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Extended Life Span of Human Endometrial Stromal Cells Transfected with Cloned Origin-Defective, Temperature-Sensitive Simian Virus 40

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Human endometrial stromal cells transfected with an origin-defective, temperature-sensitive simian virus 40 recombinant plasmid are dependent on T-antigen function for proliferation and at the permissive temperature have an extended life span in culture. Southern blot analysis indicates that the transfected gene is present in low copy number, possibly at a single integration site. Normal stromal cells are capable of 10 to 20 population doublings in culture. Transfected cultures have been carried at the permissive temperature to 80 population doublings before crisis. In the multistep model of malignant transformation of human cells, these cells represent one of the earliest stages: extended but finite life span. We have used these cells to investigate alterations in signal transduction that may be responsible for this early stage of transformation caused by the large T antigen. Temperature shift experiments indicate that the expression of ornithine decarboxylase (ODC) but not of c-*fos* is altered by the large T antigen. Induction of c-*fos* by serum or 12-*O*-tetradecanoylphorbol-13-acetate is independent of temperature. However, in the transfected cells, the induction of ODC by asparagine or serum is greatly enhanced at the permissive temperature. This result indicates that the large T antigen acts downstream of c-*fos* but upstream of ODC expression in the signal-transducing cascade.

Human cells derived from normal tissue have a limited life span in culture (27); the proliferative phase is followed by a period of senescence during which the cells no longer proliferate but remain viable. The senescent period may last for several months in vitro (33). This limited life span impedes the study of carcinogenesis, especially if the cells are derived from an adult donor. The large T antigen of the DNA virus simian virus 40 (SV40) increases the life span of human cells in culture, and transformation by SV40 has become a standard approach in producing cell lines with extended life spans (2, 52). However, even though they have increased life spans in culture, human mesenchymal cells transformed with SV40 rarely become immortal except as a low-frequency, postcrisis event (23, 28). The use of origin-defective SV40 constructs (21, 36) produces a higher rate of transformation (55) and increased frequency of production of immortalized populations (37). We have used an origin-defective construct of the temperature-sensitive mutant A209 of SV40 (4, 32) (*ts*SV40) to transfect normal adult human endometrial stromal cells. Endometrial stromal cells are hormonally responsive in vivo, have specialized functions, and represent a cell type different from the fibroblasts or epithelial cells usually reported in transformation studies. The *ts*SV40-transfected endometrial stromal cells provide the opportunity to study cells in one of the earliest phases of neoplastic transformation and may be useful in understanding the nature of carcinogenesis in the uterus.

Immortalization may be the rate-limiting step in carcinogenesis, and in SV40-transfected or -infected human cells, this step appears to consist of two stages (61). In the first stage, the cells have an extended but finite life span. These cells eventually enter a period of crisis in which the cells continue to attempt to divide. However, the population becomes static and, over a period of time that may last as long as 6 months, subsequently declines. In the second phase, a few cells may emerge from this crisis period to become capable of indefinite life span in culture. This second phase has been interpreted as the result of the loss of a dominantly acting growth repressor gene activity (41). This emerging view of immortalization itself as a multistep process is consistent with and increases our understanding of carcinogenesis as a multistep process.

Since neoplasia is a disease characterized by loss of precise homeostatic control of cell growth, the multistep nature of carcinogenesis may be understood in part as progressive alterations in the mitogenic signal transduction pathway(s). To better understand the alterations in signal transduction that may characterize the initial steps in the transformation of human endometrial stromal cells, we studied the effects of the large T antigen (reviewed in references 31 and 57) on two important components of the signal-transducing cascade, c-*fos* and ornithine decarboxylase (L-ornithine carboxylyase; EC 4.1.1.17; ODC).

The *fos* gene product is involved in the regulation of cellular growth and proliferation as well as neoplastic transformation. When complexed with the c-*jun* protein, the *fos-jun* complex transcriptionally activates genes whose products are necessary for proliferation (53). c-*fos*, often referred to as an immediate-early gene, is expressed as soon as 5 min after a growth stimulus to quiescent cells and reaches peak levels of expression within 30 to 60 min (25, 30). c-*fos* is induced by a variety of extracellular stimuli (7, 18). The *fos* oncogene (v-*fos*) induces osteogenic sarcomas in FBJ virus-infected mice (11), and the c-*fos* gene induces neoplastic transformation when transduced by retroviruses (15, 16) or when inappropriately expressed (60).

ODC is the initial enzyme in the polyamine biosynthetic

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pathway, and the polyamines are essential metabolites critical to the cell's capacity for proliferation. When cells are deprived of polyamines, DNA synthesis and cell proliferation cease (1, 8, 58). ODC is induced by all known classes of hormones acting on their target tissues (51) and by tumor-promoting phorbol esters (40), and it is generally expressed at high levels in transformed cells (51). In quiescent cells exposed to appropriate stimuli, ODC is expressed at peak levels 4 to 8 h later.

We studied the expression of c-*fos* and ODC as two important components of the proliferation-inducing cascade to determine alterations in their expression which might be characteristic of endometrial stromal cells in the earliest phases of neoplastic transformation. The difference in the time frame of induction also allows us to begin to localize the block in signal transduction which is overcome by the large T antigen. We find that the expression of c-*fos* is not altered by T-antigen activity, while ODC is induced only at the temperature permissive for large-T-antigen function. This result indicates that the large T antigen acts downstream of c-*fos* but upstream of ODC expression and that an increase in polyamine biosynthesis might be one of the early alterations in the neoplastic transformation process in human endometrial stromal cells.

MATERIALS AND METHODS

Tissue culture. Endometrial tissue was obtained and processed as previously described (48). The stromal cells were cultured in a 1:1 mixture of Opti-MEM and RPMI 1640 (GIBCO) supplemented with 1% fetal bovine serum (FBS), 3% bovine calf serum, 2 μg of insulin per ml, 4 mM glutamine, 2× nonessential amino acids, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.25 μg of amphotericin B per ml (Sigma antibiotics). The cells were routinely subcultured at a 1:4 split ratio, and the medium was changed twice weekly. Low-serum medium consisted of the same components except that the serum level was 0.2% FBS.

Transfection. The origin-of-replication-defective (21) construct of the temperature-sensitive mutant A209 (4, 32, 39) (ori− *ts*A209 SV40) cloned into plasmid pMK 16 was generously provided by S. P. Banks-Schlegel. Approximately 10^6 cells were transfected by electroporation (38, 43) with 100 μg of plasmid DNA per ml in Opti-MEM (GIBCO) at 4.0 kV/cm for 30 μs. The cells were replated, allowed to reach confluence, passaged once, and held at confluence until colonies of morphologically altered cells appeared. These were subcultured by using cloning rings. Alternatively, the entire population was passaged until the untransfected cells senesced and were overgrown.

DNA analysis. Integration of the transfected DNA was analyzed by the method of Southern (56). High-molecular-weight DNA was isolated by CsCl gradient centrifugation and purified by phenol extraction. This DNA (10 μg) was restricted with 20 U of *Eco*RI (Promega) at 37°C for 18 h. The DNA was electrophoresed in a 0.8% agarose gel at 50 mV for 20 h and transferred to nitrocellulose. The ^{32}P-labeled DNA probe was generated by random priming of the 2.6-kb *Stu*I-*Bam*HI fragment of the transfected plasmid. This fragment includes the origin of replication and sequences coding for the small t and large T antigens.

RNA analysis. Total RNA was isolated by the guanidinium isothiocyanate method and then denatured. A 20-μg sample of RNA was electrophoresed through a 1% formaldehyde gel and subsequently transferred to a nitrocellulose membrane (59). Expression of c-*fos* was determined by hybridization with the 0.6-kb *Pst*I fragment of v-*fos*, generously provided by Inder Verma (10). Hybridization was for 18 h at 42°C, and subsequently the filter was washed with 0.2× SSC at 56°C.

TABLE 1. *ts*SV40-transfected cell lines

Primary cell strain (donor)	No. of clonal lines with given DNA content		
	Diploid	Mixed	Tetraploid
87-062 (uncloned)			
88-035	8	7	1
88-038	9	0	0
88-053	0	6	14
88-054	2	12	10
89-002	0	8	0
89-003	0	0	6

Temperature shift. The *ts*SV40-transfected cells were routinely cultured at 33°C. The nonpermissive temperature was set at 39°C. Cells were incubated at the permissive or nonpermissive temperature for 3 days prior to initiation of the experiments to evaluate c-*fos* and ODC expression.

ODC assays. ODC activity was determined by the release of $^{14}CO_2$ from 1-^{14}C-ornithine. Activity was determined in a 10,000 × *g* supernatant fraction (cytosol) of cell lysate. The CO_2 released was collected on an antibiotic sensitivity disk impregnated with 15 μl of Protosol (NEN) and quantitated by liquid scintillation spectroscopy as previously described (3, 49). Enzyme activity is expressed as nanomoles of CO_2 released per milligram of protein per hour.

DNA content. Stromal cells (10^6) were harvested by trypsination, centrifuged, and resuspended in 0.1 ml of phosphate-buffered saline. Cells were fixed by addition of 1.0 ml of cold (4°C) 70% ethanol. After pelleting and removal of the ethanol, RNase A (2 mg/ml; Sigma) was added to remove RNA, and the DNA was stained with propidium iodide (50 μg/ml; Sigma) for 30 min at room temperature. The DNA content was then analyzed in an Ortho 50 H cytofluorometer, using human lymphocytes as the standard. Cell cycle analysis was performed by the Ortho 2150 QuickEstimate program.

RESULTS

Endometrial stromal cells were transfected either prior to primary culture or in low (fewer than six population doublings) passage. Cells from eight different donors were transfected, and transformants were isolated from all eight. The typical transfection resulted in 10 to 20 colonies per 100-mm dish containing approximately 2×10^6 cells, giving a transfection frequency of 0.5×10^{-5} to 1.0×10^{-5}; 83 clonal cell lines and one uncloned cell population were obtained. The number of clonal lines and the DNA content of each are described in Table 1.

Colonies of morphologically altered cells began to appear 4 to 6 weeks after transfection (Fig. 1a). The transfected cells were smaller than their normal stromal cell parents and continued to grow past confluence, eventually forming large multilayered colonies (Fig. 1b). The growth pattern of the transfected cells retained a large degree of order, however, and exhibited little criss-cross growth pattern.

Southern blot analysis of *ts*SV40-transfected cells from the uncloned 87-062 line demonstrated the presence of the SV40 sequences (Fig. 2). SV40 contains a single restriction site for *Eco*RI. Two bands were obtained from the transfected stromal cells (Fig. 2, lane 1), one at approximately 4.9

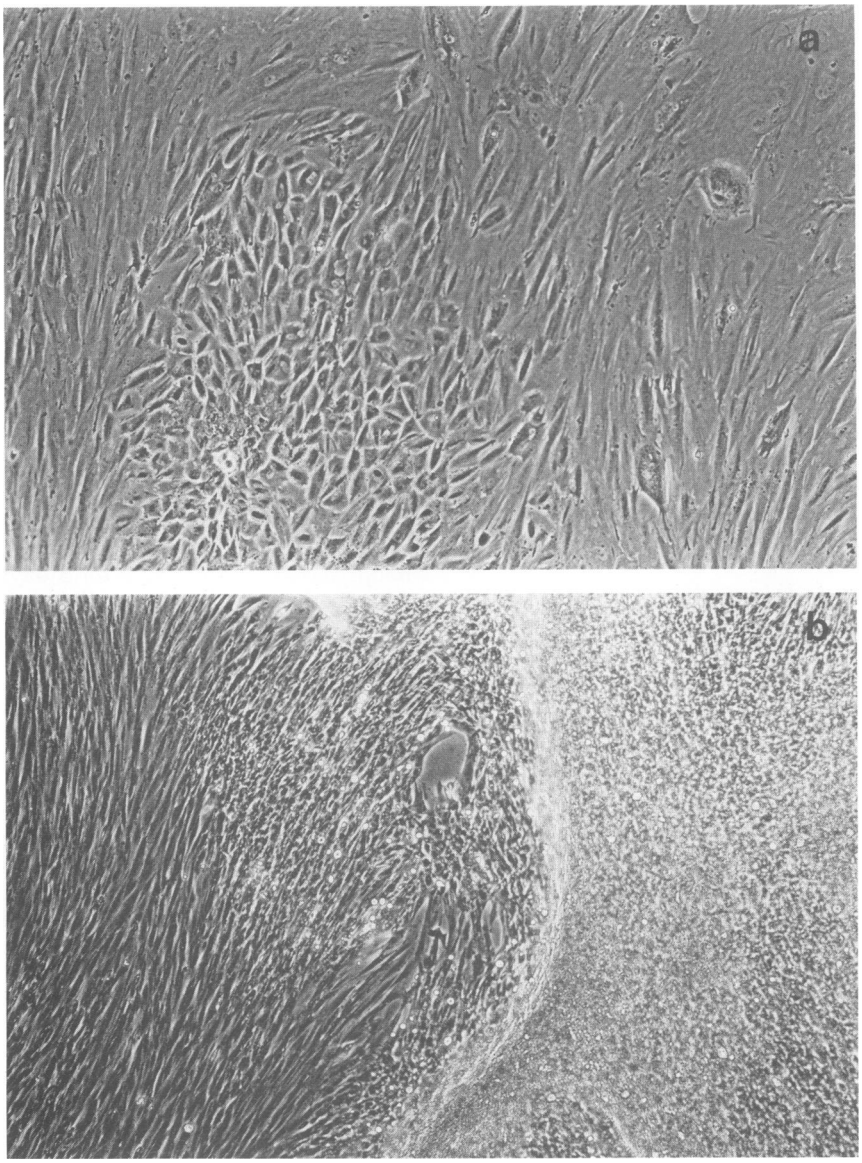

FIG. 1. Morphological alterations and focus formation by *ts*SV40-transformed human endometrial stromal cells. (a) Small colony of transformed cells demonstrating the smaller size of the transformed cells; (b) large focal area formed by the *ts*SV40-transformed cells. Magnification, ×100.

kb and a weaker band at about 2.9 kb (not shown). The two hybridization bands in the DNA of transfected stromal cells indicate a single integration site which, on the basis of the band intensity, indicates a low, possibly single copy of the transferred gene. The increased intensity and heterogeneity of the bands from the SV40 replication-competent infected fibroblast line (lane 4) may be due to the generation of tandem repeats and insertions which often occur in these cell lines. Expression of the large T antigen was observed by immunohistochemistry in more than 90% of the cells at the permissive temperature.

The normal stromal cells are typically capable of 10 to 20 population doublings under these culture conditions, with some variation between donors. Uncloned and some clonal populations of ori− *ts*A209 SV40-transfected cells are capable of 70 to 80 population doublings before entering senescence. Other clonal lines were capable of as few as 30 population doublings before becoming senescent.

The effect of temperature on growth of the ori− *ts*A209 SV40-transfected cells is demonstrated in Fig. 3. The untransfected stromal cells proliferate faster at the nonpermissive temperature (39°C) than at the permissive temperature (33°C). The ori− *ts*A209 SV40-transfected cells cease proliferation upon shift to the nonpermissive temperature when propagated past their normal life span of 20 population doublings. Earlier studies with primary cultures of rodent cells indicated that cell lines established with temperature-sensitive mutants of SV40 were growth restricted at the nonpermissive temperature (29, 42, 45), and two recent studies with human fibroblasts immortalized with controllable SV40 genes indicate a continued, postcrisis dependence on large T antigen for growth (44, 61). The transfected

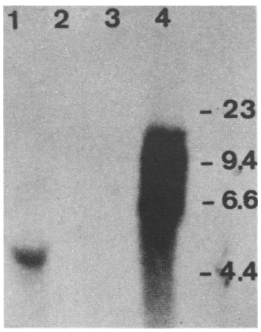

FIG. 2. Analysis of integrated SV40 DNA. Lanes: 1, DNA from *ts*SV40-transfected human endometrial stromal cells; 2, DNA from untransfected stromal cells; 3, DNA from untransfected human monocytes; 4, DNA from immortal SV40-infected human fibroblasts.

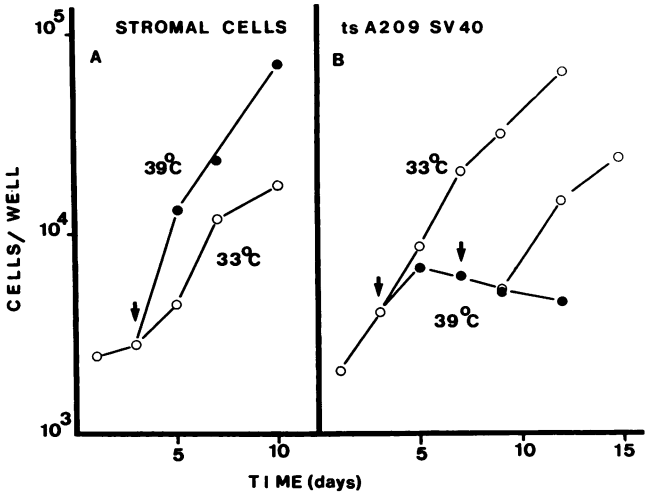

FIG. 3. Temperature-sensitive growth in *ts*SV40-transfected stromal cells. Endometrial stromal cells were plated at 33°C, and one portion of the cells was shifted to 39°C at day 3 (arrow). Untransfected stromal cells grow faster at the nonpermissive temperature (A). *ts*SV40-transfected cells proliferate rapidly at the permissive temperature (33°C) but cease proliferation at the nonpermissive temperature (39°C). When returned to the permissive temperature, the cells resume proliferation (B, second arrow).

endometrial stromal cells continue to be viable for at least 2 weeks at the nonpermissive temperature, and the cessation of growth is reversible. When the nonproliferating cells at 39°C are returned to 33°C, they resume proliferation after a lag period (Fig. 3B, second arrow).

To determine the cell cycle characteristics of ori− *ts*A209 SV40-transformed cells, parallel cultures were incubated in low-serum medium at the permissive temperature and subsequently shifted to the nonpermissive temperature (Fig. 4). At the permissive temperature, the transformed cells continue to enter the S phase of the cell cycle even under culture conditions that prevent the proliferation of normal endometrial stromal cells (Fig. 4A). The percentage of cells entering the S phase of the cell cycle is unchanged during the course of the experiment at the permissive temperature. The relief of serum dependence by SV40 transformation is a well-known phenomenon (32). At the nonpermissive temperature, the proportion of the cells entering the S phase declines from 26.6 to 16.3 and 10.5% after 24 and 48 h, respectively. However, this decline appears to be insufficient to explain the complete cessation of growth at the nonpermissive temperature (Fig. 3). The cells in S phase may be trapped there by withdrawal of T antigen but not actually progressing through the cell cycle. Alternatively, it is possible that cell replication is evenly matched with cell death so that the total cell number remains static. A third possibility is that a minority of the cells synthesize DNA but do not undergo mitosis. Other *ts*A SV40 mutants have been shown to stimulate DNA synthesis without mitosis in senescent human fibroblasts at the nonpermissive temperature (22). This result is not altered by the addition of serum (data not shown). At the nonpermissive temperature, cells accumulate in both the G_0/G_1 and G_2/M phases of the cell cycle, suggesting that there are at least two restriction points in the cell cycle which are overcome by the T antigen. Similar results have been obtained with rat embryo fibroblasts transformed with *ts*SV40 (29), and the block in G_2/M was localized to the G_2 phase of the cell cycle.

To better understand the alterations in signal transduction that may characterize the initial steps in the transformation of human endometrial stromal cells, we used temperature shift experiments to study the effects of the large T antigen on c-*fos* and ODC expression.

Serum induces a low level of c-*fos* expression in the ori− *ts*A209 SV40-transfected cells under proliferative and nonproliferative conditions (at permissive and nonpermissive temperatures). Exposure to 12-*O*-tetradecanoylphorbol-13-acetate (TPA) results in much higher levels of expression (Fig. 5). However, there is relatively little difference in the induction of c-*fos* by either agent in the presence or absence of T-antigen activity. No c-*fos* mRNA is detected in the lanes from serum-starved, unstimulated control cells.

A different picture emerges when the induction of ODC activity is examined. High levels of ODC are induced in the transfected cells at the permissive temperature, while induction at the nonpermissive temperature occurs to a much lesser extent (Fig. 6A). At the permissive temperature, ODC activity is induced by 10-fold less asparagine (1 to 2 versus 20 mM) and to a 5-fold-higher level than at the nonpermissive temperature. To control for the possibility that cells at 39°C are thermally stressed and unable to maintain ODC activity, and to compare ODC activity in ori− *ts*A209 SV40-transfected and untransfected endometrial cells, ODC induction was compared at 33, 37, and 39°C (Table 2). Serum deprivation (0.2%) reduces ODC activity to 1.0 U in ori− *ts*A209 SV40-transfected cells and below the level of detection in untransfected stromal cells. ODC activity is induced to high levels in the ori− *ts*A209 SV40-transfected cells only at the permissive temperature. The converse is true of the untransfected cells; as the temperature is increased, ODC induction is increased. Untransfected endometrial stromal cells also proliferate faster at 39°C than at 33°C (Fig. 3A). The ratio of ODC activity in transfected cells to that in normal control cells decreases from 52 after 8 h at 33°C to 0.4 at 39°C. This indicates a 130-fold difference in the ratio of ODC activity in the presence and absence of T-antigen activity at the two temperatures.

These results do not discriminate between the possibilities that the large T antigen (i) induces ODC activity autonomously and (ii) functions in a permissive role, allowing other agents to act as inducers of ODC activity. These possibilities are analyzed in the experiment shown in Fig. 6B. The ori− *ts*A209 SV40-transfected cells were shifted from the nonper-

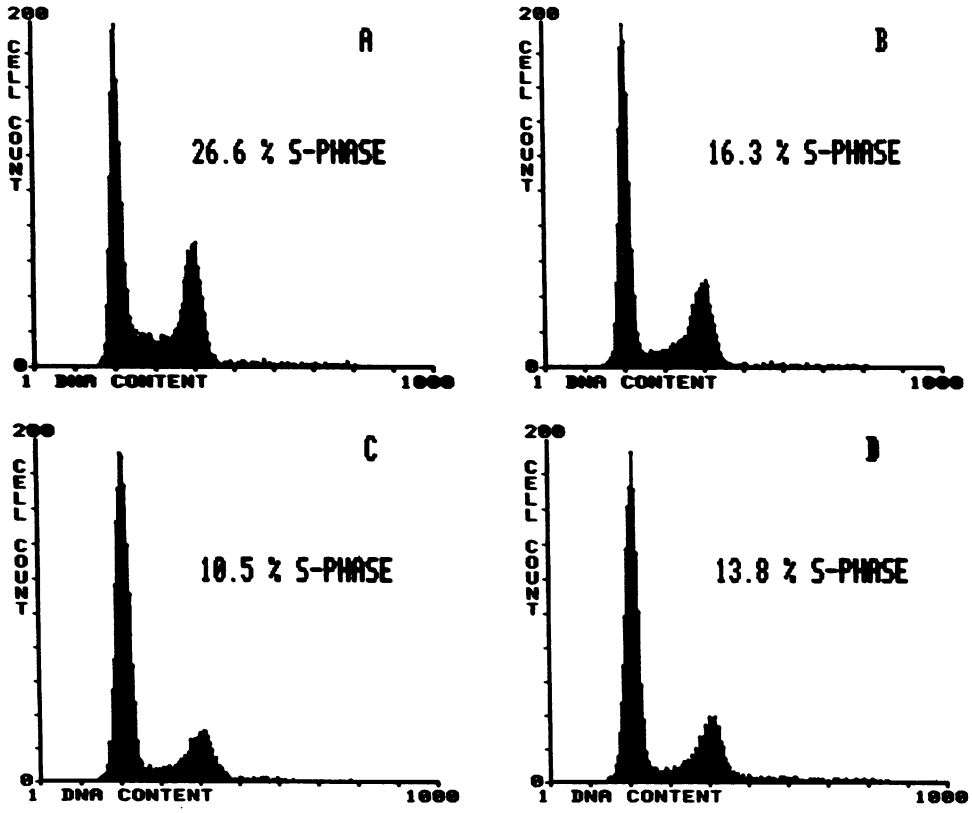

FIG. 4. Cell cycle distribution of *ts*SV40-transfected cells. The cells were incubated in RPMI 1640 supplemented with 0.2% serum at either the permissive (A) or nonpermissive temperature. The cells were harvested, and the DNA content was analyzed at days 1 (B), 2 (C), and 3 (D) after the temperature shift.

missive to the permissive temperature in the presence or absence of serum. No ODC activity was detectable in the absence of serum (data not shown). ODC was induced to much higher levels at 33°C than at 39°C (see also Table 2). Only those cells in the presence of serum express ODC, implying that the large T antigen does not induce ODC directly but permits other agents to do so. This result is also consistent with that seen in the induction of ODC by asparagine.

Putrescine was supplied to the ori− *ts*A209 SV40-transfected cells to determine whether polyamine administration could overcome the inhibition of growth at the nonpermissive temperature. Putrescine at 25 μM accelerated cell growth at the permissive temperature but had no effect at the nonpermissive temperature (data not shown), indicating that other alterations in addition to ODC activity are responsible for the lack of growth in the absence of T-antigen activity.

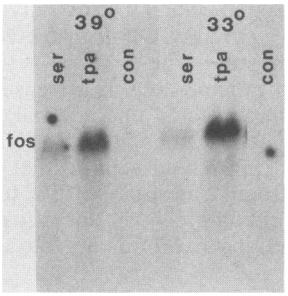

FIG. 5. Northern (RNA) blot analysis of the temperature dependence of induction of c-*fos* expression. Confluent cells were maintained at the indicated temperature for 3 days prior to analysis and were serum starved for 16 h before stimulation. The additions were 10% FBS (ser) or 10^{-7} M TPA. Control cultures (con) were unstimulated. RNA was isolated 1 h after the stimulus.

DISCUSSION

Carcinogenesis in human tissue is a complex multistep process that has proved to be difficult to model in vitro (34). However, the advantages of using human tissue for carcinogenesis studies alleviate the difficulties in extrapolating from data obtained with rodent systems as well as allowing the study of congenital dispositions for which no animal models exist (26). The conversion of normal cells capable of a limited number of population doublings to a cell line capable of indefinite life span is a critical and perhaps the rate-limiting step in the neoplastic transformation process (5, 10, 34, 47). SV40-immortalized cell lines (5, 6, 46) have been used to study the role of cooperating oncogenes in the malignant conversion of human epithelial cells (6, 47).

To create cell lines with enhanced proliferative potential for the study of the initial phases of this process, we have transfected human endometrial stromal cells with a plasmid containing a temperature-sensitive mutant SV40. The cells eventually entered a crisis but did increase their proliferative potential from the 10 to 20 population doublings characteristic of endometrial stromal cells obtained from adult donors

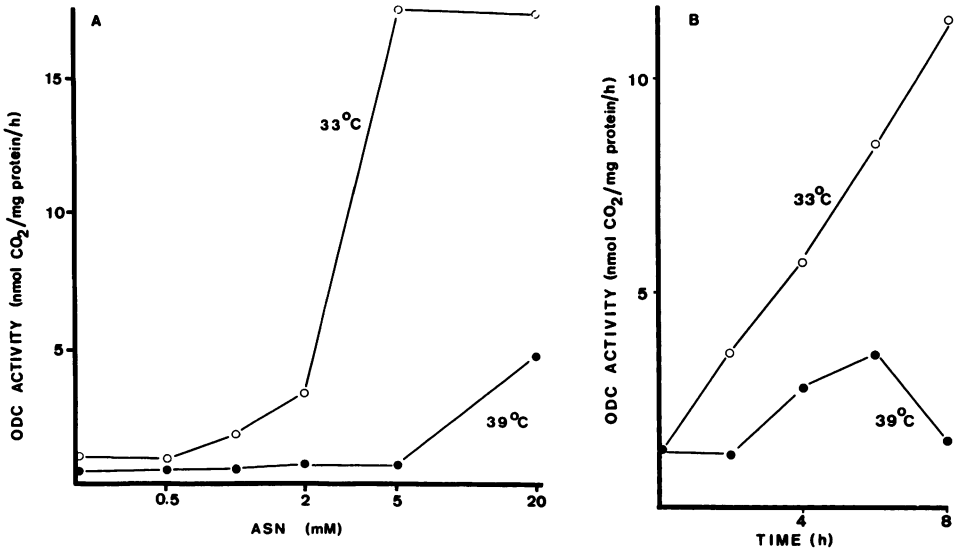

FIG. 6. Temperature dependence of induction of ODC activity. The cells were prepared for induction as for Fig. 5 and then exposed to the indicated concentrations of asparagine in Earle basal salts solution for 6 h before harvest (A) or stimulated with or without 10% FBS in RPMI 1640 and harvested at the indicated times (B). The data shown represent one of two separate experiments, each done with duplicate dishes. The variability between dishes at each point is less than 10%.

to at least 30, and in some clones to as much as 70 to 80, population doublings. This result is in agreement with previous studies indicating that human cells must pass through a senescence crisis from which a few cells may escape at very low frequency to become immortal. The ori− *ts*A209 SV40-transformed cells should be useful in studying the role of cooperating oncogenes or alterations induced by chemical carcinogens in a human cell-based system.

The analysis of SV40 sequences in the transfected cells indicated that the genes had integrated at a single site and in low, possibly single copy number. The transformation induced by a low copy number of transfected oncogenes may be more relevant to carcinogenesis in vivo than are some studies in which a high multiplicity of SV40 infection is used.

The temperature-sensitive feature of the SV40 large T antigen used in this study permits the direct comparison of cells in the presence and absence of large-T-antigen activity. It also eliminates the possibility of genetic differences due to the selection of transformed subpopulations or to the genetic drift which often occurs when cells are passaged in culture for extended periods. We have used the ability to conditionally regulate the large T antigen to begin to localize its effects on the signal transduction pathway(s). At the nonpermissive temperature, the cells cease proliferation and ODC activity is much diminished, but the induction of c-*fos* expression by serum is relatively unaffected. Membrane-associated oncogenes such as *src* and growth factor receptors possessing a tyrosine kinase activity do induce c-*fos* (see reference 19). This implies a sequence of events in which a membrane-associated tyrosine kinase phosphorylates an intermediate which acts through an undetermined series of steps to transcriptionally activate responsive elements of the *fos* gene. Our results indicate that the large T antigen acts downstream of these events. The SV40 large T antigen is a nuclear-localized phosphoprotein with no known tyrosine kinase activity (40, 61) and would therefore be unlikely to act at such an early stage in the signal transduction pathway.

Inhibition of c-*fos* expression has been reported to occur in senescent as compared with proliferative human fibroblasts (54). However, SV40 can induce DNA synthesis in these senescent cells (22). These studies differ from ours in that they compare young, proliferative cells with older, senescent cells. It is not known whether *fos* or ODC expression is blocked in senescent endometrial stromal cells. In our study, we compared age-matched cells that differ in T-antigen activity. Serum induced relatively low levels of c-*fos* mRNA at both temperatures. TPA, which is not mitogenic for these cells, induced much higher levels of c-*fos* mRNA at both temperatures. These results indicate that the extended life span conferred upon the endometrial stromal cells by SV40 is not due to an increase in c-*fos* expression, since cells at proliferative conditions (serum stimulation at the permissive temperature) do not have higher levels of *fos* expression

TABLE 2. ODC activity induced by serum in *ts*SV40-transformed or normal cells[a]

Temp (°C)	Time (h)	ODC activity[b]		Significance (*P*)[c]	Ratio, *ts*SV40-transfected to control cells
		*ts*SV40-transformed cells	Normal cells		
33	0	1.0 ± 0.29	0		
	4	3.68 ± 0.43	0.03 ± 0.06	<0.01	123
	6	9.57 ± 2.83	0.38 ± 0.19	<0.01	24
	8	15.9 ± 2.33	0.33 ± 0.14	<0.01	52
37	4	2.20 ± 0.12	0.92 ± 0.06	<0.01	2.4
	6	2.08 ± 0.48	1.74 ± 0.10	<0.30	1.2
	8	1.05 ± 0.30	3.14 ± 0.58	<0.01	0.3
39	4	2.28 ± 0.18	3.84 ± 0.54	<0.01	0.6
	6	1.55 ± 0.81	4.40 ± 0.79	<0.05	0.4
	8	1.75 ± 0.05	4.17 ± 0.44	<0.01	0.4

[a] The cells were grown to confluence, incubated at the indicated temperatures for 48 h, serum deprived in 0.2% serum at the same temperature for an additional 16 h, and stimulated with 10% FBS.

[b] Nanomoles of CO_2 released per milligram of protein per hour. Values are means ± standard deviations of triplicate dishes from one of two experiments. The control (normal stromal) cells were at six population doublings.

[c] Tested by the Student *t* test.

than cells at nonproliferative conditions (serum stimulation at the nonpermissive temperature).

In contrast, the expression of ODC in the presence of T-antigen activity is much higher than in the absence of T-antigen activity. Large-T-antigen expression itself is insufficient to induce ODC expression but is necessary to permit the high levels of induction by serum or asparagine. This result is consistent with the hypothesis that the T antigen exerts its influence, at least in part, by neutralizing the effects of an inhibitor. ODC mRNA is expressed at similar levels in senescent and proliferating fibroblasts (50, 54), but ODC activity is higher in proliferating cells (54), indicating that the decline in ODC activity observed in senescent cells may be due to regulatory events which take place posttranscriptionally. The mechanism by which the large T antigen permits expression of ODC activity is at this point a matter of conjecture. The dependence on the large T antigen for the extended life span of ori− *ts*A209 SV40-transfected stromal cells derives from the ability of the T antigen to permit the transfected cells to traverse the cell cycle and complete mitosis. The large T antigen binds to a number of cellular proteins having regulatory functions, including the AP-2 enhancer-binding protein (35), p53 (20), and the retinoblastoma (RB) susceptibility gene product (12), as well as to cellular proteins with undetermined functions (13, 14). The transforming activity of DNA viruses and perhaps some of the cellular oncogenes has been ascribed to their ability to complex with these proteins having growth-inhibitory or tumor suppressor activity (24). p53 (17) and p105-RB are the two best-characterized transformation inhibitory proteins, and the SV40 large T antigen binds to both. The binding of the large T antigen to the dephospho-RB present during the G_0/G_1 portions of the cell cycle may be responsible for the growth-promoting effects (see reference 9). Our data indicate that alleviating the suppression of ODC activity might be one important aspect of transformation by SV40.

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