Regulation of Biosynthesis of Hypusine in Chinese Hamster Ovary Cells

EVIDENCE FOR eIF-4D PRECURSOR POLYPEPTIDES*

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The effects of spermidine depletion and the effects of translation inhibition on hypusine biosynthesis were studied in Chinese hamster ovary cells. Upon depletion of cellular spermidine by treatment with DL-a-difluoromethylornithine for 42 h or longer, both the rate of deoxyhypusine + hypusine synthesis and the content of protein-bound hypusine were significantly reduced. Cycloheximide caused complete inhibition of deoxyhypusine + hypusine synthesis in untreated cells and in cells in which the spermidine level was reduced to approximately 10% of the untreated cells by incubation with DL-a-difluoromethylornithine for 24 h. In contrast, the initial synthesis of deoxyhypusine + hypusine was not arrested by cycloheximide in cells depleted of spermidine by treatment with DL-a-difluoromethylornithine for 42 h. The initial rate of deoxyhypusine + hypusine production in these spermidine-depleted cells increased 5- to 10-fold when the cellular spermidine level was restored through addition of this polyamine to the culture medium. These findings suggest that in control Chinese hamster ovary cells and in cells containing ~10% of the control level of spermidine, deoxyhypusine + hypusine synthesis occurs during or immediately after eukaryotic initiation factor 4D precursor translation. However, in cells during depletion of spermidine, there is an accumulation of an eukaryotic initiation factor 4D precursor that contains no hypusine or deoxyhypusine, and in these cells deoxyhypusine + hypusine synthesis is mainly regulated by the cellular level of spermidine.

Eukaryotic translation initiation factor 4D (eIF-4D) is the only cellular protein thus far known to contain the unusual amino acid hypusine (N\(^\alpha\)-(4-aminobutyl)lysine) (1). The biosynthesis of hypusine occurs by a novel post-translational event in which the 4-aminobutyl moiety of the polyamine spermidine is transferred to the \(\epsilon\)-amino group of a specific lysine residue of an eIF-4D precursor to form deoxyhypusine and in which the deoxyhypusine residue of this polypeptide is subsequently hydroxylated (2-4). Thus the biogenesis of eIF-4D may be divided into three stages as shown in Scheme 1: 1) Translation of eIF-4D mRNA; 2) post-translational modification, step 1; 3) post-translational modification, step 2.

The rate of hypusine formation parallels the increase in protein synthesis in mitogen-treated human peripheral lymphocytes (5) and correlates in general with the rate of cellular proliferation in various mammalian cells (6-8). The overall regulation of hypusine biosynthesis, although not clearly understood, may depend upon the rate of synthesis of eIF-4D precursor I (Scheme 1), the concentration of this precursor, the level of intracellular spermidine, and/or the activities of the enzymes involved.

The level of spermidine in mammalian cells can be manipulated by the use of DFMO (9), which is an irreversible inhibitor of ornithine decarboxylase, a regulatory enzyme in the biosynthesis of polyamines (10). The dependence of the rate of hypusine synthesis on spermidine concentration has been reported in DFMO-treated rat hepatoma (HTC) cells (8, 11). Furthermore, the presence of a pool of unmodified eIF-4D precursor in these cells was postulated on the basis of an immediate increase in the rate of hypusine synthesis following restoration of cellular spermidine (8). A similar eIF-4D precursor was also proposed to be present in resting human peripheral lymphocytes (5). However, no definitive evidence in support of these proposals has thus far been provided.

The present study was undertaken to gain insight into the overall regulation of this post-translational modification in DFMO-treated and untreated CHO cells. The experiments described here provide evidence that the initial step of post-translational modification in untreated cells occurs immediately following the translation of eIF-4D mRNA, whereas in spermidine-deprived cells, the post-translational modification (step 1 in Scheme 1) is repressed and eIF-4D precursor I, which contains no hypusine or deoxyhypusine, accumulates. In these cells, which contain a pool of eIF-4D precursor I, spermidine appears to be the major factor governing the rate of deoxyhypusine + hypusine biosynthesis through control of modification step 1 in Scheme 1. Recently Duncan and Hersey (12) proposed a coupled translation/hypusine modification of eIF-4D in HeLa cells. Our observations in CHO cells provide new information on the relationships between the translation and the two steps of post-translational modification and thus contribute a further understanding of the regulation of hypusine synthesis.

**EXPERIMENTAL PROCEDURES**

CHO cells WTB were kindly supplied by Dr. April R. Robbins (National Institutes of Health). DFMO was a gift from Merrel Dow Research Center. [terminal methylenes-\(^{3}\)H]Spermidine-3 HCl (25 Ci/mmole) was purchased from DuPont-New England Nuclear; cycloheximide from Sigma. Other materials, reagents, and the general procedures for cell culture, radiolabeling, and ion exchange chroma-

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*The abbreviations used are: eIF-4D, eukaryotic initiation factor 4D; CHO, Chinese hamster ovary; DFMO, DL-a-difluoromethylornithine; HTC, hepatoma tissue culture.
tography have been described in previous publications (2-4, 13). Specific details of each experiment are given in the Figure and Table legends.

RESULTS

The data presented in Fig. 1 show that the depletion of cellular spermidine by treatment with DFMO causes a reduction in the content of protein-bound hypusine. When CHO cells were treated with a 4 mM level of DFMO, cellular putrescine dropped to below a detectable level within 24 h. Spermidine was reduced to about 10% of that of untreated cells at 24 h and was not detectable after 48 h of treatment with DFMO (Table I). The content of protein-bound hypusine was lowered by ~20% after 24 h but dropped to approximately 30% of the value of untreated cells between 48 and 72 h of treatment (Fig. 1). This reduction in protein-bound hypusine may result from decreased spermidine synthesis, from increased degradation of eIF-4D, or from changes of both. To date, the effect of polyamine depletion on the turnover of eIF-4D has not been precisely determined. However, the marked repression of the post-translational modification upon spermidine depletion as shown in Fig. 2 suggests that decreased synthesis may be mainly responsible for the reduction in protein-bound hypusine. The reduction of hypusine was not as pronounced as that of spermidine in these DFMO-treated cells, presumably because only a minute portion of the total cellular spermidine is used for hypusine production and because the half-life of eIF-4D is relatively long (8).

The relationships between cellular spermidine levels and the initial rates of modification (step 1 in Scheme 1) were assessed in untreated cells and in those treated with DFMO (Fig. 2). In newly modified protein, that which contains radioactivity provided through a structural contribution from [3H]spermidine, the transient intermediate [3H]deoxyhypusine was seen in addition to [3H]hypusine. Parenthetically, in the digest of total cellular proteins, only hypusine was detected with the use of the fluorometric method (Fig. 1). This is the case because the quantity of the transient intermediate deoxyhypusine is very small (0.1–5 pmol/mg protein) compared to total hypusine (0.05–0.2 nmol/mg protein). The relative amounts of [3H]hypusine and [3H]deoxyhypusine in newly modified protein varied in untreated and DFMO-treated cells (Table II). The percentages of [3H]deoxyhypusine in [3H]deoxyhypusine + [3H]hypusine at 1.3 h after addition of [3H]spermidine were ~14.5% in untreated cells (Table II, part A), ~4% in cells pretreated with DFMO for 42 h after DFMO addition, cells were washed and harvested as described (3). To the pellet of cells from one dish, 0.2 ml of 6 N HCl, and hypusine in the digests was partially purified by ion exchange chromatography on Bio-Rex 70 as previously described (4). Hypusine content in the partially purified fraction was determined by ion exchange chromatography and fluorometric detection as described (4, 13).

![Fig. 1. The total content of protein-bound hypusine in untreated (●) and DFMO-treated (△) CHO cells.](image1)

**Table I**

| Polyamine content of untreated and DFMO-treated CHO cells |
|----------------------------------------------------------|
| Cells were plated in 100-mm dishes at approximately 1 x 10^6 cells/dish in the α-modification of Eagle's medium supplemented as described (3). After 1 day, DFMO (4 mM) was added. At 24, 48, and 72 h after DFMO addition, cells were washed and harvested as described (3). To the pellet of cells from one dish, 0.2 ml of 5% trichloroacetic acid was added. The polyanines in 0.1 ml of the acid supernatant were measured with the use of fluorometric detection after their ion exchange chromatographic separation employing the three-buffer system described earlier (13). |

| Cell treatment | Incubation time | Putrescine | Spermidine | Spermine |
|----------------|-----------------|------------|------------|----------|
| Untreated      | 0 h             | 1.09       | 6.25       | 6.25     |
| Untreated      | 24 h            | 2.75       | 13.10      | 11.41    |
| Untreated      | 48 h            | 1.36       | 10.32      | 7.62     |
| Untreated      | 72 h            | 0.65       | 10.91      | 8.67     |
| DFMO           | 24 h            | <0.1       | 1.35       | 11.18    |
| DFMO           | 48 h            | <0.1       | <0.1       | 9.10     |
| DFMO           | 72 h            | <0.1       | <0.1       | 7.13     |

The data presented in Fig. 1 show that the depletion of cellular spermidine by treatment with DFMO causes a reduction in the content of protein-bound hypusine. When CHO cells were treated with a 4 mM level of DFMO, cellular putrescine dropped to below a detectable level within 24 h. Spermidine was reduced to about 10% of that of untreated cells at 24 h and was not detectable after 48 h of treatment with DFMO (Table I). The content of protein-bound hypusine was lowered by ~20% after 24 h but dropped to approximately 30% of the value of untreated cells between 48 and 72 h of treatment (Fig. 1). This reduction in protein-bound hypusine may result from decreased spermidine synthesis, from increased degradation of eIF-4D, or from changes of both. To date, the effect of polyamine depletion on the turnover of eIF-4D has not been precisely determined. However, the marked repression of the post-translational modification upon spermidine depletion as shown in Fig. 2 suggests that decreased synthesis may be mainly responsible for the reduction in protein-bound hypusine. The reduction of hypusine was not as pronounced as that of spermidine in these DFMO-treated cells, presumably because only a minute portion of the total cellular spermidine is used for hypusine production and because the half-life of eIF-4D is relatively long (8).

The relationships between cellular spermidine levels and the initial rates of modification (step 1 in Scheme 1) were assessed in untreated cells and in those treated with DFMO (Fig. 2). In newly modified protein, that which contains radioactivity provided through a structural contribution from [3H]spermidine, the transient intermediate [3H]deoxyhypusine was seen in addition to [3H]hypusine. Parenthetically, in the digest of total cellular proteins, only hypusine was detected with the use of the fluorometric method (Fig. 1). This is the case because the quantity of the transient intermediate deoxyhypusine is very small (0.1–5 pmol/mg protein) compared to total hypusine (0.05–0.2 nmol/mg protein). The relative amounts of [3H]hypusine and [3H]deoxyhypusine in newly modified protein varied in untreated and DFMO-treated cells (Table II). The percentages of [3H]deoxyhypusine in [3H]deoxyhypusine + [3H]hypusine at 1.3 h after addition of [3H]spermidine were ~14.5% in untreated cells (Table II, part A), ~4% in cells pretreated with DFMO for 42 h after DFMO addition, cells were washed and harvested as described (3). To the pellet of cells from one dish, 0.2 ml of 6 N HCl, and hypusine in the digests was partially purified by ion exchange chromatography on Bio-Rex 70 as previously described (4). Hypusine content in the partially purified fraction was determined by ion exchange chromatography and fluorometric detection as described (4, 13).

![Fig. 2. The rate of deoxyhypusine + hypusine synthesis in untreated and DFMO-treated CHO cells.](image2)
Table II

| Cell treatment | Incubation time | Deoxyhypusine | Hypusine | Deoxyhypusine + hypusine |
|---------------|-----------------|---------------|----------|--------------------------|
|               | h pmol/mg protein (%) |               |          |                          |
| A. Untreated  | 1.3 0.22 (14.5) | 1.30 (85.5) | 1.52     |                          |
|               | 5.0 0.38 (6.0) | 6.0 (94.0) | 6.38     |                          |
| B. DFMO 24 h | 1.3 0.23 (20.9) | 0.87 (79.1) | 1.10     |                          |
|               | 5.0 0.42 (8.0) | 4.80 (92.0) | 5.22     |                          |
| C. DFMO 42 h | 1.3 0.05 (4.0) | 1.2 (96.0) | 1.25     |                          |
|               | 5.0 0.06 (3.0) | 2.0 (97.0) | 2.06     |                          |
| D. DFMO 42 h + SPD | 1.3 0.52 (70.0) | 2.25 (300.0) | 7.50 |                          |

h (Table II, part C), and ~70% in cells initially depleted of and then restored with spermidine (Table II, part D). The percentages of [3H]deoxyhypusine decreased with incubation time and were lower than 5% after 24 h (not shown) in all cases.

In spite of the inherent technical limitations involved in the accurate determination of hypusine and deoxyhypusine from radioactivity measurements as described in the legend of Fig. 2, the estimated rates in untreated exponentially growing CHO cells are in fair agreement with those values (1–2 pmol/mg protein/h) previously reported for CHO cells and HTC cells (6, 8). The rate of deoxyhypusine + hypusine synthesis declined only slightly after 24 h of DFMO treatment (compare A and B in Fig. 2, A). Thus, it appears that there is no significant inhibition of either the translation of eIF-4D mRNA or the modification of eIF-4D precursor I (Scheme 1) up to this point. Marked depression of deoxyhypusine + hypusine formation was found to occur after 42 h at the time when cellular spermidine was depleted (compare A and C in Fig. 2, A). This reduction in modification step 1 (Scheme 1) is probably responsible for the decrease in protein-bound hypusine (Fig. 1) and the accumulation of eIF-4D precursor I (Figs. 2 and 3) in DFMO-treated cells.

Inhibition of translation by cycloheximide caused the complete arrest of new synthesis of deoxyhypusine + hypusine in untreated CHO cells (Fig. 2A, A), and also in cells pretreated with DFMO for 24 h (Fig. 2B, A). This effect by cycloheximide was seen in cells from the early to the late exponential stage of growth irrespective of cell densities (not shown). The failure of untreated cells and cells with reduced spermidine content (DFMO 24 h) to form hypusine in the presence of cycloheximide probably results from the lack of substrate protein eIF-4D precursor I (Scheme 1), rather than from inhibition of the enzymes involved. Certainly, the enzymes involved in hypusine production are functional in the presence of cycloheximide (Fig. 2, C and D, A). Thus the indication of the lack of eIF-4D precursor I accumulation supports the notion that eIF-4D precursor I undergoes post-translational modification during or immediately following translation in control cells and even in cells with reduced spermidine content (DFMO, 24 h). Furthermore, the complete arrest of radiolabeling of eIF-4D by cycloheximide (Fig. 2, A and B, A) suggests that hypusine is produced only de novo in eIF-4D precursor I and that there is no pathway for exchange or turnover of the 4-amino-2-hydroxybutyl moiety in the hypusine residue of eIF-4D.

In contrast to control cells and cells with reduced spermidine content (DFMO, 24 h), cells virtually depleted of spermidine (DFMO, 42 h) showed essentially no inhibition of production of deoxyhypusine + hypusine by cycloheximide (Fig. 2C, A). Whereas the addition of exogenous spermidine (2.5 μM) did not cause changes in the rate of deoxyhypusine + hypusine synthesis in control cells (not shown), replenishment of this polyamine to the control level in spermidine-depleted cells (DFMO, 42 h) by addition of 2.5 μM unlabeled spermidine (8) together with [3H]spermidine caused a 5- to 10-fold elevation of the initial rate of synthesis of deoxyhypusine + hypusine (compare C and D in Fig. 2, A). The finding of no apparent inhibition by cycloheximide at 1.3 h (Fig. 2D, A) is probably due to the small amount of eIF-4D precursor I newly translated during this period compared to what may be a pre-existing pool of precursor I. The partial inhibition seen at 5 h (Fig. 2D, vertical broken arrow) is likely the result of arrest of eIF-4D precursor I translation by cycloheximide over the incubation period of 5 h. Certainly, the cycloheximide resistant labeling of deoxyhypusine + hypusine.

![Figure 3. Comparison of radiolabeling of eIF-4D from untreated and DFMO-treated CHO cells.](image)

**Fig. 3.** Comparison of radiolabeling of eIF-4D from untreated and DFMO-treated CHO cells. Untreated cells were incubated with [3H]leucine (20 μCi/ml) and [3H]spermidine (5 μCi/ml) in medium containing approximately 5% of the normal level of leucine for 2 h (A and D). Cells pretreated with DFMO for 42 h were incubated under the same conditions, except that unlabeled spermidine (2.5 μM) was added together with [3H]leucine and [3H]spermidine (B and E). DFMO-pretreated cells were also incubated under these conditions in the presence of cycloheximide (100 μg/ml) (C and F). Whole cell proteins (~200 μg) were analyzed by two-dimensional polyacrylamide gel electrophoresis (23). Radiofluorographs of portions of the gels surrounding the area occupied by eIF-4D are shown. The position of eIF-4D is denoted by the solid arrow. The portions of the gels occupied by eIF-4D in A–C were excised, the protein in each gel piece was hydrolyzed in acid, and the amounts of radioactivity in leucine and deoxyhypusine + hypusine were measured (D–F) after ion exchange chromatographic separation.
Deoxyhypusine substantiates the existence of a pool of eIF-4D precursor I in the spermidine-depleted cells. Evidently, the accumulation of this precursor occurred when the rate of modification (step 1 in Scheme 1) dropped below that of the translation of eIF-4D mRNA as a result of reduction of cellular spermidine from ~10% (DFMO, 24 h) to an undetectable level (DFMO, 42 h). The extent of eIF-4D precursor I accumulation during the period between 24 and 42 h of DFMO treatment may be estimated as approximately 9 pmol/mg of protein from the maximum value of cycloheximide-resistant synthesis of deoxyhypusine + hypusine (Fig. 2D, vertical solid arrow).

Additional evidence for accumulation of eIF-4D precursor I in spermidine-deprived cells was provided by direct comparison of labeling of \[^{[3}H\]deoxyhypusine + \[^{[3}H\]hypusine and \[^{[3}H\]leucine in eIF-4D from untreated CHO cells with that from cells pretreated with DFMO for 42 h. Labeling was carried out for 2 h with \[^{[3}H\]deoxyhypusine and \[^{[3}H\]leucine (Fig. 3). Cold spermidine (2.5 \(\mu\)M) was added to cells pretreated with DFMO (42 h) together with the two radiola beled compounds in order to maintain the specific radioactivity of the spermidine pool of these cells comparable to that of control cells. Both \[^{[3}H\]deoxyhypusine and \[^{[3}H\]hypusine were found in the spots indicated by the solid arrows in essentially the same ratios as given in Table II (A and D). The comparison of D and E in Fig. 3 reveals that the post-translational modification represented by radiolabeling in \[^{[3}H\]deoxyhypusine + \[^{[3}H\]hypusine was ~10 fold greater in cells pretreated with DFMO (42 h) than in control cells, while the rates of synthesis of eIF-4D precursor I represented by \[^{[3}H\]leucine incorporation were similar. Furthermore, cycloheximide which caused the complete inhibition of \[^{[3}H\]leucine incorporation into eIF-4D, did not block the post-translational formation of \[^{[3}H\]deoxyhypusine + \[^{[3}H\]hypusine in cells pretreated with DFMO for 42 h (Fig. 3F). No radiolabeling of eIF-4D was observed when control cells were incubated with \[^{[3}H\]spermidine and \[^{[3}H\]leucine in the presence of cycloheximide (not shown). Thus a rapid modification of a pool of eIF-4D precursor I accumulated during spermidine depletion must have occurred upon replenishment of this polyamine in the absence (Fig. 3E) or presence (Fig. 3F) of cycloheximide. The post-translational modification independent of new translation seen in E and F of Fig. 3 is consistent with the data given in Fig. 2D and strongly supports the presence of a pool of eIF-4D precursor I in spermidine-depleted cells.

**DISCUSSION**

The data presented in this paper provide strong evidence that the post-translational modification of eIF-4D precursor I (step 1 in Scheme 1) normally occurs concomitantly with or immediately after translation of eIF-4D mRNA in control CHO cells and also in cells with approximately one-tenth of their normal spermidine content (DFMO, 24 h). A coupled translation/hypusine modification has been proposed by Duncan and Hershey (12) on the basis of the effects of translation inhibition in HeLa cells on the incorporation of radioactivity from \[^{[3}H\]spermidine into eIF-4D. However, we have detected the accumulation of a small amount of deoxyhypusine-containing eIF-4D precursor II in control CHO cells and in cells with reduced spermidine content (A and B, respectively, in Table II). Thus it appears that although deoxyhypusine synthesis is coupled to translation, immediate hydroxylation may not occur following the initial step of modification. Furthermore, we have found conditions under which the translation and the post-translational modification step 1 (Scheme 1) can be uncoupled. Our data suggest that in cells which are virtually depleted of spermidine, deoxyhypusine synthesis is no longer coupled to translation, the eIF-4D precursor I that contains no deoxyhypusine or hypusine accumulates and, upon replenishment with spermidine, rapid modification of preformed eIF-4D precursor I can occur. It is obvious from the data in Fig. 2 that the rate of modification (step 1 in Scheme 1) is significantly reduced when spermidine is depleted. Whether cellular polyamines regulate the translation of eIF-4D mRNA and its gene expression is not known. However, it may be concluded that the accumulation of eIF-4D precursor I can result from the differential effects of spermidine depletion on the translation and the modification (step 1 in Scheme 1). In the cells containing a pool of eIF-4D precursor I (42 h preincubation with DFMO, C and D in Fig. 2) spermidine appears to be the major factor determining the rate of deoxyhypusine + hypusine formation (modification step 1 in Scheme 1). The spermidine concentration dependence of hypusine synthesis in rat hepatoma cells was previously reported by Gerner et al. (8). It is not clear at present whether the effect of cellular spermidine is exerted at the level of substrate concentration or at the level of activities of the enzymes involved or both. It is obvious from the data of Table II that both radiolabeled deoxyhypusine and hypusine are detected in the early times after addition of \[^{[3}H\]spermidine. Although the quantity of deoxyhypusine is small compared to the total protein-bound hypusine (Table II and Fig. 1), the levels of deoxyhypusine in newly modified protein are higher in the cells with the higher initial rates of modification (step 1, Scheme 1). This increased accumulation of unhydroxylated amino acid may result from a relatively stable rate of hydroxylation irrespective of the large increase in initial synthesis of deoxyhypusine. Alternatively, deoxyhypusine hydroxylation may also be regulated by the level of cellular polyamines and in an independent manner. This possible regulation is currently being investigated.

In spite of the strong evidence for accumulation of eIF-4D precursor I in spermidine-deficient cells, we have thus far failed to detect eIF-4D precursor I protein after separation of cellular proteins by two-dimensional gel electrophoresis. This may be due to the small quantity of this precursor, ~0.018% of the total cellular protein, and the limited resolution of our two-dimensional gels. Enrichment of this protein fraction and use of specific antibodies may provide the means for direct identification of this precursor protein.

The physiological significance of the decreases in the biosynthesis and content of protein-bound hypusine, and of the accumulation of eIF-4D precursor I in DFMO-treated cells is not known. Depletion of putrescine and spermidine by DFMO leads to depression of total cell protein synthesis in HTc cells (14) and in mitogen-activated lymphocytes (15) and causes the inhibition of growth in various mammalian cells (16-19). However, the mechanism by which polyamines contribute to the regulation of cellular proliferation is not clearly understood. In cells treated with DFMO, or in mutant cells defective in polyamine synthesis, the marked inhibition of replication is delayed by 1 to 3 generations after the depletion of putrescine and spermidine (16, 18, 20, 21). On this ground it was speculated that the inhibition of proliferation may not be a direct consequence of polyamine depletion, but rather that polyamines may participate in the biogenesis of some cellular component(s) that is required in the replication (18). Since neither the function of eIF-4D in eukaryotic protein synthesis (22) nor the role of hypusine in this factor has been elucidated, it is as yet premature to attribute inhibition of cellular proliferation to a decrease in hypusine. In a recent report, Duncan and Hershey (12) suggest that changes in eIF-4D hypusine modification or abundance are not correlated with translation repression in HeLa cells by serum depletion, heat shock, or
hypertonic shock. Although normally, eIF-4D precursor is largely modified to contain hypusine, our results show that the modification (step 1, Scheme 1) is regulated in DFMO-treated cells where cellular spermidine is drastically reduced. In this system, the modification may play a critical role in the modulation of protein synthesis and cellular proliferation. The isolation of eIF-4D precursor I from spermidine-deficient cells would provide the first step towards an understanding of the role of hypusine. Possession of this unmodified protein is certainly essential for in vitro studies of the mechanism of biosynthesis of deoxyhypusine and hypusine and its regulation.

Acknowledgments—I am grateful to Dr. J. E. Folk for his advice and encouragement throughout this work; I also thank Dr. H. L. Cooper for the two-dimensional gel electrophoretic separations.

Addendum—Since the submission of this paper, we have obtained more direct evidence for accumulation of eIF-4D precursor I in spermidine-depleted CHO cells. When these cells (DFMO for 42 h) were incubated for 6 h in the presence of [3H]lysine, no labeled eIF-4D (hypusine form, M, ~18,000; pl ~5.3) was detected by high resolution two-dimensional gel electrophoresis. Instead, a new radio-labeled cellular protein (M, ~18,000; pl ~5.1) which migrated at a position predicted for eIF-4D precursor I was seen. Peptide mapping studies are underway in an effort to confirm the identity of this protein as eIF-4D precursor I.

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