MOLECULAR AND CELLULAR BIOLOGY, Nov. 1990, p. 5814–5821
0270-7306/90/115814-08$02.00/0

Vol. 10, No. 11

Deprivation of a Single Amino Acid Induces Protein Synthesis-Dependent Increases in c-*jun*, c-*myc*, and Ornithine Decarboxylase mRNAs in Chinese Hamster Ovary Cells

PIRKKO POHJANPELTO[1]* AND ERKKI HÖLTTÄ[2]

Department of Virology[1] and Department of Pathology,[2] University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, Finland

Received 23 May 1990/Accepted 24 July 1990

Genes of higher eucaryotic cells are considered to show only a limited response to nutritional stress. Here we show, however, that omission of a single essential amino acid from the medium caused a marked rise in the mRNA levels of c-*myc*, c-*jun*, *junB* and c-*fos* oncogenes and ornithine decarboxylase (ODC) in CHO cells. There was no general accumulation of mRNAs in amino acid-starved cells, since the γ-actin, β-tubulin, protein kinase C, RNA polymerase II, and glyceraldehyde-3-phosphate dehydrogenase mRNAs and the total poly(A)$^+$ mRNA were not increased. The levels of c-*myc*, ODC, and c-*jun* mRNAs were elevated more by amino acid starvation than by inhibition of protein synthesis with cycloheximide, which is known to increase the levels of these mRNAs. Importantly, however, cycloheximide present during amino acid starvation reduced the rise in the levels of the mRNAs down to the level obtained with cycloheximide alone. This implies that protein synthesis is required for the accumulation of c-*myc*, ODC, and c-*jun* mRNAs in amino acid-deprived cells. The *junB* and c-*fos* mRNAs, instead, were increased to the same extent or less by amino acid starvation than by cycloheximide treatment. The accumulation of the c-*myc* mRNA in amino acid-starved cells was due to both stabilization of the mRNA and increase of its transcription. The rise in the c-*jun* mRNA level seemed to be caused merely by stabilization of the mRNA. Further, despite the inhibition of general protein synthesis, amino acid starvation led to an increase in the synthesis of c-*myc* polypeptide. The results suggest that mammalian cells have a specific mechanism for registering shortages of amino acids in order to make adjustments compatible with cellular growth.

Signals controlling gene expression in higher eucaryotic cells are known to differ in many respects from those operating in single-celled organisms. While procaryotes and single-celled eucaryotes can accommodate to conditions such as amino acid deprivation or variation in sugar supply by changing gene control, it has been generally assumed that animal cells cannot do so (11).

We have succeeded in isolating from Chinese hamster ovary (CHO) cells a strain, A2, which grows in serum-free medium and is therefore well suited for studies of signals effective in the control of cell proliferation (40). This cell strain overproduces ornithine decarboxylase (ODC), which is the key enzyme in polyamine biosynthesis. In an attempt to make the A2 cells quiescent, we deprived them of proline, which is an essential amino acid for these cells. As expected, omission of proline from the medium caused retardation of the growth of the A2 cells, but surprisingly there was simultaneously an increase in the ODC mRNA. This result prompted us to determine how the c-*myc* proto-oncogene, which, like ODC, is induced by various growth stimuli and belongs to the so-called early genes (27; for a review, see reference 9), would respond to amino acid deprivation. These studies showed that c-*myc* mRNA was elevated in amino acid-starved cells to a much greater degree than ODC mRNA. Since suppression of protein synthesis by various drugs can increase the levels of the mRNAs of many growth-associated genes (6, 27), and since amino acid deprivation also affects protein synthesis, one could assume that the mechanisms for elevation of the mRNA levels would be the same in both cases. We found, however, that the two phenomena are distinct. The response to amino acid starvation was also studied with the parental CHO cells and with cells from other animal species to show the generality of the phenomenon.

In light of these data, it was also of interest to study the behavior of the so-called immediate genes, the c-*jun*, *junB*, and c-*fos* proto-oncogenes, which are induced more rapidly after stimulation of cell proliferation than the early ones (42). We found that c-*jun*, at least, responded to amino acid starvation similarly to c-*myc* and ODC by increasing its expression in a protein synthesis-dependent manner.

The protein synthesis-dependent increase in the expression of certain growth-associated genes in mammalian cells during amino acid starvation is a novel finding. It shows that not only single-celled organisms but also mammalian cells have a specific system for registering lack of amino acids and are able to accommodate to amino acid deprivation by changing gene expression.

MATERIALS AND METHODS

Cell culture. CHO-K1 cells auxotrophic for proline were obtained from the American Type Culture Collection. They were cultured on petri dishes (diameter, 10 cm) in medium containing F12 nutrient mixture and minimal essential medium (MEM) in a 1:1 ratio and supplemented with 5% fetal calf serum. From this cell strain a mutant, A7, growing in serum-free medium, was isolated (41), and from these cells in turn another mutant, A2, with an amplified ODC gene overproducing ODC, was obtained (40). The A2 cells were cultured on collagen-coated petri dishes in F12-MEM without putrescine, supplemented with 0.1% bovine serum albu-

* Corresponding author.

min. The cells were regularly checked for mycoplasmas with negative results.

Amino acid starvation. In the exponential growth phase, the cultures were washed twice with Hanks balanced salt solution. The cells were then incubated for the indicated times with the medium used for culturing the cells (F12-MEM plus 0.1% bovine serum albumin without putrescine for A2 cells and F12-MEM plus 5% dialyzed fetal calf serum for the wild-type CHO cells) and lacking the amino acid under study.

Northern blot analysis of mRNAs. Polyadenylated RNA was isolated from 3×10^7 to 10×10^7 exponentially growing cells as described earlier (39). Northern (RNA) blot hybridization analysis was performed by the method of Thomas (45) as previously described (39). A linearized human c-*myc* DNA fragment carrying the second *myc* exon (Amersham International), mouse c-*myc* (pSV-c-*myc*-1) (29), mouse ODC cDNA (the pODC16 plasmid) (24), mouse *junB* cDNA (clone 465) (42), human c-*jun* cDNA (hcJ-1) (2), a human c-*fos* gene fragment (Amersham International), human β-tubulin 21-beta 3′ UT (31), human protein kinase C α-polypeptide cDNA (the prPKC-α7 plasmid) (38), the human γ-actin gene (13), rat glyceraldehyde-3-phosphate dehydrogenase cDNA (the pRGAPDH-13 plasmid) (15), and the human RNA polymerase II large subunit (pHRp 5.5) (8), were nick translated and used as probes in the hybridization. For rehybridization the previous probe was removed from the filter by boiling in distilled water for 3 min.

Autoradiographic signals were quantitated with an EDC densitometer coupled with a computer (Helena Laboratories, Beaumont, Tex.). Each experiment was repeated at least three times with similar results.

Determination of protein synthesis. Cells were cultured in the appropriate media and labeled with [^{35}S]methionine (>1,000 Ci/mmol; 5 μCi/ml) for 30 min. After the cells were washed twice with cold MEM, the proteins were precipitated with 5% trichloroacetic acid at 4°C and washed twice with cold 5% trichloroacetic acid and once with ethanol. The precipitate was then dissolved in 0.3 N NaOH, and the radioactivity was measured in a liquid scintillator.

[^{35}S]methionine labeling and immunoprecipitation of c-*myc* and ODC polypeptides. The determination of the rate of the synthesis of c-*myc* and ODC polypeptides was carried out as described earlier (20). After 30 min of labeling with [^{35}S]methionine, the cells were homogenized and the cell lysates were preadsorbed with normal rabbit serum and then precipitated with monospecific ODC immune serum (23), anti-pan-*myc* antiserum (35), or polyvalent c-*myc* immune serum prepared against β-galactosidase–human c-*myc* (second exon) hybrid protein (K. Saksela, unpublished data). The immunoprecipitates were then subjected to sodium dodecyl sulfate-polyacrylamide gel (8% polyacrylamide) electrophoresis according to standard procedures and visualized by autoradiography.

Nuclear run-on transcription assay. The procedure described by Greenberg et al. (17) was followed. For isolation of nuclei, the cells were detached with cold trypsin, centrifuged, and resuspended in lysis buffer containing 0.5% (vol/vol) Nonidet P-40. The nuclei were washed once with lysis buffer, resuspended in glycerol buffer, and washed once with glycerol buffer. The nuclei were labeled for 15 min in a reaction mixture containing 100 to 200 μCi of [α-^{32}P]UTP (3,000 Ci/mmol) and 0.5 mmol each of ATP, CTP, and GTP. The reaction kinetics were linear for 30 min. The transcripts were isolated as described previously (18). The linearized plasmid DNAs were immobilized on nylon filters (Hybond N; Amersham) and prehybridized as described above for Northern blot analysis. Hybridization with the labeled RNAs was carried out for 48 h at 40°C. The filters were washed several times in 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 60°C and twice in 0.1× SSC at 37°C and finally were treated with RNase. The filters were then dried and autoradiographed. The following plasmid DNAs were used: pSV-c-*myc*, pODC16, pRGAPDH-13, human γ-actin, pBR322, and pHSR-1 (1).

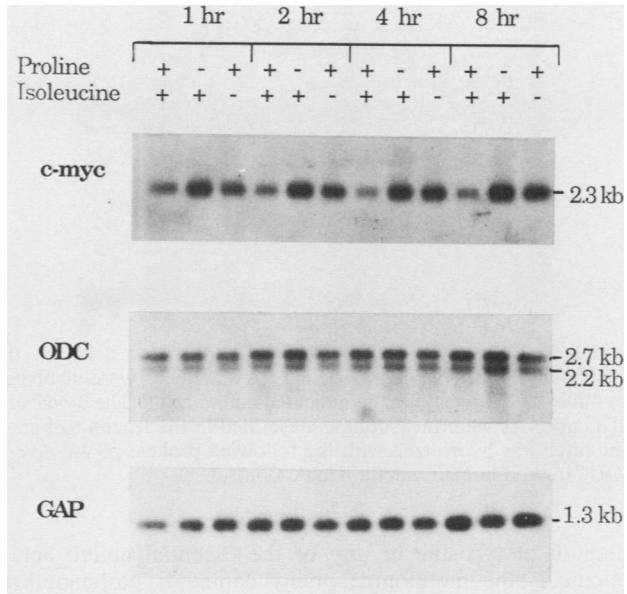

FIG. 1. Effect of length of amino acid starvation on mRNA levels of c-*myc* and ODC. Nearly confluent A2 cell cultures were incubated for different lengths of time either with complete F12-MEM or with the same medium lacking proline or isoleucine. The poly(A)$^+$ mRNA was isolated and submitted to Northern blotting as described in Materials and Methods with human c-*myc* DNA and plasmids pODC16 and pRGAPDH-13 as probes. Each sample contained 10 μg of RNA.

RESULTS

Effect of duration of amino acid starvation on accumulation of c-*myc* and ODC mRNAs. Proline starvation of the A2 cells for 2 h caused about a threefold increase (based on densitometric scanning) in the c-*myc* mRNA as compared with the nonstarved control cultures. After 4 h the increase was about fourfold, and after 8 h the increase was about sevenfold (Fig. 1). Thereafter the difference started to wane; at 12 h a small rise in the c-*myc* mRNA was still observed, but at 24 h the difference had disappeared (results not shown). Isoleucine starvation, which is commonly used for synchronization of cells, was a little less effective than proline starvation in promoting the c-*myc* mRNA accumulation, but the time course was the same. It is notable that the total poly(A)$^+$ RNA content in the different samples, calculated by the absorbance measurements, did not show any significant variation.

Proline starvation caused about a threefold increase in the ODC mRNA in A2 cells after 8 h (Fig. 1). Although the magnitude of the increase in the ODC mRNA was small and varied to some extent in different experiments, it was highly reproducible.

In addition to proline and isoleucine, omission from the

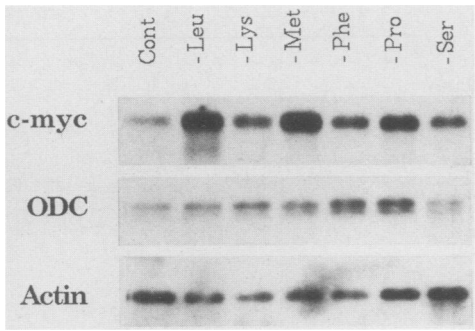

FIG. 2. Effect of deprivation of different amino acids on the accumulation of c-*myc* and ODC mRNAs. A2 cells were incubated for 4 h in medium lacking the indicated amino acids. The c-*myc* and ODC mRNAs were measured as described in the legend to Fig. 1. The filter was hybridized with the following probes: pSV-c-*myc*-1, pODC16, and human γ-actin. Cont, Control.

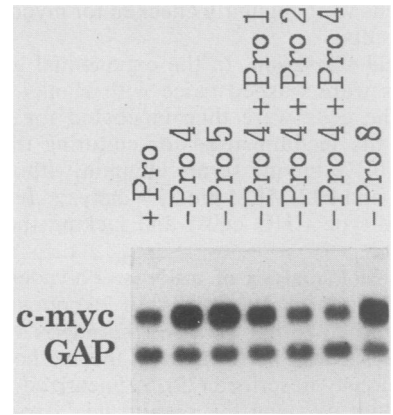

FIG. 3. Reversal of the amino acid starvation-induced rise in c-*myc* mRNA. A2 cells were starved of proline for 4 h, and then proline was added for different times. The control was incubated with complete medium. The c-*myc* mRNA was measured as described in the legend to Fig. 1. The probes were pSV-c-*myc*-1 and pRGAPDH-13. The numbers next to the amino acid designations indicate the times of incubation in hours.

medium of tyrosine or any of the essential amino acids, leucine, lysine, methionine, phenylalanine, tryptophan, threonine, or valine, increased the amount of c-*myc* mRNA in A2 cells. Deprivation of methionine and leucine for 4 h seemed to enhance the amount of c-*myc* mRNA more effectively than that of proline, causing 8- and 10-fold increases, respectively, in the c-*myc* mRNA (Fig. 2). Omission from the medium of the nonessential amino acids alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, and serine had only a very weak effect or no effect at all on the accumulation of c-*myc* mRNA. Lack of essential amino acids also increased the level of ODC mRNA. There were, however, differences in the induction of the ODC and c-*myc* mRNAs by deprivation of individual amino acids. Hybridization of the filter in succession with c-*myc* and ODC probes revealed that proline and phenylalanine deprivation of cells increased the levels of both mRNAs markedly while omission of leucine, lysine, or methionine from the medium affected the c-*myc* more than the ODC mRNA (Fig. 2). Omission of two or more essential amino acids simultaneously from the medium did not have a synergistic effect (results not shown).

The amino acid starvation-induced rise in the c-*myc* mRNA could be reversed by adding the missing amino acid to the cultures. When cell cultures were starved of proline for 4 h and proline was then added, there was a rapid decrease in the amount of c-*myc* mRNA. Within 2 h the c-*myc* mRNA returned to the control level (Fig. 3).

The accumulation of c-*myc* and ODC mRNAs following amino acid starvation was not limited to the mutant A2 cells but could also be demonstrated in the parental wild-type CHO cells, which require serum for growth. Deprivation of methionine for 6 h raised the level of c-*myc* mRNA in the wild-type CHO cells in both the absence and the presence of serum growth factors (Fig. 4). However, the increase was most pronounced under optimal growth conditions in medium containing 5% fetal calf serum. The ODC mRNA level was elevated in amino acid-starved wild-type CHO cells in a manner similar to c-*myc* mRNA with respect to serum concentrations (data not shown).

It is notable that in addition to CHO cells, human primary skin fibroblasts and Vero cells (originating from monkey kidney) responded to methionine starvation by increasing their c-*myc* mRNA levels (results not shown).

Deprivation of amino acids did not cause a general increase in the mRNA levels. Glyceraldehyde-3-phosphate dehydrogenase (GAP) and γ-actin mRNAs were not increased upon amino acid starvation. This is shown by Fig. 1, 2, and 4, in which the filters used for hybridization with the c-*myc*, ODC, c-*jun*, and *junB* probes were rehybridized with GAP or γ-actin probes (see also Fig. 10 and 11). The levels of protein kinase C, β-tubulin, and RNA polymerase II mRNAs also remained unchanged when the wild-type CHO cells were starved of methionine for 6 h (results not shown).

Protein synthesis. In order to assess the importance of protein synthesis for the amino acid starvation-induced increase in the levels of c-*myc* and ODC mRNAs, the rates of protein synthesis in cells starved of various amino acids and in cells treated with cycloheximide were determined. The cultures were starved of any one of the amino acids, and the incorporation of [^{35}S]methionine into the acid-precipitable fraction was measured. Cells deprived of any of the essential amino acids or tyrosine for 4 h showed a 62 to 78% reduction in protein synthesis, while cells starved of any of the remaining nonessential amino acids showed a 7 to 46% reduction in protein synthesis. It is notable that cycloheximide inhibited protein synthesis much more effectively than amino acid starvation, causing 98% inhibition in 1 h.

Effect of cycloheximide on the levels of c-*myc* and ODC

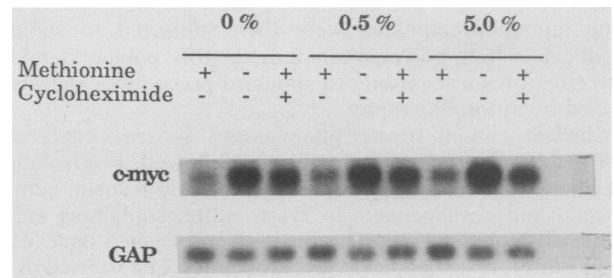

FIG. 4. Effect of amino acid starvation and cycloheximide on the accumulation of c-*myc* mRNA in wild-type CHO cells. The cells were starved of methionine or treated with cycloheximide (10 μg/ml) for 6 h in the presence of 0, 0.5, or 5.0% dialyzed fetal calf serum. The c-*myc* mRNA was measured as described in the legend to Fig. 1. The probes were pSV-c-*myc*-1 and pRGAPDH-13.

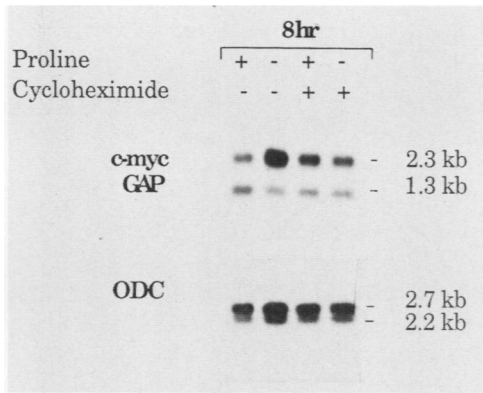

FIG. 5. Effect of cycloheximide on the amino acid starvation-induced rise in c-*myc* and ODC mRNAs. A2 cells were incubated for 4 or 8 h, either with medium lacking proline or isoleucine or with complete medium in the presence or absence of cycloheximide (10 μg/ml). The c-*myc* and ODC mRNAs were measured as described in the legend to Fig. 1. The probes were human c-*myc* DNA, pODC16, and pRGAPDH-13.

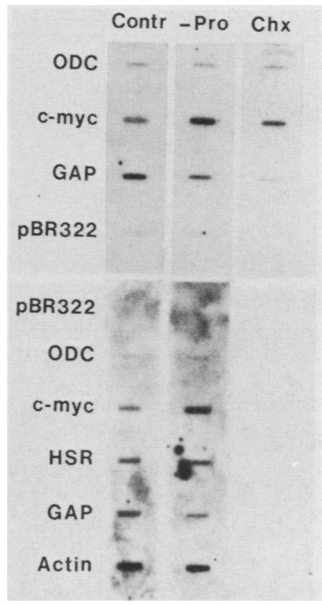

FIG. 6. Effect of amino acid starvation and cycloheximide on transcription of the c-*myc* and ODC genes. A2 cells were incubated for 2 h either with complete medium in the presence or absence of cycloheximide (Chx; 10 μg/ml) or in medium lacking proline. Nuclei were isolated, and transcription was allowed to proceed in the presence of [^{32}P]UTP (150 μCi/ml) for 15 min. The RNAs were isolated and hybridized with specific gene sequences immobilized to a nylon filter as described in Materials and Methods. The upper panel and the lower panel represent two independent experiments. Contr, Control.

mRNAs. In agreement with earlier investigations, cycloheximide alone was found to increase the amount of c-*myc* mRNA in A2 cells. However, the rise was less than that found in cultures starved of specified amino acids (Fig. 5) in spite of the fact that cycloheximide inhibited protein synthesis much more effectively than amino acid starvation did. Because of the possibility that a moderate inhibition of protein synthesis would promote the accumulation of mRNAs more effectively than the nearly complete blocking attained by 10 μg of cycloheximide per ml, different concentrations of the inhibitor were tested. It appeared that the lower concentrations of cycloheximide, which caused about the same degree of inhibition of protein synthesis as proline starvation caused, did not increase the accumulation of c-*myc* mRNA (results not shown). Not only was cycloheximide less effective than amino acid starvation in increasing the c-*myc* mRNA, but more importantly, cycloheximide present during amino acid starvation prevented a rise in the c-*myc* and ODC mRNAs above the level obtained with cycloheximide alone (Fig. 5; see also Fig. 11).

Transcription of c-*myc* and ODC genes. To study the role of transcription in the enhancement of the c-*myc* and ODC mRNAs, run-on transcription assays were carried out. Proline starvation and cycloheximide treatment increased the rate of transcription of the mRNA hybridizing with the coding second and third exons of the mouse c-*myc* gene (Fig. 6) or the human c-*myc* (second exon) DNA (data not shown). However, the mRNA hybridizing with the human c-*myc* DNA (pHSR1) containing all three exons showed very little or no increase upon amino acid starvation. The rate of transcription of ODC mRNA did not seem to change after amino acid starvation or cycloheximide treatment. However, it is notable that in the nuclear run-on assays the GAP mRNA and actin mRNA gave stronger signals in the control than in the proline-starved cultures despite the fact that in Northern blotting the amounts of these mRNAs were the same. This cannot be explained by a relative reduction in the synthesis of GAP and actin mRNAs in relation to the total RNAs, since the overall transcriptional efficiency, as measured by incorporation of [^{32}P]UTP into total RNA, did not increase in the amino acid-starved or cycloheximide-treated cells. In this connection it should be borne in mind that nuclear run-on assays may not always faithfully reflect the conditions in intact cells. Thus, one could speculate that the rate of ODC transcription would also show a slight increase in amino acid-starved cells if expressed in relation to GAP.

When the nuclear run-on transcription assay was carried out with wild-type CHO cells, it appeared that in these cells deprivation of methionine increased transcription of the c-*myc* mRNA only slightly (results not shown).

Stability of c-*myc* and ODC mRNAs. To find out whether prolongation of the half-lives of the c-*myc* and ODC mRNAs contributed to the mRNA accumulation, transcription was blocked with dactinomycin, and the rates of decay of the mRNAs were determined. After 4 h of incubation of wild-type CHO cells with dactinomycin, four times more c-*myc* mRNA was left in the methionine-starved cultures than in the control cultures (Fig. 7). Methionine starvation also increased the stability of c-*myc* mRNA in A2 cells, but less than in wild-type CHO cells (results not shown).

Dactinomycin did not cause a detectable decrease in the ODC mRNA in 8 h in A2 cells (Fig. 8) or in wild-type CHO cells (results not shown). This indicates either that the half-life of ODC mRNA is very long or that the machinery for ODC mRNA degradation is very sensitive to the blocking of mRNA synthesis.

Dactinomycin is also known to inhibit protein synthesis and thus has effects unrelated to transcriptional inhibition (28). It is unlikely, however, that this inhibition played a role in our experiments, since dactinomycin at the concentration used, 0.5 μg/ml, inhibited protein synthesis by less than 10% in 4 h.

Synthesis of c-*myc* and ODC polypeptides. The rates of synthesis of the c-*myc* and ODC polypeptides in the proline-starved and control cultures were determined by giving the cells a [^{35}S]methionine pulse and precipitating the cellular

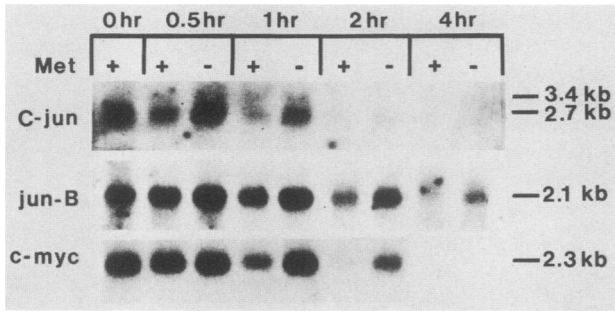

FIG. 7. Effect of amino acid starvation on the degradation of c-*myc*, c-*jun*, and *junB* mRNAs. Wild-type CHO cells were incubated for the indicated times in the presence of dactinomycin (0.5 μg/ml) either in complete medium or in medium lacking methionine. Northern blotting was carried out as described in the legend to Fig. 1. The probes were pSV-c-*myc*-1, c-*jun* (hcJ-1), and *junB* (clone 465).

homogenates with specific antisera. It appeared that the rate of synthesis of c-*myc* polypeptide was increased in the proline-starved cultures, although the overall protein synthesis was decreased (results not shown). When the immunoprecipitation was carried out with cellular lysates with equal amounts of radioactivity, there was a marked increase in the synthesis of c-*myc* polypeptide, while the synthesis of ODC polypeptide remained the same or showed only a slight increase during amino acid starvation (Fig. 9).

Effect of amino acid starvation on the expression of c-*jun*, *junB*, and c-*fos*. Next we studied the effect of methionine starvation on accumulation of c-*jun*, *junB*, and c-*fos* mRNAs (Fig. 10). Amino acid starvation increased the levels of all these mRNAs, and the rises were higher after 6 h than after 2 h of starvation. The highest increase, 10- to 15-fold, was found with *junB* mRNA. Of the two species of c-*jun* mRNA (2), the 2.7-kb band showed a three- to fivefold increase and the 3.4-kb band showed five- to sevenfold increase in amino acid-starved cells. Cycloheximide also enhanced the mRNAs of the three genes. There were, however, distinct differences in the responses. The two species of c-*jun* mRNA were elevated less (two- to threefold) by cycloheximide than by amino acid starvation, while the *junB* mRNA was increased equally by both. The c-*fos* mRNA, in turn, was increased much more by cycloheximide than by amino acid deprivation.

In order to see if the increases in the mRNAs in amino acid-deprived cells were dependent on protein synthesis, the

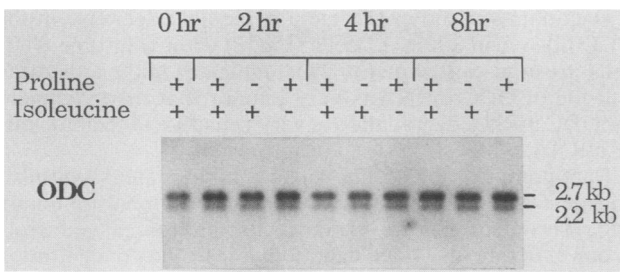

FIG. 8. Effect of amino acid starvation on the degradation of ODC mRNA. A2 cells were incubated for the indicated times in the presence of dactinomycin (0.5 μg/ml) either in complete medium or in medium lacking proline or isoleucine. Northern blotting was carried out as described in the legend to Fig. 1. The probe was pODC16.

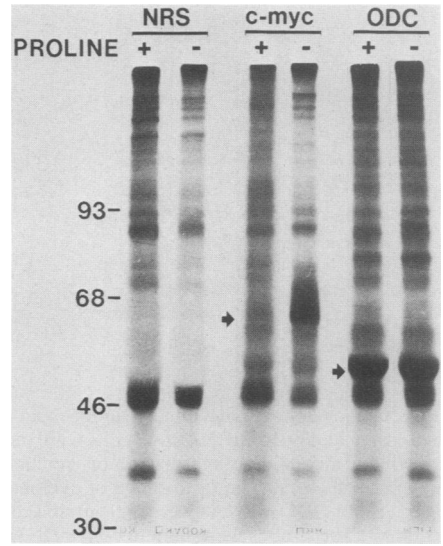

FIG. 9. Effect of amino acid starvation on synthesis of c-*myc* and ODC polypeptides. The A2 cells were incubated for 4 h either with complete medium or without proline. Afterward, [^{35}S]methionine (5 μCi/ml) was added, and 30 min later the cells were harvested. The cellular homogenates with equal amounts of radioactivity were precipitated with either normal rabbit serum (NRS), anti-pan-*myc* immune serum, or monospecific ODC immune serum and submitted to sodium dodecyl sulfate-polyacrylamide gel (8% polyacrylamide) electrophoresis.

cells were incubated without methionine in the presence and absence of cycloheximide for 6 h (Fig. 11). It appeared that cycloheximide decreased the accumulation of c-*jun* mRNA to the level obtained with cycloheximide alone. In this respect, the expression of c-*jun* resembles that of c-*myc* and the ODC genes.

Analysis of the synthesis of c-*jun* and *junB* mRNAs in wild-type CHO cells revealed no significant differences between the methionine-starved and the control cells in nuclear run-on assays (results not shown).

The potential role of mRNA stabilization in the accumulation of c-*jun* and *junB* mRNAs in the amino acid-starved cells was studied with dactinomycin. After 0.5 h of incubation with dactinomycin, about two times more c-*jun* mRNA

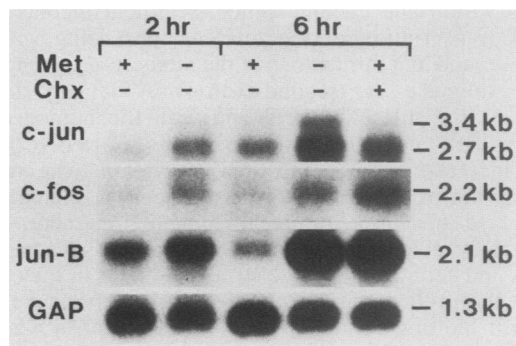

FIG. 10. Effect of amino acid starvation and cycloheximide on mRNA levels of c-*fos*, c-*jun*, and *junB*. Wild-type CHO cells were incubated for 2 or 6 h either in complete medium containing 5% dialyzed fetal calf serum or in the same medium lacking methionine or containing cycloheximide (Chx; 10 μg/ml). The filters were hybridized with the following probes: c-*jun* (hcJ-1), *junB* (clone 465), human c-*fos*, and pRGAPDH-13.

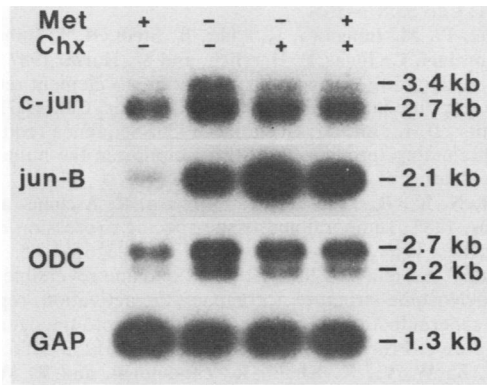

FIG. 11. Effect of cycloheximide on the amino acid-induced rise in c-*jun*, *junB*, and ODC mRNAs. Wild-type CHO cells were incubated in complete medium or in medium lacking methionine in the presence or absence of cycloheximide (Chx; 10 μg/ml) for 6 h. The filters were hybridized with the following probes: c-*jun* (hcJ-1), *junB* (clone 465), pODC16, and pRGAPDH-13.

remained in the methionine-starved cultures than in the control cultures (Fig. 7). This indicates that amino acid starvation causes prolongation of the half-life of c-*jun* mRNA. The *junB* mRNA was also more stable in the amino acid-starved cultures than in the control cultures (Fig. 7).

DISCUSSION

In this paper we demonstrate that depriving mammalian cells of a single essential amino acid induces rises in the levels of the mRNAs of several growth-associated genes: the gene for ODC, c-*myc*, c-*jun*, *junB*, and c-*fos*. This is not a general phenomenon, since mRNAs of the GAP, γ-actin, β-tubulin, RNA polymerase II, and protein kinase C genes, as well as the total poly(A)$^+$ RNA, were not elevated during amino acid starvation. The specificity of the phenomenon is also supported by our finding that amino acid starvation has an opposite effect on the mRNA of ribonucleotide reductase M2, which decreases after deprivation of an essential amino acid (unpublished results). Also, this is not a question of general response to stress, since heat shock and cadmium do not affect the expression of c-*myc* in the same way as amino acid starvation (unpublished results).

It is well known that inhibition of protein synthesis by cycloheximide or by other drugs increases the accumulation of the mRNAs of many genes, including ODC, c-*myc*, and c-*fos* (6, 7, 10, 27, 30). This has been ascribed to increased transcription and to increased stability of the mRNAs apparently brought about by inhibition of the synthesis of some short-lived regulatory protein(s) (7, 10, 21). As expected, amino acid deprivation also suppressed protein synthesis, and lack of an essential amino acid had a stronger suppressor effect than lack of a nonessential amino acid. In concert with this finding, deprivation of nonessential amino acids (except tyrosine) had very little effect on accumulation of the c-*myc* and ODC mRNAs. However, the degree of inhibition of protein synthesis caused by individual essential amino acids was not correlated with the level of mRNA accumulation. Also, the transcripts of c-*myc* and ODC were elevated more by deprivation of an essential amino acid than by cycloheximide, in spite of the fact that cycloheximide suppressed protein synthesis more effectively than starvation with any of the amino acids did. More importantly, cycloheximide reduced the rises in the ODC, c-*myc*, and c-*jun* mRNAs in the amino acid-starved cells to the level found in the cells incubated with cycloheximide alone. The conclusion is that amino acid deprivation causes accumulation of the ODC, c-*myc*, and c-*jun* mRNAs by a mechanism that requires protein synthesis. This conclusion implies that, although amino acid starvation suppresses protein synthesis, the triggering signal cannot be inhibition of protein synthesis as such. Instead, the results give reason to believe that cells sense a reduced pool of an amino acid or a low level of a charged tRNA.

The c-*fos* mRNA level was increased more by cycloheximide than by amino acid deprivation, and it is likely that in both cases the mere inhibition of protein synthesis was responsible for the elevation of the c-*fos* mRNA. If this is true, it means that the increase in c-*fos* mRNA during amino acid starvation does not require de novo protein synthesis. The rises in the *junB* mRNA were about the same in cycloheximide-treated and in amino acid-starved cells. This similarity made it difficult to demonstrate whether protein synthesis was required for the amino acid starvation-induced elevation of the level of *junB* transcripts.

Cells can regulate mRNA levels by varying both synthesis and turnover, and in many instances both of these mechanisms operate. For example, accumulation of c-*myc* mRNA during normal T-cell activation is based on both of these mechanisms (33). This also appears to be true with c-*myc* mRNA in amino acid-starved CHO cells. However, there seem to be some differences between the two CHO strains. In the wild-type CHO cells,the increase in the stability of the c-*myc* mRNA seemed to play a more important role than the elevation of transcription, while in the mutant A2 cells the increased transcription appeared to be relatively more important. The mechanisms behind the accumulation of different mRNAs may also differ. It appears that the rise in the c-*jun* mRNA in the amino acid-deprived cells may be due solely to the prolongation of the half-life of the mRNA.

The assays of c-*myc* transcription gave different results when the labeled mRNAs were hybridized to c-*myc* mRNAs containing only the second and the third exons, compared with c-*myc* DNA containing all three exons (pHSR-1). In the former case amino acid starvation increased the rate of c-*myc* transcription, but that was not true in the latter case. There is indication that in certain instances, synthesis of c-*myc* mRNA may be regulated by blocking the transcription after the first exon (5, 33, 34, 37, 47). Correspondingly, release from transcriptional blocking has been found to operate in the activation of c-*myc* transcription (12, 33, 36), and it might also be responsible for the increased synthesis of c-*myc* mRNA in the amino acid-starved A2 cells. Our preliminary run-on transcription experiments with sense and antisense RNA probes (generated from plasmids containing either the first or the second c-*myc* exon) support this interpretation. The sense transcription of exon 1 was higher than that of exon 2. Moreover, the transcription of exon 1 was not increased but instead slightly decreased during amino acid starvation.

It is of interest that yeast cells respond to amino acid starvation by increasing the amount of the enzymes required for the synthesis of the missing amino acid and of certain other amino acids (25). Transcription of the respective genes is stimulated by a positive regulatory protein, GCN4, which binds to a specific nucleotide sequence (22, 44). In *Neurospora crassa* there is also a general control of amino acid biosynthesis, but in this organism mutations in a gene involved in the derepression of the amino acid-synthesizing enzymes also affect transcription of other genes not con-

nected with amino acid synthesis (14). Transcription factor Jun/AP-1 in mammalian cells binds to the same nucleotide sequences as GCN4 in the yeast genome (2, 4, 32, 43, 46). It is interesting that the three genes found to respond to amino acid starvation by increasing their mRNAs in a protein synthesis-dependent manner all contain Jun-binding sequences. Screening the nucleotide sequences in the human c-*myc* genome (16) revealed the consensus Jun-binding sequence at positions 1378 to 1384 upstream of the gene. A potential Jun-binding sequence is also located 0.3 kb upstream of the c-*myc* gene (19). Sequencing of the 5′ region of the mouse ODC gene has also revealed four Jun-binding sequences (26). Further, it was recently found that transcription of c-*jun* is stimulated by its own product binding to a high-affinity sequence located in the promoter region (3). However, it is not very likely that Jun is the common regulator of the expression of these genes in mammalian cells during amino acid starvation, since in most cases an increase in the mRNA stability rather than an increase in the rate of transcription appears to be the main factor causing accumulation of the mRNAs. In general, the direct comparison of the response mechanisms to amino acid starvation in lower and higher eucaryotes may not be entirely appropriate because higher eucaryotes cannot adapt to the lack of an essential amino acid by increasing its synthesis.

The fact that protein synthesis is needed for the rise in the c-*myc*, ODC, and c-*jun* mRNA levels in amino acid-deprived cells suggests that some protein(s) inhibiting the mRNA degrading machinery or protein(s) stimulating transcription must be synthesized.

Synthesis of the c-*myc* polypeptide increased and that of the ODC polypeptide remained the same or increased slightly in amino acid-starved cells. Given the short half-life of both polypeptides, the results seem to indicate that by increasing the c-*myc* and ODC mRNA levels the cells can selectively compensate for the depressing effect amino acid deprivation has on protein synthesis.

In conclusion, contrary to the general assumption, the results presented in this paper show that mammalian cells can adjust to amino acid deprivation by altering specific gene expression. This may be a compensatory reaction by which cells try to continue the synthesis of certain vital proteins in unfavorable growth conditions. For animals, the phenomenon might have physiological significance during periods of inadequate dietary supply of essential amino acids.

ACKNOWLEDGMENTS

We thank Irma Lantinen and Anne Aronta for competent technical assistance. We also thank Olli A. Jänne for ODC antiserum and the pODC16 plasmid, Michael Karin for the c-*jun* plasmid, Daniel Nathans for the *junB* plasmid, Larry Kedes for the γ-actin plasmid, and Philip Fort for the pRGAPDH-13 plasmid.

This work was supported by grants from the Medical Research Council of the Finnish Academy of Sciences, the Finnish Cancer Society, and the Sigrid Jusélius Foundation.

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