JOURNAL OF VIROLOGY, Oct. 1975, p. 818-823

Vol. 16, No. 4
Printed in U.S.A.

Transformation of Primary Rat Kidney Cells by Fragments of Simian Virus 40 DNA

P. J. ABRAHAMS, C. MULDER,[1] A. VAN DE VOORDE, S. O. WARNAAR, AND A. J. VAN DER EB*

Laboratory for Physiological Chemistry, State University of Leiden, Leiden, The Netherlands; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724; and Laboratory for Molecular Biology, University of Gent, Gent, Belgium*

Received for publication 10 June 1975

Linear simian virus 40 (SV40) DNA molecules of genome length and DNA fragments smaller than genome length were prepared with restriction endonucleases and tested for transforming activity on primary cultures of baby rat kidney cells. The linear molecules of genome length (prepared with endonucleases R·*Eco*RI, R·*Bam*HI, and R·*Hpa*II or R·*Hap*II), a 74% fragment (*Eco*RI/*Hpa*II or *Hap*II-A), and a 59% fragment (*Bam*HI/*Hap*II-A) could all transform rat kidney cells with the same efficiency as circular SV40 DNA. All transformed lines tested contained the SV40-specific T-antigen in 90 to 100% of the cells, which was taken as evidence that the transformation was SV40 specific. The DNA fragments with transforming activity contained the entire early region of SV40 DNA. Endo R·*Hpa*I, which introduced one break in the early region, apparently inactivated the transforming capacity of SV40 DNA, since no transformation was observed with any of the three *Hpa*I fragments tested. Attempts were made to rescue infectious virus from some of the transformed lines by fusion with permissive BSC-1 cells. Infectious virus was only recovered from the cells transformed by circular form I DNA. No infectious virus could be isolated from any of the other types of transformed cells.

We reported recently that rat and mouse cells can be transformed by DNA of simian virus 40 (SV40) by the calcium technique (2). This technique appears to be particularly suitable for demonstrating transforming activity of DNA of several viruses, such as adenoviruses (10), herpesvirus (29), and BK virus (J. v. d. Noordaa, submitted for publication). It was observed that transformation could be obtained not only with intact adenovirus 5 DNA but also with fragments of the DNA (11). The latter result indicated that the integrity of the adenovirus DNA is not required for transformation, although it is essential for infectivity, and it suggested the possibility of isolating a specific DNA fragment with transforming potential. By using bacterial restriction endonucleases, we recently succeeded in isolating a small specific fragment of adenovirus 2 and 5 DNA, which represents 7% of the genome (molecular weight 1.6×10^6) and which apparently contains the information necessary for inducing and maintaining transformation in cultured cells (8). The availability of such DNA segments with transforming potential will provide several possibilities for studying the viral genes involved in the process of transformation.

In the case of SV40, studies with temperature-sensitive mutants have indicated that one class of mutants, the only early complementation group A, is defective in initiation and phenotypic expression of transformation (5, 18, 24, 27), suggesting that only one viral gene is involved in these processes. Studies with nondefective adenovirus 2-SV40 hybrids have shown that different SV40-specific nonstructural antigens are expressed depending on the size of the SV40 DNA segment present in the adenovirus genome (17, 21). Thus the question arises of whether the whole early region of SV40 is required for transformation or only a part of it.

To determine which part of the SV40 genome is required for transformation, we isolated specific fragments of SV40 DNA and tested their ability to transform primary baby rat kidney (BRK) cells. It will be shown that

[1] Present address: Department of Pharmacology and Microbiology, University of Massachusetts Medical School, Worcester, Mass. 01605.

linearized SV40 DNA molecules of genome size as well as fragments of the DNA are capable of transforming cells with an efficiency comparable to that of circular DNA. Preliminary results of this study were reported elsewhere (8).

MATERIALS AND METHODS

Virus and viral DNA. SV40 virus, clone 307 L, obtained from S. Kit, was grown in confluent monolayers of BSC-1 cells at an input multiplicity of about 10^{-3} PFU/cell.

Viral DNA was extracted from infected cells by the Hirt procedure (13) and was purified by at least two cycles of banding in CsCl gradients (density 1.56 g/cm^3) containing 200 μg of ethidium bromide per ml.

Only the form I DNA in the denser band was used. All viral DNA preparations were usually lightly labeled with [^{3}H]thymidine (1.5×10^3 to $5.0\ 10^3$ counts/min per μg of DNA).

Assay of transforming activity. The transformation assays were carried out on primary kidney cell cultures of 6- to 7-day-old Wistar rats, as described previously (2, 9). These cells are further designated BRK cells.

Cleavage of SV40 DNA with restriction endonucleases. Restriction endonuclease *Eco*RI was isolated as described before (20). Incubations were carried out in 90 mM Tris-hydrochloride (pH 7.9) and 10 mM $MgCl_2$.

Endonucleases R·*Hpa*I and *Hpa*II were isolated as described by Sharp et al. (26). The incubation mixtures used for these enzymes were as follows: for *Hpa*I, 30 mM Tris-hydrochloride (pH 7.6), 5 mM $MgCl_2$, 100 mM KCl, and 1 mM β-mercaptoethanol (β-ME); for *Hpa*II, 10 mM Tris-hydrochloride (pH 7.6), 10 mM $MgCl_2$, 6 mM KCl, and 2 mM β-ME.

Endo R·*Hap*II, an isoschizomer of endo R·*Hpa*II, was used in some experiments to replace endo R·*Hpa*II. The enzyme was isolated according to M. Takanami (*in* A. L. Laskin and J. A. Last, ed., *Methods of Molecular Biology*, in press), and the incubation mixture contained 100 mM Tris-hydrochloride (pH 7.6), 70 mM $MgCl_2$, and 70 mM β-ME.

Endonuclease R·*Bam*HI was purified according to G. Wilson and F. Young (manuscript in preparation). Incubation with this enzyme was carried out in 6 mM Tris-hydrochloride (pH 7.6), 6 mM $MgCl_2$, 15 mM KCl, and 6 mM β-ME.

After incubation with a restriction endonuclease, the extent of conversion was monitored by electrophoresis in 1.5% agarose gels, using superhelical and nicked circular SV40 DNA as markers.

The DNA solutions were deproteinized by extraction with chloroform-isoamylalcohol (24:1), and the DNA was fractionated by electrophoresis in 1.5 or 3% agarose gels. To obtain a double digest, the DNA solution was deproteinized after the first endonuclease digestion, and either the solution was dialyzed overnight in the buffer for the second enzyme or the DNA was precipitated with ethanol and redissolved in the second incubation buffer.

Incubations with S1 single-strand-specific endonuclease were carried out for 10 min at 37 C in 30 mM sodium acetate buffer (pH 4.6), 300 mM NaCl, 1 mM $ZnSO_4$, and 5% glycerol. The reaction was terminated by addition of Tris-hydrochloride (pH 8.1) and EDTA to final concentrations of 100 and 10 mM, respectively. The DNA solution was deproteinized by digestion with 20 μg of proteinase-K (Merck) per ml, followed by extraction with chloroform-isoamyl alcohol (24:1) and dialysis in 0.1× SSC (SSC = 0.15 M NaCl plus 0.015 M sodium citrate).

Gel electrophoresis. DNA fragments were purified by electrophoresis in cylindrical or slab gels of 1.5 or 3.0% agarose (Sigma Chemical Co., electrophoresis grade) (22). The DNA bands were visualized by staining the gels in a solution of ethidium bromide (0.5 μg/ml) in electrophoresis buffer and illuminating with a long-wave UV lamp (1). The DNA was extracted from the agarose as described elsewhere (22).

Immunofluorescence. Transformed BRK cells were grown on cover slips, fixed with cold acetone, and tested for the presence of SV40-specific T-antigen by the indirect immunofluorescence method (25), using serum from tumor-bearing hamsters and fluorescein-isothiocyanate-conjugated swine anti-hamster serum.

Rescue of SV40 from transformed cells. Transformed cells were fused with permissive BSC-1 cells by using β-propiolactone-inactivated Sendai virus (28) in order to rescue SV40. For each experiment, approximately 10^6 BSC-1 cells and 10^6 transformed cells were used. The extent of fusion was monitored by growing a small aliquot of the cells on cover slips and counting the percentage of bi- and multinucleated cells after fixing and staining with Giemsa. The fused cells were incubated at 37 C for 11 to 14 days. The cultures were then freeze-thawed and tested for the presence of infectious virus by plaque titration on BSC-1 cells.

Electron microscopy of DNA. Electron microscopy of DNA was carried out according to a modification of the Kleinschmidt protein monolayer technique (3).

RESULTS

Transformation by linear SV40 DNA molecules of genome size. It has been shown that fragments of adenovirus DNA can transform primary BRK cells. These results suggested that it might similarly be possible to isolate a fragment of SV40 DNA with transforming activity. Obviously, this would only be possible if linear SV40 DNA molecules can transform cells. To investigate this, linear molecules of genome size were prepared by incubating form I SV40 DNA with restriction endonucleases that introduce one double-stranded break per DNA molecule. The following enzymes were used for this purpose: endo R·*Eco*RI, which introduces one cleavage at a position defined as 0 (20, 23);

endo R·*Bam*HI, which cleaves at 0.15 (Mulder and Greene, unpublished data); and endo R·*Hpa*II and *Hap*II, which both cleave SV40 DNA at 0.74 (26). The linear molecules were separated from residual circular molecules by electrophoresis in 3% agarose gels and the DNA, recovered from the agarose, was tested for transforming activity on BRK cells.

Cleavage of DNA by endo R·*Eco*RI results in the formation of short single-stranded ends with a complementary base sequence (12, 19). Therefore, the possibility existed that some molecules would be converted to circular molecules as a consequence of ligase activity within the cell. This mechanism was used to explain the observed low infectivity of *Eco*RI linear SV40 DNA (19). To prevent recircularization, the *Eco*RI linear molecules were treated with single-strand-specific S1 endonuclease. The effectiveness of this treatment was tested by spreading the linear molecules for electron microscopy before and after treatment with S1 at 0 C. The results indicated that without S1 treatment the majority of the molecules occurred as relaxed circles, whereas after treatment with S1 endonuclease only 2 to 3% were found as circles. In addition, no infectivity was found in the S1-treated DNA preparation.

*Bam*HI linear molecules were not treated with S1 endonuclease; therefore it could not be ruled out that in this case some of the cells were transformed by recircularized molecules. This is not very likely, however, since no relaxed circular molecules were observed either by electron microscopy or by electrophoresis at 0 C, possibly due to a slight contamination of the endo R·*Bam*HI preparation with a single-strand-specific nuclease. Linear molecules produced by endo R·*Hpa*II were similarly not treated with S1 endonuclease since the single-stranded ends produced by this enzyme consist of only two nucleotides.

The cleavage sites of the three restriction endonucleases are shown in Fig. 1, and the results of the transformation assays with the linear SV40 molecules of genome size are presented in Table 1. It can be seen that all the three types of linear molecules had approximately the same specific transforming activity as the circular SV40 DNA molecules.

Transformation by SV40 DNA fragments. The positive results that were obtained with linear DNA molecules suggested that smaller fragments of SV40 DNA could transform cells. First, fragments were made by incubating SV40 DNA with two enzymes that each cleaved the DNA only once. Thus, successive cleavage with

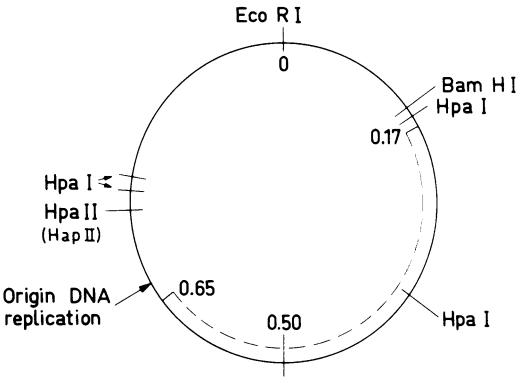

FIG. 1. *Map of the SV40 genome showing the cleavage sites of endonucleases R EcoRI, BamHI, HpaI, and HpaII (or HapII). The positions of the R·HpaI and HpaII (HapII) cleavage sites are taken from Danna et al. (6) and Sharp et al. (26). The position of the R·BamHI cleavage site has been determined by Mulder and Greene (unpublished data), and the position of the replication origin has been determined by Fareed et al. (7). The location of the early region, indicated by the dashed line, is taken from Khoury et al. (14, 15).*

endo R·*Eco*RI and *Hpa*II (*Hap*II) yielded two fragments, A and B, of 74 and 26%. These were separated, purified, and tested for transforming activity. Similarly, a 41% and a 59% fragment were obtained by digesting SV40 DNA successively with endo R·*Bam*HI and *Hap*II. SV40 DNA was also cleaved by endo R·*Hpa*I into three specific fragments (20, 38, and 42%, respectively) (see Fig. 1).

The results of the transformation assays with these SV40 DNA fragments are also shown in Table 1. As can be seen, the 74% *Eco*RI/*Hpa*II (*Hap*II) A and the 59% *Bam*HI/*Hap*II A fragments were both capable of transforming primary BRK cells with approximately the same efficiency as circular DNA. No activity was found with any of the other fragments.

Presence of SV40-specific T-antigen. Several transformed colonies, which appeared in the cultures infected with the linear SV40 DNA molecules and with the 59% *Bam*HI/*Hap*II A and the 74% *Eco*RI/*Hpa*II A DNA fragments, were isolated and established as cell lines. Two or three cell lines of each type were tested for the presence of SV40-specific T-antigen by indirect immunofluorescence. All cell lines tested were found to contain the nuclear fluorescence characteristic for SV40 T-antigen in approximately 90 to 100% of the cells.

Attempts to rescue infectious virus from transformed cells. To investigate whether infectious virus could be rescued from cells

TABLE 1. *Transforming activity of SV40 DNA and DNA fragments*

SV40 DNA	μg of DNA/dish (genome equivalent)	Foci/dish	Avg no. of foci/μg of genome equivalent
Form I[a]	0.5	18, 13, 12	28.7
	1	30, 26, 20	25.3
	2	40, 43, 40	20.5
	4	85, 75, 74	19.5
*Eco*RI linear[b]	0.5	14, 18, 11	28.7
	1	15, 30, 13	19.3
*Hpa*II linear	0.5	10, 13	23
*Bam*HI linear	1	45, 30	37.5
	2	37, 40	19.2
*Eco*RI/*Hap*II A 74% fragment	1.3	14, 15, 17	11.8
*Eco*RI/*Hpa*II A 74% fragment	1	12, 13	12.5
*Eco*RI/*Hpa*II B 26% fragment	2	0, 0	0
*Bam*HI/*Hap*II A	1	22, 26	24
59% fragment	2	25, 46	17.7
	4	67, 43	13.7
*Bam*HI/*Hap*II B	1	0, 0	0
41% fragment	2	0, 0	0
	4	0, 0	0
*Hpa*I			
Fragment A (42%)	1, 4	0, 0	0
Fragment B (38%)	1, 4	0, 0	0
Fragment C (20%)	1, 4	0, 0	0

[a] Data taken from Abrahams and van der Eb (2).
[b] *Eco*RI linear was treated with single-strand-specific S1 endonuclease.

transformed by the linear SV40 DNAs and the DNA fragments, one cell line of each type was fused with permissive BSC-1 cells, using inactivated Sendai virus as fusing factor. One day after fusion, the percentage of bi- and multinucleated cells in the cultures was 70 to 80%. After an incubation of 11 to 14 days at 37 C the cultures were tested for the presence of infectious virus by direct plaque titration on BSC-1 cells. Infectious virus could only be rescued from the cell line transformed by form I SV40 DNA (Table 2). No infectivity was found in any of the other fused cultures. Although no infectious virus could be detected, a cytopathic effect was observed in the cells transformed by the *Eco*RI and *Hpa*II linear DNAs and by the 74% *Eco*RI/*Hpa*II (or *Hap*II) A fragment, after fusion with BSC-1 cells (Table 2). This cytopathic effect was similar to that observed after a lytic infection of BSC-1 cells with SV40, or after fusion of BSC-1 cells with BRK cells transformed by form I SV40 DNA. The cytoplasmic vacuolization in the fused cultures was detected 8 to 11 days after fusion.

DISCUSSION

The results presented here show that linearized SV40 DNA molecules and specific DNA fragments, prepared by cleavage of SV40 DNA with restriction endonucleases, can transform primary BRK cells with the same efficiency as circular SV40 DNA. The presence of SV40-specific T-antigen in all transformed cell lines tested and the fact that the transformed foci were found to be morphologically indistinguishable from colonies obtained by transformation with intact virus were taken as evidence that the observed transformation was SV40 specific.

The question may be asked whether the transformation observed in the cultures infected with the linearized SV40 DNA and with the DNA fragments is indeed caused by these molecules and not by contaminating circular molecules. Three observations argue in favor of transformation by linear molecules. First, the efficiency of transformation by the linear DNAs is approximately the same as that of circular

TABLE 2. *Rescue of SV40 virus from transformed BRK cells by fusion with BSC-1 cells*

Transformed line[a]	Appearance of CPE[b]	Infectious virus[c]
BRK SV form I	+	+[d]
BRK SV *Eco*RI linear	+	−
BRK SV *Hpa*II linear	+	−
BRK SV *Bam*HI linear	−	−
BRK SV *Eco*RI/*Hap*II 74% fragment	+	−
BRK SV *Eco*RI/*Hpa*II 74% fragment	+	−
BRK SV *Bam*HI/*Hap*II 59% fragment	−	−

[a] Primary BRK cells transformed by circular form I SV40 DNA, linearized SV40 DNA, and SV40 DNA fragments.
[b] Fused cultures were checked at regular intervals for the appearance of a cytopathic effect (CPE). CPE was usually detected after 8 to 11 days.
[c] Cultures were tested for the presence of infectious virus by plaque titration on BSC-1 cells.
[d] 2.3×10^8 PFU/ml of culture fluid.

DNA, indicating that the transforming activity observed for the linear DNAs is probably not caused by a (minor) contamination with circular DNA. No detectable contamination with circular DNA was found by electrophoretic analysis; therefore, if any circular DNA was present, it must have been less than 2%. Second, no infectious virus could be rescued from any of the cell lines transformed by linear DNAs. Finally, measurements of the viral DNA sequences present in transformed cells by reassociation kinetics have shown that cells transformed by the 74% *Eco*RI/*Hpa*II (or *Hap*II) A fragment contain only that fragment or part of it (Sambrook, Abrahams, and Van der Eb, manuscript in preparation).

The linear DNA molecules and the specific fragments with transforming activity were prepared with the restriction endonucleases *Eco*RI, *Bam*HI, and *Hpa*II or *Hap*II. The finding that cleavage with these endonucleases does not inactivate the transforming activity implies that these enzymes do not cause a break in a position of the DNA that is essential for transformation. Figure 1 shows that all three enzymes cleave SV40 DNA in the late region and that the two DNA fragments with transforming activity (the 74% *Eco*RI/HpaII [*Hap*II] A fragment and the 59% *Bam*HI/*Hap*II A fragment) both contain the entire early region. These results correlate with those of Khoury et al. (15) and Botchan et al. (4), which show that only early SV40 mRNA sequences (and in some cases also some anti-late mRNA) are transcribed in SV40-transformed cells. They also agree with studies on temperature-sensitive mutants, which indicate that the transformation-defective class A mutants map in the early region (16).

The observed lack of transforming activity in the three *Hpa*I fragments was not unexpected since this enzyme introduces one break in the early region in addition to two breaks in the late region (6). Of course, this does not prove that the whole early region is essential for transformation; more work is needed to clarify this point.

Table 1 shows that the efficiency of transformation of the linear SV40 DNA molecules and DNA fragments is approximately the same as that of circular DNA. This result raises the question of which mechanism causes the integration of the circular and linear molecules, assuming that the viral DNA indeed integrates into the cellular DNA during induction of transformation. It is often speculated that a circular DNA structure would be the most efficient intermediate for integration since only one reciprocal crossing-over would be required. The finding that linear SV40 DNA transforms with approximately the same efficiency as circular DNA could mean that both types of molecules integrate via a similar mechanism. Whether this involves a circular or a linear structure (or both) is still an open question, although it is not clear how a DNA fragment could efficiently circularize.

After fusion of the various types of transformed cell lines with permissive BSC-1 cells, infectious virus could only be readily rescued from the lines transformed with circular form I DNA. The method used to detect infectious virus consisted of a direct plaque titration of the frozen and thawed fused cultures on BSC-1 cells; thus it cannot be excluded that very small amounts of infectious virus were produced in the negative cultures, which would have been detected only after blind passages in permissive cells. In fact, it would not have been surprising if infectious virus was rescued from some of the lines transformed with linear molecules of genome size. More extensive tests to rescue virus from such transformed cells are in progress.

The observation that a cytopathic effect, resembling the SV40 cytopathic effect, appeared in some of the fused cultures can be explained in several ways. First, the effect may have been caused by a factor not related to SV40. Alternatively, it may have been a consequence of expression of some late SV40 function(s) from the integrated DNA, or by replication and expression of excised viral DNA. The fact that all the linear DNA molecules and DNA fragments with transforming capacity contain the origin of DNA replication as well as the whole early region might enable them to replicate. This point is presently under investigation.

In summary, we have shown that linear SV40 DNA molecules of genome size as well as specific DNA fragments can cause transformation of primary BRK cells. The smallest fragment with transforming capacity represents 59% of the genome, and this is only approximately 20% greater than the early region. Further work is in progress to identify the segment of SV40 DNA that is minimally required for transformation.

ACKNOWLEDGEMENTS

We thank W. Fiers for stimulating discussions and helpful suggestions, F. A. J. de Vries for providing endonuclease R·*Eco*RI, H. L. Heijneker for S1 single-strand specific endonuclease, and Ronni Greene for valuable technical assistance. One of us (P. J. A.) thanks F. L. Graham for introducing him to the calcium technique.

This work was supported in part by funds from the Institute of Radiopathology and Radiation protection, Euratom and the Foundation for Medical Research of the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) and (for C.M.) by Public Health Service grant from the CA13106 from the National Cancer Institute and grant DRG 1090 from the Damon Runyon Memorial Fund for Cancer Research, and (for A. v. d. V.) by the Kanferfonds van de Algemene Spaar- en Lijfrentekas (Belgium).

LITERATURE CITED

1. Aay, C., and P. Borst. 1972. The gel electrophoresis of DNA. Biochim. Biophys. Acta **269**:192-200.
2. Abrahams, P. J., and A. J. van der Eb. 1975. In vitro transformation of rat and mouse cells by DNA from simian virus 40. J. Virol. **16**:206-209.
3. Borst, P., E. F. J. van Bruggen, G. J. C. M. Ruttenberg, and A. M. Kroon. 1967. Mitochondrial DNA. II. Sedimentation analysis and electron microscopy of mitochondrial DNA from chick liver. Biochim. Biophys. Acta **149**:156-172.
4. Botchan, M., B. Ozanne, B. Sugden, P. A. Sharp, and J. Sambrook. 1974. Viral DNA in transformed cells. III. The amounts of different regions of the SV40 genome present in a line of transformed mouse cells. Proc. Natl. Acad. Sci. U.S.A. **71**:4183-4187.
5. Brugge, J. S., and J. S. Butel. 1975. Role of simian virus 40 gene A function in maintenance of transformation. J. Virol. **15**:619-635.
6. Danna, K. J., G. H. Sack, and D. Nathans. 1973. Studies of simian virus 40 DNA. VII. A cleavage map of the SV40 genome. J. Mol. Biol. **78**:363-376.
7. Fareed, G. C., C. F. Garon, and N. P. Salzman. 1972. Origin and direction of Simian virus 40 deoxyribonucleic acid replication. J. Virol. **10**:484-491.
8. Graham, F. L., P. J. Abrahams, C. Mulder, H. Heijneker, S. O. Warnaar, F. A. J. de Vries, and A. J. van der Eb. 1974. Studies on in vitro transformation by DNA and DNA fragments of human adenoviruses and simian virus 40. Cold Spring Harbor Symp. Quant. Biol. **39**:637-650.
9. Graham, F. L., and A. J. van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456-467.
10. Graham, F. L., and A. J. van der Eb. 1973. Transformation of rat cells by DNA of human adenovirus 5. Virology **54**:536-539.
11. Graham, F. L., A. J. van der Eb, and H. L. Heijneker. 1974. Size and location of the transforming region in human adenovirus type 5 DNA. Nature (London) **251**:687-691.
12. Hedgpeth, J., H. M. Goodman, and H. W. Boyer. 1972. DNA nucleotide sequence restricted by the R1 endonuclease. Proc. Natl. Acad. Sci. U.S.A. **69**:3448-3452.
13. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365-369.
14. Khoury, G., M. A. Martin, T. N. H. Lee, K. J. Danna and D. Nathans. 1973. A map of simian virus 40 transcription sites expressed in productively infected cells. J. Mol. Biol. **78**:377-389.
15. Khoury, G., M. A. Martin, T. N. H. Lee, and D. Nathans. 1975. A transcriptional map of the SV40 genome in transformed cell lines. Virology **62**:263-272.
16. Lai, C. J., and D. Nathans. 1974. Mapping temperature sensitive mutants of simian virus 40: rescue of mutants by fragments of viral DNA. Virology **60**:466-475.
17. Lebowitz, P., T. J. Kelly, Jr., D. Nathans, T. N. H. Lee, and A. M. Lewis, Jr. 1974. A colinear map relating the simian virus 40 (SV40) DNA segments of six adenovirus-SV40 hybrids to the DNA fragments produced by restriction endonuclease cleavage of SV40 DNA. Proc. Natl. Acad. Sci. U.S.A. **71**:441-445.
18. Martin, R. G., and M. Y. Chou. 1975. Simian virus 40 functions required for the establishment and maintenance of malignant transformation. J. Virol. **15**:599-612.
19. Mertz, J. E., and R. W. Davis. 1972. Cleavage of DNA by RI restriction endonuclease generates cohesive ends. Proc. Natl. Acad. Sci. U.S.A. **69**:3370-3374.
20. Morrow, J. F., and P. Berg. 1972. Cleavage of simian virus 40 DNA at a unique site by a bacterial restriction enzyme. Proc. Natl. Acad. Sci. U.S.A. **69**:3365-3369.
21. Morrow, J. F., P. Berg, T. J. Kelly, Jr., and A. M. Lewis, Jr. 1973. Mapping of simian virus 40 early functions on the viral chromosomes. J. Virol. **12**:653-658.
22. Mulder, C., J. R. Arrand, H. Delius, W. Keller, U. Pettersson, R. J. Roberts, and P. A. Sharp. 1974. Cleavage maps of DNA from adenovirus types 2 and 5 by restriction endonucleases Eco·RI and HpaI. Cold Spring Harbor Symp. Quant. Biol. **39**:397-400.
23. Mulder, C., and H. Delius. 1972. Specificity of the break produced by restriction endonuclease RI in simian virus 40 DNA, as revealed by partial denaturation mapping. Proc. Natl. Acad. Sci. U.S.A. **69**:3215-3219.
24. Osborn, M., and K. Weber. 1975. Simian virus 40 gene A function and maintenance of transformation. J. Virol. **15**:636-644.
25. Pope, J. M., and W. P. Rowe. 1964. Detection of specific antigen in SV40 transformed cells by immuno-fluorescence. J. Exp. Med. **120**:124-128.
26. Sharp, P. A., B. Sugden, and J. Sambrook. 1973. Selection of two restriction endonuclease activities in Haemophilus parainfluenzae using analytical agarose ethidium bromide electrophoresis. Biochemistry **12**: 3055-3063.
27. Tegtmeyer, P. 1975. Function of simian virus 40 gene *A* in transforming infection. J. Virol. **15**:613-618.
28. Watkins, J. F. 1971. Fusion of cells for virus studies and production of cell hybrids, p. 1-32. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, vol. V. Academic Press Inc., New York.
29. Wilkie, N. M., J. B. Clements, J. C. M. Macnab, and J. H. Subak-Sharpe. 1974. The structure and biological properties of herpes simplex virus DNA. Cold Spring Harbor Symp. Quant. Biol. **39**:657-666.