EXPERIMENTAL AND MOLECULAR PATHOLOGY 47, 59–68 (1987)

Characterization of a Mutant Polyoma That Expresses in F9 Embryonal Carcinoma Cells: Morphology, Tumorigenicity, and Restriction Enzyme Analysis

JOHN M. LEHMAN,[1,4] JILL MCMAHON, KAREN HALES,[2] and KATRINA TREVOR[3]

[1]*Department of Microbiology and Immunology, Albany Medical College, Albany, New York 12208, and Department of Pathology, University of Colorado School of Medicine, Denver, Colorado;* [2]*Department of Microbiology and Immunology, University of Michigan School of Medicine, Ann Arbor, Michigan;* [3]*La Jolla Cancer Research Foundation, La Jolla, California*

Received December 18, 1986

A mutant polyoma virus (TT340), which replicates in F9 embryonal carcinoma (EC) cells and contains 2500 base pairs (bp) of additional DNA located in the early noncoding region of the genome, was analyzed to determine the DNA origin of the mutant insertion. Two fragments, representing repeated units of the 2500-bp insert, were isolated from TT340, labeled, and hybridized to the parental wild-type viral DNA. A *Bgl*I 500-bp unit, of which there are approximately five copies within the 2500-bp insert, contains sequences homologous to regions on the early and late side of the viral origin of replication. A *Hpa*II 400-bp repeated fragment shows homology to sequences on the early side with little hybridization to the late side. Removal of the 2500-bp insert results in the loss of infectivity on F9 EC cells but not on 3T6 or mouse embryo fibroblasts. Insertion of the *Bgl*I 500-bp repeat element into wild-type DNA at the *Bgl*I site allows replication of the constructed virus in F9 cells. The mutant virions were tumorigenic in newborn Syrian hamsters and the morphology of the virus was that of wild-type as assayed by electron microscopy.

INTRODUCTION

The stem cell of the murine teratocarcinoma, embryonal carcinoma (EC), restricts the expression of the Simian virus 40 and polyoma. However, the differentiated cells obtained from the embryonal carcinoma will express polyoma permissively and SV40 nonpermissively (Swartzendruber and Lehman, 1975; Boccara and Kelly, 1978). Recently, a number of mutant polyoma viruses have been isolated which replicate in certain embryonal carcinoma cell lines (Fujimura *et al.*, 1981; Katinka *et al.*, 1981; Katinka *et al.*, 1980; Sekikawa and Levine, 1981; Vasseur *et al.*, 1982). Analysis of these mutants may be useful in defining the block to wild-type polyoma virus expression in the stem cell. The majority of these mutants had alterations near and to the late side of the origin of viral DNA replication in a noncoding region which contains the enhancer region of polyoma (Herbomel *et al.*, 1984). These mutants include single base pair changes, small deletions and duplications and sequence duplications that encompass single-point mutations.

Another mutant polyoma (TT340) was isolated that had a large insertion (2500 bp) on the early side of the genome in a noncoding region between the two *Hae*II sites at positions 84 and 95 (Trevor and Lehman, 1983). This mutant was of interest due to the large size of the DNA (7700 bp) and the fact that the change was in the early noncoding side of the genome.

[4] To whom correspondence and reprint requests should be addressed at Department of Microbiology and Immunology, Albany Medical College, Albany, NY 12208.

0014-4800/87 $3.00

In this paper TT340 viral DNA was analyzed to determine the DNA origin of the 2500-bp insertion and whether this insertion is necessary for virus expression and replication in F9 EC cells. In addition, tumorigenicity and morphology of TT340 virus were examined and compared to the parental wild-type virus.

MATERIALS AND METHODS

The cell lines utilized in this study were F9 EC carcinoma obtained from Dr. E. E. Moore from a subline line originated by Bernstine *et al.* (1973), 3T6 cells from American Type Tissue Culture, and mouse embryo fibroblasts (MEF) established from 15-day-old Swiss–Webster mouse embryos (Defendi and Lehman, 1965). These cells were maintained in Eagle's minimal essential medium (MEM) (Grand Island Biological Co., Grand Island, NY), supplemented with 2× amino acids, vitamins, and antibiotics (100 μg/ml penicillin and 50 μg/ml streptomycin). All cells were grown in 5% fetal calf serum (Microbiological Associates) and passaged twice weekly with 0.25% trypsin in Versene and maintained at 37°C in 5% CO_2.

The wild-type (wt) polyoma virus used in these studies was the Toronto large plaque (TOR) strain, obtained from Dr. I. Macpherson, which was plaque-purified and titered (Defendi and Lehman, 1965).

The mutant polyoma TT340 has been previously described (Trevor and Lehman, 1983) and was harvested from 3T6 or MEF-infected cells. Cl14 was constructed as previously described (Trevor and Lehman, 1983) and consists of TT340 inserted into the *Bam*HI site of pBR322. PY6 which consists of wt PY DNA (A_2 strain) inserted into the *Bam*HI site of pBR322 was kindly provided by Dr. L. P. Villarreal. Cells and virus were assayed and found negative for mycoplasma. Transfections were performed by the calcium phosphate precipitation technique of Graham and Van der Eb (1973).

Anti-polyoma tumor (T) antigen serum was obtained by injecting PYB4 cells into the peritoneal cavity of brown Norwegian rats and harvesting the ascites fluid (Silver *et al.*, 1978). Anti-polyoma viral capsid serum was produced by injection of purified virus into rabbits. Cells were stained by the indirect immunofluorescent method as previously described (Lehman and Defendi, 1970). The fluorescent-labeled anti-rat or rabbit antibody was obtained from Antibodies, Inc. (Davis, CA). Whole cell DNA was isolated as previously described (Friedrich and Lehman, 1981) and viral DNA was harvested by the Hirt procedure (1967). Isolation of PY6 and Cl14 plasmids was performed by the methods of Kohn *et al.*, 1979.

The restriction endonucleases were purchased from Bethesda Research Laboratories (Rockville, MD) or Worthington Biochemical Co. (Freehold, NJ). Two to four units of enzyme per microgram of DNA in the manufacturer's buffer were used. Reactions were incubated at 37°C for 3.5 hr, terminated by the addition of 0.5% SDS, 10 m*M* EDTA, 0.1% bromphenol blue, and 3% glycerol, and immediately placed onto an agarose gel for electrophoretic separation. DNA was isolated from agarose gels by one of the following methods: (1) low melting point agarose by the method of Weislander (1979), (2) electroelution as described by Maniatas *et al.* (1982), and (3) electroelution onto DE81 paper as described by Dretzen *et al.* (1981). For DNA analysis from gels, the DNA was transferred to DBM or nitrocellulose paper by the method of Southern (1975). The DNA was assayed by ^{32}P-labeled DNA probes prepared by the procedure of Rigby *et al.*, (1977). End

labeling of DNA was performed by digestion of DNA followed by labeling with the Klenow fragment of *Escherichia coli* DNA polymerase (Maniatas *et al.*, 1982).

Ligation of TT340-Derived 500-bp Length to wt Polyoma

Toronto large plaque DNA was isolated from infected MEF cells by the Hirt procedure (1967) and digested by *Bgl*I endonuclease. The Cl14 DNA was digested by *Bgl*I and separated on 1% agarose. The 500-bp band was isolated by electroelution onto DE81 paper and suspended in double distilled water at a concentration of 0.810 μg/μl. Various numbers of the 500-bp fragments were ligated to *Bgl*I linearized wt PY DNA as described by Dugaicyzyk *et al.* (1975).

Tumorigenicity

Newborn (<24 hr) LSH Syrian Hamsters were inoculated with 0.1 ml of wild-type Toronto strain polyoma, TT340 virus, and Cl14 DNA subcutaneously into the suprascapular region. The virus was inoculated at approximately 10^4 plaque-forming units/0.1 ml. The animals were examined once a week until tumors appeared and were autopsied when the tumors reached a size of 2–3 cm in diameter. The tissues were placed in Tellyesniczky's fixative and the tissue sections were stained with hematoxylin and eosin. Certain tumors were excised and placed into tissue culture (Defendi and Lehman, 1966).

Electron Microscopy

The virus suspensions were examined by placing a suspension on a colloidion-coated grid and allowed to stand for 15 min in a humidified chamber. The grid was washed with 3 drops of phosphotungstic acid (2%, pH 6–8), and then a thin layer of phosphotungstic acid was left for 5 min and dryed with filter paper. The grids were examined and photographed with a Phillips 201 transmission microscope. Between 15 and 20 particles were measured.

RESULTS

Viral Origin of Insert

In initial studies to determine whether the 2500-bp TT340 mutant insert was derived from cellular or viral sequences, mouse cellular DNA was probed with the Cl14 plasmid which contains the entire mutant viral genome inserted into pBR322. No homology to cellular sequences was observed (data not shown), suggesting that the insert was of viral DNA origin. Restriction enzyme analysis of TT340 DNA indicated that the 2500 bp had been inserted on the early side of the TOR strain polyoma genome. *Hae*II, as well as *Bgl*I, digestion resulted in the appearance of approximately five 500-bp fragments which accounted for the 2500-bp insertion (Trevor and Lehman, 1983). *Hae*II restricts polyoma at nucleotide positions 84 and 95 in the early noncoding regions, while *Bgl*I restricts at position 87 between the two *Hae*II sites (Tooze, 1980). *Hpa*II digestion of TT340 DNA gave 400- and 100-bp repeated fragments, which most likely derive from the 500-bp repeats, as well as two fragments migrating at approximately 500 bp and 200 bp (data not shown). The later two fragments may be rearranged flanking sequences which are not repeated.

To analyze the viral DNA origin of the 500-bp repeats comprising the 2500-bp insertion, TT340 DNA was digested with *Bgl*I, generating the complete 500-bp

unit, or with *Hpa*II, generating 400- and 100-bp fragments of the unit. The 500- and 400-bp fragments were isolated, nick translated, and used to probe *Hpa*II digested wild-type TOR DNA. As shown in Fig. 1, hybridization of TOR DNA to ^{32}P-labeled Cl14 plasmid DNA detects several of the fragments expected from *Hpa*II digestion of wild-type polyoma DNA (Tooze, 1980). Smaller fragments were not seen and most likely were run off the gel during electrophoresis. After hybridization of the ^{32}P-labeled *Bgl*I 500-bp fragment, strong signals were observed for the *Hpa*II-3 and *Hpa*II-5 fragments (Fig. 1). The other fainter bands were probably due to remaining counts on the filter which were insufficiently washed off prior to addition of the probe. A similar result was obtained by utilizing a probe of the *Hae*II 500-bp fragments derived from TT340 DNA (data not shown). When the 400-bp *Hpa*II fragment was used to probe TOR DNA, a strong hybridization to the *Hpa*II-5 fragment was seen and a much weaker signal was observed for the *Hpa*II-3 fragment. The *Hpa*II-3/*Hpa*II-5 fragment junction lies very near or at the viral origin of DNA replication. The *Hpa*II-3 fragment is on the late side of the origin and the *Hpa*II-5 fragment lies on the early side (Tooze, 1980). These results suggest that the *Bgl*I 500-bp repeated element in the mutant

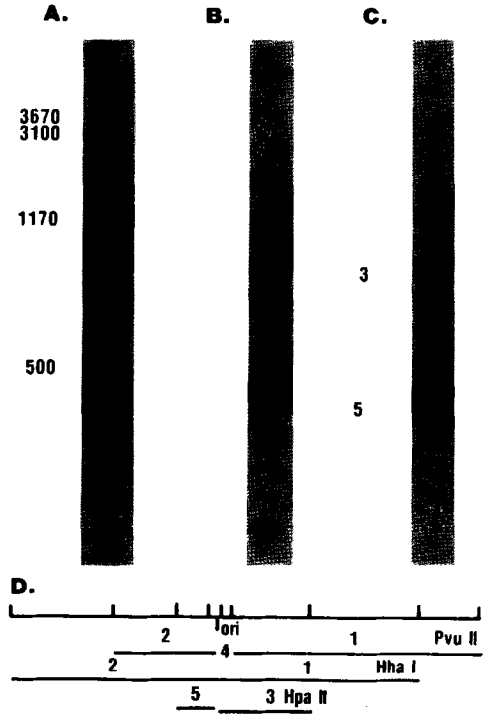

FIG. 1. Viral DNA origin of the repeat element present in TT340 mutant polyoma DNA. Wild-type TOR polyoma DNA was digested with *Hpa*II, separated on a 2% agarose gel, and transferred to a nitrocellulose filter. Filters were hybridized with ^{32}P-labeled Cl14 plasmid DNA (A), the *Bgl*I 500-bp repeat fragment (B) on the *Hpa*II (C) fragment. The *Hpa*II-3 and *Hpa*II-5 fragments are denoted on the right. Filters used for the *Bgl*I-500 and the *Hpa*II-400 hybridizations were previously probed with Cl14. The probe was removed by heating the filters to 95°C in 0.1 × SSC, 0.1% SDS and then cooled to room temperature; most of the counts were washed off. (D) Diagram of the region surrounding the origin of replication, including restriction fragments *Hpa*II-3 and *Hpa*II-5, *Hha*I-1 and *Hha*I-2, and *Pvu*II-1, *Pvu*II-2, and *Pvu*II-4.

TT340 DNA may consist of 400 bp from the early side of the viral origin of DNA replication, most of which are in the *Hpa*II 400-bp fragment, and 100 bp from the late side.

Function of Insert

Since maintenance of the additional 2500-bp insert may play a role in the mutant's ability to replicate in embryonal carcinoma cells, the insert was removed and the resulting virus was assayed for ability to replicate on F9 cells. Cloned TT340 DNA was excised from the plasmid and used to transfect F9 cells. The viral DNA was extracted 4 weeks posttransfection when the cells were expressing T antigen at a level of 30–40%. This DNA was then cleaved with *Bgl*I to excise the 500-bp repeats, gel purified, and used to transfect F9, MEF, and 3T6 cells. By 6 weeks, the 3T6 and MEF cells had undergone a complete lytic infection whereas the F9 cells remained negative. Subsequent infection of F9 cells with supernatants from transfected 3T6 and MEF cells did not result in the expression of virus. This indicates that the 500-bp repeat was required for the expression of TT340 cells but that removal of the insert does not impair the viability of the virus in cells other than EC cells.

A further series of experiments demonstrated that insertion of the 500-bp sequence into wild-type TOR conveyed the ability of this virus to replicate in F9 cells. The fragments were purified on a 1% agarose gel and ligated to TOR DNA cleaved at the *Bgl*I site. The ligation mixture was used to transfect F9 and 3T6 cells. Within 3 weeks, the 3T6 cells were 100% T antigen-positive and the F9 cells were 15–20% T antigen-positive. As seen in Fig. 2, the viral DNA extracted from the F9 cells did contain the 500-bp repeats although less than five copies were inserted. The linearized virus migrated just below TT340 and lost some sequences, possibly of the five repeats. This is supported by the *Bgl*I digestion, which generated one fragment migrating like wt PY and a second fragment at 500 bp, indicating that no sequence had been lost within the wild-type virus. The *Hpa*II cleavage pattern was similar to TT340, which further substantiates this conclusion.

Morphology and Tumorigenicity

The mutant particles photographed and measured were similar to the wild-type PY and SV40 viruses. Figure 3 shows representatives of the various viruses measured that demonstrate approximately a 5–10% variation in size.

Tumors appeared in 8/8 animals injected with TT340 and half the animals had palpable tumors 1 month following inoculation. The wild-type polyoma induced tumors in 6/6 animals but were palpable at 2 months postinoculation. All tumors, mutant and wild-type, were encapsulated, undifferentiated fibrosarcomas with no evidence of invasion or metastasis. Morphologically, the tumors were indistinguishable. Serum obtained from the autopsied animals had antibodies to polyoma T antigen but no V antibodies were detected. Cell cultures were initiated from the tumors (Defendi and Lehman, 1966) and all grew with the phenotype of transformed cells. These tumor-derived cells were producing the polyoma T antigen but not the V antigen. DNA, isolated from three lines induced by the mutant virus, when assayed by Southern analysis demonstrated mutant DNA (data not shown). One of the 10 newborns injected with mutant polyoma DNA developed a tumor similar in morphology to those described above.

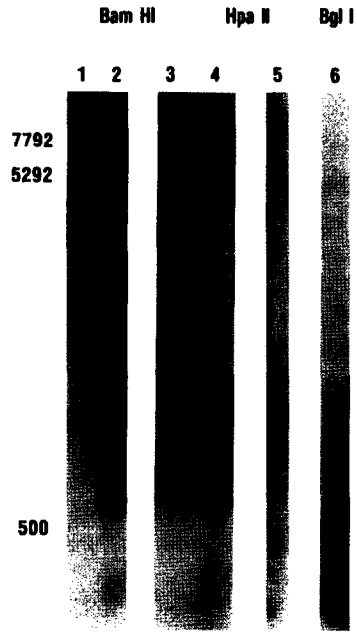

FIG. 2. Enzyme cleavage patterns of wild-type PY with the inserted *BglI* 500-pb insert. The constructed virus was isolated from a F9 cell, in which it was replicating, and was cut with *Bam*HI (lane 3), *Bgl*(lane 6), and *Hpa*II (lane 4). Lanes 2 and 5 contain Cl14 and lane 6 contains Py6 DNA for comparison. The fragments were separated in a 1% agarose gel, transferred, and hybridized to a Cl14 probe.

DISCUSSION

The observation that embryonal carcinoma cells are refractory to infection with SV40 and polyoma virus has provided an opportunity to characterize those events and factors which may play a role in viral expression in a mammalian cell. Identification and characterization of these factors may be useful in proposing mechanisms for cellular gene expression and differentiation.

The mutant discussed in this paper is of interest since it contains a 2500-bp insert on the early noncoding side of the viral genome, which can be cut by *BglI*, *Hpa*II, and *Hae*II restriction enzymes resulting in a single band migrating at 400–500 bp. Such fragments are present at greater than equivalent concentrations and appear to represent separate units. When the *BglI* 500-bp repeat unit was hybridized to wild-type polyoma, homology was seen to sequences in the virus DNA which included both the early and late sides of the origin of replication. Hybridization of the 400-bp *Hpa*II repeat fragments to TOR DNA indicated homology to the early region of the wild-type genome and little homology to the late side. The repeating unit composing the 2500-bp insertion may contain approximately 400 bp derived from the early side of the viral genome and 100 bp from the late side. Each unit would be repeated approximately five times.

To characterize whether the insertion was necessary for viral replication in F9 cells, this fragment was excised. This virus was no longer able to infect F9 cells, however, 3T6 cells replicated the virus. In another series of experiments, different copy numbers of the *BglI* 500-bp fragment from TT340 were inserted into the *BglI* sites of wt polyoma. When transfected onto F9 and 3T6 cells, this virus

FIG. 3. All three virus pools were prepared as described under Materials and Methods and photographed. (A) Wild-type (TOR) polyoma, (B) mutant polyoma (TT340), and (C) wild-type SV40.

produced T antigen within 1 week in both cells. Further, the virus recovered from the F9 cells migrated just below the TT340 virus demonstrating that the 500-bp repeat was present but in fewer copies than the original TT340 virus. These results suggest that the insert, or a portion of it, is necessary for infection and replication in F9 cells.

When the morphology of the virions was examined, the mutant virus had a

morphology similar to the wt virus. This is one of the few embryonal carcinoma mutant viruses that have been tested for tumorigenicity and the results showed an enhanced tumor-forming capability when the virions were utilized; however, the tumors were similar to tumors induced by wt virus. It is also interesting to note that mutant DNA resulted in a tumor in one animal. Further, when the tumor cells were characterized, they exhibited morphological characteristics of transformed cells and the presence of viral antigens and T antigen. When probed for mutant sequences, the tumor lines contained the mutant DNA.

This mutant, TT340, may be different than the other mutants which have been described to replicate in various embryonal carcinoma cell lines. A number of models have been put forward suggesting a possible mechanism for replication of the various mutants in the embryonal carcinoma cells. These include (1) splicing (Sekikawa and Levine, 1981), (2) factors which stabilize the secondary structure of the late region making it inaccessible to RNA polymerase II, may be altered by a length of AT inserts which may interfere with this factor binding (Vasseur *et al.*, 1982), and (3) modifications in the enhancer region, for which there is evidence (Herbomel *et al.*, 1984). In fact, evidence suggests that most mutants have changes in the noncoding region on the late side of the origin which contains the enhancer. Possibly cellular factors recognize the mutated enhancer but are unable to recognize the wt enhancer until the cells differentiate. Recent evidence has suggested that the PY enhancer is not active in F9 cells but is active in differentiated cells (Linney and Donerly, 1983; Herbomel *et al.*, 1984). However, the SV40 enhancer is active in both differentiated and undifferentiated cells (Sassone-Corsi *et al.*, 1985). Possibly there are positive and negative factors which interact with the viral enhancers to allow the function of this region (Hen *et al.* 1986). Further, these factors may be involved in cellular differentiation.

The mutant discussed in this paper is interesting since it contains five repeats overlapping the origin of replication, mostly on the early side but also some sequences on the late side. Contained within the insert are five extra copies of the origin of replication, promoter sequences for T antigen binding and transcription, and, possibly, the enhancer region on the late side of the origin. The further characterization of the TT340 mutant may give some further insight into the blocking of polyoma replication in embryonal carcinoma cells. In addition, this information may be useful in defining how the cell regulates gene expression and differentiation.

ACKNOWLEDGMENTS

The authors wish to thank Dr. A. Vatter and Mr. H. Mitchell for their advice concerning the electron microscope studies, Ms. P. Ledbury for technical assistance, and Ms. Jo Ann D'Annibale for preparation of this manuscript. This work was supported by Grants CA 16030 (41608), CA 15823, and CA 09157 from the National Cancer Institute and by a grant from R. J. Reynolds Industries.

REFERENCES

BERNSTINE, E. G., HOOPER, M. L., GRANDCHAMP, S., and EPHRUSSI, B. (1973). Alkaline phosphatase activity in mouse teratoma. *Proc. Natl. Acad. Sci. USA*, **70**, 3899–3903.

BOCCARA, M., and KELLY, F. (1978). Expression of polyoma virus in heterokaryons between embryonal carcinoma cells and differentiated cells. *Virology* **90**, 147–150.

DEFENDI, V., and LEHMAN, J. M. (1965). Transformation of hamster embryo cells *in vitro* by polyoma virus: Morphological, karyological, immunological, and transplantation characteristics. *J. Cell. Physiol.* **66**, 351–410.

DEFENDI, V., and LEHMAN, J. M. (1966). Biological characteristics of primary tumors induced by polyoma virus in hamsters. *Int. J. Cancer* **1**, 524–540.

DRETZEN, G., BELLARD, M., SASSONE-CORSI, P., and CHAMBERS, P. (1981). A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. *Anal. Biochem.* **112**, 195–198.

DUGAICYZYK, A., BOYER, H. W., and GOODMAN, H. M. (1975). Ligation of *Eco*RI, endonuclease-generated DNA fragments into linear and circular structure. *J. Mol. Biol.* **96**, 171–184.

FRIEDRICH, T. D., and LEHMAN, J. M. (1981). The state of the Simian virus 40 DNA in the embryonal carcinoma cells of the murine teratocarcinoma. *Virology* **110**, 159–166.

FUJIMURA, F. K., DEININGER, P. L., FRIEDMANN, T., and LINNEY, E. (1981). Mutation near the polyoma DNA replication origin permits productive infection of F9 embryonal carcinoma cells. *Cell* **23**, 809–814.

GRAHAM, F. L., and VAN DER EB, A. S. (1973). A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–467.

HEN, R., BASSELLI, E., FROMENTAL, C., SASSONE-CORSI, P., and CHAMBERS, P. (1986). A mutated polyoma virus enhancer which is active in undifferentiated embryonal carcinoma cells is not repressed by adenovirus-2 EIA products. *Nature (London)* **321**, 244–251.

HERBOMEL, P., BOURACHAT, B., and YANIV, M. (1984). Two distinct enhancers with different cell specificities co-exist in the regulatory region of polyoma. *Cell* **39**, 653–662.

HIRT, B. (1967). Selective extraction of polyoma DNA from infected mouse cell culture. *J. Mol. Biol.* **26**, 365–369.

KATINKA, M., VASSEUR, M., and BLANGY, D. (1980). Expression of polyoma early functions in mouse embryonal carcinoma cells depends on sequence rearrangements in the beginning of the late regions. *Cell* **20**, 393–399.

KATINKA, M., YANIV, M., VESSEUR, M., MONTREAU, N., YANIV, M., and BLANGY, D. (1981). Polyoma DNA sequences involved in control of viral gene expression in murine embryonal carcinoma cells. *Nature (London)* **290**, 720–722.

KOHN, M., KOLTER, R., THOMAS, C., FIGURSKI, D., MEYER, R., REMANT, E., and HELSINKI, D. R. (1979). Plasmid cloning vehicles derived from plasmids ColEl,F,R6K, and RK2. *In* "Methods in Enzymology" (R. Wu, Ed.), Vol. 68, pp. 268–280. Academic Press, New York.

LEHMAN, J. M., and DEFENDI, V. (1970). Changes in deoxyribonucleic acid synthesis regulation in Chinese hamster cells infected with Simian virus 40. *J. Virol.* **6**, 738–749.

LEHMAN, J. M., KLEIN, I. B., and HACKENBERG, R. M. (1975). The response of murine teratocarcinoma cells to infection with DNA and RNA virus. *In* "Symposium on Teratomas and Differentiation" (M. Sherman and D. Solter, Eds.), pp. 289–301. Academic Press, New York.

LINNEY, E., and DONERLY, S. (1983). DNA fragments from F9PYEC mutants increase expression of heterologous genes in transfected F9 cells. *Cell* **35**, 693–699.

MANIATAS, T., FRITSCH, E. F., and SAMBROOK, J. (1982). "Molecular Cloning. A Laboratory Manual." pp. 1–545. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

RIGBY, P. W. J., DIECKMAN, M., RHODES, C., and BERG, P. (1977). Labeling deoxyribonucleic acid and high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**, 237–251.

SEKIKAWA, K., and LEVINE, A. J. (1981). Isolaton and characterization of polyoma in host range mutants that replicate in nullipotential embryonal carcinoma cells. *Proc. Natl. Acad. Sci. USA* **78**, 1100–1104.

SILVER, J., SCHOFFHAUSEN, B., and BENJAMIN, T. (1978). Tumor antigens included by non-transforming mutant of polyoma virus. *Cell* **15**, 485–496.

SOUTHERN, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**, 503–517.

SWARTZENDRUBER, D. E. and LEHMAN, J. M. (1975). Neoplastic differentiation: Interaction of Simian virus 40 and polyoma virus with murine teratocarcinoma cells. *J. Cell. Physiol.* **85**, 179–188.

SASSONE-CORSI, P., DUBOULE, D., and CHAMBERS, P. (1985). Viral enhancer activity in teratocarcinoma cells. *Cold Spring Harbor Symp. Quant. Biol.* **50**, 747–752.

TOOZE, J. (1980). "DNA tumor viruses: Molecular biology of Tumor Viruses." 2nd ed. Part II. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

TREVOR, K. and LEHMAN, J. M. (1982). The interaction of polyoma virus with F9 embryonal carcinoma cells: Fate of the viral DNA and expression of viral antigens. *J. Cell. Physiol.* **2**(Suppl.), 69–83.

TREVOR, K. and LEHMAN, J. M. (1983). Characterization of a mutant polyoma virus that expresses in

F9 embryonal carcinoma cells. *In* "Cold Spring Harbor Conferences on Cell Proliferation." Vol. 10, pp. 327–339. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

VASSEUR, M., KATINKA, M., HERBOMEL, D., YANIV, M., and BLANGY, D. (1982). Physical and biological features of polyoma virus mutants able to infect embryonal carcinoma cell lines. *J. Virol.* **43**, 800–808.

WEISLANDER, L. (1979). A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. *Anal. Biochem.* **98**, 305–309.