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POLYAMINE METABOLISM AND CELL-CYCLE-DEPENDENT GENE EXPRESSION IN IMR-90 HUMAN DIPLOID FIBROBLASTS DURING SENESCENCE IN CULTURE

KUANG YU CHEN,[1,2] ZEE-FEN CHANG,[2] JONG-HWEI PANG,[2] GUO-SHUN HE[1] and ALICE Y-C LIU[3]

[1]Department of Chemistry, [2]Department of Biochemistry and [3]Department of Biological Sciences, Rutgers — The State University of New Jersey, New Brunswick, New Jersey 08903

Abstract — Aging of IMR-90 human diploid fibroblasts in culture is accompanied by specific changes of polyamine metabolism including: (a) a fivefold decrease of serum-induced activity of ornithine decarboxylase (ODC[1] EC 4.1.1.17); (b) a six to tenfold increase of polyamine catabolism; and (c) a reduction of putrescine uptake. These changes apparently led to a significant reduction of putrescine accumulation in senescent cells following serum stimulation. Since the induction of ODC is a mid-G_1 event, the change of polyamine metabolism may be related to changes of expression of other cell-cycle-dependent genes during cellular aging. In addition to ODC gene, we have examined the expression of two early G_1 genes, *c-erb*B and *c-myc*, and one late G_1/S gene thymidine kinase, at mRNA levels, in both young and old IMR-90 cells. We have also compared the enzyme activities of two late G_1/S genes, thymidine kinase and thymidylate synthetase, in young and old cells following serum stimulation. We did not observe significant changes of *c-erb*B, *c-myc*, and ODC mRNA levels during cellular senescence. However, we found that serum-induced mRNA level of thymidine kinase gene in old IMR-90 cells was significantly reduced compared to that in the young cells. Results also demonstrate that aging of IMR-90 cells was accompanied by significant decrease of both thymidine kinase and thymidylate synthetase activities. In view of the recognized importance of polyamines in growth regulation, it is possible that alteration of polyamine metabolism may contribute to the impairment of expression of some key G_1/S genes and such impairment may contribute to the ultimate loss of dividing potential in senescent cells.

Key Words: polyamines, ornithine decarboxylase, *c-myc*, thymidine kinase, cell cycle, cellular aging

INTRODUCTION

POLYAMINES ARE naturally occurring cations widely distributed in living organisms. Putrescine $[NH_2(CH_2)_4NH_2]$, spermidine $[NH_2(CH_2)_4NH(CH_2)_3NH_2]$, and spermine

Correspondence to: K.Y. Chen.

Abbreviations used: ODC, ornithine deacarboxylase; PDL, population doubling level; TK, thymidine kinase; DFMO, -α- difluromethyl ornithine.

$[NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2]$ constitute the polyamine pool in mammalian cells. Abundant literature evidence suggests that polyamines play an important role in growth regulation (for reviews see Tabor and Tabor, 1976, 1984). Ornithine decarboxylase (ODC, EC 4.1.1.17) is the initial and rate-controlling enzyme for the biosynthesis of polyamines in mammalian cells (Pegg and Williams-Ashman, 1969). Mammalian ODC activity increases dramatically and early in response to a whole array of hormonal, developmental, and growth-related stimulation (e.g., Russell and Durie, 1978; Canellakis *et al.*, 1979; Tabor and Tabor, 1984). Increases in ODC activity and polyamine accumulation generally precede DNA synthesis (Heby *et al.*, 1975; Boynton *et al.*, 1976). Inhibition of ODC induction and polyamine biosynthesis by specific inhibitors such as α-difluoromethyl ornithine (DFMO) and methylglyoxal bis- (guanylhydrazone) (MGBG) invariably leads to reduced DNA synthesis and growth cessation (Prakash *et al.*, 1980; Poso and Pegg, 1982). That polyamines may be directly linked to DNA synthesis is further suggested by the observation that exogenously added polyamines can reverse the inhibitory action of these inhibitors on DNA synthesis (Boynton *et al.*, 1976; Poso and Pegg, 1982; Cheetham and Bellet, 1982).

The hallmark of cellular aging is the failure of senescent cells to synthesize DNA (or to enter S phase of the cell cycle) and thus loss of dividing potential in these cells (Hayflick, 1965; Cristofalo and Sharf, 1973; Hayflick, 1979). In view of the importance of ODC/polyamines in growth regulation and their possible involvement in the regulation of DNA synthesis, it is reasonable to speculate that changes in polyamine metabolism may occur during senescence of human diploid fibroblasts and that these changes may actually contribute to the senescence of cells.

Polyamine metabolism involves complicated pathways (Tabor and Tabor, 1984; see Fig. 1). To investigate the role of polyamines in cellular aging, we have focused on three aspects of polyamine metabolism, namely, the regulation of the ODC gene, the biodegradation of putrescine, and polyamine transport.

ODC regulation

Recent development of methods to specifically label the ODC molecule with [^{3}H]DFMO, an enzyme-activated irreversible inhibitor of ODC (Pritchard et al., 1981), the construction of cDNA probes for ODC mRNA (McConlogue *et al.*, 1984; Kontula *et al.*, 1984; Kahana and Nathans, 1984) and the availability of both monoclonal (Pegg *et al.et al.*, 1984) and polyclonal antibodies (Persson, 1981; Isomaa *et al.*, 1983) against ODC has made it possible for us to study the regulation of ODC gene at both the transcriptional and translational level in IMR-90 human diploid fibroblasts as a function of their PDL[1].

Polyamine degradation

Most of the studies on polyamine metabolism emphasize biosynthesis rather than biodegradation. Thus, the physiological function of polyamine biodegradation is largely unclear. In mammalian cells, putrescine can be oxidized and converted to GABA. GABA can then be channeled into the Krebs cycle and form various amino acids (Fig. 1). Thus the amino acid pool in a cell can become radioactively labeled if [^{3}H] or [^{14}C]putrescine is added to the cell. General protein synthesis using these radioactive amino acids can be taken as an indirect measurement of the degree of putrescine degradation (Chen and Liu, 1981).

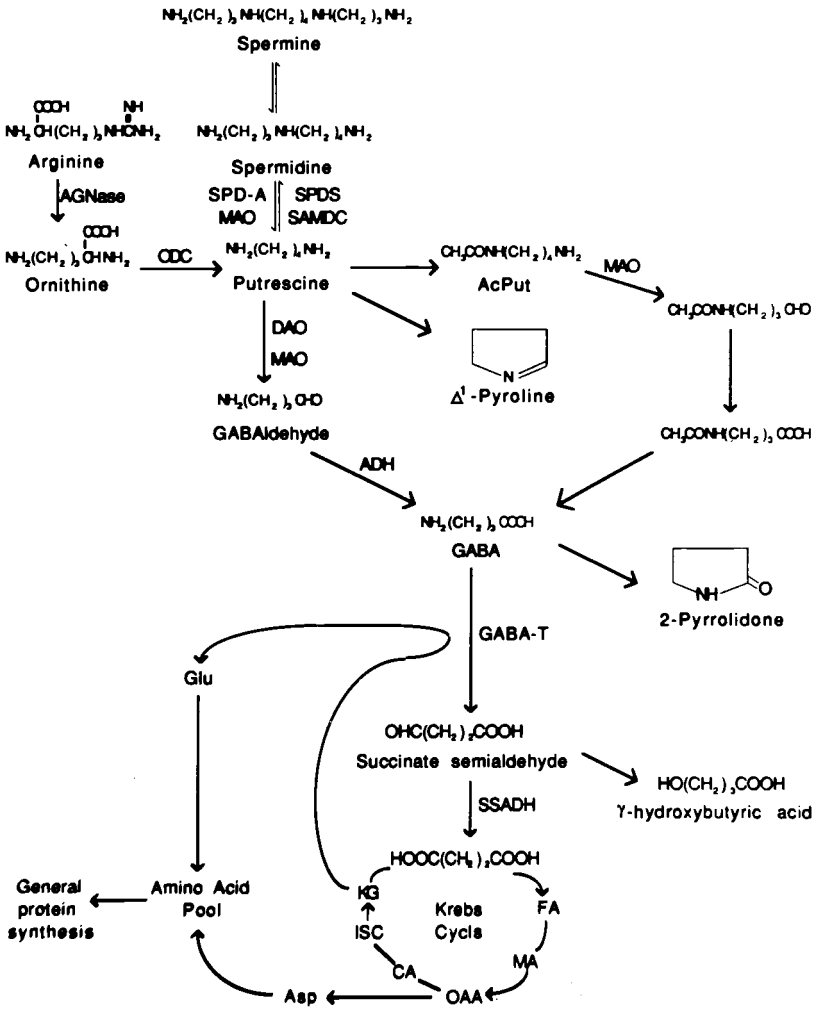

FIG. 1. Polyamine metabolic pathway. ODC, ornithine decarboxylase; AGNase, arginase; MAO, monoamine oxidase; DAO, diamine oxidase; SPDS, spermidine synthase; SAMDC, S-adenosyl methionine decarboxylase; SPD-A, spermidine acetylase; AcPut, acetyl putrescine; GABA-T, GABA transaminase; SSADH, succinate semialdehyde dehydrogenase; ADH, aldehyde dehydrogenase.

Polyamine transport

We have previously identified and characterized a unique polyamine transporter system in mouse neuroblastoma cells (Rinehart and Chen, 1984). Due to the presence of polyamines in serum, polyamine transport may play a role in regulating the polyamine content in cells.

Once the differences in polyamine metabolism between young and senescent cells are established, it is logical to ask whether and how these differences contribute to the loss of dividing potential in human diploid fibroblasts during aging. Because the induction of ODC activity is a mid-G_1 event during cell cycling, one approach is to examine whether other

cell-cycle-dependent genes change their expression at either mRNA or protein level during cell senescence and to investigate how these changes are related to changes of polyamine metabolism. Therefore, we have examined the expression, at the mRNA level, of two early G_1 genes *c-erb*B and *c-myc*, a mid-G_1 gene ODC, and a late G_1/S gene thymidine kinase (TK) in IMR-90 cells during cellular aging. At the enzyme level, we have determined activities of thymidine kinase and thymidylate synthetase in addition to ODC.

MATERIALS AND METHODS

Materials

All tissue culture media and sera were obtained from Gibco, Grand Island, NY. [α-^{32}P]dCTP (>3000 Ci/mmol), [2-^{14}C]thymidine (60 mCi/mmol), [5-^{3}H]dUMP (10 Ci/mmol), and [1,4-^{14}C]putrescine 2HC1 (122 mCi/mmol) were purchased from Amersham, Arlington Heights, IL. The cloned human *c-myc* gene was a gift of Dr. P. Leder (Harvard Medical School). Dr. P.L. Deininger (Louisiana State University Medical Center, New Orleans, LA), Dr. Olle Janne (Rockfeller University, NY), and Dr. L.F. Johnson (Ohio State University) provided plasmids, pTK11, pODC54, and pTS8, respectively.

Cell Culture

Low passage IMR-90 human embryonic lung diploid fibroblasts (passage 5, PDL[1] = 12) and progeria strains were obtained from Coriell Institute for Medical Research, Camden, NJ. The conditions of growth and serum stimulation have been described previously (Chen *et al.*, 1986; Chang and Chen, 1988).

Isolation of Poly(A) $^{+}$RNA and Northern Blot Hybridization

The detailed procedures for the preparation of poly(A) $^{+}$RNA from both young and old IMR-90 cells and Northern blot analysis have been reported (Chang and Chen, 1988). For all the Northern blot analyses, β-actin probe was also used as an internal control.

Enzyme assays

The activity of ornithine decarboxylase was determined according to a procedure previously described (Chen and Canellakis, 1977). Thymidine kinase activity was determined according to procedures reported by Bradshaw (1983). Thymidylate synthetase activity was measured as described by Robert (1966).

Polyamine transport

The rate of putrescine uptake was determined by procedures previously described (Rinehart and Chen, 1984).

RESULTS

Regulation of ornithine decarboxylase gene in IMR-90 cells

Confluent monolayer cultures of IMR-90 cells at different PDL were serum-deprived for at

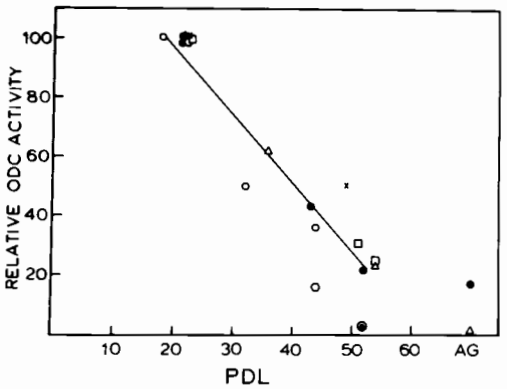

FIG. 2. Inverse linear relationship between serum-induced peak ODC activity and PDL of IMR-90 human diploid fibroblasts. Data from seven separate experiments, indicated by different symbols, are expressed as percentage of maximal ODC activity in young cells (PDL 18~23). Each data point represents an average of duplicate assay with an error of less than 5%. The peak ODC activities of progeria AG1178 cells (●) and AG1139 cells (△) were also included as data points and are located above AG on the PDL coordinate.

least 24 h to ensure quiescent state. Cells were then stimulated to re-enter the cell cycle by replenishing the exhausted medium with fresh Dulbecco's medium containing 10% fetal bovine serum. Maximal ODC activity was induced 8 to 10 h after serum addition, whereas the peak activity of DNA synthesis, measured by thymidine incorporation, occurred 22 to 24 h after serum stimulation (Chen *et al.*, 1986). Whereas the kinetics of ODC induction was the same for IMR-90 cells at different PDL's, and for two progeria cell lines derived from patients with Hutchinson–Gilford syndrome, the magnitude of induced ODC activity was clearly PDL-dependent. Figure 2 summarizes the results from seven separate experiments on ODC induction in IMR-90 cells at early, middle and late passages and in progeria cells (AG 1178 strain and AG 3198 strain). The peak ODC activity was inversely related to the PDL of the culture. We have employed Northern blot analysis to compare the ODC mRNA levels between young (PDL = 22) and old (PDL = 52) IMR-90 cells. Results from several experiments show that ODC mRNA (size 2.3 kb) was serum-inducible, and there was no difference in ODC mRNA levels between young and old IMR-90 cells (Fig. 3; also see Chang and Chen, 1988). Using affinity labeling technique (Erwin *et al.*, 1983), we have demonstrated a threefold decrease of active ODC molecules in old IMR-90 cells as compared to young cell (Chang and Chen, 1988). These data suggest that the decrease of ODC activity in old IMR-90 cells is due to alterations of control at the translational and/or posttranslational level. In accordance with this notion, we have found that the half-life of ODC activity in young IMR-90 cells is more than twofold longer than that in the old cells (Fig. 4). Pulse-chase labeling experiments using monospecific anti-ODC antiserum have further confirmed that the newly synthesized ODC molecules were more stable in young cells as compared to that in old cells (Data not shown).

Polyamine biodegradation

As shown in Fig. 1, putrescine can be oxidized to GABA which can then be channeled through GABA shunt into Krebs cycle. Such catabolic conversion can lead to amino acid

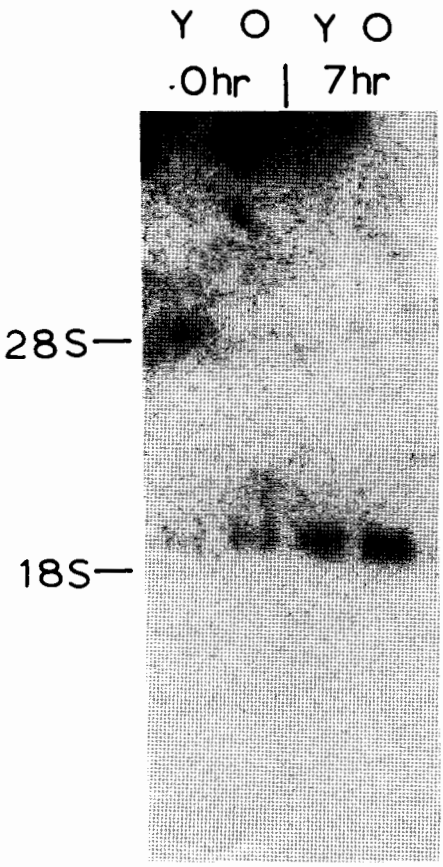

FIG. 3. Effect of serum stimulation on the induction of ornithine decarboxylase mRNA in young (PDL = 22); and senescent (PDL = 52) IMR-90 cells. Quiescent cultures of young cells (Y) and old cells (O) were serum-stimulated for 7 h. Poly(A) $^{+}$RNA was isolated and fractionated on a formaldehyde-agarose gel, blotted, and hybridized with ^{32}P-labeled plasmid pODC54 as described previously (Chang and Chen, 1988). The specific activity of the labeled probe was about 1×10^8 cpm/μg DNA. The radioactivity was visualized by autoradiography.

formation using carbon skeleton derived from putrescine. Since the amino acids formed can be utilized for general protein synthesis, the specific radioactivity of labeled cellular proteins (or acid-insoluble radioactivity), using [^{14}C]putrescine as a precursor, provides a convenient means for estimating the degree of polyamine biodegradation. Figure 5 illustrates that, using this approach, we have found that putrescine catabolism increased by more than eightfold in old IMR-90 cells (also see Chen and Chang, 1986).

Polyamine transport

The polyamine transport system, ubiquitously present in mammalian cells, appears to be responsive to growth stimulation (Pohjanpelto, 1976; Chen and Rinehart, 1981; Rinehart and

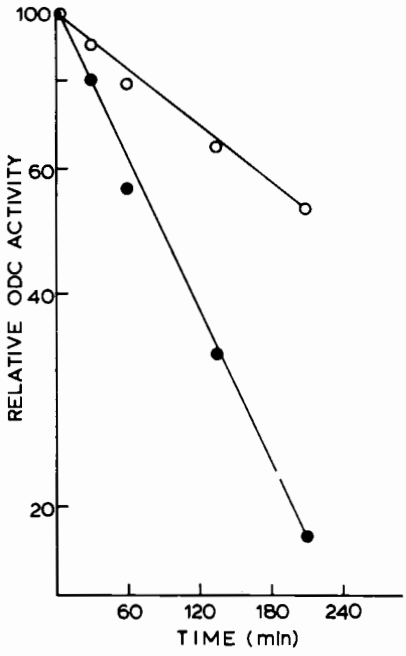

FIG. 4. Loss of ODC activity in young and old IMR-90 cells after treatment with cycloheximide. Quiescent young (PDL = 22, -○-) and old (PDL = 54, -●-) IMR-90 cells were stimulated with 10% fetal bovine serum for 8 h prior to the addition of cycloheximide (final concentration 0.18 mM) at time-zero. At the designated time, cells were harvested and assayed for ODC activity. Specific activity of ODC at time zero was 8 units/mg protein for young cells and 2 units/mg protein for old cells.

Chen, 1984). We have compared the rate of transport of putrescine in young and old cells after serum stimulation. In addition, we have examined other nutrient transport systems (amino acid, carbohydrate, and nucleoside) in IMR-90 cells during cellular aging. Our studies indicated significant differences in putrescine uptake and thymidine uptake between young and old cells (Fig. 6). No clear difference in deoxyglucose and aminoisobutyrate uptake existed between young and old cells (Fig. 6). The reduced uptake of putrescine in the old cells is consistent with the notion that the activity of polyamine transport correlated with proliferation. It should be noted that in these studies only the initial rate of transport was determined.

Early G_1 gene expression

The results shown in Fig. 7 indicated that the expressions of two proto-oncogenes, *c-erb*B and *c-myc* genes were serum-inducible, and that there was no significant difference in the expression of these two genes between young and old IMR-90 cells. These data, together with our study of ODC gene expression, suggest that there is no difference in cell-cycle-dependent gene expression during the first half of G_1 phase between young and old cells after they enter the cell cycle.

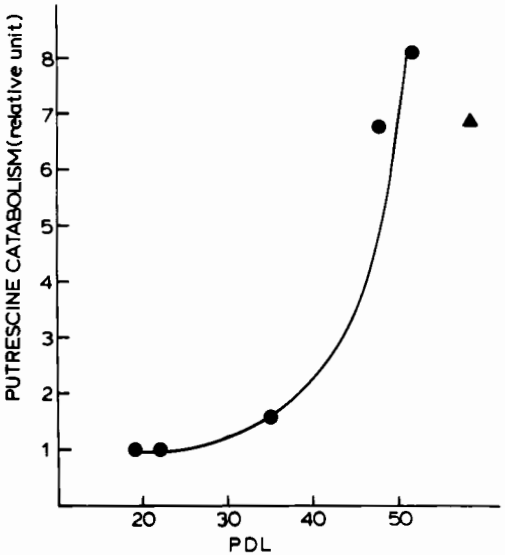

FIG. 5. Putrescine biodegradation in IMR-90 human diploid fibroblasts as a function of PDL. Putrescine catabolism in IMR-90 cells at various PDL was determined as a ratio of protein synthesis measured by metabolic labeling of proteins using [^{14}C]putrescine to that measured by [^{3}H]leucine incorporation (Chen and Chang, 1986). The ratio obtained for cells at PDL = 18 was arbitrarily set as 1 for comparative purpose. The data point expressed by the closed triangle (▲) is for progeria cell strain AG1178.

Late G_1/S gene expression

The difference in the thymidine kinase mRNA levels between young and old IMR-90 cells has been demonstrated by Northern blot analysis using the 1.5 kb *Bam*H1 fragment of pTK11 plasmid as a probe (Chang and Chen, 1988; also see Fig. 8). The decrease of TK mRNA level in old IMR-90 cells appears to be sufficient to account for the decrease of TK activity in old IMR-90 cells (Fig. 8). It can also be noted that the time course of the induction of TK activity and TK mRNA correlated well with DNA synthesis (Fig. 8). However, since TK is a salvage enzyme, whether decreases of TK mRNA and enzyme activity are directly responsible for the decrease of DNA synthesis in the old cells remains to be investigated. To further examine whether this striking age-dependent change of gene expression is unique for thymidine kinase gene, or may be more general for some other G_1/S or S genes, we have compared thymidylate synthetase activity in both young (PDL = 21) and old (PDL = 52) cells after serum stimulation. Figure 9 shows that serum-induced thymidylate synthetase activity was PDL-dependent, indicating that decrease of G_1/S gene expressions is not limited only to thymidine kinase.

DISCUSSION

The importance of polyamines in growth regulation is well documented (e.g., Tabor and Tabor, 1976, 1984; Pegg, 1986). Basal levels of ODC activity and polyamine content in various

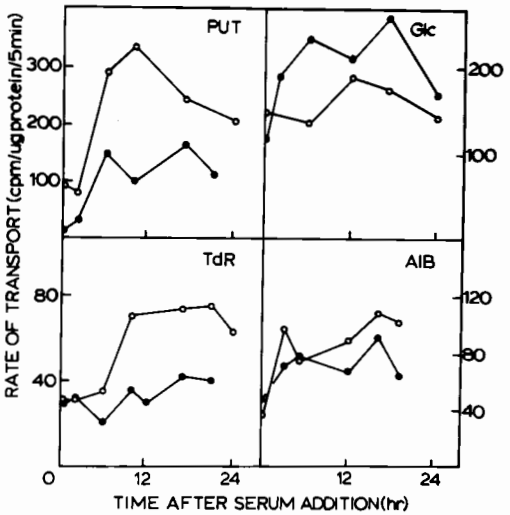

FIG. 6. Transport of putrescine and other nutrients into IMR-90 cells after serum stimulation. Quiescent cultures of IMR-90 cells (young cells, -○-; old cells, -●-) were treated with 10% fetal calf serum. At various time point after serum stimulation, cells were washed and reincubated in Earle's balanced salt solution (37 °C). The uptake was initiated by adding various nutrients ([^{3}H]putrescine, 1.85 μM, 1 μCi/ml; [^{3}H]thymidine, 0.5 μM, 1 μCi/ml; [^{3}H]deoxyglucose, 3 mM, 5 μCi/ml; AIB, 91 μM, 0.9 μCi/ml) to the culture and the radioactivity taken up into the cells was determined at 5-min intervals during the initial 20-min period. The initial rate was linear and was expressed as cpm per 5 min per μg protein. Put, putrescine; TdR, thymidine; Glc, deoxyglucose; AIB, α-aminoisobutyric acid.

organs have been measured in aging rats (Ferioli *et al.*, 1976; Shain and Moss, 1981). Whether changes of polyamine metabolism may be involved in cellular aging has not been systematically studied before. The results presented above, together with out previous studies have indicated that changes of specific polyamine metabolism do occur during senescence of IMR-90 human diploid fibroblasts in culture. These changes include: (a) a fivefold decrease in serum-induced peak ODC activity (Fig. 2), (b) a lack of serum-induced putrescine accumulation (Chen *et al.*, 1986); (c) a more than eightfold increase of putrescine catabolism (Fig. 5); and (d) a two to threefold decrease of putrescine uptake (Fig. 6). Despite these changes, spermidine and spermine content in both young and old IMR-90 cells are in the range of 6 to 10 nmol per mg protein and do not show significant fluctuation after serum addition (Chen et al., 1986). These results, taken together, suggest that after serum addition, due to the combination of low ODC activity and high putrescine catabolism, the transient surge of putrescine accumulation that was generally associated with serum stimulation does not occur in the old cells.

The results shown in Fig. 3 also suggest that the difference in serum-induced ODC activity between young and old IMR-90 cells is due to difference of control at a translational and/or posttranslational level. There have been reports that ODC activity can be regulated by translation efficiency (Kahana and Nathans, 1984), formation of an inactive complex with a specific inhibitor antizyme (Heller *et al.*, 1976), or protein stability (Isomaa *et al.*, 1983). The difference in the half-life of ODC activity in the young and old cells (Fig. 4) supports the notion that there may be changes in posttranslational control of ODC enzyme during cell senescence.

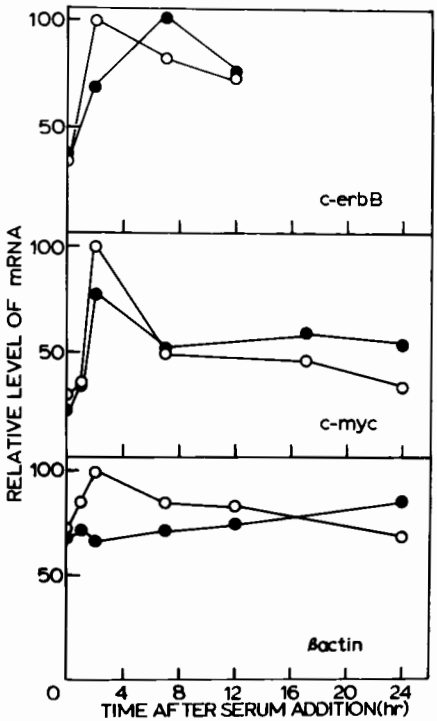

FIG. 7. Effect of serum stimulation on *c-erb*B and *c-myc* mRNA expression in young (PDL = 22) and senescent (PDL = 52) IMR-90 cells. Quiescent cultures of both young (-○-) and senescent (-●-) cells were serum stimulated for various times and poly(A) $^{+}$RNA was isolated for Northern blot hybridization as previously described (Chang and Chen, 1988). Relative levels of mRNA were determined by densitometric tracing of the autoradiogram. β-actin mRNA level was also determined using the same blot to ensure an internal control.

The precise mechanism underlying the age-associated decrease of ODC activity remains to be elucidated.

The induction the ODC gene is a mid-G_1 event (Canellakis *et al.*, 1979; Tabor and Tabor, 1976, 1984). The decrease of ODC activity and changes of polyamine metabolism during cellular senescence may have a causal relationship with the expression of other cell-cycle-dependent genes. In this regard, it is of interest to note that Cheetham and Bellet (1982) have reported that α-methylornithine and methylglyoxal bis-(guanylhydrazone), both inhibitors of polyamine biosynthesis, inhibit serum-induced thymidine kinase activity in quiescent rodent cells. Our study so far indicates no significant difference in either the time course or the magnitude of expression of *c-myc*, *c-erb*B, and ODC gene between young and old IMR-90 cells after serum stimulation (Fig. 3 and 7). These results are consistent with the notion that cell cycle progression is not arrested at G_0 or early- or mid-G_1 phase in senescent cells (Rittling *et al.*, 1986). In contrast to these findings, we found that the serum-induced expression of the TK gene, a late G_1/S gene, is significantly reduced in old IMR-90 cells.

DNA synthesis in vitro is generally measured by the incorporation of either [^{3}H] or [^{14}C]thymidine into acid-insoluble material. Thymidine taken up into the cell has to be phosphorylated by thymidine kinase (TK) before it can be used for DNA synthesis. Despite the

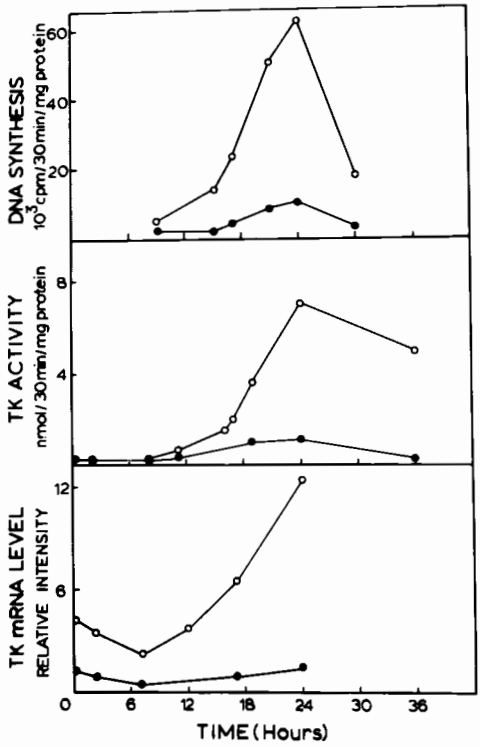

FIG. 8. Time course of the rate of DNA synthesis, the induction of TK activity and relative TK mRNA content in young and old IMR-90 human fibroblasts. Confluent cultures of young (PDL = 22, -○-) and old (PDL = 52, -●-) cells were serum deprived, stimulated by 10% fetal calf serum (Fig. 1). The rate of DNA synthesis was determined by pulse labeling cells with 10 μCi/ml of [methyl-^{3}H]thymidine (20 Ci/mmol) for 30 min at 37°C. The radioactivity recovered in the trichloroacetic acid-precipitable material indicated the amount of incorporated thymidine. The activity of thymidine kinase was determined according to the procedure described by Bradshaw (1983). Relative levels of TK mRNA were determined by densitometric tracings of the autoradiograms.

fact that TK is a salvage enzyme, close correlation of TK activity and growth rate has been demonstrated in many cell types (Kit and Jorgensen, 1976; Johnson *et al.*, 1982). TK activity increases in cells during DNA synthesis and is tightly coupled to DNA synthesis (Artishevsky *et al.*, 1987). Reddy and Pardee (1980) have proposed that when entering S phase of the cell cycle, the nucleus of eukaryotic cells contains a multienzyme complex termed *replitase* for DNA replication, and that its assembly may signal the initiation of S phase. The functional integrity of this complex, of which TK is a component, will be impaired if one or several of its components is inactivated or lacking. That a complex of enzymes or structural components is required for DNA synthesis has also been suggested in T4 phage-infected cells (Wovcha *et al.*, 1973). Thus the reduced TK gene expression and TK activity in senescent IMR-90 cells may block the formation of an active replitase complex and prevent the cells from entering S phase. Although the molecular mechanism subserving the decreased TK gene expression in senescent IMR-90 cells remains to be elucidated, TK activity and ODC activity in IMR-90 cells are temporally related and both are inversely proportional to the PDL of IMR-90 cells, suggesting

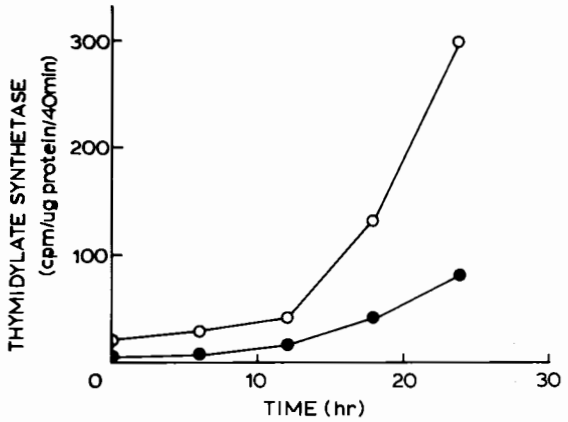

FIG. 9. Time course of the induction of thymidylate synthetase activity in young and old IMR-90 human diploid fibroblasts. Confluent cultures of young (PDL = 21, -○-) and old (PDL = 52, -●-) IMR-90 cells were serum deprived for 48 h and then stimulated with 10% fetal bovine serum. Cells were harvested at designated time for enzyme assay.

that they may be either causally related or coordinately regulated. Indeed, there have been reports which provided evidence of a relatedness of ODC induction, TK induction and DNA synthesis in living organisms (e.g., Boynton *et al.*, 1976; Poso and Pegg, 1982; Cheetham and Bellet, 1982). It is possible that the drastic reduction of putrescine accumulation after serum addition in senescent cells may contribute to the decrease of TK gene expression and thus DNA synthesis, and we are currently examining this possibility.

It is also of interest to examine whether the suppression of gene expression during cellular

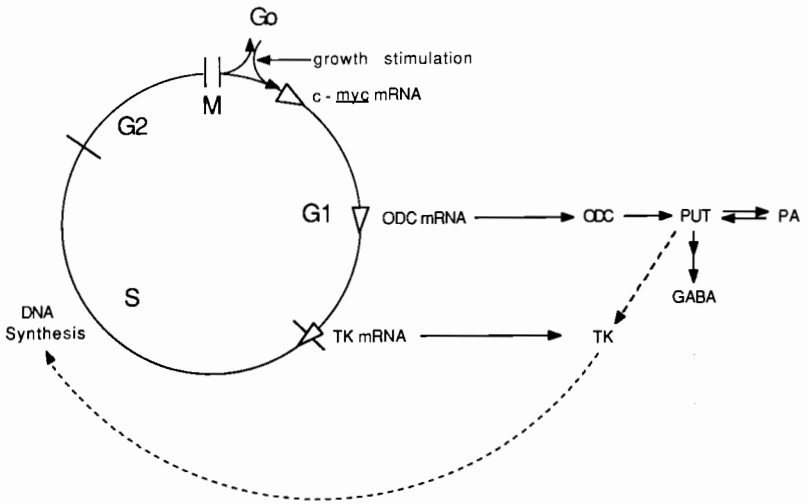

FIG. 10. Proposed model for cellular aging of IMR-90 cells. ODC, ornithine decarboxylase; TK, thymidine kinase; Put, Putrescine; PA, polyamines. The dashed lines indicate that putrescine (PUT) may affect TK activity which in turn may be linked to DNA synthesis.

senescence is limited only to the TK gene or represents a more global phenomenon involving other G_1/S genes. This is particularly relavant because TK is known as a salvage enzyme. Along this line, we have found another component of the replitase, thymidylate synthetase, showed reduced activity in senescent cells (Fig. 9). Whether thymidylate synthetase mRNA in IMR-90 cells also shows age-dependent suppression is currently being examined. It should be noted that Rittling *et al.* (1986) have reported that both TK and histone H3 gene expression are not suppressed in senescent WI-38 cells. The cause for the discrepancy is not clear. Based on the hypothesis of Pardee (Reddy and Pardee, 1980), a defect of any component in the multienzyme complex for DNA synthesis can impair DNA synthesis. It is therefore possible that there may be several target sites at the G_1/S boundary or deep in S phase that can be regulated during aging, and that these target sites are cell type specific. In any event, our data with thymidylate synthetase suggest that the suppression of G_1/S gene expression, at least in IMR-90 cells, is not unique to TK gene.

Based on our studies on polyamine metabolism and cell-cycle-dependent gene expression in IMR-90 human diploid fibroblasts, we have proposed a working model on cellular aging (Fig. 10). This model emphasizes that serum factors are effective in stimulating both young and old cells to enter the cell cycle, at least equally well till mid-G_1 phase. Alteration of polyamine metabolism, particularly the induction of ODC activity, occurs during cell senescence. The combination of low ODC activity and increased putrescine degradation results in a lack of putrescine accumulation in the old cells after serum addition. The model also illustrates a significant reduction of TK gene expression and TK activity when cells traverse to G_1/S boundary after serum stimulation. It is possible that the lack of serum-induced putrescine accumulation may affect expressions of genes which are temporally located at late G_1 or G_1/S boundary of the cell cycle (e.g., TK gene). Based on this model, ongoing research in our laboratory is focused on the following questions: (a) What is the molecular basis for the decreased ODC activity and increased putrescine catabolism in the old IMR-90 cells? (b) Is the decreased gene expression during cellular aging limited to the TK gene or is it a more global phenomenon involving other G_1/S genes? and (c) Does putrescine accumulation directly or indirectly affect the expression of TK and/or other G_1/S genes?

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