JOURNAL OF CELLULAR PHYSIOLOGY 128:149–154 (1986)

Increased Level of cAMP-Dependent Protein Kinase in Aging Human Lung Fibroblasts

ALICE Y.-C. LIU,* ZEE-FEN CHANG, AND KUANG YU CHEN

Departments of Biological Sciences (A.Y.-C.L.) and Chemistry and Biochemistry (Z.-F.C., K.Y.C.), Rutgers University, Piscataway, New Jersey 08854

Regulation of the expression of cAMP-dependent protein kinase in cellular aging was studied using the IMR-90 diploid human lung fibroblasts. The level of cAMP-dependent protein kinase present in cell extracts was monitored by 1) photoactivated incorporation of 8-N_3-[^{32}P]cAMP into the 47,000- and 54,000-dalton regulatory subunits of the type I and type II cAMP-dependent protein kinases, respectively; 2) cAMP-dependent phosphorylation of histone II AS catalyzed by the catalytic subunit of the kinase; and 3) fractionation and analysis of the type I and type II cAMP-dependent protein kinase by DEAE-Sephacel column chromotography. Our results showed an approximately two- to threefold increase in the level of the type I cAMP-dependent protein kinase and a somewhat smaller increase in the type II kinase in extracts of the "old" IMR-90 cells (population doubling >48) as compared to that of the "young" cells (PDL 22–27). The timing of the increase in cAMP-dependent protein kinase coincided with a significant decrease in the proliferative potential of the cells. This result together with previously demonstrated effects of cAMP in the control of cell growth and differentiation and the increased expression of cAMP-dependent protein kinase during terminal differentiation of the murine preadipocytes (3T3-L1) and myoblast (L-5, L-6, and C_2C_{13}) suggests that regulation of the levels of cAMP and cAMP-dependent protein kinase plays a significant role in the control of cell growth and differentiation.

Human diploid cells have a limited proliferative potential in vitro. The phenomenon, initially described more than 20 years ago (Hayflick, 1965; Hayflick and Moorhead, 1961), is well established and accepted. Thus, starting with a primary culture of cells from a sample of tissue, the cells multiply at a constant rate for many cell generations, but they finally enter a senescent condition culminating in death of the culture. The remarkable consistency of normal cells in culture to express a limited in vitro replicative life span inversely related to the age of the donor from which the cell culture was initiated and the species specificity of this in vitro replicative potential (Hayflick, 1975) have led to their utilization as models for cellular aging. The biochemical or molecular basis of this proliferative limit of normal diploid cells remains an important unsolved problem in cell biology.

cAMP appears to play an important role in the regulation of cell growth, differentiation, and transformation (for reviews, see Friedman, 1976; Lockwood et al., 1981; Pastan and Willingham, 1978; Prasad, 1975). Pastan and his colleagues, in a series of studies, demonstrated an increase in cAMP concentration in contact-inhibited fibroblasts and a decrease in cAMP concentration in transformed cells. With cells transformed by mutant virus that were temperature-sensitive for transformation, they found that the levels of cAMP were low in cells grown at the permissive temperature and normal at the restrictive temperature (Carchman et al., 1974). Furthermore, exogenous addition of derivatives of cAMP to virally transformed fibroblasts causes a slowing of cell growth and suppresses the expression of transformed phenotypes.

In addition to regulation of the level of cAMP, regulation of the level of cAMP-dependent protein kinase also appears to be linked intimately to events involved in the control of cell growth and differentiation. In previous studies, we have reported on the increased expression of the type I cAMP-dependent protein kinase during terminal differentiation of the adipogenic 3T3-L1 cells (Liu, 1982) and myogenic L-5, L-6, and C_2C_{13} cells (Kamalakannan and Liu, 1985; Liu and Chen, 1983). In mouse neuroblastoma cells, there is an increase in expression of the R_I cAMP-binding protein concomitant with cell differentiation (Liu et al., 1980, 1981). Studies from other laboratories have demonstrated increased cAMP-binding and cAMP-dependent protein kinase activities during endodermal differentiation of the F-9 teratocarcinoma cells (Plet et al., 1982), during erythroid differentiation of Friend leukemic cells (Schwartz and Rubin, 1983), and during tumor regression of the Walker mammary carcinoma cells (Cho-Chung et al., 1977). The

Received November 6, 1985; accepted April 9, 1986.

An abstract describing part of this work has previously been published (Liu, A.Y.-C., Chang, Z.-F., and Chen, K.Y. (1985) Fed. Proc., *44*:465).

*To whom reprint requests/correspondence should be addressed.

increase in cAMP-dependent protein kinase may be central to the expression of differentiated phenotypes, the loss of proliferative capacity, or a combination of various events in the differentiating cells. To define better the role of cAMP-dependent protein kinase in the regulation of cell growth and differentiation, we examined the expression of cAMP-dependent protein kinase during aging of the IMR-90 cells, a strain of human diploid fibroblasts that loses its proliferative potential with successive passages in vitro without undergoing global changes in cell structure and function. We report on the increased expression of cAMP-dependent protein kinase in aging IMR-90 cells.

MATERIALS AND METHODS

Materials

All tissue culture supplies were obtained from GIBCO (Grand Island, NY). 8-N_3-[^{32}P]cAMP, [^{3}H]cAMP, and [r^{32}P]ATP were from ICN (Irvine, CA). Other biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

Cell Culture

Early-passage IMR-90 human diploid lung fibroblasts were obtained at population doubling levels (PDLs) ranging from 12 to 20 from the Institute for Medical Research, Camden, NJ. Cells were grown as monolayer culture in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum in the presence of 50 U/ml penicillin and 50 μg/ml streptomycin in a water-jacketed CO_2 incubator at 37°C. Cells were routinely passaged at confluency using 0.25% trypsin in calcium- and magnesium-free Earle's balanced salt solution at a 1:2 or 1:4 split ratio, and cells were refed with fresh medium every 5–7 days. Failure to grow to confluency, defined as the inability of cells to fill a 100–mm tissue culture plate within 2 weeks with weekly media changes, occurred at PDLs of 45–55 in these studies.

Preparation of cell extracts

Confluent monolayer cultures were rinsed twice with 5 ml of phosphate-buffered saline (PBS; 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4), and cells were removed from the substratum by incubating with PBS containing 1.5 mM EDTA for about 5 min at room temperature. Occasionally, the monolayer cultures were serum-deprived for 24 hr prior to harvesting to ensure that the difference in level of cAMP-dependent protein kinase reflected a difference in the population doubling level of the cell cultures rather than the actual growth status of the cells used.

For each of the experimental conditions, cells from four to six 100-mm plates were used. Cells were pelleted and washed once with PBS. The cell pellet was resuspended in 1.5–2 ml of 10 mM Tris HCl (pH 7.4) containing 1 mM EDTA and 50 μg/ml phenylmethylsulfonyl fluoride (PMSF). Cells were broken by forcing the cell suspension through a 25-gauge needle. The 100,000g supernatant obtained from cell homogenate was defined as the cytosol fraction. Cell extracts were dialyzed against 10 mM Tris·HCl (pH 7.4) containing 1 mM EDTA, 50 μm/ml PMSF, and 1 mM dithiothreitol prior to analysis for cAMP-dependent protein kinase. Protein concentration was determined by the method of Lowry et al. (1955) using bovine serum albumin as the standard.

Photoactivated incorporation of 8-N_3-[^{32}P]cAMP

Covalent incorporation of 8-N_3-[^{32}P]cAMP was performed as described previously (Liu, 1980, 1982; Walter et al., 1977). The standard reaction mixture (final volume 100 μl) contained 50 mM 2-(N-morpholinol)ethane sulfonate (pH 6.2), 10 mM $MgCl_2$, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM 8-N_3-[^{32}P]cAMP (specific activity 4–10 Ci/mmol), and 100–200 μg protein. The pattern and amount of radioactivity incorporated were analyzed by SDS-PAGE and autoradiography; sections of the dried gel containing radioactive bands were excised and the amounts of radioactivity determined by liquid scintillation spectrometry.

Partial purification and analysis of the type I and type II cAMP-dependent protein kinase by DEAE-Sephacel column chromatography

DEAE-Sephacel was preequilibrated with 10 mM Tris HCl (pH 7.4) containing 1 mM dithiothreitol and 50 μg/ml phenylmethylsulfonyl fluoride and was packed into columns (1.5 × 6 cm). Cytosol preparations from young and old IMR-90 cells containing the same amount of protein were loaded onto two identical columns. The columns were washed with 5 ml of the equilibration buffer and the kinases eluted with a linear gradient of NaCl (0–300 mM; total volume 20 ml) in the equilibration buffer. Fractions of 0.5 ml were collected and aliquots assayed for histone kinase activity in the absence and presence of 5 μM cAMP.

Other methods

Histone kinase activity was measured by the method of Witt and Roskoski (1975) as previously described (Liu, 1980). [^{3}H]cAMP-binding activity present in cell extracts was assayed according to the method of Gilman (1970); all results were corrected for nonspecific binding determined from the amount of radioactivity retained in the presence of 50 μM cAMP.

RESULTS

In this study, we examined the regulation of expression of cAMP-dependent protein kinase as a function of the population doubling level of IMR-90 diploid human lung fibroblasts. The specific activity of cAMP-dependent protein kinase present in homogenates and in 100,000g cytosol fractions of both young (PDL 22–27) and old (PDL 48–55) IMR-90 diploid fibroblasts was studied, and the respective activities were compared. Photoactivated incorporation of 8-N_3-[^{32}P]cAMP into the 47,000- and 54,000-dalton proteins was used to quantitate the level of regulatory subunits of the type I (R_I) and type II (R_{II}) cAMP-dependent protein kinase (Liu, 1980, 1982); cAMP-dependent phosphorylation of histone II AS, that inhibitable by a partially purified inhibitor of the kinase (Walsh et al., 1971; Ashby and Walsh, 1972, 1973), was used to quantitate the level of catalytic subunit activity of the kinase present in cell extracts (Liu, 1980, 1982). Results shown in Figure 1 and Table 1 demonstrate a significant increase in the incorporation of the 8-N_3-[^{32}P]cAMP into the 47,000-dalton R_I and a somewhat smaller increase in the incorporation into R_{II} in both the homogenate and cytosol fractions of the old cells compared to that of the young cells. The ratio of R_{II} to R_I is higher in the homogenate fraction than the cytosol; this result is consistent with the notion that

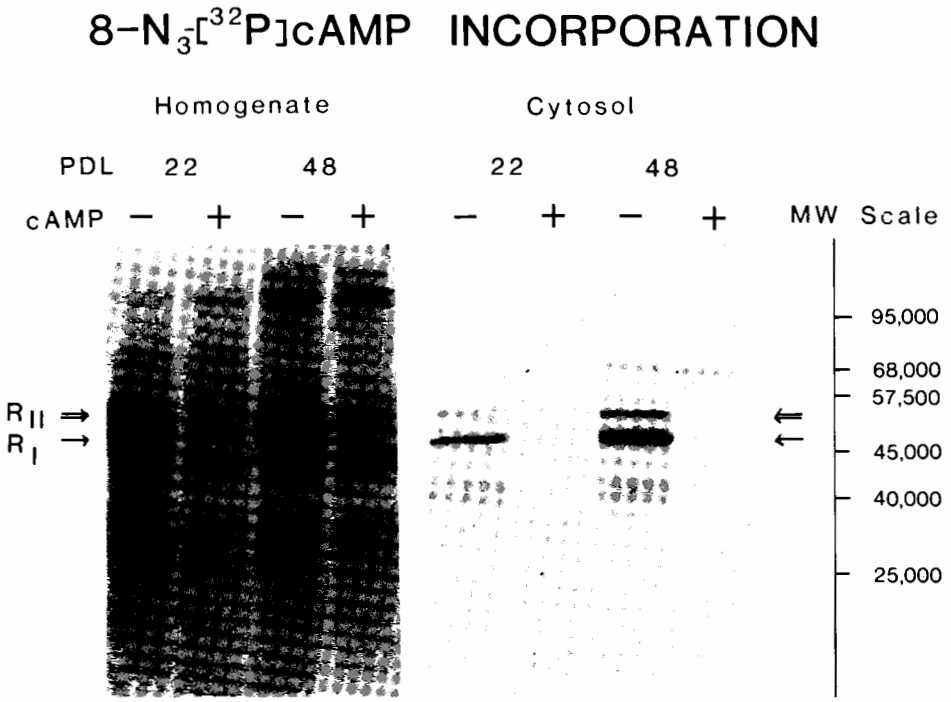

Fig. 1. Autoradiographs illustrating the incorporation of 8-N_3-[^{32}P]cAMP into homogenate and cytosol fractions of young (PDL 22) and old (PDL 48) IMR-90 diploid fibroblasts. Cells were harvested at confluency and cell extracts prepared according to methods described in the text. The incorporation of 8-N_3-[^{32}P]cAMP was carried out under standard conditions in the absence and presence of 50 μM cAMP (as indicated by the minus and plus signs at the tops of the autoradiographs). After a 60 min incubation at 4°C in the dark, the samples were photolyzed with a Mineralite UVS-11 handlamp for 10 min at 4°C. The pattern and amount 8-N_3-[^{32}P]cAMP incorporated were analyzed by subjecting the samples to SDS-polyacrylamide (5–15%) gel electrophoresis and autoradiography as previously described (Liu, 1982). The arrows identify the positions on the gel of the 54,000-dalton R_{II} and the 47,000-dalton R_I.

TABLE 1. Quantitation of the regulatory and catalytic subunit activity present in cytosol fractions of the young (PDL 22) and old (PDL 48) IMR-90 diploid fibroblasts[1]

Subunit	PDL 22 ("young")	PDL 48 ("old")
Regulatory		
8-N_3-[^{32}P]cAMP incorporation (pmol/mg protein)		
47,000-Dalton R_I	0.42 ± 0.09	0.95 ± 0.12
54,000-Dalton R_{II}	0.31 ± 0.07	0.55 ± 0.09
[^{3}H]cAMP binding (pmol/mg protein)	1.14 ± 0.22	2.79 ± 0.31
Catalytic		
Histone kinase activity (pmol ^{32}P transferred/min/mg)		
−cAMP	12.0 ± 2.4	10.0 ± 4.6
+cAMP	94.0 ± 12.9	188.0 ± 16.8

[1]Cells were harvested at confluency and the cytosol fractions prepared. Regulatory subunit activity was measured both by the covalent incorporation of 8-N_3-[^{32}P]cAMP into the 47,000-dalton R_I and the 54,000-dalton R_{II} and by the amount of [^{3}H]cAMP bound (22). Catalytic subunit activity of the kinase was determined by phosphorylation of histone II AS. Results represent means of four independent measurements ± SD.

the type II cAMP-dependent protein kinase is preferentially localized in membrane fractions of cell extracts (Corbin and Keely, 1977). Occasionally, as in the homogenate fraction illustrated in Figure 1, there were other protein bands with molecular weights both higher and lower than that of R_{II} and R_I, which incorporated 8-N_3-[^{32}P]cAMP. These bands do not represent specific cAMP-receptor proteins; the inclusion of a 50× excess of cAMP in the binding assay mixture did not displace the incorporation of 8-N_3-[^{32}P]cAMP into these proteins (Fig. 1).

Assay of the catalytic subunit activity of the kinase using the cAMP-dependent phosphorylation of histone II AS also demonstrated an approximately twofold increase in the enzyme activity present in the 100,000g cytosol fraction of old cells (PDL 48) compared to that of the young cells (PDL 22). As a reaffirmation that the activity assayed was specific to the cAMP-dependent protein kinase, we showed that this activity was inhibited by a partially purified inhibitor (Ashby and Walsh, 1972, 1973; Walsh et al., 1971) of the kinase (Fig. 2). The concomitant increase in regulatory and catalytic subunit activities of cAMP-dependent protein kinase in aging IMR-90 cells suggests an increase in the expression of the holoenzyme rather than its subunit proteins.

The increase in cAMP-dependent protein kinase activity in aging IMR-90 diploid fibroblasts as depicted in Figure 1 and Table 1 represents an increase in the level of the enzyme as indicated by results of the following two experiments. In experiment 1, measurement of the 8-N_3-[^{32}P]cAMP dose-dependent labeling of R_I and R_{II} demonstrated an increase in the maximal level of labeling of the proteins in old cells (PDL 48) compared to that in young cells (PDL 22) (Fig. 3). There was no significant difference in the apparent affinity, measured by EC_{50}, of the incorporation. The EC_{50} values of the incorporation of 8-N_3-[^{32}P]cAMP into R_I and R_{II} of the IMR-90 cells were in the ranges of 1–3 × 10^{-8} M and 1–2 × 10^{-7} M, respectively (Fig. 3); these values were in agreement with values obtained from similar experiments of other tissues and cell types (Liu, 1982; Walter et al., 1977). In experiment 2, fractionation of the kinases by DEAE-Sephacel column chromatography demonstrated a significant increase in the type I kinase and a small

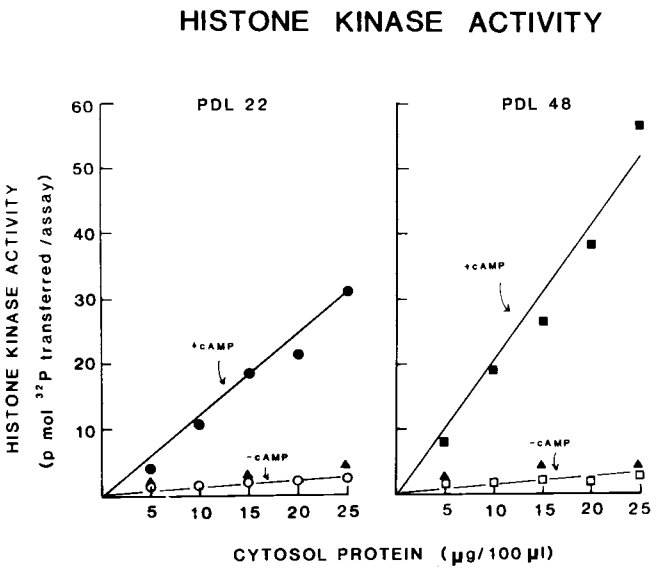

Fig. 2. cAMP-dependent protein kinase activity present in cytosol fractions of young (PDL 22) and old (PDL 48) IMR-90 diploid fibroblasts. Confluent cell cultures were harvested and used to prepare cytosol fractions according to methods described in the text. The assay mixture (final volume 100 µl) contained 50 mM 2(N-morpholino)ethane sulfonate (pH 6.2), 10 mM $MgCl_2$, 40 µg histone II AS, 40 µM [r^{32}P]ATP (specific activity 1–5 × 10^5 cpm/nmol), and various amounts of cytosol protein as indicated. The reaction was carried out for 10 min at 30°C in the absence (open circles) and presence (closed circles) of 5 µM cAMP. To validate that the activity measured represents that of the catalytic subunit cAMP-dependent protein kinase, assays were also carried out in the presence of 5 µM cAMP and a partially purified inhibitor of the catalytic subunit protein (closed triangle). The amount of radioactivity incorporated into histone II AS was determined by the method of Witt and Roskoski (1975) as previously described (Liu, 1980, 1982).

increase in the type II kinase in the column eluate of old diploid cells as compared to that of the young cells (Fig. 4).

The proliferative characteristics of human diploid fibroblasts can be roughly divided into three phases, phase I for the initial explanation period, phase II for a rapid proliferation with cells dividing logarithmically, and phase III for a period of declining proliferative capacity with granular cell morphology and little or no cell division. It was of interest to us to determine whether the increase in cAMP-dependent protein kinase activity in aging IMR-90 cells could be correlated with entrance into phase III of their life span. To examine this possibility, extracts were prepared from cells with PDLs of 27, 40, 48, and 53. Under our experimental conditions, cell senescence, or failure to grow to confluency with a 1:4 split ratio in 2 weeks with weekly media changes, occurred at PDLs of about 45–55. Examination of the pattern and amount of incorporation of 8-N_3-[^{32}P]cAMP into proteins present in extracts of cells with different PDLs revealed that the most significant increase occurs between PDL 40 and 48 (Fig. 5, Table 2). Similar results were obtained with the catalytic subunit activity of the kinase (Table 2). These results suggest that the increase in expression of cAMP-dependent protein kinase is specific to those cells approaching phase III of their life span.

DISCUSSION

Results in this study demonstrated an increase in the level of cAMP-dependent protein kinase in aging IMR-90 human diploid lung fibroblasts. Increases in the expression of cAMP-dependent protein kinase have been observed during terminal differentiation of a number of

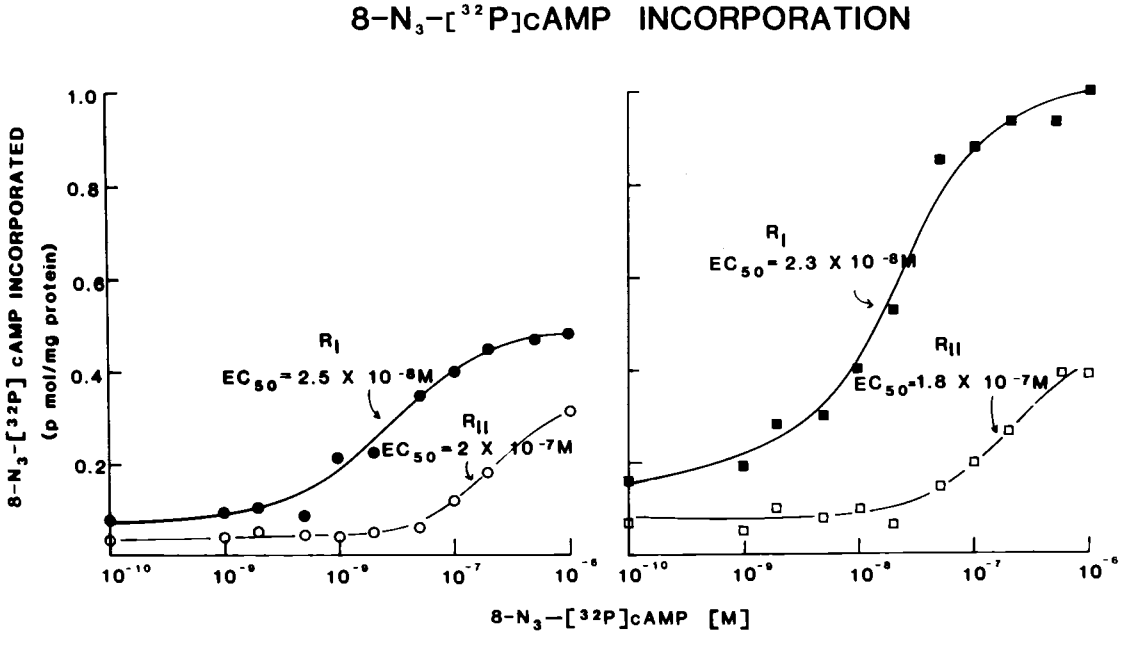

Fig. 3. Semilog dose-response plots illustrating the concentration-dependent incorporation of 8-N_3-[^{32}P]cAMP into the 47,000-dalton R_I and 54,000-dalton R_{II} present in cytosol of young (PDL 22; left) and old (PDL 48; right) IMR-90 diploid fibroblasts. The assay was carried out under standard conditions using 200 µg cytosol protein and various concentrations of 8-N_3-[^{32}P]cAMP as indicated. The amount of radioactivity incorporated into R_I and R_{II} was quantitated by liquid scintillation counting of excised gel slices. EC_{50} is defined as the concentration of 8-N_3-[^{32}P]cAMP needed to give a half-maximal level of incorporation into R_I or R_{II}.

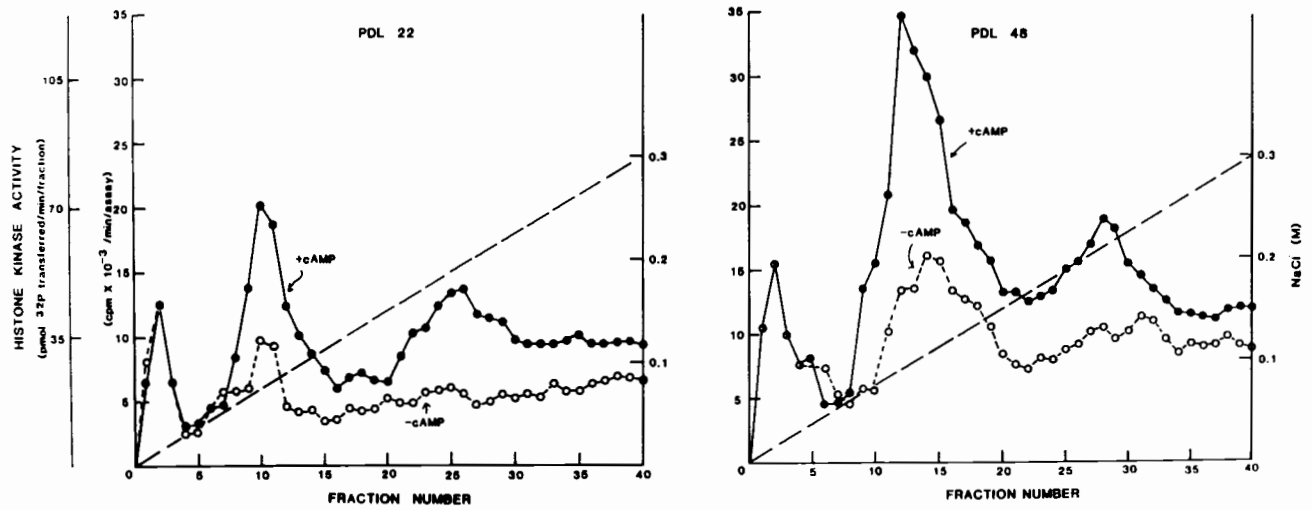

Fig. 4. Elution profile from DEAE-Sephacel columns of cAMP-dependent protein kinase of young (PDL 22) and old (PDL 48) IMR-90 cells. Cytosol preparations (1.2 ml) from young and old cell cultures containing 5 mg protein were loaded onto two identical DEAE-Sephacel columns (1.5 × 6 cm) and the columns developed with a linear gradient of NaCl (0–300 mM; total volume 20 ml) according to methods described in the text. Fractions of 0.5 ml were collected, and 50 μl aliquots were used for analysis of histone kinase activity in the absence (○) and presence (●) of 5 μM cAMP.

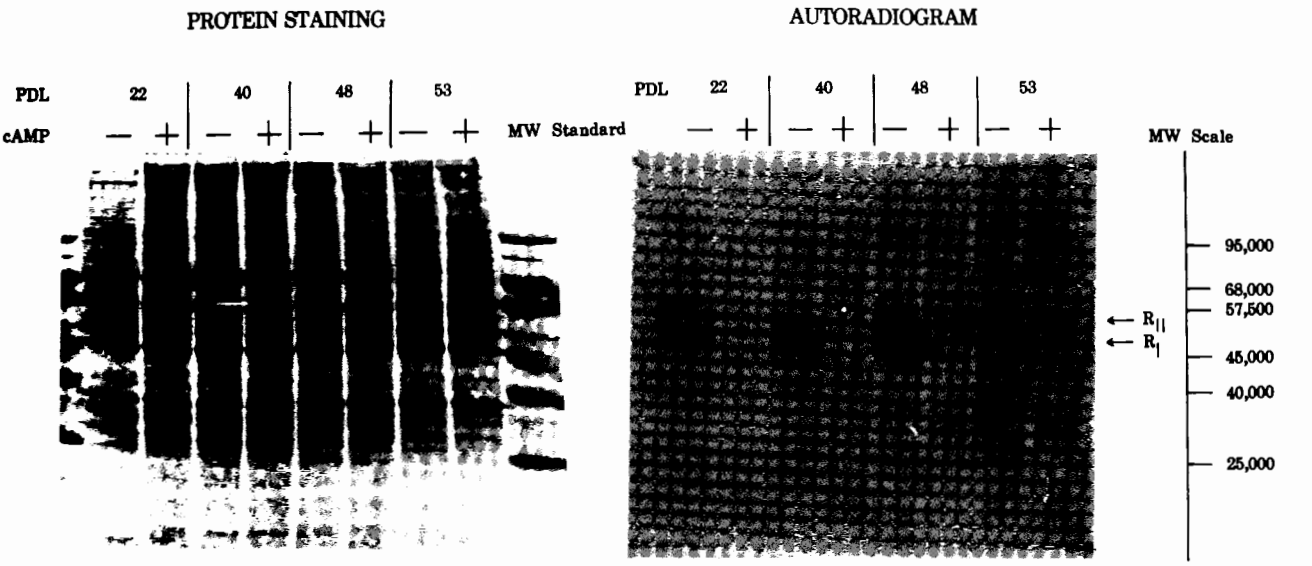

Fig. 5. Pattern of (left) Coomassie blue-stained SDS-polyacrylamide slab gel and (right) the corresponding autoradiograph illustrating the incorporation of 8-N_3-[^{32}P]cAMP cAMP into proteins present in extracts of IMR-90 with PDLs of 22, 40, 48, and 53. Confluent cell cultures were harvested and homogenates prepared. The incorporation of 8-N_3-[^{32}P]cAMP was carried out under standard conditions. Each lane contained 200 μg protein. The minus and plus signs at the tops of the gel and autoradiograph indicate the absence and presence, respectively, of 50 μM cAMP in the binding assay mixture. The protein standards (and their molecular weights) used were phosphorylase a (95,000), bovine serum albumin (68,000), catalase (57,000), ovalbumin (45,000), aldolase (40,000), chymotrypsinogen (25,000), and cytochrome c (12,500).

different cell types, including adipogenic 3T3-L1 cells (Liu, 1982), myogenic L-5, L-6, and C2 cells (Kamalakannan and Liu, 1985, Liu and Chen, 1983), and Friend erythroleukemic cells (Schwartz and Rubin, 1983). Together, these results implicate a significant role of cAMP-dependent protein kinase in regulating the proliferative potential of these cells.

One important consideration in comparing the level of cAMP-dependent protein kinase of young and old diploid fibroblasts is their difference in growth. Thus, although a significant proportion of the cells in aging IMR-90 culture is permanently growth-arrested, the majority of cells in early passage culture are capable of undergoing cell division. The possibility exists that the increased expression of cAMP-dependent protein kinase serves merely as an index of a specific part of the cell cycle. In this connection, it is noteworthy that all the experiments described in this study were carried out using growth-arrested confluent cell cultures to minimize growth differences between young and old cells. Furthermore, there was little or no significant difference in the cAMP-dependent protein kinase activity present in

TABLE 2. Measurement of the incorporation of 8-N_3-[^{32}P]cAMP into R_I and R_{II} and the cAMP-dependent histone kinase activity as a function of the population doubling level of IMR-90 diploid fibroblasts[1]

Population doubling level	8-N_3-[^{32}P]cAMP incorporation (pmol/mg protein)		Histone kinase (pmol ^{32}P/min/mg)
	R_I (47,000-d)	R_{II} (54,000-d)	
22	0.14	0.24	40
40	0.23	0.33	62
48	0.63	0.61	144
53	0.61	0.59	138

[1]Cells were harvested at confluency and homogenates prepared. The photoactivated incorporation of 8-N_3-[^{32}P]cAMP and histone kinase assay were carried out under standard conditions. The amount of 8-N_3-[^{32}P]cAMP incorporated into R_I and R_{II} was quantitated by liquid scintillation counting of excised gel slices. Results on the histone kinase activity represent the difference in activity between that obtained in the presence and absence of 5 μM cAMP. Results are representative of three separate experiments.

extracts of cells at logarithmic phase of growth vs. that of cells at the stationary phase of growth. The results of these experiments suggest that the increased expression of cAMP-dependent protein kinase activity in aging IMR-90 cells is not simply secondary to the slowing or cessation of cell growth.

Another important consideration in comparing the level of cAMP-dependent protein kinase in young and old diploid fibroblasts is their difference in size and protein content (Pool and Metter, 1984). In this connection, we determined the ratio of protein to DNA content in young and old IMR-90 diploid cells. Our results revealed that this ratio was approximately two to three times higher in the average old cells than in the average young cells. In other words, the amount of cellular protein is about two to three times higher in the old cell than in the young cell. As a consequence, the increase in cAMP-dependent protein kinase activity in the old cells would translate from a two- to threefold increase when expressed on a per milligram of protein basis to a five- to sevenfold increase when expressed on a per unit cell basis.

The precise function of the increased cAMP-dependent protein kinase in the control of cell growth and differentiation remains to be determined. Certainly, with the availability of techniques of microinjecting purified macromolecules into living somatic cells (Stacey, 1981), one of the possible approaches to this problem is to introduce purified cAMP-dependent protein kinase and its subunits into the diploid fibroblast and to evaluate the intracellular activity of the injected protein in the physiology of the target cell.

ACKNOWLEDGMENTS

The authors are indebted to Alfred Kulik and Jeffiner Kim for their excellent help during the course of this work. This research was supported in part by grants from the NSF (PCM 84–41349; DCB 84–17775), NIH (AG 03578), the New Jersey State Commission on Cancer Research, and the Busch Memorial Funds.

LITERATURE CITED

Ashby, C.D. and Walsh, D.A. (1972) Characterization of the interaction of a protein inhibitor with adenosine 3′:5′-monophosphate-dependent protein kinases. I. Interaction with the catalytic subunit of the protein kinase. J. Biol. Chem., *247*:6637–6642.

Ashby, C.D., and Walsh, D.A. (1973) Characterization of the interaction of a protein inhibitor with adenosine 3′:5′-monophosphate-dependent protein kinases. II. Mechanism of action with the holoenzyme. J. Biol. Chem., *248*:1255–1261.

Carchman, R.A., Johnson, G.S., and Pastan, I. (1974) Studies on the levels of cyclic AMP in cells transformed by wild-type and temperature-sensitive Kirsten sarcoma virus. Cell, *1*:59–64.

Cho-Chung, Y.S., Clair, T., and Porper, R. (1977) Cyclic AMP-binding proteins and protein kinase during regression of Walker 256 mammary carcinoma. J. Biol. Chem., *252*:6342–6348.

Corbin, J.D., and Keely, S.L. (1977) Characterization and regulation of heart adenosine 3′:5′-monophosphate-dependent protein kinase isozymes. J. Biol. Chem., *252*:910–918.

Friedman, D.L. (1976) Role of cyclic nucleotides in cell growth and differentiation. Physiol. Rev. *56*:652–708.

Gilman, A.G. (1970) A protein binding assay for adenosine 3′:5′-cyclic monophosphate. Proc. Natl. Acad. Sci. USA, *67*:305–312.

Hayflick, L. (1965) The limited in vitro lifetime of human diploid cell strains. Exp. Cell Res. *37*:614–636.

Hayflick, L. (1975) Current theories of biological aging. Fed. Proc. *34*:9–13.

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

Kamalakannan, V., and Liu, A.Y.-C. (1985) Increased expression cAMP-dependent protein kinase during myogenesis. J. Cell Biol., *101*:356a.

Liu, A.Y.-C. (1980) Role of cAMP-dependent protein kinase in the induction of tryosine aminotransferase. J. Biol. Chem., *255*:4421–4429.

Liu, A.Y.-C. (1982) Differentiation-specific increase of cAMP-dependent protein kinase in the 3T3-L1 cells. J. Biol. Chem., *257*:298–306.

Liu, A.Y.-C., Chan, T., and Chen, K. Y. (1981) Induction of the regulatory subunit of type I adenosine cyclic 3′:5′-monophosphate-dependent protein kinase in differentiated N-18 mouse neuroblastoma cells. Cancer Res., *41*:4579–4587.

Liu, A.Y.-C., and Chen, K.Y. (1983) The increase of a cAMP-dependent protein kinase during myogenesis. Fed. Proc., *42*:2915 (abstract).

Liu, A.Y.-C., Fiske, W.W., and Chen, K.Y. (1980) Regulation of cyclic adenosine 3′:5′-monophosphate-binding protein in N-18 mouse neuroblastoma cells. Cancer Res. *40*:4100–4108.

Lockwood, A.H., Trivette, D.D., and Pendergast, M. (1981) Molecular events in cAMP-mediated reverse transformation. Cold Spring Harbor Symp. Quant. Biol., *46*:909–919.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1955) Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193*:265–275.

Pastan, I., and Willingham, M. (1978) Cellular transformation and the 'morphologic phenotype' of transformed cells. Nature, *274*:645–650.

Plet, A., Evain, D., and Anderson, W.B. (1982) Effect of retinoic acid treatment of F9 embryonal carcinoma cells on the activity and distribution of cyclic AMP-dependent protein kinase. J. Biol. Chem., *257*:889–893.

Pool, T.B., and Metter, J.D. (1984) New concepts in regulation of the lifespan of human diploid fibroblasts in vitro. In Aging and Cell Structure. J.E. Johnson, Jr., ed. Plenum Press, New York, Vol. 2, pp. 89–112.

Prasad. K. N. (1975) Differentiation of neuroblastoma cells in culture. Biol. Rev., *50*:129–165.

Schwartz, D.A., and Rubin, C.S. (1983) Regulation of cAMP-dependent protein kinase subunit levels in Friend erythroleukemic cells. J. Biol. Chem., *258*:777–784.

Stacey, D.W. (1981) Microinjection of mRNA and other macromolecules into living cells. Meth. Enzymol. *79*:76–88.

Walsh, D.A., Ashby, C.D., Gonzalez, C., Calkins, D., Fischer, E.H., and Krebs, E.G. (1971) Purification and characterization of a protein inhibitor of adenosine 3′:5′-monophosphate protein kinases. J. Biol. Chem., *246*:1977–1985.

Walter, U., Uno, E., Liu, A.Y.,-C., and Greengard, P. (1977) Identification, characterization, and quantitative measurement of cyclic AMP receptor proteins in cytosol of various tissues using a photoaffinity ligand. J. Biol. Chem., *252*:6494–6500.

Witt, J.J., and Roskoski, R. (1975) Rapid protein kinase assay using phosphocellulose paper absorption. Anal. Biochem., *66*:253–258.