# Dramatic Attenuation of Hypusine Formation on Eukaryotic Initiation Factor 5A During Senescence of IMR-90 Human Diploid Fibroblasts

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Deoxyhypusine synthase catalyzes the conversion of lysine to deoxyhypusine residue on the eukaryotic initiation factor 5A (eIF-5A) precursor using spermidine as the substrate. Subsequent hydroxylation of the deoxyhypusine residue completes hypusine formation on eIF-5A. Hypusine formation is one of the most specific polyamine-dependent biochemical events in eukaryotic cells. Although changes in polyamine metabolism have been demonstrated in human diploid fibroblasts during senescence (Chen and Chang, 1986, J. Cell. Physiol., 128:27–32.), it is unclear whether or not polyamine-dependent hypusine formation itself is an age-dependent biochemical event. In the present study, hypusine-forming activity was measured by a radiolabeling assay in cells whose polyamines have been depleted by prior treatment of α-difluoromethyl ornithine (DFMO). In addition, an in vitro cross-labeling assay was developed for simultaneous measurement of the deoxyhypusine synthase activity and protein substrate (eIF-5A precursor) amount. We showed that the hypusine-forming activity in low-passage presenescent IMR-90 cells [population doubling level (PDL) = 15–23, termed young cells] was prominently induced by serum whereas little or no hypusine-forming activity could be detected in late-passage senescent cells (PDL = 46–54, termed old cells). The striking difference in hypusine-forming activity between young and old cells was due to changes in both deoxyhypusine synthase activity and eIF-5A precursor amount in IMR-90 cells during senescence. However, Northern blot analysis showed no significant difference in the eIF-5A messenger RNA (mRNA) between young and old cells, suggesting that the age-dependent attenuation of eIF-5A precursor protein may be regulated at either translational or post-translational level. **J. Cell. Physiol. 170:248–254, 1997.** © 1997 Wiley-Liss, Inc.

Free hypusine (*N*-(4-amino-2-hydroxybutyl)lysine) was first discovered in bovine brain (Shiba et al., 1971). This unusual amino acid appears to be derived from eukaryotic initiation factor 5A (eIF-5A), the only cellular protein that is known to contain hypusine residue (reviewed in Park et al., 1993). The hypusine residue on eIF-5A is formed through (i) an nicotinamide-adenine dinucleotide ($NAD^+$)-dependent oxidative cleavage and transfer of the 4-aminobutyl moiety from spermidine to a single lysine residue on eIF-5A precursor to form deoxyhypusine ($N^{\varepsilon}$-(4-aminobutyl)lysine), and (ii) a hydroxylation of the deoxyhypusine to form hypusine residue (Park et al., 1984; Chen and Dou, 1988; Park and Wolff, 1988). The highly conserved nature of eIF-5A, the responsiveness to growth stimulation, and the specificity of hypusine formation (Cooper et al., 1982; Chen, 1983; Park et al., 1993), together with the recognized importance of polyamines in growth regulation (Cohen, 1971; Tabor and Tabor, 1984) suggest that hypusine formation may have an important role in cell physiology. Disruption of the two eIF-5A genes or deoxyhypusine synthase gene in yeast has been shown to be lethal (Schnier et al., 1991; Sasaki et al., 1996). Recent studies have also demonstrated that eIF-5A may be the cellular target of the human immunodeficiency virus type I Rev (Ruhl et al., 1993; Bevec et al., 1996) and human T-cell leukemia virus type I Rex (Katahira et al., 1995), suggesting a role for eIF-5A in premessenger RNA (mRNA) processing. Other reports suggest that hypusine formation may be involved in cell proliferation (Park et al., 1994) and cell cycling (Hanauske-Abel et al., 1995). We have shown recently that the inhibition of hypusine formation could affect tumor differentiation (Chen et al., 1996).

Normal diploid fibroblasts have limited doubling po-

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tential in tissue culture (Hayflick and Moorhead, 1961) and most probably in vivo (reviewed by Campisi et al., 1995; Smith and Pereira-Smith, 1996). The hallmark of cellular senescence is an inability to initiate DNA synthesis after growth stimulation (Cristofalo and Sharf, 1973). Studies have shown that the expression of most, if not all, G1/S genes involved in DNA synthesis is significantly attenuated in senescent cells, even after serum stimulation (Chang and Chen, 1988; Seshardri and Campisi, 1990; Stein et al., 1991; Pang and Chen, 1994). However, several mid-G1 genes, including ornithine decarboxylase, a key enzyme for polyamine biosynthesis, are induced fully by serum in senescent cells (Chang and Chen, 1988; Chen et al., 1989; Seshardri and Campisi, 1990), suggesting that old cells still retain the ability to enter the cell cycle after serum stimulation. Nevertheless, the ornithine decarboxylase activity is significantly diminished in old cells (Chen et al., 1986; Chen et al., 1989; Seshardri and Campisi, 1990), suggesting that polyamine biosynthesis is affected by cell senescence (Chen et al., 1986; Chen et al., 1989). Some earlier studies have suggested that polyamine biosynthesis is required for the initiation of DNA synthesis (Boynton et al., 1976; Sunkara et al., 1979). Since hypusine formation on eIF-5A precursor is one of the most specific polyamine-dependent processes known and has been implicated to be coupled tightly to cell proliferation (Park et al., 1994; Chen et al., 1996), cell cycling (Hanauske-Abel et al., 1995), and tumor differentiation (Chen et al., 1996), it is tempting to speculate that hypusine formation may have a role in replicative senescence. Using both in vitro labeling assay and cross-labeling assay, we showed in this study that cellular senescence is accompanied with significant attenuation of hypusine-forming activity.

## MATERIALS AND METHODS

### Chemicals

All tissue culture supplies were obtained from Gibco (Grand Island, NY). Spermidine, $NAD^+$, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Molecular biological grade of sodium dodecylsulfate (SDS) acrylamide were from ICN Chemical, Radioisotope Division (Irvine, CA). [1,8-$^3$H]-Spermidine (17.6 Ci/mmol) was obtained from DuPont NEN Research Products (Boston, MA). All other chemicals were of standard reagent grade.

### Cell culture and serum stimulation

Low-passage IMR-90 human embryonic lung fibroblasts were obtained from the Coriell Institute for Medical Research, Camden, New Jersey. Cells were maintained in Dulbecco's medium supplemented with 10% fetal bovine serum at 37°C in a water-jacketed Forma $CO_2$ incubator. Cell cultures were expanded through subculturing to achieve a higher population doubling level (PDL) as described previously (Chang and Chen, 1988). In this paper, young cells refer to cell cultures at a PDL = 25 or less, and old cells refer to cell cultures at a PDL = 45 or above; they do not indicate the chronological age of cells in a particular culture. Serum deprivation and serum stimulation were carried out as described previously (Pang and Chen, 1993, 1994). To deplete cellular polyamine pools, cultures at 80% confluence were serum-deprived for 48 hr in the presence of 5 mM α-difluoromethyl ornithine (DFMO). Dialyzed fetal bovine serum (10% final concentration) was added to the cultures to initiate growth stimulation. Cells were harvested afterward at various times as indicated.

### In vitro labeling assay of deoxyhypusine-forming activity

At various times after serum stimulation, cells were harvested by washing three times with phosphate-buffered saline (pH 7.2), suspended and homogenized in 0.1 M glycine buffer (pH 9.5) containing 5% glycerol by a brief sonication (5–10 sec) at 4°C. The supernatant fraction was passed through a Sephadex G-50 spun-column to remove small molecules and free polyamines. The eluent from the spun-column was used for polyamine quantitation, protein determination, and in vitro labeling assay. The assay was carried out in a 25-μl of 0.1 M glycine buffer containing 40 μg of cytosolic proteins, 1 mM $NAD^+$, 1 μCi [$^3$H]spermidine, 1 mM dithiothreitol (DTT), and 5% glycerol at 37°C for 2 hr. At the end of incubation, the reaction was terminated by adding 5 μl of SDS-stop solution and heated at 100°C for 3 min. The samples were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography (Dou and Chen, 1990).

### In vitro cross-labeling assay

To determine the deoxyhypusine synthase activity, the exogenously added 21,000-dalton *Neurospora* eIF-5A precursor (Yang et al., 1990) was used as the substrate in the in vitro labeling assay mixture as described above. The labeling intensity of the 21,000-dalton protein band, measured by either densitometric tracing or liquid scintillation counting, was used to represent the deoxyhypusine synthase activity in the sample. Alternatively, deoxyhypusine synthase activity was measured by metal chelate chromatography as described (Tao et al., 1994). The relative amount of eIF-5A precursor in young and old cells was estimated by using heat-treated IMR-90 cell lysates as the substrate for *Neurospora* deoxyhypusine synthase. In this case, the labeling intensity of the 18,000-dalton band represents only the amounts of eIF-5A precursor because the endogenous deoxyhypusine synthase activity has been heat inactivated.

### Western blot analysis

Twenty micrograms of whole cell extracts were resolved in a 4–10% gradient SDS-PAGE, and transferred onto a piece of Immobilon-P transfer membrane (Millipore, Bedford, MA) using Mini Trans-Blot Electrophoresis Transfer Cell (Bio-Rad, Hercules, CA). Nonspecific protein binding sites on the membrane were blocked by incubation with 5% nonfat dry milk. The membrane was probed with polyclonal anti-eIF-5A

*Abbreviations*

| | |
|---|---|
| eIF-5A | eukaryotic initiation factor 5A |
| DFMO | α-difluoromethyl ornithine |
| PDL | population doubling level |

antibody (1:500 dilution). The antigen-antibody complex was detected by enhanced chemiluminescence (ECL, Amersham, Arlington Heights, IL).

### Polyamine quantitation and other biochemical assays

Individual polyamine contents were determined by dansylation procedure and high-performance liquid chromatography (HPLC) analysis as described before (Chen et al., 1983). Protein was determined by a modified Lowry's method using bovine serum albumin as the standard (Bradford, 1976).

## RESULTS

### Hypusine-forming activity in DFMO-treated IMR-90 cells

We have shown previously that metabolic labeling of the 18,000-dalton eIF-5A precursor protein with [$^3$H]-spermidine is about twofold greater in young (PDL = 22) cells than in old cells (PDL = 48) after serum stimulation (Chen and Chang, 1986). Due to the presence of endogenous polyamines and the large accumulation of putrescine in young cells following serum stimulation (Chen et al., 1986), the difference in metabolic labeling may not represent the true difference in hypusine-forming activity between young and old cells. Hypusine-forming activity depends not only on spermidine level, but also on the amounts of eIF-5A precursor and deoxyhypusine synthase activity (Gerner et al., 1986; Park, 1987; Dou and Chen, 1990). Because of tight coupling between eIF-5A precursor biosynthesis and hypusine formation (Park, 1987), little or no eIF-5A precursor could be detected in cultured cells, making it difficult to estimate the overall hypusine-forming activity in living cells. Several studies have demonstrated that depletion of polyamine pools by DFMO causes an uncoupling of eIF-5A biosynthesis and hypusine formation and thus allows direct measurement of hypusine-forming activity by an in vitro labeling assay (Gerner et al., 1986; Park, 1987; Chen and Dou, 1988; Dou and Chen, 1990). We first compared hypusine-forming activity, as determined by radiolabeling of the 18,000-dalton eIF-5A precursor, in young and old cells after their polyamine pools were depleted. Figure 1 shows the time course of hypusine-forming activity in polyamine-depleted young (PDL = 22) and old (PDL = 52) cells following serum stimulation. The results demonstrated that hypusine-forming activity was serum responsive in young cells. The long delay of the appearance of hypusine-forming activity, prominent only after 24–36 hr after serum stimulation, is most likely due to the effect of severe polyamine depletion. In contrast, almost no hypusine-forming activity could be detected in old cells throughout the time course examined. A parallel Commassie blue-stained protein gel of identical samples was included to serve as an internal control and emphasize the dramatic difference in hypusine-forming activity between young and old cells following polyamine depletion. The hypusine-forming activity in young cells, as estimated from radioactive counting of the labeled eIF-5A bands, was more than 30-fold greater than that in old cells.

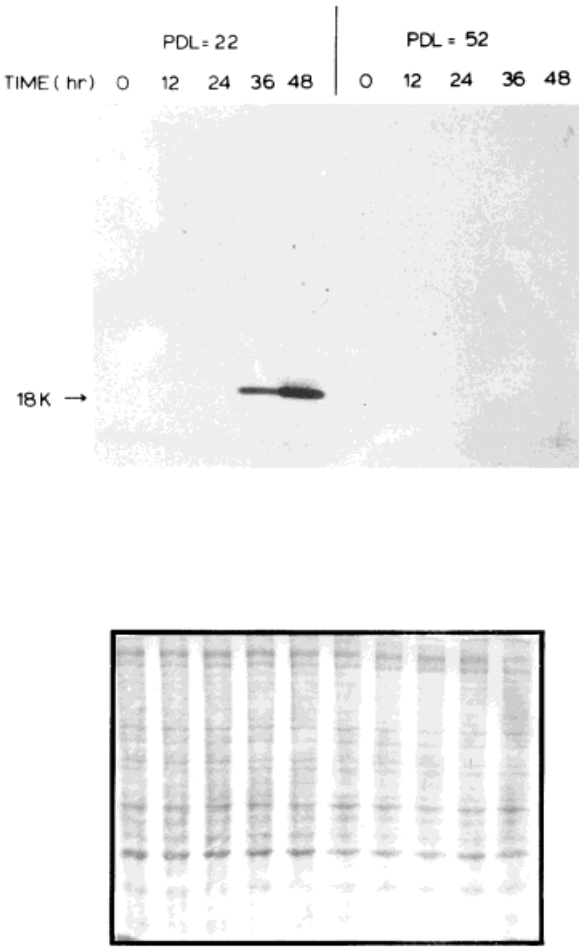


Fig. 1. Hypusine formation in young (PDL = 22) and old (PDL = 52) IMR-90 human diploid fibroblasts. Confluent cultures in both young and old cells were serum deprived for 24 hr followed by incubation with 5 mM DFMO for another 48 hr. Cultures were stimulated with 20% dialyzed fetal bovine serum for various times as indicated. Cells were then harvested for in vitro labeling assay. Each assay contained 75 μg cytosolic proteins, 1 mM $NAD^+$, 5 μCi [$^3$H]spermidine in a total volume of 100 μl (0.1 M glycine-NaOH, pH 9.5) and the reaction was carried out at 37°C for 5 hr. The reaction mixtures were analyzed by SDS-PAGE and fluorography on a 12% gel (Top panel). Each lane contained 50 μg of protein. The lower panel was a Commassie blue-stained gel pattern of the same samples except that each lane contained 25 μg of protein.

### Deoxyhypusine synthase and substrate activity in DFMO-treated cells

Hypusine-forming activity as determined by in vitro labeling assay (Fig. 1) is a function of the amounts of substrate (i.e., eIF-5A precursor) and deoxyhypusine synthase activity. Thus, the difference in hypusine-forming activity observed in Fig. 1 could be due to difference in either one or both of these two variables. The eIF-5A precursor in *Neurospora crassa* has an apparent molecular mass of 21,000- dalton, instead of 18,000-dalton as seen in all mammalian cells examined (Yang et al., 1990). Both of the 21,000-dalton and the 18,000-dalton eIF-5A precursor are heat stable and can serve as a substrate for deoxyhypusine synthase from either animal or fungal source (Dou and Chen, 1990). Taking

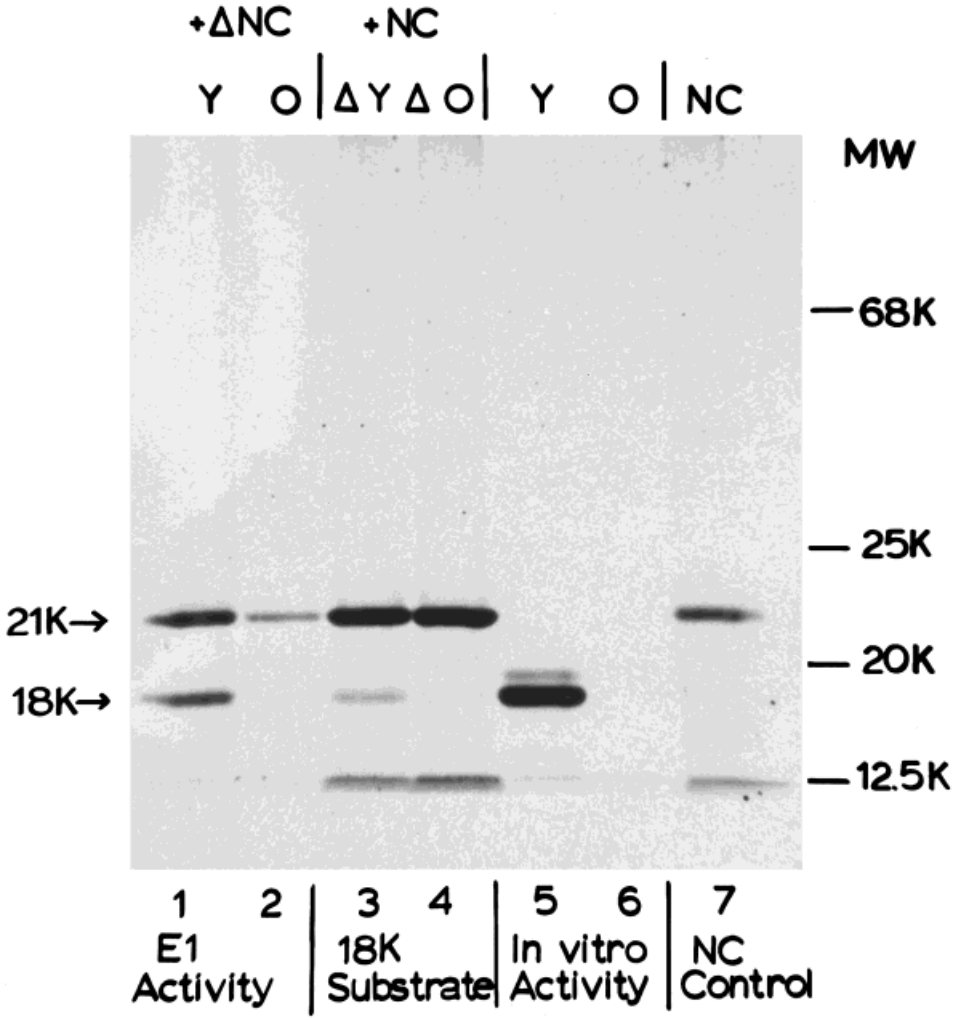


Fig. 2. Deoxyhypusine synthase and eIF-5A precursor in IMR-90 cells. Both young (PDL = 23) and old (PDL = 52) cells at confluent state were serum deprived for 24 hr. Cells were treated with 5 mM DFMO in fresh Dulbecco's medium for another 48 hr. During this period, cells were stimulated with 20% dialyzed fetal bovine serum for 36 hr. Cells were then harvested for in vitro cross-labeling assay in a glycine buffer (0.1 M, pH 9.5) as described in Materials and Methods. Heat-treated *Neurospora* lysates (ΔNC) were used as the source for the 21,000-dalton eIF-5A precursor. Deoxyhypusine synthase in *Neurospora* lysates (NC) were used to label the 18,000-dalton eIF-5A precursor in heat-treated IMR-90 cell lysates. Cross-labeling was initiated by adding [$^3$H]spermidine to the mixture of 75 μg of IMR-90 cell lysates plus 75 μg of heat-treated *N. crassa* lysates (lanes 1 and 2) or to the mixture of 75 μg of heat-treated IMR-90 cell lysates plus 75 μg of *N. crassa* lysates (lanes 3 and 4). The reaction was carried out at 37°C for 4 hr. In vitro labeling assay was carried out using either 75 μg of IMR-90 cell lysate (lanes 5 and 6) or *N. crassa* lysates (lane 7). All reaction mixtures were analyzed by SDS-PAGE (12% acrylamide) and fluorography. Each lane contained 75 μg of protein. Y, young IMR-90 cells (PDL = 22); O, old IMR-90 cells (PDL = 52); NC, *N. crassa,* aga strain; Δ, heat treated at 85°C for 6 min.

advantage of these findings we have developed an in vitro cross-labeling assay that allows us to measure the levels of eIF-5A precursor and deoxyhypusine synthase simultaneously in one sample. In this assay, the labeling of an exogenously added 21,000-dalton eIF-5A precursor with [$^3$H]spermidine in IMR-90 cell lysates was used to estimate the deoxyhypusine synthase activity in human cells, whereas the labeling of the endogenous 18,000-dalton eIF-5A precursor in heat-treated IMR-90 cell lysates by exogenous added deoxyhypusine synthase was used to estimate the amounts of substrate. Using this assay, we showed in Fig. 2 that (i) the level of deoxyhypusine synthase activity in young and old cells differed by at least fivefold (lane 1 vs. lane 2) and (ii) the eIF-5A precursor in young cells was about tenfold higher than that in old cells (lane 3 vs. lane 4). Taken together, the decreases in both deoxyhypusine synthase activity and eIF-5A precursor in old cells were sufficient to account for a striking difference in hypusine-forming activity between young and old cells (lane 5 vs. lane 6). It can be noted that the labeling intensity of the 18,000-dalton band by *Neurospora* deoxyhypusine synthase (Fig. 2, lane 3) was lower than that by human enzyme (Fig. 2, lane 1). This is most likely due to the fact that the optimal reaction temperature for *Neurospora* enzyme was ~25°C instead of 37°C (Tao and Chen, 1995).

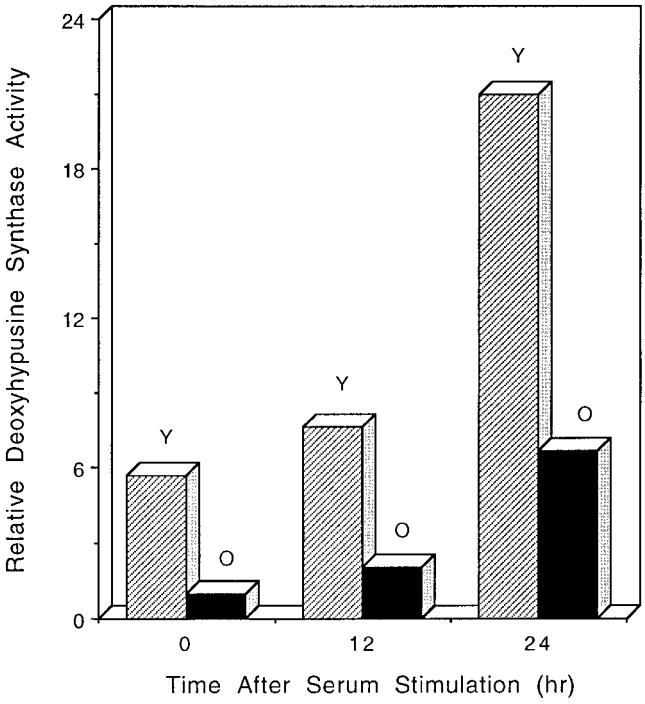


Fig. 3. Deoxyhypusine synthase activity in serum-stimulated young and old IMR-90 cells. Confluent cultures of young (Y, PDL = 22) and old (O, PDL = 52) IMR-90 cells were serum deprived for 48 hr. Cells were then stimulated by fresh dialyzed fetal bovine serum (20%) to enter the cell cycle. The G-50 lysates were prepared from cultures at various times after serum stimulation. *Neurospora* eIF-5A precursor protein (21,000-dalton) was used as the substrate. The reaction mixture contained 75 μg IMR-90 cell lysates, 2 μg *Neurospora* eIF-5A precursor, 1 mM $NAD^+$, 5 μCi [$^3$H]spermidine in a total volume of 100 μl. The reaction mixtures (100 μl) were analyzed by SDS-PAGE and fluorography. Densitometric tracing of the labeled 21,000-dalton band was used to estimate the relative deoxyhypusine synthase activity. The deoxyhypusine synthase activity in the old cells at time zero was taken as one for comparative purpose.

### Deoxyhypusine synthase activity and the expression of eIF-5A gene

To ascertain that the difference in enzyme activities that we observed is not an artifact caused by DFMO-treatment, we have measured also deoxyhypusine synthase activity in IMR-90 cells without prior DFMO treatment. Figure 3 shows that serum stimulation induced an increase in deoxyhypusine synthase activity in both young and old cells. The magnitude of deoxyhypusine synthase activity in young cells was three- to fivefold greater than that in old cells throughout the time course of serum stimulation. Similar results were obtained when deoxyhypusine synthase was measured by metal chelate affinity chromatographic assay (data not shown). These results indicated that deoxyhypusine synthase activity was serum inducible and that there was a three- to fivefold decrease in deoxyhypusine synthase activity in IMR-90 cells during senescence with or without prior polyamine depletion. Although we cannot determine hypusine-forming activity in cells without prior DFMO treatment (Gerner et al., 1986; Park, 1987; Chen and Dou, 1988; Dou and Chen, 1990), we can still measure the eIF-5A at both mRNA and protein level in these cells. Figure 4A shows the eIF-5A mRNA levels were significantly enhanced in both young and old cells within 12 hr after serum stimulation, suggesting that the expression of eIF-5A gene at mRNA level was a mid-G1 event and appeared to be age independent. In contrast, the late G1/S genes such as thymidine kinase, dihydrofolate reductase, and thymidylate synthase were not expressed at all in the old cells even after serum stimulation (data not shown), as we have reported previously (Pang and Chen, 1994). Quantitation of the hybridized bands indicated that the eIF-5A mRNA level in young cells was only slightly higher than that in old cells during serum stimulation. Figure 4B shows that the eIF-5A protein level was also serum inducible in both young and old cells, but the amount of eIF-5A protein in young cells was at least threefold higher than that in old cells 24 hr after serum stimulation. Because of the long half-life of eIF-5A protein in mammalian cells (Dou and Chen, 1990; Park et al., 1993), the eIF-5A protein level, although low, was still detectable in young IMR-90 cells at quiescent state (Fig. 4B, time 0). However, incubation of IMR-90 cells in serum-free medium for a longer period (>72 hr) could result in almost complete disappearance of eIF-5A (data not shown). Taken together, the results shown in Fig. 4 suggested that the age-dependent decrease in the eIF-5A protein level is likely controlled at posttranscriptional or translational/posttranslational level.

## DISCUSSION

Table 1 summarizes our studies on the comparison of the hypusine-forming activities in young and old human cells, with or without prior polyamine depletion. These results indicate: (i) there exists a striking attenuation of hypusine-forming activity in IMR-90 cells during cell senescence (Fig. 1), (ii) the decreases in deoxyhypusine synthase activity and the amount of eIF-5A precursor were sufficient to account for the attenuation of hypusine-forming activity in old cells (Figs. 2 and 3), (iii) the induction of eIF-5A gene at mRNA level appeared to be a mid-G1 event and this induction did not exhibit any significant age-dependent attenuation (Fig. 4A), and (iv) the age-dependent decrease in the amount of eIF-5A, either the precursor form or the mature form, was likely to be regulated at translational/posttranslational level (Fig. 4B). The significant reduction of hypusine-forming activity in cells during senescence is consistent with the notion that hypusine may have a role in controlling cell replication (Park et al., 1993) and cell differentiation program (Chen et al., 1996). However, the precise physiological function of eIF-5A in cell proliferation, differentiation, and senescence remains to be investigated.

Although eIF-5A was considered initially as a protein translation initiator (Kemper et al., 1976), more recent work has demonstrated that depletion of eIF-5A in yeast does not affect general protein synthesis (Kang and Hershey, 1994), suggesting that translation initiation may not be the true function of eIF-5A. Recent findings that eIF-5A interacts with Rev, an HIV-1 viral mRNA binding protein (Bevec et al., 1996), and Rex, an HLTV-1 viral mRNA binding protein (Katahira et al., 1995), suggest that eIF-5A may be involved in pre-mRNA processing. It is possible that eIF-5A may interact with certain cellular RNA binding proteins that

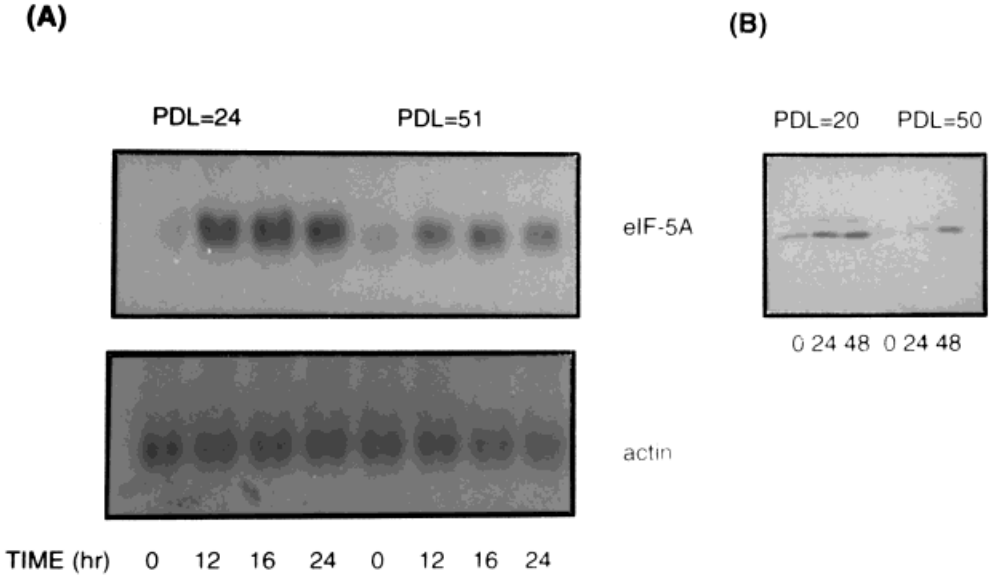


Fig. 4. Expression of eIF-5A gene in IMR-90 cells. (**A**) Northern blot analysis. Both young (PDL = 24) and old (PDL = 51) cells at confluent state were serum deprived for 48 hr. Cells were then stimulated to re-enter cell cycle by fresh fetal bovine serum (20%). Total RNA was isolated from the young and old IMR-90 cells at indicated time for Northern blot hybridization using $^{32}$P-labeled eIF-5A cDNA as the probe. The same membrane was rehybridized with $^{32}$P-labeled β-actin cDNA as an internal control. Each lane contained 20 μg of total cellular RNA. (**B**) Western blot analysis. Confluent cells were serum deprived for 48 hr and then serum stimulated for various time. Cells were harvested and whole cell extracts were prepared at indicated time for SDS-PAGE and Western blot analysis using anti-*Neurospora* eIF-5A at 500-fold dilution. Each lane contained 25 μg of cell extract protein.

TABLE 1. Comparison of the biochemistry of hypusine formation in young and old IMR-90 human diploid fibroblasts with and without prior DFMO treatment

| | −DFMO | | +DFMO | |
|---|---|---|---|---|
| | Young | Old | Young | Old |
| Hypusine-forming activity[1] | —[6] | — | >30[7] | 1 |
| Deoxyhypusine synthase[2] | 3 | 1 | 5 | 1 |
| eIF-5A mRNA[3] | 1.3 | 1 | 1.3 | 1 |
| eIF-5A precursor[4] | — | — | 10 | 1 |
| eIF-5A[5] | 5 | 1 | n.d.[8] | n.d. |

[1]Hypusine-forming activity at 36 hr after serum stimulation was compared.
[2]Deoxyhypusine synthase activity measured at 24 and 36 hr, respectively, after serum stimulation for cells without and with prior DFMO treatment.
[3]eIF-5A mRNA at 24 hr after serum stimulation was compared.
[4]eIF-5A precursor at 36 hr after serum stimulation was compared.
[5]eIF-5A at 24 hr after serum stimulation was compared.
[6]—, undetectable.
[7]The number indicates relative value for each parameter in young cells as compared with old cells.
[8]n.d. = not determined.

resemble Rev/Rex under normal physiological conditions. It is therefore possible that eIF-5A, though not essential for general protein synthesis, could still be involved in the translation of a subset of special mRNAs (Kang and Hershey, 1994). Indeed, it has been suggested that eIF-5A, with its unique hypusine residue, may be required for the expression of a small number of key growth-related genes (Kang and Hershey, 1994; Hanauske-Abel et al., 1995). Recent studies that eIF-5A is essential for cell survival (Schnier et al., 1991), and that inhibition of hypusine formation leads to either growth arrest (Park et al., 1993; Chen et al., 1996; Shi et al., 1996) or changes in differentiation program (Chen et al., 1996) also support the notion that, in addition to being essential for cell survival, eIF-5A plays an important role in growth regulation. Our studies showed that eIF-5A gene expression appears to be a mid-G1 event and that the age-dependent attenuation of eIF-5A may occur translationally or posttranslationally at mid-G1 phase. Thus, if eIF-5A may affect certain gene expression as suggested previously, then the target candidates are likely to be late G1/S genes, particularly those that are essential for DNA replication. If this is the case, it will be tempting to propose that the striking age-dependent attenuation of hypusine-forming activity in normal cells may have some causal relationship to the global attenuation of cell cycle-associated gene expression at late G1/S boundary during cellular senescence (Pang and Chen, 1994).

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## LITERATURE CITED

Bevec, D., Jaksche, H., Oft, M., Wohl, T., Himmelspach, M., Pacher, A., Schebesta, M., Koettnitz, K., Dobrovnik, M., Csonga, R., Lottspeich, F., and Hauber, J. (1996) Inhibition of HIV-1 replication in lymphocytes by mutants of the Rev cofactor eIF-5A. Science, *271:*1858–1860.

Boynton, A.L., Whitfield, J.F., and Isaacs, R.J. (1976) A possible involvement polyamines in the initiation of DNA synthesis by hu-

man WI-38 and mouse BALB/3T3 cells. J. Cell. Physiol., *89:*481–488.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., *72:*248–254.

Campisi, J., Dimri., G.P., and Hara, E. (1995) Control of replicative senescence. In: Handbook of the Biology of Aging. E. Schneider and J. Rowe, eds. Academic Press, New York, pp. 121–149.

Chang, Z.F., and Chen, K.Y. (1988) Regulation of ornithine decarboxylase and other cell cycle-dependent genes during senescence of IMR-90 human diploid fibroblasts. J. Biol. Chem., *263:*11431–11435.

Chen, K.Y. (1983) An 18,000-dalton protein metabolically labeled by polyamines in various mammalian cell lines. Biochim. Biophys. Acta, *756:*395–402.

Chen, K.Y., and Chang, Z.F. (1986) Age dependency of the metabolic conversion of polyamines into amino acids in IMR-90 human embryonic lung diploid fibroblasts. J. Cell. Physiol., *128:*27–32.

Chen, K.Y., and Dou, Q.P. (1988) $NAD^+$ stimulated the spermidine-dependent hypusine formation on the 18 kDa protein in cytosolic lysates derived from NB-15 mouse neuroblastoma cells. FEBS Lett., *229:*325–328.

Chen, K.Y., Nau, D., and Liu, A.Y.-C. (1983) Effect of inhibitors of ornithine decarboxylase on the differentiation of mouse neuroblastoma cells. Cancer Res., *43:*2812–2818.

Chen, K.Y., Chang, Z.F., and Liu, A.Y.-C. (1986) Changes of serum-induced ornithine decarboxylase activity and putrescine content during aging of IMR-90 human diploid fibroblasts. J. Cell. Physiol., *129:*142–146.

Chen, K.Y., Chang, Z-F., Pang, J.H., He, G.S., and Liu, A.Y.-C. (1989) Polyamine metabolism and cell cycle-dependent gene expression in IMR-90 human diploid fibroblasts during senescence in culture. Exp. Gerontol., *24:*523–537.

Chen, Z.P., Yan, Y.P., Ding, Q., Knapp, S., Potenza, J.A., Schugar, H.J., and Chen, K.Y. (1996) Effects of inhibitors of deoxyhypusine synthase on the differentiation of mouse neuroblastoma and erythroleukemia cells. Cancer Lett., *105:*233–239.

Cristofalo, V.J., and Sharf, B.B. (1973) Cellular senescence and DNA synthesis. Exp. Cell Res., *76:*419–427.

Cohen, S.S. (1971) Introduction to Polyamines. Prentice-Hall, Englewood Cliffs, NJ, pp. 1–179.

Cooper, H.L., Park, M.H., and Folk, J.E. (1982) Posttranslational formation of hypusine in a single major protein occurs generally in growing cells and is associated with activation of lymphocyte growth. Cell, *29:*791–797.

Dou, Q.P., and Chen, K.Y. (1990) Characterization and reconstitution of cell free system for NAD+-dependent deoxyhypusine formation on the 18kDa eIF-4D precursor. Biochim. Biophys. Acta, *1036:*128–137.

Gerner, E.W., Mamont, P.S., Bernhardt, A., and Siat, M. (1986) Post-translational modification of the protein-synthesis initiation factor eIF-4D by spermidine in rat hepatoma cells. Biochem. J., *239:*379–386.

Hayflick, L., and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res., *25:*585–621.

Hanauske-Abel, H.M., Slowinska, B., Zagulska, S., Wilson, R.C., Staiano-Coico, L., Hanauske, A.R., McCaffrey, T., and Szabo, O. (1995) Detection of a sub-set of polysomal mRNAs associated with modulation of hypusine formation at the G1-S boundary proposal of a role for eIF-5A in onset of DNA replication. FEBS Lett., *366:*92–98.

Kang, H.A., and Hershey, J.W.B. (1994) Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae.* J. Biol. Chem., *269:*3934–3940.

Kemper, W.M., Berry, K.W., and Merrick, W.C. (1976) Purification and properties of rabbit reticulocyte protein synthesis initiation factors M2α and M2β. J. Biol. Chem., *251:*5551–5557.

Katahira, J., Ishizaki, T., Sakai, H., Adachi, A., Yamamoto, K., and Shida, H. (1995) Effects of translational initiation factor eIF-5A on the functioning of human T-cell leukemia virus type I Rex and human immunodeficiency virus Rev inhibited trans-dominantly by a Rex mutant deficient in RNA binding. J. Virol., *69:*3125–3133.

Pang, J.P., and Chen, K.Y. (1993) A specific CCAAT-binding protein, CBP/tk, may be involved in the regulation of thymidine kinase gene expression in human IMR-90 diploid fibroblasts during senescence. J. Biol. Chem., *268:*2909–2916.

Pang, J.P., and Chen, K.Y. (1994) Global change of gene expression at late G1/S boundary may occur in human IMR-90 diploid fibroblasts during senescence. J. Cell. Physiol., *160:*531–538.

Park, M.H. (1987) Regulation of biosynthesis of hypusine in Chinese hamster ovary cells. J. Biol. Chem., *262:*12730–12734.

Park, M.H., and Wolff, E.C. (1988) Cell-free synthesis of deoxyhypusine. J. Biol. Chem., *263:*15264–15269.

Park, M.H., Liberato, D.J., Yergey, A.L., and Folk, J.E. (1984) The biosynthesis of hypusine ($N^{\varepsilon}$-(4-amino-2-hydroxybutyl)lysine). J. Biol. Chem., *259:*12123–12127.

Park, M.H., Wolff, E.C., and Folk, J.E. (1993) Is hypusine essential for eukaryotic cell proliferation? TIBS, *18:*475–479.

Park, M.H., Wolff, E.C., Lee, Y.B., and Folk, J.E. (1994) Antiproliferative effects of inhibitors of deoxyhypusine synthase. J. Biol. Chem., *269:*27827–27832.

Ruhl, M., Himmelspach, M., Bahr, G.M., Himmerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Farrington, G.K., Probst, H., Bevec, D., and Hauber, J. (1993) Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type I Rev activation domain mediating trans-activation. J. Cell Biol., *123:*1309–1320.

Sasaki, K., Abid, M.R., and Miyazaki, M. (1996) Deoxyhypusine synthase gene is essential for cell viability in the yeast Saccharomyces cerevisiae. FEBS Lett., *384:*151–154.

Schnier, J., Schwelberger, H.G., Smit-McBride, Z., Kang, H.A., and Hershey, J.W.B. (1991) Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast *Saccharomyces cerevisiae.* Mol. Cell. Biol., *11:*3105–3114.

Seshadri, T., and Campisi, J. (1990) Repression of *c-fos* transcription and an altered genetic program in senescent human fibroblasts. Science, *247:*205–209.

Shi, X.P., Yin, K.C., Ahern, J., Davis, L.J., Stern, A.M., and Waxman, L. (1996) Effects of N1-guanyl-1,7-diaminoheptane, an inhibitor of deoxyhypusine synthase, on the growth of tumorigenic cell line in culture. Biochim. Biophys. Acta, *1310:*119–126.

Shiba, T., Mizote, H., Kaneko, T., Nakajima, T., and Kakimoto, Y. (1971) Hypusine, a new amino acid occurring in bovine brain: Isolation and structural determination. Biochim. Biophys. Acta, *244:*523–531.

Smith, J.R., and Pereira-Smith, O.M. (1996) Replicative senescence: implications for in vivo aging and tumor suppression. Science, *273:*63–67.

Stein, G.H., Drulliner, L.F., Robetorye, R.S., Pereira-Smith, O.M., and Smith, J.R. (1991) Senescent cells fail to express cdc2, cycA, and cycB in response to mitogen stimulation. Proc. Natl. Acad. Sci. U.S.A., *88:*11012–11016.

Sunkara, P.S., Pargac, M.B., Nishioka, K., and Rao, P.N. (1979) Differential effects of inhibition of polyamine biosynthesis on cell cycle traverse and structure of the prematurely condensed chromosomes of normal and transformed cells. J. Cell. Physiol., *98:*451–457.

Tabor, C.W., and Tabor, H. (1984) Polyamines. Annu. Rev. Biochem., *53:*749–790.

Tao, Y., and Chen, K.Y. (1995) Purification of deoxyhypusine synthase from Neurospora crassa to homogeneity by substrate elution affinity chromatography. J. Biol. Chem., *270:*383–386.

Tao, Y., Skrenta, H.M., and Chen, K.Y. (1994) Deoxyhypusine synthase assay based on the use of polyhistidine-tagged substrate and metal chelate-affinity chromatography. Anal. Biochem., *221:*103–108.

Yang, Y.C., Chen, K.Y., Seyfzadeh, M., and Davis, R.H. (1990) Deoxyhypusine/hypusine formation on a 21,000-dalton cellular protein in a Neurospora crassa mutant in vivo and in vitro. Biochim. Biophys. Acta, *1033:*133–138.