we have isolated radioactive FHP from acid hydrolyzates of UV-irradiated *Escherichia coli* DNA labeled with [methyl-^{3}H]thymine.

The overall yield of TA* in a particular DNA will depend on how frequently T-A doublets occur within its sequence. It is evident that TA* does not constitute a major photoproduct in UV-irradiated DNA, since the quantum yield for its formation is only 10^{-2} to 10^{-3} times the quantum yields quoted for pyrimidine photodimerization or the formation of bipyrimidine (6–4) photoadducts (*1*). However, its biological significance will be determined not only by its abundance but also by its susceptibility to cellular DNA repair mechanisms and the functional importance of the target sites. In the latter respect, it is noteworthy that the sequence T-A-T-A is a conserved feature of many prokaryotic and eukaryotic promoters (*9*). Both of the tandem base substitutions observed in a recent study (*10*) of the specificity of UV mutagenesis in the *lac* promoter of M13-*lac* hybrid phage DNA were found to occur at T-A sites. Because T-A is a self-complementary sequence, the photoproduct TA* could be formed at the same site on both strands of a DNA duplex. Such an event would cause a permanent loss of genetic information unless biological reversal of the photoreaction could be accomplished.

SAMARENDRA N. BOSE
R. JEREMY H. DAVIES
Department of Biochemistry, Queen's University of Belfast, Belfast BT9 7BL, Northern Ireland
SATINDER K. SETHI
JAMES A. MCCLOSKEY
Departments of Medicinal Chemistry and Biochemistry, University of Utah, Salt Lake City 84112

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Gene Transfer by Polyoma-Like Particles Assembled in a Cell-Free System

Abstract. *Empty capsids of polyoma virus interact with DNA in a cell-free system to form polyoma-like particles (PLP). The DNA in these particles is protected from the action of pancreatic deoxyribonuclease. Transfer of genetic information by PLP has been accomplished by using a restriction fragment containing the transforming sequences of polyoma DNA as a model gene. Infection of rat F111 cells by PLP containing these sequences results in DNA-mediated cellular transformation. Gene transfer by PLP is 50 to 150 times more efficient than by the calcium phosphate precipitation method.*

Gene transfer studies are important in investigating fundamental questions of molecular biology, including the control of gene expression in mammalian cells. Genes have been introduced into cells by coprecipitation with calcium phosphate (*1, 2*), by microinjection (*3*), fusion (*4*), liposomes (*5*), the use of facilitating agents such as DEAE-dextran (*6*), and by electric pulses (*7*). Viral vectors, assembled intracellularly with the aid of coinfecting wild-type particles, have also been used (*8*). The most extensively applied method is calcium phosphate coprecipitation, but the efficiency of gene transfer by this method is close to the rate of spontaneous mutation (*9*), making it difficult in some cases to distinguish between revertant cells and cells that have acquired the exogenous genetic material.

The cell-free assembly of polyoma-like particles (PLP) from DNA and purified polyoma empty capsids has been described (*10, 11*). The DNA contained in these particles is approximately 1.2×10^6 daltons in size and is protected by the capsid from hydrolysis by pancreatic deoxyribonuclease. The particles are stable in solutions of high salt concentration. The formation of PLP is independent of the primary, secondary, or superhelical tertiary structure of the DNA substrate. Purified DNA, from a variety of cellular and viral sources, has been used in the preparation of PLP. A model for the mechanism by which empty capsids and DNA interact to form PLP has been proposed (*11*).

Whether PLP can transfer genetic information to cells has been investigated by using, as a model gene, a restriction fragment of polyoma DNA that has measurable biological activity. This fragment is approximately 1.2×10^6 daltons in size and extends clockwise from the Bcl I site to the Eco RI site on the conventional polyoma map (Fig. 1). It is the smallest of several restriction fragments that contain the genetic information necessary for the induction and maintenance of transformation (*12*) in rat cells (*13–15*). The rest of the polyoma genome, which includes the distal part of the early region and extends clockwise from the Eco RI site to the Bcl I site, has no oncogenic activity (*13*). These two fragments of DNA will be referred to as the Bcl I–Eco RI transforming fragment and the Eco RI–Bcl I nontransforming fragment of polyoma DNA, respectively.

Rat embryo F111 cells were infected with PLP containing either the Bcl I–Eco RI transforming fragment or, as a control, the Eco RI–Bcl I nontransforming fragment of polyoma DNA (Fig. 2). The production of dense foci was used as an indication of oncogenic transformation and, therefore, of gene transfer and expression. Dense foci were found after

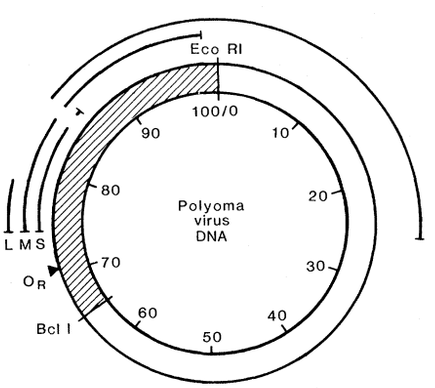

Fig. 1. Physical map of the polyoma virus genome divided into 100 units. The origin of DNA replication (O_R) and the coding locations of the early viral proteins small (*S*), middle (*M*), and large (*L*) tumor antigens are indicated. The fragment of DNA extending clockwise from the Bcl I site to the Eco RI site (shaded area) contains the coding sequences of the small and the middle tumor antigens and is sufficient for the induction of the transformed phenotype (*13*). The other fragment, which extends clockwise from the Eco RI site to the Bcl I site, has no oncogenic activity (*13*). These two fragments of DNA were generated by treating ^{3}H-labeled polyoma DNA with Bcl I and Eco RI (New England BioLabs) followed by electrophoretic separation on a 1.5 percent agarose gel (*11*). Each DNA fragment was purified from agarose with two rounds of KI density gradient centrifugation (*18*) or by electroelution into a dialysis bag.

infection with PLP containing the Bcl I–Eco RI transforming sequences (Fig. 2A) or with polyoma virions (Fig. 2B), or by treating the cells with a coprecipitate of the Bcl I–Eco RI transforming fragment and calcium phosphate (Fig. 2C). Transformation was found only when the Bcl I–Eco RI transforming fragment was transferred by PLP, by calcium phosphate coprecipitation, or as a part of the polyoma genome by polyoma virus. When the transforming fragment was absent, or a mechanism for its transfer was not provided, transformation did not occur (Fig. 2, D to H). Transfer by PLP requires the prior formation of PLP by reaction of polyoma empty capsids with DNA (*10, 11*), since an unreacted mixture of empty capsids and Bcl I–Eco RI transforming DNA failed to induce dense foci production (Fig. 2H).

Induction of the transformed phenotype requires the delivery of a DNA fragment containing the required genetic information and is not the result of penetration of the cells by empty capsids or contamination by virions of the empty capsid preparations used to make PLP. This is indicated by the following: Transformation was not observed when cells were infected with empty capsids (Fig. 2F) or with PLP made with the Eco RI–Bcl I nontransforming polyoma fragment (Fig. 2E) or with the replicative form of DNA from the bacteriophage ϕX174 (data not shown). Nor was oncogenic transformation observed when ten times the amount of empty capsids was used as a control (data not shown). In addition, when empty capsid preparations or preparations of PLP containing the Bcl I–Eco RI transforming fragment were assayed for plaque-forming activity at the multiplicity of infection used in Table 1, no plaques were detected.

In the individual experiments performed, infection with the PLP gene transfer system produced dense foci 50 to 150 times more efficiently than did transfection with the calcium phosphate method (Table 1). This corresponds to a frequency range of 5.6 to 6.5 $\times 10^3$ transformants per 10^6 cells per microgram of DNA for PLP and 0.04 to 0.12 $\times 10^3$ per 10^6 cells per microgram of DNA for the calcium phosphate method.

No differences were noted in the frequency of dense foci induction under conditions in which deoxyribonuclease was present in the PLP preparations as compared to the standard conditions in which PLP were purified before being used for cell infection. This indicates that gene transfer by PLP is not sensitive to deoxyribonuclease. Transfer of the polyoma oncogenic sequences by PLP, as with calcium phosphate, results in a stable expression of the transformed phenotype, since cell cultures established from individual dense foci induced by either method displayed the growth properties and morphology characteristic of transformed cells.

Our results demonstrate that PLP containing the Bcl I–Eco RI transforming fragment of polyoma DNA can transfer this DNA fragment to rat cells. The genetic information of the fragment is then expressed and cellular transformation results. Although limited to some extent by the size of gene that can be encapsidated (*10, 11*) and therefore

Table 1. Transfer of the oncogenic sequences of polyoma DNA by PLP or calcium phosphate coprecipitation as measured by dense foci production on rat F111 cells. The number of dense foci per flask is the average of five separate experiments. Slightly subconfluent cultures of rat F111 cells (1×10^6) were infected or exposed to various agents as described in the legend to Fig. 2. Each of the five experiments was performed with approximately 2.5×10^4 particles or molecules of DNA per cell. Inoculum for the unreacted mixture of polyoma empty capsids plus the Bcl I–Eco RI fragment was prepared by mixing empty capsids (2.5×10^{10} particles) and the DNA fragment (3.5×10^{10} molecules, 0.07 μg) into 0.5 ml of double-strength Dulbecco's modified Eagle's medium with antibiotics and adding PLP buffer to obtain a final volume of 1.0 ml. Ca/PO_4 refers to the use of the calcium phosphate coprecipitation method.

Inoculum	Number of dense foci per flask
PLP (Bcl I–Eco RI transforming fragment)	303 ± 23
PLP (Eco RI–Bcl I nontransforming fragment)	0
Ca/PO_4 (Bcl I–Eco RI transforming fragment)	4 ± 2
Ca/PO_4 (Eco RI–Bcl I nontransforming fragment)	0
Bcl I–Eco RI transforming fragment	0
Empty capsids	0
Empty capsids + Bcl I–Eco RI transforming fragment	0
Polyoma virus	1195 ± 73

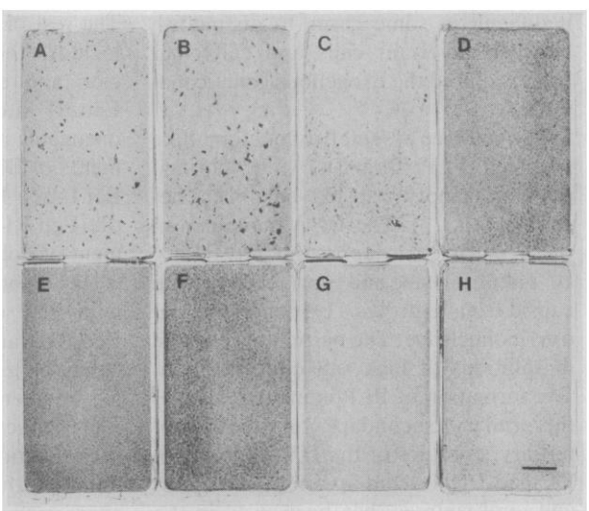

Fig. 2. Macroscopic dense foci production by infection of rat cells with PLP containing the Bcl I–Eco RI transforming fragment. Slightly subconfluent rat F111 cells (1×10^6 cells) were infected or treated with (A) PLP containing the Bcl I–Eco RI transforming fragment (2.5×10^{10} particles containing 0.05 μg of DNA), (B) polyoma virus (0.5×10^{10} particles), (C) Bcl I–Eco RI transforming fragment coprecipitated with calcium phosphate (10×10^{10} molecules or 0.2 μg of DNA), (D) Bcl I–Eco RI transforming fragment as naked DNA (2.5×10^{10} molecules or 0.05 μg of DNA), (E) PLP containing Eco RI–Bcl I nontransforming sequences (2.5×10^{10} particles containing 0.05 μg of DNA), (F) polyoma empty capsids (2.5×10^{10} particles), (G) Eco RI–Bcl I nontransforming fragment coprecipitated with calcium phosphate (10×10^{10} molecules or 0.37 μg of DNA), and (H) an unreacted mixture of polyoma empty capsids (2.5×10^{10} particles) and Bcl I–Eco RI transforming fragment (3.5×10^{10} molecules or 0.07 μg). Scale bar, 1.0 cm. PLP reactions were carried out as previously described (*11*) except that the mass ratio of empty capsids to DNA was 12:1, the 10 to 22 percent sucrose gradients were formed in PLP buffer [10 m*M* tris-HCl (*p*H 7.5), 10 m*M* NaCl, 1 m*M* EDTA, and bovine serum albumin (100 μg/ml)], and the CsCl cushion was replaced with 0.5 ml of 66 percent (by weight) sucrose in PLP buffer. Fractions of PLP at the peak (*10*) were pooled, concentrated by sedimentation onto a 66 percent sucrose cushion with the Beckman SW41 rotor (35,000 rev/min, 4°C, 4 hours), and dialyzed against PLP buffer. Rat embryo F111 cells (*14*) were grown in T25 flasks (Falcon) at 37°C in a humidified incubator with a 10 percent CO_2 atmosphere using Dulbecco's modified Eagle's medium (DME) supplemented with 10 percent fetal calf serum and antibiotics. Before infection or transfection by the calcium phosphate method as modified by Loyter *et al.* (*2*), cells were washed with Dulbecco's phosphate-buffered saline (PBS). Solutions for infection were prepared by mixing 0.5 ml of the infecting agent in PLP buffer with 0.5 ml of double-strength DME and antibiotics. Infection was carried out at 37°C for 2 hours with gentle rocking of flasks every 15 minutes. Medium was added without removal of inoculum and changed every 3 or 4 days. Twenty-one days after infection or transfection, cells were fixed with 10 percent glutaraldehyde in PBS and stained with 0.03 percent Giemsa in water. The macroscopic, darkly stained, dense foci were then counted.

transferred, PLP, as a gene transfer system, offers greater efficiency than the widely used calcium phosphate coprecipitation method. In addition, the PLP system provides a novel capability for application to gene transfer studies in vitro and, potentially, in vivo.

STEVE N. SLILATY
H. VASKEN APOSHIAN
Department of Cellular and Developmental Biology, University of Arizona, Tucson 85721

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Passive Transfer of Diabetes in the BB/W Rat

Abstract. *Severe diabetes with insulitis was produced in young diabetes-prone BB/W rats by passive transfer of concanavalin A–treated spleen cells from BB/W animals with acute diabetes. Spleen cells alone or in combination with lymph node cells were active in transferring disease.*

Autoimmunity may play an important role in the pathogenesis of insulin-dependent diabetes (*1*). The spontaneously diabetic Bio Breeding (BB) rat constitutes an important model for the study of autoimmune diabetes. These nonobese animals generally first manifest hyperglycemia between 60 and 120 days of age, with a mean age of onset close to 90 days. Approximately 30 to 50 percent become affected (*2*). Once diabetic, the animals require daily injections of insulin for survival.

A salient feature of the disease is severe insulitis in animals with acute diabetes (*3*). Other abnormalities of the immune system include lymphocytic thyroiditis and the presence of antibodies to the islet cell surface as well as to lymphocytes and other tissues (*4–6*). Administration of an antiserum to lymphocytes prior to or at the time of onset of hyperglycemia is useful in preventing the development of permanent diabetes, as is neonatal thymectomy (*7, 8*) and bone marrow transplantation (*9*).

These studies in the BB rat and other experimental systems suggest a role for cell-mediated immunity in the pathogenesis of diabetes. Strong support for this hypothesis would be provided by the demonstration that diabetes or insulitis can be produced by the passive transfer of cells from affected animals. This has proved difficult to do (*10–12*). A recent study described the passive transfer of mild insulitis from BB rats with acute diabetes to nude mice by means of spleen and peripheral blood cells (*13*): another described the transfer of insulitis to x-irradiated BB recipients (*9*). Other attempts to transfer insulitis from diabetic BB/W rats to nude rats or mice were unsuccessful (*14*).

It seemed possible that the efficiency of transfer might be enhanced by culturing donor cells with the T cell mitogen concanavalin A (Con A) (*15*), as is the case for another autoimmune disease in rodents, experimental allergic encephalomyelitis (*16*). We therefore attempted to transfer diabetes in the BB/W rat using spleen and lymph node cells cultured with Con A (*17*). We first injected cells from BB/W donors with acute diabetes into young, 30- to 40-day-old diabetes-prone BB/W recipients (*18*). We reasoned that passive transfer would produce early appearance of diabetes, and that a much higher than expected percentage would become diabetic.

The results (Table 1) supported these assumptions. Spleen cells alone or in combination with smaller numbers of lymph node cells produced severe hyperglycemia in 19 of 21 recipients (*19*). In some recipients, hyperglycemia appeared within 7 days after transfer, with a mean interval of 12 ± 1 days (± standard error of the mean). The age of onset was 50 ± 1 days in the 19 recipients which became diabetic. This is significant since spontaneous diabetes rarely appears before 60 days of age. Within the range of cell numbers used in these experiments (50×10^6 to 120×10^6) there

Table 1. Passive transfer of diabetes by means of cells cultured with Con A. The recipients received Con A–treated spleen cells (50×10^6 to 100×10^6) alone or in combination with lymph node cells (5×10^6 to 20×10^6). Plasma glucose concentrations in rats were determined from blood samples from a tail vein before the donors were killed and at least three times weekly in recipients by means of a Beckman glucose analyzer. The values, in milligrams per deciliter, were (mean ± standard error of the mean): donors with acute diabetes, 399 ± 24; Wistar Furth, 124 ± 4; diabetes-prone rats prior to transfer of cells or before being injected with medium alone, 126 ± 4; low-incidence rats, 125 ± 3; and diabetes-prone rats after transfer of cells from rats with acute diabetes, 520 ± 40 (when killed). Glycosuria in nude mice (BALB/c and SWR, *nu/nu*) was determined with Testape.

Donor	Recipient	Number of diabetic rats at 60 days of age	Number tested
BB/W with acute diabetes	Diabetes-prone BB/W	19	21
BB/W with acute diabetes	Low-incidence BB/W	0	14
BB/W with acute diabetes	Wistar Furth	0	8
BB/W with acute diabetes	Nude mice	0	8
Diabetes-prone BB/W	Diabetes-prone BB/W	0	6
Low-incidence BB/W	Diabetes-prone BB/W	0	5
Wistar Furth	Diabetes-prone BB/W	0	4
None (RPMI-1640 medium alone)	Diabetes-prone BB/W	0	30