 1982. Decreases in tubulin and actin gene expression prior to morphological differentiation of 3T3 adipocytes. Cell **29**:53–60.
30. **Stark, R., L. F. Liebes, D. Nevrla, M. Conklyn, and R. Silber.** 1982. Decreased actin content of lymphocytes from patients with chronic lymphocytic leukemia. Blood **59**:536–541.
31. **Taylor, I. W.** 1980. A rapid single step staining technique for DNA analysis by flow microfluorimetry. J. Histochem. Cytochem. **28**:1021–1024.
32. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
33. **Traganos, F., Z. Darzynkiewicz, T. Sharpless, and M. R. Melamed.** 1977. Simultaneous staining of ribonucleic and deoxyribonucleic acids in unfixed cells using acridine orange in a flow cytofluorometric system. J. Histochem. Cytochem. **25**:46–56.
34. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.