JOURNAL OF VIROLOGY, July 1973, p. 90-98

Vol. 12, No. 1
Printed in U.S.A.

Transcription of Simian Virus 40

II. Hybridization of RNA Extracted from Different Lines of Transformed Cells to the Separated Strands of Simian Virus 40 DNA

BRAD OZANNE, PHILLIP A. SHARP, AND JOE SAMBROOK
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Received for publication 13 February 1973

The amount of simian virus 40 (SV40) DNA present in various SV40-transformed mouse cell lines and "revertants" isolated from them was determined. The number of viral DNA copies in the different cell lines ranged from 1.35 to 8.75 copies per diploid quantity of mouse cell DNA and from 2.2 to 14 copies per cell. The revertants had the same number of viral DNA copies per diploid quantity of mouse cell DNA as their parental cell lines. (However, they showed an increased number of viral DNA copies per cell due to their increased amount of DNA.) By using separated strands of SV40 DNA, the extent of each DNA strand transcribed into stable RNA species was determined for the transformed and "revertant" cell lines. From 30 to 80% of the "early" strand and from 0 to 20% of the "late" strand was present as stable RNA species in the cell lines tested. There was no alteration in the pattern of the stable viral RNA species present in three concanavalin A-selected revertants, whereas in a fluorodeoxyuridine-selected revertant there appeared to be less viral-specific RNA present in the cells.

During infection of permissive cells by simian virus 40 (SV40), two distinct classes of stable species of SV40-specific RNA are synthesized. One class is present at all times after infection and corresponds to about 35% of the sequences of one of the strands (the E strand) of SV40 DNA. The other class, which appears only after the onset of SV40 DNA synthesis, is complementary to 65 to 70% of the sequences of the L strand of SV40 DNA (6, 15). Thus at late times after infection, 50% of the total sequences of SV40 DNA are present in stable species of RNA.

Mouse cells stably transformed by SV40 contain several copies of viral DNA (3, 24) in an integrated state (16). Virus-specific RNA is present (see review 14) which in the one line of SV3T3 cells so far examined comprises about 0.002% of the total RNA of the cells and is complementary to 55 to 60% of the sequences of the E strand and 15 to 20% of the sequences of the L strand of SV40 DNA (15). Thus, more sequences of RNA complementary to the E strand of SV40 DNA are detectable in transformed cells than at any time during lytic infection. Clearly it is of interest to examine viral RNA sequences in other lines of transformed cells. We are particularly curious about the virus-specific RNA present in revertant cell lines, for the possibility exists that these cells have resumed a normal phenotype as a consequence of a change in the expression of the integrated viral genome. In this paper, we report data on: (i) the pattern of viral RNA species present in several different mouse lines transformed by SV40 and revertants derived from them; (ii) the amount of SV40 DNA in each of the cell lines; (iii) the total amount of DNA per cell; and (iv) the chromosome number of the cells. Part of this work has already been published in a preliminary form (10).

MATERIALS AND METHODS

Cell lines. The BS-C-1 line of African green monkey kidney cells (5) obtained from T. Benjamin was cultured in plastic dishes in Dulbecco's modification of Eagle medium (2) supplemented with 10% fetal bovine serum. SV3T3 clone 9 cells were obtained from W. Eckhart; SV101 and F1SV101 cells from R. Pollack; SVT2, SVPy11 and SVuv30 from S. Aaronson. SVB30 is a line of SV40-transformed Balb/c 3T3 cells made in this laboratory by Brad Ozanne. CA^{r}30, CA^{r}32 and CA^{r}41 are concanavalin A (Con A)-resistant variants derived from SV3T3 clone 9 cells as described in the accompanying paper (9). All the lines of transformed cells were cultured as described above except that the medium was supplemented with 10% calf serum.

Virus. SV40, strain 777 (4), a single stock lysate of four times plaque-purified virus was used throughout.

The titer of the stock determined by plaque assay on BS-C-1 monolayers was 10^9 PFU/ml.

Preparation of ^{32}P-labeled SV40 DNA and separation of the strands. The preparation of ^{32}P-labeled SV40 DNA, the conditions of synthesis of complementary RNA from SV40 DNA by *Escherichia coli* DNA-dependent RNA polymerase, and the method used to separate the strands of SV40 DNA have all been published elsewhere (15).

Preparation of RNA from transformed cells. RNA was extracted from log-phase transformed cells by the hot phenol method (17). After precipitation with ethanol, the RNA was dissolved in 0.01 M sodium acetate (pH 5.5), 10^{-3} M $MgCl_2$ and treated with DNase I (25 μg/ml, electrophoretically pure (Worthington Biochemicals, Freehold, N.J.), for 30 min at 37 C. The mixture was extracted with phenol, and the RNA was concentrated by ethanol precipitation. The RNA was then dissolved in a small volume of 0.01 M sodium acetate (pH 5.5), 0.001 M EDTA and passed through a column of G75 Sephadex equilibrated with the same buffer. The RNA eluting in the void volume was stored at −20 C.

Preparation of DNA from cells. DNA was extracted from cells as described elsewhere (11). Calf thymus DNA and salmon sperm DNA were purchased from Worthington. Before use in hybridization assays, the DNA was sheared by two passages through a French press at 20,000 lb/in^2. The size of the DNA fragments was approximately 500 to 700 nucleotide pairs.

Hybridization conditions. All hybridizations were carried out at 68 C in 10^{-3} M Tris (pH 7.0), 0.4% sodium dodecyl sulfate (SDS), and 1 M NaCl. Before addition to the hybridization mixture, native DNA was denatured for 10 min at room temperature with 0.2 N NaOH. In experiments to measure the kinetics of hybridization, samples were withdrawn at intervals, diluted at least 10-fold into 0.14 M phosphate buffer (pH 6.8) containing 0.4% SDS, and stored at 4 C before the proportion of single- and double-stranded DNA was assayed by chromatography on hydroxyapatite columns.

Hydroxyapatite chromatography. Hydroxyapatite chromatography was carried out in disposable plastic syringes in a water bath at 60 C as described earlier (15). The eluates containing ^{32}P-labeled SV40 DNA from the hydroxyapatite columns were mixed with 2.5 vol of Aquasol (New England Nuclear, Boston, Mass.) and were counted directly.

Karyotype analysis. Karyotype analysis was carried out as described by Pollack, Vogel, and Wollmann (13).

The amount of DNA per cell was determined by the method of Kraemer, Peterson, and VanDilla (7).

RESULTS

Amount of SV40 DNA present in different lines of transformed cells. The proportion of SV40 sequences present in DNA extracted from different transformed cell lines was determined by reassociation kinetics essentially as described by Gelb, Kohne, and Martin (3). The results are shown in Fig. 1a and b and in summary form in Table 1. The rate of reannealing of viral DNA was always faster in the presence of DNA from transformed cells than in the presence of control cell DNA. Figure 1a shows that the rate of reannealing of ^{32}P-labeled SV40 DNA was increased eightfold when 1.25 equivalents of viral DNA per diploid quantity (3.9×10^{12} daltons; 20) of mammalian DNA were reannealed in the presence of DNAs from SV3T3 clone 9, SV101, F1SV101, CA^{r}32.6, and CA^{r}41.6. Thus these cells must contain about (8×1.25) − 1.25 = 8.75 copies of SV40 DNA per diploid mammalian genome. Similarly, the data in Fig. 1b show that SVB30 contains 6.1 copies of viral DNA and that SVPy11 and SVT2 cells contain, respectively, 1.35 and 2.2 copies of viral DNA per diploid quantity of mammalian DNA—values that agree quite well with the previously published estimates of Gelb, Kohne, and Martin (3). These results show that both the F1SV101 line, which was isolated as a flat revertant of SV101 by Pollack, Green, and Todaro (12), and the con A-resistant variants of SV3T3 clone 9 all contain amounts of SV40 DNA per diploid quantity of cell DNA which are indistinguishable from the transformants from which they were originally derived. Thus, revertants of SV40-transformed cells, like those of polyoma virus-transformed cells (18), have not suffered significant loss of viral DNA sequences.

Gelb, Kohne, and Martin (3) reported that they were able to detect the presence of 0.45 copy of SV40 DNA per diploid quantity of host DNA in untransformed 3T3 cells. We were unable to confirm this observation, and in our hands the rate of reannealing of ^{32}P-labeled SV40 DNA was identical in the presence of DNA from 3T3 cells, adenovirus 2-transformed rat cells, calf thymus, HeLa cells, and salmon sperm. The reason for this discrepancy is unknown.

Total amount of SV40 DNA per cell. To calculate the amount of SV40 DNA present per cell, it is necessary to estimate the DNA content of the nuclei of transformed cells. These measurements were kindly carried out for us by Arthur Vogel in the laboratory of D. Peterson, Los Alamos, New Mexico, using the method described by Kraemer, Peterson, and VanDilla (7). In brief, preparations of fixed cells were stained with a fluorescent Feulgen dye and were then passed rapidly through a narrow laser beam of blue light. The amount of DNA per nucleus is proportional to the height of the pulse

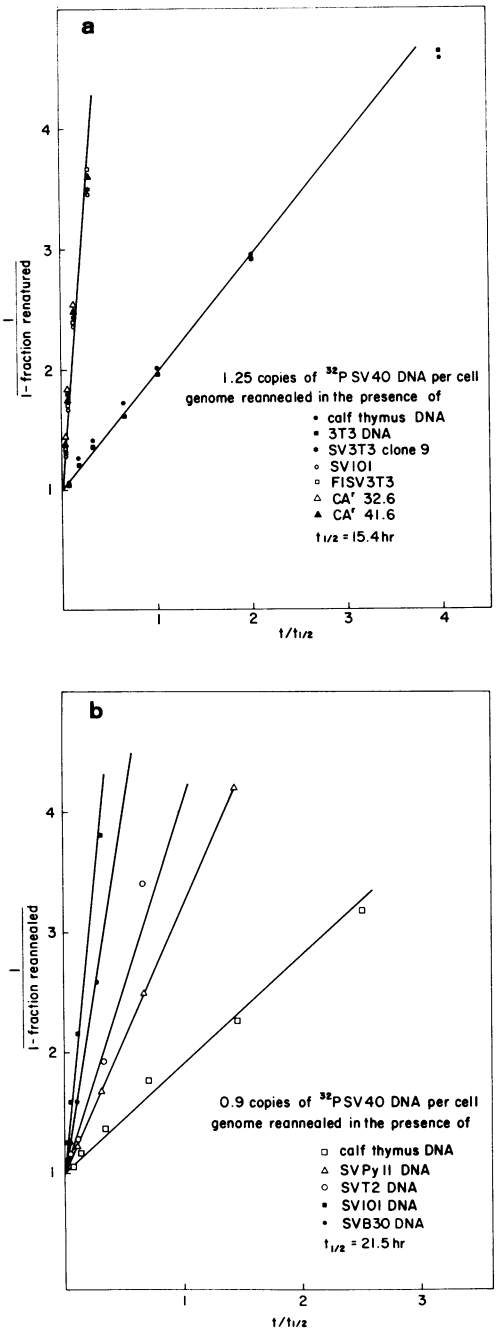

FIG. 1. *Kinetics of reassociation of ^{32}P-labeled SV40 DNA in the presence of unlabeled DNAs from transformed and control cells. The reaction mixtures for the experiment shown in Fig. 1a contained 4.5 × 10^{-5} optical density units (OD)/ml of sheared ^{32}P-labeled SV40 DNA (specific activity 9 × 10^5 counts per min per μg), 0.0002 M EDTA, 1.0 M NaCl, and 41.5 OD/ml of sheared DNA from calf thymus (●), 3T3 cells (■), SV3T3 clone 9 cells (✳), SV101 cells (○), F1SV101 cells (□), CAr32.6 cells (△), and CAr41.6 cells (▲). In Fig. 1b, the reaction mixtures contained 3 × 10^{-5} OD/ml of ^{32}P-labeled SV40 DNA (1.2 × 10^6 counts per min per μg), 0.0002 M EDTA, 1.0 M NaCl, and 38.5 OD/ml of calf thymus DNA (□), SVPyll DNA (△), SVT2 DNA (○), SVB30 DNA (●), or SV101 DNA (■). The DNAs were heat-denatured and incubated at 68 C. Samples of 0.3 ml were removed at intervals, and the proportion of reassociated ^{32}P-labeled DNA was determined by chromatography on hydroxyapatite. Each point represents a total of 600 to 700 counts/min. The reannealing data is expressed in a standard second order reaction plot (see Wetmur and Davidson [25]). This plot gives a straight line for a bimolecular reaction and the slope of the line is directly proportional to the rate of the reaction. The abscissa (t/t, 1/2) is the time of incubation of each mixture divided by the time required for 1/2 of the ^{32}P-labeled SV40 DNA to reanneal in the presence of carrier DNA (calf thymus DNA or 3T3 DNA).*

of light emitted by the cell. Typical results are shown in Fig. 2. Using mouse embryo fibroblast cells as a standard, it can be seen (Table 1) that all the cell lines except SVT2 contain more DNA per cell than is present in normal diploid mouse cells, that in general, transformed cells contain more DNA than their untransformed parents, and that revertants in turn contain more DNA than the transformants from which they were derived. Using the figure of 3.9 × 10^{12} daltons of DNA per diploid mouse cell (20) and knowing both the proportion of the DNA sequences of transformed cells that are complementary to SV40 DNA and the total amount of DNA in the cells relative to control cell DNA, it is possible to calculate the number of SV40 genomes in some of the transformed cell lines. These estimates, which range from a low of 2.2 genomes per cell for SVT2 cells to a high of 26 to 32 genomes per cell in F1SV101 cells, are shown in Table 1.

Chromosome numbers. The chromosome numbers of some of the transformed cells used in this study have already been published by other workers (1, 13), and we obtained values not significantly different from these published estimates. Histograms showing the number of chromosomes present in cells of the other lines are shown in Fig. 3. The modal chromosome number for each cell line is also shown in Table 1. All of the transformed lines except SVT2 show an increased number of chromosomes compared with the parental 3T3 cells. The three con A-resistant cells show an additional small increase in chromosome number compared with the SV3T3 clone 9 cells from which they were derived. The increase in the amount of DNA in the cells is greater than the increase in the

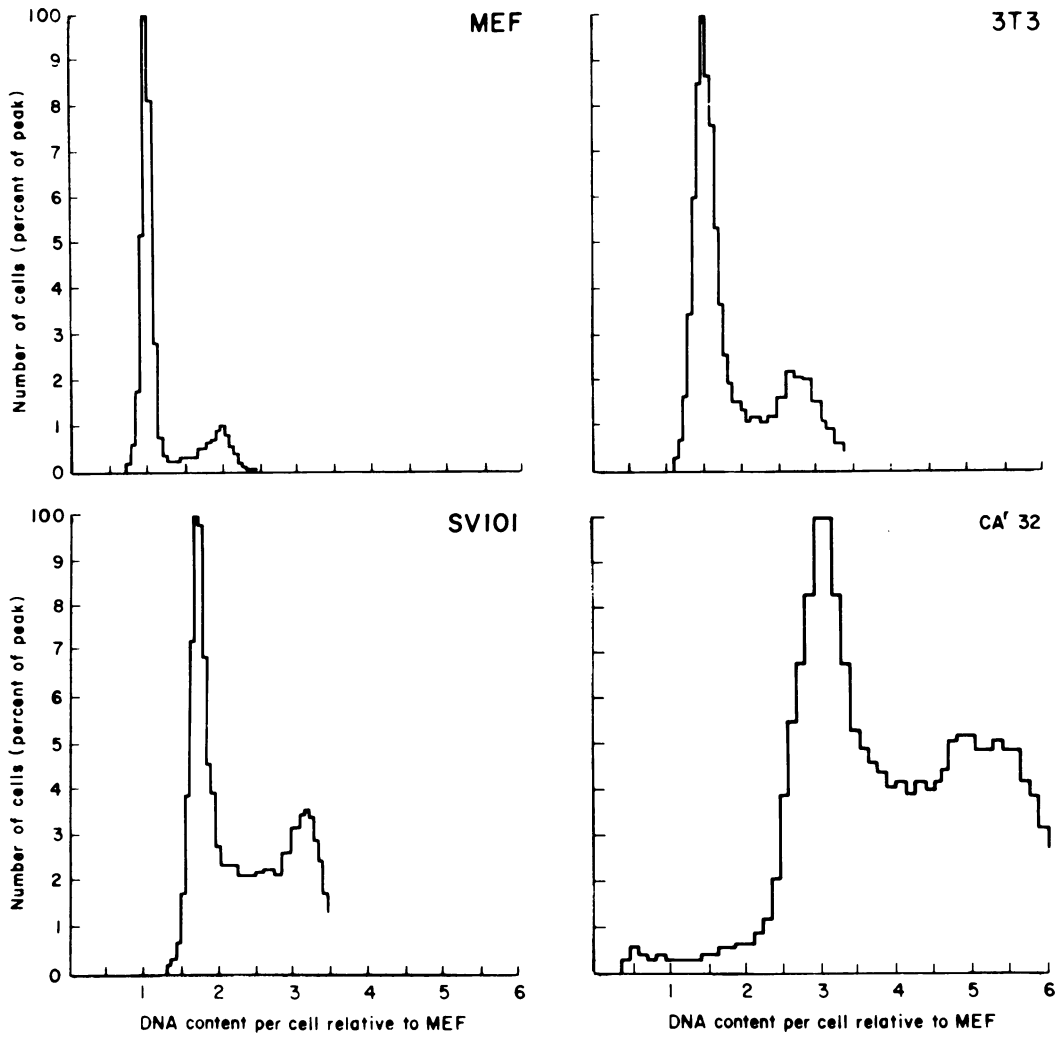

FIG. 2. *DNA content per cell was determined by the method of Kraemer, Peterson, and Van Dilla (7). Fixed cells were stained with a fluorescent Feulgen dye and passed through a narrow beamed blue light from an argon ion laser. As each cell passed through the beam, it emitted a pulse of fluorescent light which was observed by a photomultiplier. After being amplified, the output from the photomultiplier was analyzed by a multichannel pulse-height analyzer which compiled a histogram representing the Feulgen-DNA distribution of the measured cells. Typically 100,000 to 200,000 cells are measured. To calibrate the machine, a known diploid cell type was analyzed. This gives a G_1 peak representing the number of cells analyzed with a 2c amount of DNA and a second smaller peak giving the number of cells in $G_2 + M$ having a 4c content of DNA. Mouse embryo fibroblast (MEF) cells were used as the diploid cell line, and their G_1 peak was given the value of 1. The G_1 DNA content of the other cell line was expressed relative to that of the MEF cells. The drift towards the lower end of the scale seen in the figure representing CA'32 is variable and probably is due to cellular debris and was not plotted for the other cell lines.*

number of chromosomes (see Table 1). The reason for this is not understood, but it must mean either that there has been a duplication of those chromosomes that contain more DNA, or that there is a general increase in the amount of DNA in each chromosome of these cells.

Viral RNA sequences present in transformed cells. Increasing amounts of unlabeled RNA extracted from transformed cells were hybridized to a constant amount of ^{32}P-labeled DNA of each of the separated viral DNA strands. The amount of DNA that enters into hybrid at saturating concentrations of RNA is a measure of the proportion of the sequences of

TABLE 1. *Amount of SV40 DNA per cell*

Cell line	Phenotype	No. of copies of SV40 DNA per diploid quantity[a] cell DNA	Relative amt of DNA per G_1 cell compared with mouse embryo fibroblasts	No. of copies of viral DNA per G_1 cell	Modal chromosome no.	Percentage of viral genome transcribed	
						E strand	L strand
SV3T3 clone 9	Transformed	8-9	1.6	13-14	70	73	15
SV101	Transformed	8-9	1.6	13-14	74[b]	77	3
SVT2	Transformed	2.2 (1.56)[c]	1.0	2.2	45[d]	58	0
SVB30	Transformed	6.1	ND	ND	ND	65	10
SVPy11	Transformed	1.3 (1.42)[c]	ND	ND	ND	65	0
SVuv30	Transformed	ND	ND	ND	ND	24[e]	22[e]
F1SV101	Revertant	8-9	3.5	26-32	118[b]	32[e]	3
CA^{r}30.4	Revertant	8-9	2.9	23-26	72	70	10
CA^{r}32.6	Revertant	8-9	2.9	23-26	75	75	12
CA^{r}41.6	Revertant	8-9	2.7	22-24	76	78	10
3T3	Untransformed	0	1.5	0	70 (67[d])	0	0

[a] The quantity of 3.9×10^{12} daltons of DNA per diploid mammalian genome was used throughout (20).
[b] From Pollack, Vogel, and Wollman (13).
[c] Figures in parentheses were obtained by Gelb, Kohne, and Martin (3).
[d] From Culp and Black (1).
[e] This figure is a minimal estimate because of difficulties in reaching saturating levels of RNA.

the strand that are present in RNA. After hybridization in the presence of 400 μg of RNA extracted from untransformed 3T3 cells, less than 0.5% of the radioactivity of E and L strand DNA behaved as hybrid on hydroxyapatite columns; however, virus-specific RNA was detected in all transformed cell lines that we examined. Typical results are shown in Fig. 4, 5, and 6. In most experiments involving hybridization with E strand DNA we had no difficulty in reaching saturating levels of RNA, and in these cases we can provide accurate estimates of the percentages of the sequences of the strand that appear in RNA. For different cell lines examined in these experiments this value varies between 58 and 78%. Most of the cell lines examined also contain RNA which is complementary to between 4 and 20% of the L strand sequences. However, we always found it difficult to obtain saturation of the L strand DNA with RNA from transformed cells. Thus it seems that RNA complementary to this strand is present in much lower concentrations in transformed cells than is the RNA which is complementary to the E strand. It may be that considerably more of the L strand sequences than we estimate are present in RNA, but at such a low concentration that we were unable to detect them by our hybridization technique.

All of the con A-resistant variants contained RNA complementary to approximately the same proportions of both E and L strand DNA as the parental line, and from the shape of the saturation curves (Fig. 4) it seems there is little difference in the concentrations of viral-specific sequences in the RNA preparations from the two sorts of cells.

However, in F1SV101 cells the concentration of viral RNA sequences is at least fivefold less than in the parental SV101 line (Fig. 5), and we were never able to add enough RNA from F1SV101 cells to reach saturation of either E or L strand DNA. Similar problems occurred with SVuv30 (Fig. 6), and so for these two cell lines we do not know exactly how much of the viral DNA is transcribed. But we can provide minimum estimates, and these are shown in Table 1 together with data from all the other transformed cell lines.

DISCUSSION

This work confirms earlier observations (3, 24) that the genomes of different lines of mouse cells transformed by SV40 contain different amounts of viral DNA. The five independently transformed lines examined in this paper contain quantities of viral DNA which range between 1.35 copies and 8.75 copies per diploid amount of host DNA. In every case except that of SVT2, the number of copies of SV40 DNA *per cell* is greater because cells of most of the transformed lines contain more than one diploid equivalent of host DNA. In spite of the wide

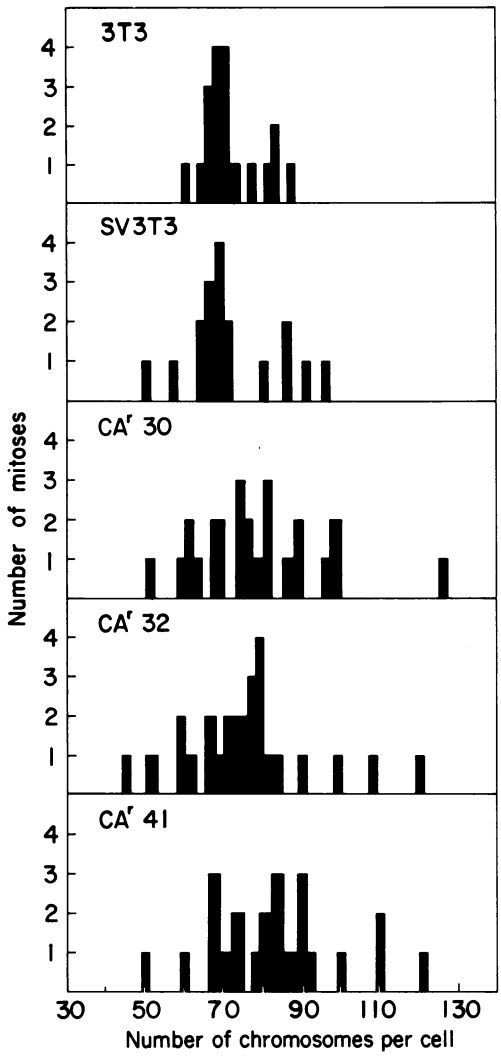

FIG. 3. *Cytogenetic analysis of 3T3, SV3T3 clone 9, CA^{r}30.4, CA^{r}32.6, and CA^{r}41.6. Chromosomes were counted in colcemid-treated cells as described by Pollack, Vogel, and Wollmann (13).*

range in the amount of viral genetic material in different cells, it is clear for two reasons that there is no correlation between the expression of the transformed phenotype and the number of copies of viral DNA. Firstly, cells like SVT2 display a phenotype which is as characteristic of transformed cells as that shown by SV3T3 clone 9 cells which contain about seven times more viral DNA. Secondly, revertant cells which express the transformed phenotype very weakly contain the same high number of copies of SV40 DNA per diploid quantity of host DNA as do the parental cells. From these data we can say that, while the presence of viral DNA may be necessary for transformation, it is by no means sufficient. A similar conclusion has already been reached on the basis of experiments with SV40 "cryptic" transformants (19) and with revertants of cells transformed by polyoma virus (18).

We have used the separated strands of SV40 DNA to detect viral RNA in different lines of transformed cells. All the transformed cell lines examined contain more RNA sequences complementary to the early strand of SV40 DNA than are ever expressed during lytic infection. In productively infected cells, not more than 35% of the sequences of the E strand appear in

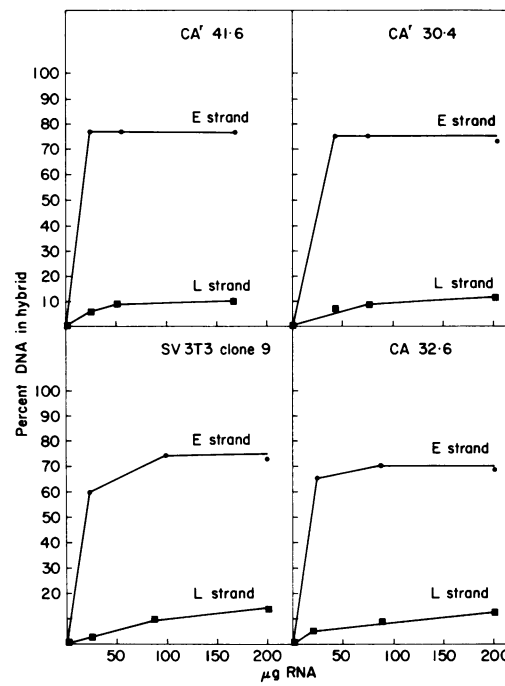

FIG. 4. *Hybridization of the separated strands of SV40 DNA to RNA isolated from transformed cells. Hybridization mixtures contained 2 × 10^{-3} μg of ^{32}P-labeled E or L strand DNA (specific activity 8 × 10^5 counts per min per μg) prepared as described earlier (15) and different amounts of RNA in a total volume of 0.125 ml of 1 M NaCl, 0.0002 M EDTA, 0.001 M Tris (pH 7.5). After incubation for 36 h at 68 C, the samples were diluted with 2.0 ml of 0.14 M phosphate buffer (pH 6.8) containing 0.4% SDS and assayed on hydroxyapatite. In all experiments, the largest quantity of transformed cell RNA used after alkaline hydrolysis gave no hybridization to either the ^{32}P-labeled E or L strand.*

stable species of RNA. However, in transformed mouse cells the percentage ranges from about 30% (SVuv30) to about 80% (SV101). This result means that a considerable fraction of the viral RNA sequences are "anti-late." Why this RNA which presumably is non-informational should be present in transformed cells is unknown. Neither do we understand the reason why different transformed cell lines show different patterns of viral RNA. There does not appear to be a correlation between the proportion of the sequences of the E strand that are present in RNA and the number of viral genomes per cell or the ease with which they can be rescued by heterokaryon formation.

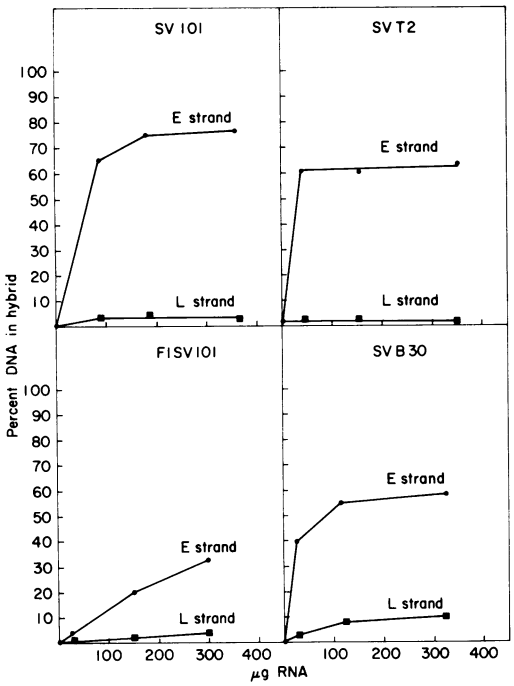

FIG. 5. *Hybridization of separated strands of SV40 DNA to RNA isolated from tranformed cells. For experimental details, see legend to Fig. 4.*

In transformed cells SV40 sequences occur both in heterogeneous nuclear and in stable cytoplasmic RNA (8, 21). If we assume that our hybridization technique detects only the stable cytoplasmic and not the less abundant, transient nuclear RNA, then it is possible to propose a model to explain the variable amount of hybridization of the E strand of SV40 to RNA extracted from transformed cells. In this model we suggest that, in different lines of cells, SV40 is integrated with breaks at different positions in the viral genome (see Fig. 7). The orientation of synthesis of RNA along the integrated genome could be host → virus or virus → host, and there is little evidence to help us decide between these two alternatives. However, in transformed cells SV40-specific sequences are present in large molecules of RNA in the nucleus and in smaller molecules in the cytoplasm (8, 21). The nuclear molecules are considerably longer than a single strand of SV40 DNA and they contain covalently linked host and viral sequences (22).

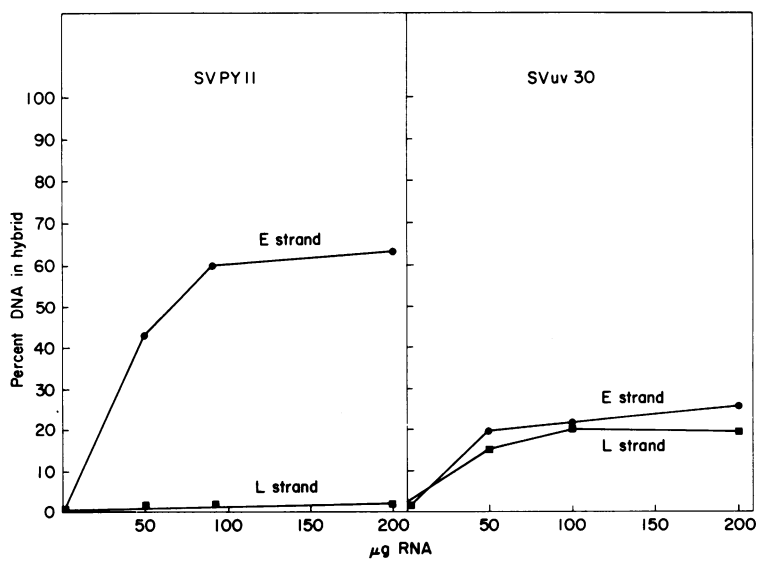

FIG. 6. *Hybridization of separated strands of SV40 DNA to RNA isolated from transformed cells. For experimental details, see legend to Fig. 4.*

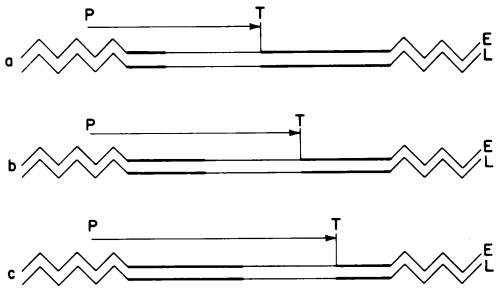

FIG. 7. *Model for SV40 transcription. a, b, and c represent SV40 transcription in different transformants. P = promoter; T = terminator; (∿∿) host DNA; (═══) early sequences of SV40; (═══) "late" sequences of SV40.*

By contrast, the cytoplasmic species consist only of SV40 sequences (22) and contain polyadenylic acid residues, presumably at their 3' ends (23). Thus, during processing of the nuclear RNA, it appears that the polyadenylic acid residues and the SV40 sequences are conserved while host sequences are not. In all probability then, the host sequences are present at the 5' end of the large nuclear RNA, so that the direction of synthesis of this RNA is host → virus. The model does not predict whether control of stable SV40 RNA occurs at the level of transcription or processing. In the case of transcriptional control, the length of the E strand that appears in stable species of RNA will be a function of the distance of the terminator site from the point of integration. If control is mediated through processing, the terminator shown in Fig. 7 becomes a cleavage site. All viral RNA sequences to the left of the site will be conserved, and all those to the right will be degraded.

In addition to E strand sequences, most lines of SV40-transformed mouse cells contain RNA complementary to the late strand of SV40 DNA. As in the case of the E strand, the proportion of the L strand sequences that are present in RNA seems to vary in different cell lines. However, it is difficult to make firm statements about the exact percentages because of problems in reaching saturating levels of RNA. For the same reason, it is difficult to provide models to account for L strand transcription. There seems to be a correlation between the presence of this RNA in transformed cells and the number of virus genomes per cell. Only those cell lines with high numbers of SV40 genomes contain RNA complementary to the late strand of SV40 DNA. It is not known whether the RNA that binds to the L strand is transcribed from true "late" or from "anti-early" sequences of viral DNA, but whatever its origin, it is unlikely that RNA plays any role in maintaining the transformed state, in view of the fact that it is not detectable in SVT2 or SV101 cells. Most probably then, expression of the transformed phenotype requires RNA coded only by the early strand of SV40 DNA. However, the presence of this RNA in cells is by no means sufficient to guarantee expression of transformation because con A-resistant cells which show a partially revertant phenotype nevertheless contain high concentrations of RNA transcribed from the E strand of SV40 DNA. Finally, it is interesting to note that F1SV101 cells, which contain a high number of SV40 genomes but which display a revertant phenotype, yield RNA preparations which contain SV40 sequences at much lower concentration than fully transformed cells. This is a provocative result, because it could provide an explanation for the change in the phenotype of the cells. However, given our present data, it is also possible to argue that the reduction in viral RNA concentration is a consequence of the cells' phenotype rather than a cause of it. To resolve this question it will be necessary to examine SV40 RNA sequences that are present in other revertants and in the "spontaneous" transformants that occasionally arise in the revertant population.

ACKNOWLEDGMENTS

We would like to thank Art Vogel for measuring the DNA content per cell, Mirka Lurye for assistance in cell culturing, and Bill Sugden for SV40 complementary RNA.

This work was supported by Public Health Service research grant CA 11432 from the National Cancer Institute.

ADDENDUM

A report which appeared after this manuscript was submitted (G. Khoury, J. C. Byrne, K. K. Takemoto, and M. A. Martin. 1973. Patterns of simian virus 40 deoxyribonucleic acid transcription. II. In transformed cells. J. Virol. **11**: 54–60) confirms the previous finding for the pattern of transcription in transformed mouse cells (15). In addition, the authors present data for the transcription patterns of other lines of SV40-transformed cells which are in general agreement with our findings.

LITERATURE CITED

1. Culp, L. A., and P. H. Black. 1972. Contact-inhibited revertant cell lines isolated from simian virus 40-transformed cells. III. Concanavalin A selected revertant cells. J. Virol. **9**:611–620.
2. Dulbecco, R., and G. Freeman. 1959. Plaque production by the polyoma virus. Virology **8**:396–397.
3. Gelb, L. D., D. E. Kohne, and M. A. Martin. 1971. Quantitation of simian virus 40 sequences in African green monkey, mouse and virus-transformed cell genomes. J. Mol. Biol. **57**:129–145.

4. Gerber, P. 1962. An infectious deoxyribonucleic acid derived from vacuolating virus (SV40). Virology **16**:96-98.
5. Hopps, H. E., B. C. Bernheim, A. Nisalak, J. H. Tjio, and J. E. Smadel. 1963. Biologic characteristics of a continuous kidney culture derived from the African green monkey. J. Immunol. **91**:416-424.
6. Khoury, G., J. C. Byrne, and M. A. Martin. 1972. Patterns of simian virus 40 DNA transcription after acute infection of permissive and non-permissive cells, Proc. Nat. Acad. Sci. U.S.A. **69**:1925-1928.
7. Kraemer, P., D. Petersen, and M. VanDilla. 1971. DNA constancy in heteroploidy and the stem line theory of tumors. Science **174**:714-717.
8. Lindberg, U., and J. E. Darnell. 1971. SV40-specific RNA in the nucleus and polyribosomes of transformed cells. Proc. Nat. Acad. Sci. U.S.A. **65**:1089-1096.
9. Ozanne, B. 1973. Variants of simian virus 40-transformed 3T3 cells that are resistant to concanavalin A. J. Virol. **12**:79-89.
10. Ozanne, B., A. Vogel, P. A. Sharp, W. Keller, and J. Sambrook. 1973. Transcription of SV40 DNA in different transformed cell lines. *In* L. G. Sylvestri (ed.), 4th Lepetit Colloquium on Biology and Medicine. North Holland, Amsterdam.
11. Pettersson, U., and J. Sambrook, 1973. The amount of viral DNA in the genome of cells transformed by adenovirus type 2. J. Mol. Biol. **73**:125-130.
12. Pollack, R. E., H. Green, and G. Todaro. 1968. Growth control in cultured cells: selection of sublines with increased sensitivity to contact inhibition and decreased tumor producing ability. Proc. Nat. Acad. Sci. U.S.A. **60**:126-133.
13. Pollack, R. E., A. Vogel, and S. Wollman. 1970. Reversion of virus-transformed cell lines: hyperploidy accompanies retention of viral genes. Nature (London) **228**:938, 967-70.
14. Sambrook, J. 1972. Transformation by polyoma virus and simian virus 40. Adv. Cancer Res. **16**:141-180.
15. Sambrook, J., P. A. Sharp, and W. Keller, 1972. Transcription of simian virus 40. I. Separation of the strands of SV40 DNA and hybridization of the separated strands to RNA extracted from lytically infected and transformed cells. J. Mol. Biol. **70**:57-71.
16. Sambrook, J., H. Westphal, P. R. Srinivasan, and R. Dulbecco. 1968. The integrated state of SV40 DNA in transformed cells. Proc. Nat. Acad. Sci. U.S.A. **60**:1288-1295.
17. Scherrer, K. 1969. Isolation and sucrose gradient analysis of RNA, p. 413-432. *In* K. Habel and N. P. Salzman (ed.), Fundamental techniques in virology. Academic Press Inc., New York.
18. Shani, M., Z. Rabinowitz, and L. Sachs. 1972. Virus deoxyribonucleic acid sequences in subdiploid and subtetraploid revertants of polyoma transformed cells. J. Virol. **10**:456-461.
19. Smith, H. S., L. D. Gelb, and M. A. Martin. 1972. Detection and quantitation of simian virus 40 genetic material in abortively transformed BALB/3T3 clones. Proc. Nat. Acad. Sci. U.S.A. **69**:152-156.
20. Sober, H. (ed.). 1968. Handbook of biochemistry, p. 58. Chemical Rubber Co., Cleveland.
21. Tonegawa, S., G. Walter, A. Bernardini, and R. Dulbecco. 1970. Transcription of the SV40 genome in transformed cells and during lytic infection. Cold Spring Harbor Symp. Quant. Biol. **35**:823-831.
22. Wall, R., and J. E. Darnell, 1971. Presence of cell- and virus-specific sequences in the same molecules of nuclear RNA from virus-transformed cells. Nature N. Biol. **232**:73-77.
23. Weinberg, R. A., Z. Ben-Ishai, and J. A. Newbold. 1972. Poly A associated with SV40 messenger RNA. Nature N. Biol. **238**:111-113.
24. Westphal, H., and R. Dulbecco. 1968. Viral DNA in polyoma and SV40-transformed cell lines. Proc. Nat. Acad. Sci. U.S.A. **59**:1158-1165.
25. Wetmur, J. G., and N. Davidson. 1968. Kinetics of renaturation of DNA. J. Mol. Biol. **31**:349-370.