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Mutant Strain of Chinese Hamster Ovary Cells with No Detectable Ornithine Decarboxylase Activity

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We previously described an arginase-deficient, polyamine-dependent Chinese hamster ovary cell line which grows in serum-free medium. From this strain we isolated a new mutant strain that has no detectable catalytic ornithine decarboxylase activity. The mutant cells contain, however, immunoreactive ornithine decarboxylase-like protein roughly in the same quantity as the parent strain. The mutant and the parent cell line strains also contain similar amounts of ornithine decarboxylase-mRNA hybridizable to a specific cDNA. If polyamines are omitted from the medium, proliferation of the mutant cells is considerably retarded and ceases in 6 to 10 days. Addition of ornithine or α-difluoromethylornithine, a specific inhibitor of ornithine decarboxylase, has no effect on these cells. Putrescine and spermidine decreased in the mutant cells to undetectable levels during polyamine starvation, whereas spermine was reduced to 1/5th of that found in the control cultures. Polyamines appear to be indispensable for the mutant strain, but this was obvious only after the amount of polyamines, found as impurities in bovine serum albumin used in the medium, was reduced by dialysis to 10^{-12} M. Because sera contain polyamines, the ability of the mutant strain to grow in serum-free medium is a great advantage in elucidation of the mechanisms of polyamine function.

The polyamines putrescine, spermidine, and spermine are found in all cells, and they appear to be required for optimal growth of both bacteria and eucaryotes. Bacterial mutants that are completely devoid of polyamines have been isolated. These mutants have a considerably retarded rate of growth, but they can replicate indefinitely in the absence of polyamines (4). However, some yeast cell mutants unable to synthesize polyamines will gradually stop replicating (28, 31).

We previously isolated a strain of Chinese hamster ovary cells (CHO), designated A7, which grows under serum-free conditions and has deficient arginase activity. Hence, it is unable to synthesize ornithine, the precursor of polyamines. If ornithine and polyamines are omitted from the growth medium, the cells are depleted of polyamines, and their growth is gradually retarded (8, 21). At the same time, the cells lose most of their actin filaments and microtubules (21), and they also develop major chromosome aberrations (14, 19, 20). Thus, polyamine starvation of the A7 cells affects structures characteristic of eucaryotic cells. Steglich and Scheffler later isolated an ornithine decarboxylase-deficient mutant of CHO cells, designated C54, with 3% of the ornithine decarboxylase activity of the wild type (26). These cells, however, showed only a slight retardation of growth when polyamines were omitted from the growth medium based on the otherwise rich nutrient mixture F10 (25).

In this communication we report isolation from A7 cells of a mutant strain designated P22 that lacks catalytically active ornithine decarboxylase enzyme and thus cannot synthesize putrescine. When external sources of polyamines were excluded, the P22 cells gradually ceased replication and did not resume growth in a subsequent passage. Thus, polyamines seemed to be indispensable for replication of these cells. It is notable that serum invariably contains small quantities of polyamines, and therefore, the ability of the P22 cells to grow in serum-free medium is a valuable asset when the importance of polyamines for higher eucaryotes is evaluated.

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MATERIALS AND METHODS

Cell culture. The cells were cultured on collagen-coated petri dishes in a 1:1 (vol/vol) mixture of minimal essential medium (MEM) and nutrient mixture F12 supplemented with 0.1% (wt/vol) bovine serum albumin (BSA) (21). Nutrient mixture F12 contains 10^{-6} M putrescine, but F12 without putrescine was used in the experiments. Unless otherwise stated, the BSA used in the experiments was dialyzed as a 10% (wt/vol) solution against 1 M NaCl–0.2 M $MgCl_2$ and finally against 0.15 M NaCl. Routine cultures were performed with medium containing undialyzed BSA. Cells were regularly checked for mycoplasmas with negative results.

Method for selection of a putrescine auxotroph. The method of suicide selection introduced by Steglich and Scheffler (26) was used for obtaining the putrescine auxotroph. For mutagenesis, nearly confluent cultures were incubated with ethyl methane sulfonate (440 μg/ml) for 18 h. After being washed, the cultures were split in a 1:5 ratio, cultured for 1 day, and reseeded in ornithine- and polyamine-free medium. The medium was replaced the next day with fresh medium of the same composition, and the cultures were incubated for 24 h with L-[2,3-^{3}H]ornithine (2 μCi/ml). The cells were then collected by trypsinization, stored in liquid nitrogen for 10 days, and subjected to a second round of [^{3}H]ornithine treatment. Cell colonies were isolated from the survivors and screened for eventual putrescine auxotrophs. For this purpose, 20 to 40 cells per cm^2 were plated on petri dishes and grown in medium containing 0.9% (wt/vol) agar, 0.2% (wt/vol) BSA, and 10^{-6} M putrescine. In ca. 2 weeks, when the cell colonies were large enough for further propagation, they were picked up with Pasteur pipettes, cultured, and tested for putrescine auxotrophy by being grown in the presence and absence of ornithine and putrescine.

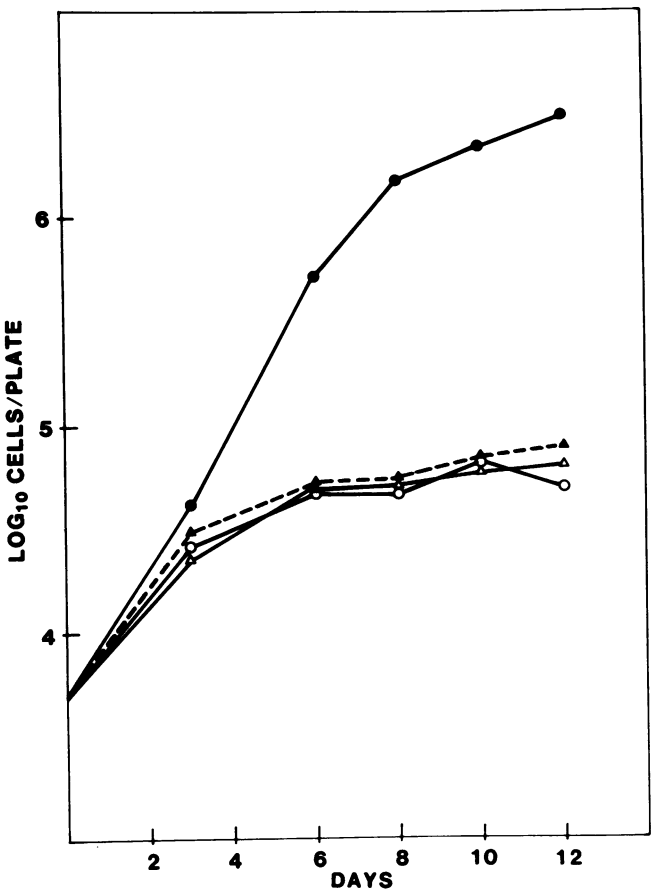

FIG. 1. Effect of putrescine, ornithine, and α-difluoromethyl ornithine on growth of the P22 cells. Five thousand cells were seeded on each plate (diameter, 3 cm) in polyamine-free medium. Part of the cultures received putrescine (10^{-6} M), ornithine (10^{-4} M), or α-difluoromethylornithine (5×10^{-4} M). At different time intervals, cells were detached with trypsin and counted in an electric counter (Coulter counter). The medium was changed on days 3, 6, and 10. Each value represents the mean of duplicate dishes. Symbols: ○, no additions; ●, putrescine; △, α-difluoromethylornithine; ▲, ornithine.

Chemicals. Putrescine dihydrochloride and BSA (Cohn fraction V) were obtained from Sigma Chemical Co., St. Louis, Mo. Spermidine and spermine were from Calbiochem-Behring, San Diego, Calif. L-Ornithine monohydrochloride was obtained from Fluka AG, Buchs, Swizerland, and it was purified as described previously (8). DL-α-Difluoromethylornithine was a gift from the Merrell Research Center, Cincinnati, Ohio. DL-[1-^{14}C]ornithine (specific activity, 58 mCi/mmol) and L-[*U*-^{14}C]ornithine (specific activity, 280 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, United Kingdom. L-[2,3,-^{3}H]ornithine (specific activity, 15.2 Ci/mmol) was from New England Nuclear Corp., Boston, Mass.

Determination of enzyme activities. The activity of ornithine decarboxylase was measured as described by Jänne and Williams-Ashman (11) with minor modifications. The assay mixture (0.25 ml) consisted of 50 mM Tris-hydrochloride buffer (pH 7.4), 3 μCi of DL-[1-^{14}C]ornithine, 0.05 mM L-ornithine, 0.1 mM pyridoxal phosphate, 4 mM dithiothreitol, 4 mM EDTA, and a 100,000 × *g* supernatant fraction of the cells. *S*-Adenosylmethionine decarboxylase activity was determined in the presence of 2.5 mM putrescine with 0.2 mM *S*-adenosyl-L-[1-^{14}C]methionine (specific activity, 10 mCi/mmol) as the substrate (12).

Radioimmunoassay of ornithine decarboxylase. The ornithine decarboxylase concentration was measured by radioimmunoassay with antiserum raised in rabbits against the enzyme protein purified to homogeneity from mouse kidneys (10). The results are expressed relative to the homogeneous mouse renal ornithine decarboxylase used as the standard in the radioimmunoassay. For each measurement 10^8 cells were used.

Polyacrylamide gel electrophoresis. One-dimensional sodium dodecyl sulfate-gel electrophoresis was performed by the system of Laemmli (16).

[^{35}S]methionine labeling and immunoprecipitation of ornithine decarboxylase. Cells in exponential growth, attached to plates, were incubated with MEM without methionine and labeled with [^{35}S]methionine as described (see the legend to Fig. 3). Incorporation was stopped by washing the cells twice with cold MEM containing methionine. To prepare cell lysates for immunoprecipitation, cells were scraped in homogenization buffer (50 mM Tris, 150 mM NaCl, 5 mM EDTA, 0.5% [vol/vol] Nonidet P-40, 2 mM methionine [pH 7.4] at 20°C) and sonicated. The immunoprecipitation with monospecific ornithine decarboxylase antiserum (10) and with normal rabbit serum was carried out as described previously (15).

Northern blot analysis of ornithine decarboxylase-mRNA. Total RNA from CHO cells was isolated by the lithium chloride-urea method (1) and enriched for polyadenylate-containing RNA by oligodeoxythymidylate-cellulose (2) as previously described (15). Northern blot hybridization analysis was performed essentially as described by Thomas (29), except that the final wash was performed at a lower stringency (0.3 M NaCl, 0.03 M sodium citrate, 0.1% sodium dodecyl sulfate; 1 h at 50°C). Nick-translated plasmid pODC16 (13) was used as the hybridization probe. This cDNA (ca. 1.3 kilobases) was prepared from ornithine decarboxylase-mRNA from the kidneys of androgen-treated mice (13, 15).

Analysis of polyamines. The cells were collected by scraping, centrifuged, and extracted with 0.2 M perchloric acid. Polyamine levels were determined by the dansylation method of Seiler (23) by using alkaline ether extraction of the dansyl derivatives (3) as described (7). The dansylated amines were separated on aluminium oxide thin-layer plates in the solvent system chloroform-dioxan-butanol (48:1:1 [vol/vol/vol]). For determining polyamines bound to BSA (before and after dialysis), 100 mg of albumin was extracted with perchloric acid and subjected to dansylation analysis. For determining serum polyamines, 10 to 20 ml of serum was first precipitated with 5% (wt/vol) trichloroacetic acid. After removal of the acid with ether, the polyamines were extracted from alkaline solution into *n*-butanol, which was then evaporated to dryness according to the method of Raina (22). The residue was dissolved in perchloric acid, and the amines were analyzed by the dansylation method as described above.

All the results were confirmed in two to four separate experiments. The cell numbers, the enzyme activities, and the polyamine contents were determined in each experiment from duplicate dishes, with less than 10% variation.

RESULTS

Selection of a putrescine auxotroph. The suicide selection method introduced by Steglich and Scheffler (26) was used

for obtaining the putrescine auxotroph. After mutagenization, the cells were subjected to two rounds of [^{3}H]ornithine treatment to enrich for cells which failed to incorporate the labeled compound, thus escaping the radiation suicide. Of the survivors, 39 cell clones were isolated and tested for putrescine auxotrophy. Two of the clones grew only in the presence of putrescine but not in the presence of ornithine. One of them, designated P22, was recloned twice and used in further studies. This cell line strain has since then been cultured for over a year without a change in its requirement for putrescine.

Polyamine requirement for growth. When polyamines were omitted from the medium, the proliferation of the P22 cells ceased gradually in 6 to 10 days (Fig. 1). The final density reached in the polyamine starved cultures was 1/40th to 1/100th of that in the control cultures supplied with putrescine. Purified ornithine had no effect on growth, even when its concentration was raised up to 5×10^{-4} M. α-Difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, did not influence the growth rate of this cell line (Fig. 1). Spermidine and spermine at a concentration of 10^{-6} M could replace putrescine for supporting the growth of the cells (data not shown).

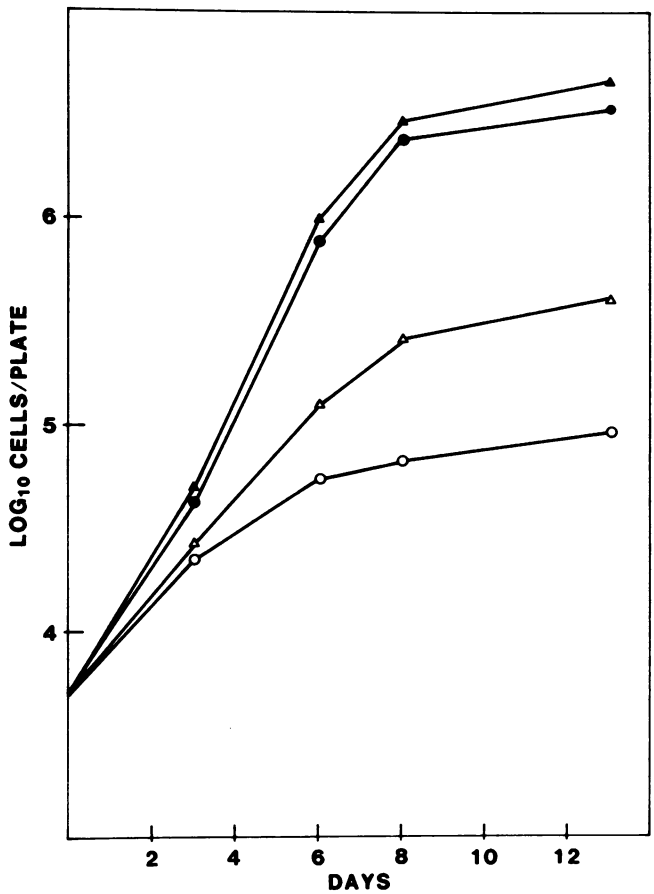

FIG. 2. Effect of dialysis of BSA on the growth of the A7 cells. Half of the cells were suspended in polyamine-free medium containing 0.1% dialyzed BSA, whereas the other half was suspended in medium containing 0.1% nondialyzed BSA. Five thousand cells were seeded on each plate, and 10^{-6} M putrescine was added to half of the cultures. The cells were counted at the indicated time points (see Fig. 1). The medium was changed on days 3, 6, and 10. Each value represents the mean of duplicate dishes. Symbols: ○, dialyzed BSA without putrescine; △, undialyzed BSA without putrescine; ●, dialyzed BSA plus putrescine; ▲, undialyzed BSA plus putrescine.

TABLE 1. Activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylases in the A7 and P22 cells[a]

Cell line	Ornithine decarboxylase		S-Adenosylmethionine decarboxylase (nmol/mg of protein per h)
	pmol/mg of protein per h	cpm/2 × 10^7 cells	
A7	500	64,000	3.0
P22	<0.04	<5	2.6

[a] The A7 and P22 cells grown to near confluency in putrescine-containing medium were split in a ratio of 1:5 and cultured in ornithine- and polyamine-free medium for 2 days. The cells (10^8 cells per 20 dishes; diameter, 10 cm) were collected by scraping, centrifuged, and suspended in 25 mM Tris-hydrochloride (pH 7.1)–0.1 mM EDTA–1 mM dithiothreitol buffer. The cells were disintegrated by sonication. The homogenate was centrifuged at 100,000 × *g* for 20 min, and the supernatant fraction was used as a source of the enzymes.

When the P22 cells were starved of polyamines for 3 days, addition of putrescine increased the growth rate almost to the same level as in the cultures grown continuously with putrescine. If the starvation lasted for 7 days before the addition of putrescine, the growth rate recovered more slowly, and after a 10-day starvation, polyamine depletion had already caused irreversible damage. Addition of putrescine at this stage no longer revived the cells (data not shown). When cultures started with 5,000 cells per dish (diameter, 3 cm) were starved of polyamines for 14 days and then replated, very few cells attached to the dish, and these cells subsequently died without replicating. On rare occasions, however, some cells gave rise to small colonies of 5 to 50 cells after several medium changes.

Effect of polyamines found as impurities in the medium. BSA, which is a component of the medium used in the experiments, was found to contain polyamines. The medium contained 0.1% BSA, giving a final concentration of polyamines in the medium of ca. 10^{-9} M. The polyamine content of the medium could, however, be reduced to 10^{-12} M by dialyzing the BSA against a solution of 1 M NaCl–0.2 M $MgCl_2$ and finally against 0.15 M NaCl. Figure 2 demonstrates that this reduction of polyamine concentration had a considerable effect on the growth of the cells. Without exogenous polyamines, the final cell density of the A7 cells was about five times higher in the medium containing undialyzed BSA than in the cultures with dialyzed BSA. On the other hand, the growth of the cultures supplied with putrescine was not significantly affected by the use of dialyzed BSA.

Enzyme activities. No catalytic ornithine decarboxylase activity was detected in the P22 cells, whereas readily measurable enzyme activity was found in the A7 cells (Table 1). Lack of measurable ornithine decarboxylase activity in the P22 cells was also confirmed by finding insignificant formation of polyamines from unlabeled ornithine (Table 2) or from L-[*U*-^{14}C]ornithine (data not shown) during several days of culture. Another key enzyme of polyamine biosynthesis, *S*-adenosylmethionine decarboxylase, was clearly measurable in both the P22 and A7 cells (Table 1).

Ornithine decarboxylase polypeptide. The ornithine decarboxylase protein was measured from the soluble cytosol extracts by radioimmunoassay (10). The P22 cells contained protein with ornithine decarboxylase-like immunoreactivity in amounts comparable to those found in the parental A7 cell

TABLE 2. Polyamine levels (nmol/10^7 cells) in A7 and P22 cells grown in the absence and presence of putrescine and ornithine[a]

Cell line (incubation time)	Addition	Amt (nmol/10^7 cells) of:			
		Putrescine	Spermidine	Aminopropyl-cadaverine	Spermine
A7 (3 days)	None	<0.02	0.05	0.14	1.5
	Putrescine	0.20	2.9		2.0
	Ornithine	0.13	2.3		3.8
A7 (6 days)	None	<0.02	0.04	0.46	1.8
	Putrescine	0.06	1.7		2.7
	Ornithine	0.06	2.8		6.8
P22 (3 days)	None	<0.02	0.03	<0.02	1.1
	Putrescine	0.28	2.5		2.0
	Ornithine	<0.02	0.03		1.0
P22 (6 days)	None	<0.02	<0.02	<0.02	0.66
	Putrescine	0.07	2.5		3.8
	Ornithine	<0.02	<0.02		0.85

[a] Cells were grown on petri dishes (diameter 10 cm) in polyamine-free medium, and putrescine (10^{-6} M) or ornithine (10^{-4} M) was added to the indicated cultures. The medium was changed on the second and fourth days. After the cultures were washed twice with MEM, the cells were collected by trypsinization, centrifuged, and extracted with perchloric acid for polyamine analysis.

line, 1.3 versus 1.2 mg/ml of cytosol protein. The ornithine decarboxylase-like protein was analyzed also by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis from cells labeled with [^{35}S]methionine. Figure 3 shows that both the A7 and the P22 displayed bands of nearly equal intensity, with a molecular weight of ca. 51,000.

Ornithine decarboxylase-mRNA levels. Polyadenylate-containing RNA from the P22 and A7 cells contained similar amounts of RNA that hybridized to a nick-translated cDNA clone (pODC16; 13) for ornithine decarboxylase-mRNA (Fig. 4). Both cell lines had two species of hybridizable mRNA with molecular sizes of 2.2 and 2.7 kilobases (Fig. 4).

Polyamine levels. Omission of polyamines and ornithine from the culture medium caused depletion of polyamines in both the A7 and the P22 cells (Table 2). High levels of polyamines were found in the A7 cells in the presence of both ornithine and polyamines, but the P22 cells had high levels of polyamines only when cultured in polyamine-containing medium; ornithine had no effect on the polyamine concentrations in these cells. Starvation of the cells of polyamines for 3 days decreased the putrescine concentration to undetectable levels in both cell types. After 6 days of starvation, spermidine was not detectable in the P22 cells, but traces of it were found in the A7 cells. On the other hand, spermine was found in both cell lines, although its concentration clearly decreased during polyamine starvation. The spermine content of the A7 cells fell in 6 days to about two-thirds of that in the control cells grown in putrescine-containing medium, whereas the corresponding fraction was

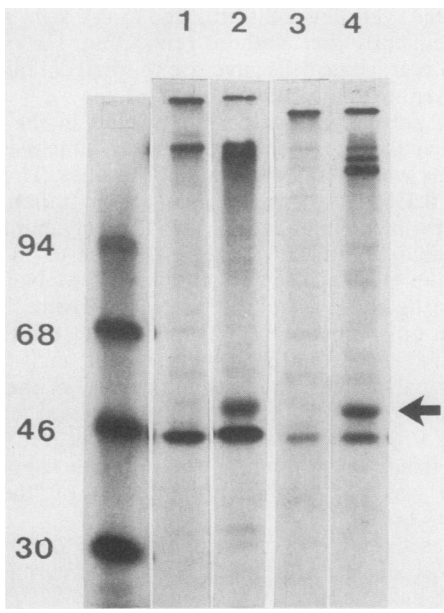

FIG. 3. Immunoprecipitation of [^{35}S]methionine-labeled extracts from the A7 and P22 cells. After the cells were incubated for 30 min with MEM without methionine, [^{35}S]methionine (80 μCi/ml) was added, and the incubation was continued for 1 h. Cytosolic extracts were prepared, and a portion was immunoprecipitated with anti-ornithine decarboxylase or with normal rabbit serum. The immunoprecipitated proteins were fractionated by one-dimensional sodium dodecyl sulfate-gel electrophoresis in 8% polyacrylamide and visualized by fluorography. Lanes: 1, A7 extract precipitated with normal rabbit serum; 2, A7 extract immunoprecipitated with anti-ornithine decarboxylase; 3, P22 extract precipitated with normal rabbit serum; 4, P22 extract immunoprecipitated with anti-ornithine decarboxylase.

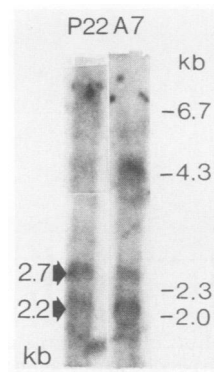

FIG. 4. Northern blot analysis of ornithine decarboxylase-mRNA in the A7 and P22 cells. Polyadenylate-containing RNA from 2×10^8 to 3×10^8 cells was isolated and fractionated on 1% agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized to nick-translated pODC16 (1.5×10^8 cpm/μg of DNA). Both samples contained 18 μg of RNA. Lambda DNA cleaved with restriction enzyme *Hin*dIII was used as the molecular-size marker. Exposure time at −70°C was 72 h with Kodak XAR film.

less than 1/5th in the P22 cells. The results indicate that polyamine depletion was more severe in P22 than in A7 cells. Prolonged polyamine starvation did not reduce the polyamine concentrations any further. This is understandable, as replication of the polyamine-starved cells had almost ceased in 6 days. As a matter of fact, during prolonged starvation and after several replenishments with fresh medium, polyamine levels in the cells even tended to increase a little, probably because the cells were able to take up traces of polyamines found as impurities in the medium.

As shown previously (9), the polyamine-starved A7 cells accumulated aminopropylcadaverine, an unusual polyamine derived from lysine through decarboxylation by ornithine decarboxylase to cadaverine that is subsequently converted to a higher polyamine analog in a spermidine synthase-catalyzed reaction. No cadaverine or its higher derivatives were detected in the P22 cells (Table 2).

DISCUSSION

This paper describes a putrescine auxotroph, designated P22, which was derived from the arginase-deficient CHO strain A7 growing in serum-free medium (8, 21). The P22 cells had no measurable ornithine decarboxylase activity and are therefore unable to transform ornithine into putrescine. Except for a brief mention of the isolation of two mutants of CHO cells without ornithine decarboxylase activity (25), no strain of higher eucaryotes without detectable ornithine decarboxylase activity has been reported. The ornithine decarboxylase-deficient strain C54 described by Steglich and Scheffler (26) differs from the P22 cells in several ways. The C54 cells have 3% of the ornithine decarboxylase activity found in the wild-type cells, whereas the P22 cells show no ornithine decarboxylase activity. The C54 cells require serum for growth, whereas the P22 cells do not. The growth of polyamine-starved C54 cells is significantly retarded only in MEM; in the richer F10 medium omission of polyamines has only a small effect on the growth of the cells (25). The P22 cells show marked growth retardation and finally complete cessation of growth in the absence of polyamines in an otherwise optimal medium consisting of MEM and the nutrient mixture F12 in a ratio of 1:1 plus 0.1% BSA. It is notable that the P22 cells are not able to grow in serum-free medium when the nutrient mixture F12 is omitted.

Despite no detectable ornithine decarboxylase activity, the P22 cells contained protein that reacted in radioimmunoassay with antibodies prepared against purified ornithine decarboxylase and had in polyacrylamide gel electrophoresis the same mobility as the enzyme from the parental A7 cells. The quantity of the immunologically reactive ornithine decarboxylase-like protein in the P22 cells was also comparable to that found in the A7 cells. In addition, these two cell lines contained similar amounts of ornithine decarboxylase-mRNA with identical molecular weights. These data suggest that the P22 cells have a mutation in the structural gene of ornithine decarboxylase, causing a change in the structure of the catalytic center of the enzyme protein.

There is evidence suggesting that polyamines might be associated with chromatin. Lack of polyamines causes retardation of DNA synthesis (5, 6, 18, 24), and it also damages chromosomes (14, 19, 27, 30). Moreover, there is an excellent stereospecific fit between polyamines and the DNA double helix (17). If we assume that polyamines are components of the chromatin, the radiation from the labeled polyamines derived from radioactive ornithine would effectively damage the DNA and kill the cell. This would explain the relative ease with which putrescine auxotrophs have been obtained.

The medium used in the experiments was supplied with BSA that was found to contain polyamines as contaminants, giving a final concentration in the medium of ca. 10^{-9} M, sufficient to support the growth of the cells to a certain extent. Dialysis of BSA reduced the final polyamine concentration in the medium to 10^{-12} M and abolished its ability to support continuous growth of the cells. When dialyzed BSA was used in the medium, the growth of the cells ceased in 6 to 10 days, and after 10 days they could not be revived anymore by adding polyamines. This is expected in view of the grave chromosomal abnormalities found in the cells after polyamine starvation (14, 19). It is of note that serum is a component of most tissue culture media and contains polyamines in variable quantities. We found that the total polyamine concentration in human and calf serum is 10^{-6} to 10^{-7} M, a concentration which is likely to affect cellular growth in medium supplemented with 5 to 10% serum. It is, therefore, essential to use serum-free medium to evaluate the whole impact of polyamine depletion on the cells.

The P22 cells contained spermine even after prolonged starvation of polyamines, although its concentration was reduced to ca. 20% of that found in the control cells. It is possible that spermine is indispensable for the cells and that cells stop replicating as soon as the concentration of spermine falls below a certain limit.

The results presented in this paper show that polyamines are indispensable for the P22 cells. This may be true for all eucaryotes, although it can be difficult to prove because polyamines as impurities are hard to exclude from the tissue culture media. Serum presents a particular problem. The putrescine auxotroph P22, which grows in serum-free medium, can therefore provide a valuable tool for the evaluation of the importance of polyamines for higher eucaryotes.

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LITERATURE CITED

1. **Auffray, C., and F. Rougeon.** 1980. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. Eur. J. Biochem. **107**:303–314.
2. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. **69**:1408–1412.
3. **Dreyfuss, G., R. Dvir, A. Harell, and R. Chayen.** 1973. Determination of polyamines in urine. Clin. Chim. Acta **49**:65–72.
4. **Hafner, E. W., C. W. Tabor, and H. Tabor.** 1979. Mutants of *Escherichia coli* that do not contain 1,4-diaminobutane (putrescine) or spermidine. J. Biol. Chem. **254**:12419–12426.
5. **Harada, J. J., and D. R. Morris.** 1981. Cell cycle parameters of Chinese hamster ovary cells during exponential, polyamine-limited growth. Mol. Cell. Biol. **1**:594–599.
6. **Heby, O., G. Andersson, and J. W. Gray.** 1978. Interference with S and G2 phases progression by polyamine synthesis inhibitor. Exp. Cell Res. **111**:461–464.
7. **Hölttä, E., J. Jänne, and T. Hovi.** 1979. Suppression of the formation of polyamines and macromolecules by DL-difluoromethylornithine and methylglyoxal bis(quanylhydrazone) in

phytohaemagglutinin-activated human lymphocytes. Biochem. J. **178**:109–117.
8. **Hölttä, E., and P. Pohjanpelto.** 1982. Polyamine dependence of Chinese hamster ovary cells in serum-free culture is due to deficient arginase activity. Biochim. Biophys. Acta **721**:321–327.
9. **Hölttä, E., and P. Pohjanpelto.** 1983. Polyamine starvation causes accumulation of cadaverine and its derivatives in a polyamine-dependent strain of Chinese hamster ovary cells. Biochem. J. **210**:945–948.
10. **Isomaa, V. V., A. E. I. Pajunen, C. W. Bardin, and O. A. Jänne.** 1983. Ornithine decarboxylase in mouse kidney. Purification, characterization and radioimmunological determination of the enzyme protein. J. Biol. Chem. **258**:6735–6740.
11. **Jänne, J., and H. G. Williams-Ashman.** 1971. On the purification of ornithine decarboxylase from rat prostate and effects of thiol compounds on the enzyme. J. Biol. Chem. **246**:1725–1732.
12. **Jänne, J., and H. G. Williams-Ashman.** 1971. Dissociation of putrescine-activated decarboxylation of *S*-adenosyl-methionine from the enzyme synthesis of spermidine and spermine by purified prostatic preparations. Biochem. Biophys. Res. Commun. **42**:222–229.
13. **Jänne, O. A., K. K. Kontula, V. V. Isomaa, and C. W. Bardin.** 1984. Ornithine decarboxylase mRNA in mouse kidney: a low abundancy gene product regulated by androgens with rapid kinetics. Ann. N.Y. Acad. Sci. **438**:72–84.
14. **Knuutila, S., and P. Pohjanpelto.** 1983. Polyamine starvation causes parallel increase in nuclear and chromosomal aberrations in a polyamine-dependent strain of CHO. Exp. Cell Res. **145**:222–226.
15. **Kontula, K. K., T. K. Torkkeli, C. W. Bardin, and O. A. Jänne.** 1984. Androgen induction of ornithine decarboxylase mRNA in mouse kidney as studied by complementary DNA. Proc. Natl. Acad. Sci. U.S.A. **81**:731–735.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Liquori, A. M., V. Costantino, V. Crescenzi, V. Elia, E. Giglio, R. Puliti, M. De Santis Savino, and V. Vitagliano.** 1967. Complexes between DNA and polyamines: a molecular model. J. Mol. Biol. **24**:113–122.
18. **Pohjanpelto, P.** 1975. Putrescine shortens the S-period in human fibroblasts. Biomedicine **23**:350–352.
19. **Pohjanpelto, P., and S. Knuutila.** 1982. Polyamine deprivation causes major chromosome aberrations in a polyamine-dependent Chinese hamster ovary cell line. Exp. Cell Res. **141**:333–339.
20. **Pohjanpelto, P., and S. Knuutila.** 1984. Induction of major chromosome aberrations in Chinese hamster ovary cells by α-difluoromethylornithine. Cancer Res. **44**:4535–4539.
21. **Pohjanpelto, P., I. Virtanen, and E. Hölttä.** 1981. Polyamine starvation causes disappearance of actin filaments and microtubules in polyamine auxotrophic CHO cells. Nature (London) **293**:475–477.
22. **Raina, A.** 1963. Studies on the determination of spermidine and spermine and their metabolism in the developing chick embryo. Acta Physiol. Scand. Suppl. **218**:1–81.
23. **Seiler, N.** 1970. Use of the dansyl reaction in biochemical analysis. Methods Biochem. Anal. **18**:259–337.
24. **Seyfried, C., and D. Morris.** 1979. Relationship between inhibition of polyamine biosynthesis and DNA replication in activated lymphocytes. Cancer Res. **39**:4861–4867.
25. **Steglich, C., J. Choi, and I. E. Scheffler.** 1983. Mammalian cell mutants with altered levels of ornithine decarboxylase activity. Adv. Polyamine Res. **4**:591–602.
26. **Steglich, C., and I. E. Scheffler.** 1982. An ornithine decarboxylase-deficient mutant of Chinese hamster ovary cells. J. Biol. Chem. **257**:4603–4609.
27. **Sunkara, P. S., C. C. Chang, and N. J. Prakash.** 1983. Role of polyamines during chromosome condensation of mammalian cells. Cell Biol. Int. Rep. **7**:455–465.
28. **Tabor, C. W., H. Tabor, A. K. Tyagi, and M. S. Cohn.** 1982. The biochemistry, genetics, and regulation of polyamine biosynthesis in *Saccharomyces cerevisiae*. Fed. Proc. **41**:3084–3088.
29. **Thomas, P. J.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
30. **Tofilon, J. P., S. M. Oredsson, D. F. Deen, and L. J. Marton.** 1982. Polyamine depletion influences drug-induced chromosomal damage. Science **217**:1044–1046.
31. **Whitney, P. A., and D. R. Morris.** 1978. Polyamine auxotrophs of *Saccharomyces cerevisiae*. J. Bacteriol. **134**:214–220.