Changes of Serum-Induced Ornithine Decarboxylase Activity and Putrescine Content During Aging of IMR-90 Human Diploid Fibroblasts

KUANG YU CHEN,* ZIEFEN CHANG, AND ALICE Y. L. LIU
Departments of Chemistry and Biochemistry (K.Y.C., Z.C.-L.) and Biological Sciences (A.Y.-L.), Wright and Riemann Laboratories, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903

The roles of ornithine decarboxylase (ODC, EC 4.1.1.17) and polyamines in cellular aging were investigated by examining serum-induced changes of these parameters in quiescent IMR-90 human diploid fibroblasts as a function of their population doubling level (PDL) and in human progeria fibroblasts. Serum stimulation caused increases of ODC and DNA synthesis in IMR-90 human diploid fibroblasts, with maximal values occurring, respectively, 10 hr and 22 hr after serum stimulation. Both serum-induced ODC activity and DNA synthesis in IMR-90 cells were found to be inversely related to their PDL. Maximal ODC activity and DNA synthesis in young cells (PDL = 1–20) were, respectively, five-fold and six-fold greater than that in old cells (PDL = 50–55), which in turn were comparable or slightly higher than that in progeria fibroblasts. Polyamine contents (putrescine, spermidine, and spermine) in quiescent IMR-90 cells did not show significant PDL-dependency. The putrescine and spermine contents in quiescent progeria cells were comparable to those in quiescent IMR-90 cells. The spermidine content in quiescent progeria cells, however, was extremely low, less than half of that in quiescent IMR-90 cells. Serum stimulation caused a marked increase in putrescine content in young cells but not in old cells or in progeria cells. The spermidine and the spermine content in IMR-90 cells, either young or old, and in progeria cells did not change significantly after serum stimulation. Our study indicated that aging of IMR-90 human diploid fibroblasts was accompanied by specific changes of polyamines metabolism, namely, the serum-induced ODC activity and putrescine accumulation. These changes were also observed in progeria fibroblasts derived from patients with Hutchinson-Gilford syndrome.

Human embryonic diploid fibroblasts undergo 50 ± 10 doublings in tissue culture and then cease to divide (reviewed by Hayflick, 1979; Crestfield and Stanulie-Pasquez, 1982). This phenomenon, initially described by Hayflick and Moorhead (1961), is to be contrasted with tumor cell or transformed cell lines, which have unlimited dividing potential in vitro. Progeria fibroblasts derived from patients with Hutchinson-Gilford syndrome or other premature aging classes exhibit a much shorter life span as compared to normal fibroblasts from age-matched controls (reviewed by Goldstein, 1979). These results together with the observation that the in vitro life span of human diploid fibroblasts is inversely related to the age of donor (Hayflick, 1965; Martin et al., 1970; Martin, 1977) have made human diploid fibroblasts a useful in vitro model for studying the biochemical basis of cellular aging.

Polyamines (putrescine, spermidine, and spermine) are naturally occurring organic cations ubiquitously distributed in living organisms. Abundant evidence has been reported in literature indicating the importance of polyamines in growth regulation (for reviews see Jonge et al., 1977; Pegg and McCann, 1982; Tabor and Tabor, 1984). The induction of ornithine decarboxylase (ODC, EC 4.1.1.17), the rate-controlling enzyme for polyamine biosynthesis, and the subsequent accumulation of polyamines generally precede DNA synthesis (Heby et al., 1975; Russell and Stambrook, 1975; Boynton et al., 1976). Inhibition of polyamine biosynthesis by specific inhibitors such as N-(deoxyguaninyl)ornithine (Manore et al., 1978; Prakash et al., 1986; Pegg and Pegg, 1980) and methionine sulfoximine (McMurray et al., 1973) (Boynton et al., 1976) invariably leads to reduced DNA synthesis and cessation of cell growth. Despite extensive studies of the regulation and function of polyamines in cell proliferation and cell differentiation (reviewed by Cohen, 1971; Broach, 1973; Tabor and Tabor, 1976, 1984), the possible involvement of polyamines in cellular aging has received April 27, 1986, accepted July 3, 1986.

*To whom reprint requests/correspondence should be addressed.

© 1986 ALAN R. LISS, INC.
received very little attention. Studies in animal tissues have indicated changes in polyamine content with increased age of the animal (reviewed by Scalabrin and Feron, 1984), but possible changes of polyamine contents in human diploid fibroblasts during aging have not been previously investigated. Duffy and Kromer (1977) have reported that the onset of the in vitro cellular senescence of WI-38 human fibroblasts is associated with reduced ODC activity in response to the stimulus of fresh culture medium. These studies, together with the consideration of the importance of polyamines in growth regulation, prompted us to initiate a systematic study of the regulation of polyamine metabolism in IMR-90 human diploid fibroblasts as a function of the PDL over their entire life span in culture. In addition, to ensure that changes of polyamine metabolism in IMR-90 cells after serial passage in vitro are truly age dependent, we also examined polyamine metabolism in progenitor cell strains derived from patients suffering from Hutchinson-Gilford syndrome, a premature aging disease that has certain aspects in common with normal aging (Goldstein, 1979).

MATERIALS AND METHODS

Materials
Dulbecco's modified Eagles medium, fetal bovine serum, diethylstilbestrol, trypsin-EDTA solution, and gentamycin were purchased from Gibco, Grand Island, NY, [1-14C]-putrescine monohydrochloride (59 mCi/mmol) was obtained from Amersham, Arlington Heights, IL, Putrescine, spermine, L-lysine, 5-dimethyl amino-1-naphthalene sulfonic chloride (dansyl chloride), and phenylmethylsulfonyl fluoridc were purchased from Sigma Chemical Co., St. Louis. High pressure liquid chromatography (HPLC) grade solvents were obtained from Baker Chemical Co. Other chemicals were of standard reagent grade.

Cell culture
Low passage (PDL = 12, passage number 5) IMR-90 human embryonic lung fibroblasts, and the AG1178 and AG3198 progenitor cell strains were obtained from the Institute for Medical Research, Camlens, NJ. The low passage cultures of IMR-90 cells were expanded through subculturing at 1:4 or 1:2 split ratio to obtain cultures of higher population doubling level (PDL) (Nolan and Parker, 1974). Cells were grown in Dulbecco's modified Eagles medium (with 4,500 mg glucose per liter without sodium pyruvate) supplemented with 10% fetal bovine serum at 37°C in a water-jacketed CO2 incubator (95% air, 5% CO2). The apparent PDL of an IMR-90 human fibroblast culture was determined by the cumulative number of cell doubling; the number of doublings for each subcultivation (passage) was estimated from the split ratio (Nolan and Parker, 1974). Except when indicated, confluent monolayer cultures were used for all experiments described in this study. The progenitor cells were used when less than 50% of the in vitro life span was consumed. They were also used at confluent state.

Growth stimulation
Confluent cultures of human fibroblasts were serum-deprived in fresh Dulbecco’s medium for 24 hr to ensure that cells were in a quiescent state. Growth stimulation was initiated by refusirhishing the quiescent cultures with fresh Dulbecco’s medium supplemented with 10% fetal bovine serum. At various times after serum stimulation, cells were harvested in a 50 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM EDTA, 50 mM pyridoxal phosphate, and 5 mM diethystrtolate. The cell homogenates were used for determining ODC activity and polyamine content.

Assay of ODC activity
ODC activity was determined using the cytochal fraction obtained from cell homogenate according to procedures previously described (Chen et al., 1976). One unit of ODC activity is defined as 1 nmol CO2 evolved per 60 min. Protein concentration was determined by a modified Lowry method using bovine serum albumin as the standard (Bens & Sillit, 1973).

Quantitation of cellular polyamine content
Polyamines were dianalyzed by the method of Seiler and Wochmann (1970). The dianalyzed polyamines were separated by high pressure liquid chromatography (Beckman) using a reverse-phase column (RP-18, 7 µm ODS column), and quantitated by a Schiiofle spectrometer (Model FSP970) as previously described (Chen et al., 1982).

DNA synthesis
The incorporation of L[3H]thymidine into acid-insoluble material was used to estimate the rate of DNA synthesis. At indicated time intervals after serum stimulation, [3H]thymidine (2 µCi/ml) was added to the cultures, and the metabolic incorporation was carried out at 37°C in a CO2 incubator for 1 hr. Cells were then washed three times with cold phosphate-buffered saline (PBS, pH 7.2) and harvested in a 10 mM Tris-HCl (pH 7.4) buffer containing 1 mM EDTA and 50 µg/ml phenylmethylsulfonyl fluoride. The amount of radioactivity incorporated into acid-insoluble material present in homogenates was determined according to procedures previously described (Chen and Canelakes, 1977).

RESULTS
Figure 1 shows the effect of age on the induction of ODC activity and DNA synthesis in IMR-90 human diploid fibroblasts and AG3198 progenitor human fibroblasts. IMR-90 cell cultures at PDL of 22 (young), 36 (middle age), and 54 (old) were used in this experiment. Results indicated that the magnitudes of the serum-induced ODC induction and DNA synthesis in IMR-90 cells were inversely related to the PDL of the cultures. Maximal ODC activity and the rate of DNA synthesis in young cells (PDL = 22) were five- and six-fold higher than the respective values in old cells (PDL = 54). The increases of both ODC activity and rate of DNA synthesis in progenitor cells were insignificant as compared to that in normal diploid fibroblasts. Analysis of the kinetics of the serum-induced increase in ODC activity and DNA synthesis indicated little difference between young and old IMR-90 cells and between normal diploid fibroblasts (IMR-90) and progenitor fibroblasts (AG1178). In all cases studied, the increase of ODC activity was detectable 3 hr after serum stimulation, reaching a maximal value ~ 6-10 hr after serum stimulation. DNA synthesis in both normal and progenitor fibroblasts, however, showed a lag period of about
13 hr, characteristic of synchronized cultures. Maximal increase of DNA synthesis in these cells occurred at about 22 hr after serum stimulation.

Results from seven separate experiments demonstrated an inverse linear relationship between serum-induced maximal ODC activity and PDL of the cell culture data not shown). Thus, four- to five-fold decrease of serum-induced ODC activity was consistently observed during aging of IMR-90 cells. The serum-induced ODC activity in progeria cells was either comparable to or less than that in old IMR-90 cells.

ODC is the rate-controlling enzyme for the biosynthesis of polyamines. In view of this consideration and to gain a better understanding of the possible role of polyamines in cellular aging, we determined the basal levels of putrescine, spermidine, and spermine in quiescent IMR-90 cells as a function of their PDL (Fig. 2). In addition, we determined the time course of the effect of serum stimulation on polyamine contents in both quiescent young (PDL = 28) and old (PDL = 51) IMR-90 cells (Fig. 3). The data points represent an average of duplicate samples. PUT, putrescine; SPD, spermidine; SPM, spermine; AD, progeria cells.

Fig. 2. Polyamine content in IMR-90 human diploid fibroblasts at various PDL and in progeria cells (AG1398). Individual polyamine content (putrescine, spermidine, and spermine) in the confluent cultures of IMR-90 cells at different PDL was determined by HPLC analysis as described in "Materials and Methods." Each data point represents an average of duplicate samples. PUT, putrescine; SPD, spermidine; SPM, spermine; AD, progeria cells.

Fig. 3. Time course of changes of polyamine content in quiescent IMR-90 cells and progeria cells AG1398 after serum stimulation. Confluent cultures of IMR-90 cells at two different PDL (PDL = 28, ○; PDL = 51, ●) and progeria cells AG1398 (-) were serum deprived for 24 hr and then stimulated with fresh Dulbecco's medium containing 10% fetal bovine serum. Polyamine content was determined at various time points after serum stimulation. PUT, putrescine; SPD, spermidine; SPM, spermine.
was extremely low as compared to IMR-90 cells, and consequently the spermidine/spermine ratio in progenitor cells was only 0.37, distinctly different from that of the IMR-90 cells. The difference in sperminined content between normal fibroblasts and progenitor fibroblasts suggests that additional changes in polyamine metabolism (e.g., difference in spermidine synthesis activity) may have occurred in progenitor cells.

Figure 3 shows that serum stimulation caused a striking increase of putrescine content in young IMR-90 cells but not in old IMR-90 cells. No putrescine accumulation in progenitor cells was detected either after serum stimulation. The increase of putrescine content in response to serum stimulation in low passage WI-38 human diploid fibroblasts has been reported by Hely et al., (1975). They also found that after serum stimulation putrescine level remains elevated for an extended period of time in WI-38 cells. In contrast, the spermidine and the spermine content in young and old IMR-90 cells and in progenitor cells did not show a significant fluctuation after serum stimulation.

DISCUSSION

Monolayer cultures of quiescent human fibroblasts can be stimulated to enter the proliferative cell cycle by the addition of fetal bovine serum to the cultures or by refeeding the serum-deprived medium with fresh serum containing growth medium (Wolbel and Baserga, 1969; Roversa and Baserga, 1973; Hely et al., 1975). In this study, we have employed quiescent confluent IMR-90 human diploid fibroblasts at various PDL and human progenitor diploid fibroblast to examine possible age-associated changes in polyamine metabolism in these cells after serum stimulation.

Our results indicated that serum-stimulated induction of ODC activity and putrescine accumulation in young IMR-90 cells (PDL = 18-22) were, respectively, approximately two- to threefold greater than that in old IMR-90 cells (PDL ≥ 50) (Figs. 1 and 3). The spermidine and spermine content in young IMR-90 cells, however, did not differ significantly from that in old IMR-90 cells after serum stimulation (Fig. 3). The differences in putrescine content between young and old IMR-90 cells appeared to be only associated with growth stimulation. When the basal cellular content of polyamines in quiescent IMR-90 cells was determined, no significant changes were observed as the PDL of the cultures increased (Fig. 2). Similar results in the old IMR-90 cells, serum stimulation caused only a minimal induction of ODC activity and no detectable putrescine accumulation in progenitor cells (Figs. 1 and 3). The spermidine content in progenitor cells was, however, extremely low when compared to that in either young or old IMR-90 cells. The calculated spermidine/spermine ratio was 0.94 for IMR-90 cells and was found to be independent of PDL, whereas the spermidine/spermine ratio for progenitor cells was only 0.37. It has been suggested that high spermidine/spermine ratio is typical of tissues undergoing rapid growth or hypertrophy, and low spermidine/spermine ratio is generally typical of highly differentiated tissues with low biosynthetic activity (James et al., 1964; Russell and Durie, 1978). Since the spermidine/spermine ratio in IMR-90 cells was independent of PDL, this parameter could not be related to cellular aging. Thus, the low value of the spermidine/spermine ratio in progenitor cells suggests that, in addition to age-related changes in polyamine metabolism (i.e., serum-induced ODC activity and putrescine accumulation), there may be other changes in polyamine metabolism associated with premature aging diseases.

The reduction of serum-induced ODC activity and putrescine accumulation in old IMR-90 cells and progenitor cells correlated well with the reduction of DNA synthesis in these cells (Figs. 1, 3). Hely et al. (1975) have shown that by varying the composition of the stimulating medium, the magnitude of putrescine accumulation correlated with the rate of DNA synthesis in primary WI-38 human diploid fibroblasts (PDL = 24-28). Pose and Pegg (1982) have shown that the administration of diaminodureneethylamine (400 mg/kg to rats) following partial hepatectomy reduces putrescine level to less than 2 nmol/g and causes a 70% inhibition of DNA synthesis. They also showed that the inhibitory action of diaminodureneethylamine can be reversed by exogenously added putrescine. In view of these observations, it seems likely that the induction of ODC and putrescine accumulation in IMR-90 cells after serum stimulation may be involved in the initiation of DNA synthesis and that decreases of these responses may contribute to the reduced DNA synthesis in old IMR-90 cells and in progenitor cells. The molecular mechanism for the alteration of serum-induced ODC activity in IMR-90 cells during aging is currently under investigation.

Polyamine metabolism involves many metabolic pathways, and polyamines themselves interact with many biological molecules (e.g., DNA, RNA, and protein(s) (see Tabor and Tabor, 1976, 1984). Thus, it is possible that changes in the other aspects of polyamine metabolism may also play a role in aging of human diploid fibroblasts. In this connection, we have recently found that the catalytic conversion of putrescine to amino acids via the GABA shunt and Krebs cycle in IMR-90 cells increased by more than eightfold during aging (Chen and Chang, 1986).

ACKNOWLEDGMENTS

This research was supported by grant AG00078 from the National Institute of Aging, NIH and a grant from Charles and Johanna Buch Memorial Fund. The very able assistance of Ms. Terry Maddata during the initial phase of this research is greatly appreciated.

LITERATURE CITED


60:401-406.
orthonucleotidase/case activity and polyamine content upon differ-
centiation of mouse NR-1 cells into melanoma cells. J. Cell. Physiol.
Cristobal, V.J., and Bannister, O.M. (1982) Cellular senescence and
Cristobal, V.J., and Bannister, O.M. (1982) Cellular senescence and
Cristobal, V.J., and Bannister, O.M. (1982) Cellular senescence and
98:645-649.
Goldstein, N. (1927) Studies of age-related diseases in cultured skin
Hayflick, L. (1963) The limited in vitro lifetime of human diploid cell
70:8-14.
Changes in polyamine metabolism in WI-38 cells stimulated to proliferate
Changes in polyamine metabolism in WI-38 cells stimulated to proliferate
Changes in polyamine metabolism in WI-38 cells stimulated to proliferate
Changes in polyamine metabolism in WI-38 cells stimulated to proliferate
Changes in polyamine metabolism in WI-38 cells stimulated to proliferate
Changes in polyamine metabolism in WI-38 cells stimulated to proliferate
Changes in polyamine metabolism in WI-38 cells stimulated to proliferate