Bovine polyomavirus, a cell-transforming virus with tumorigenic potential

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The early region of bovine polyomavirus (BPyV) was tested for its cell transformation potential employing an assay of dense focus formation. Dense foci of morphologically transformed cells were observed upon transfection of primary rodent cells with a plasmid construct encoding the complete early region of BPyV under the transcriptional control of the long terminal repeat of Rous sarcoma virus. No transformation of primary rodent cells was observed upon transfection of these cells with a plasmid encoding the complete early region of BPyV under the control of its own transcriptional regulatory sequences. In BPyV-transformed cells, the viral sequences had become integrated into the cellular genome, and expression of large T antigen could be detected in a high percentage of cells. The transformed cells were demonstrated to be capable of anchorage-independent growth and to be oncogenic in immunocompromised newborn rats. Therefore BPyV should be considered as a potentially tumorigenic polyomavirus. Since many commercial batches of calf serum have been shown to be contaminated with BPyV, our observations may have implications for the use of calf serum in cell culture.

Introduction

Bovine polyomavirus (BPyV) was originally isolated from a cell culture derived from tertiary Macaca fascicularis kidney cells (Wognum et al., 1984) and the complete nucleotide sequence has recently been reported (Schuurman et al., 1990; EMBL/GenBank/DDBJ accession number D00755). The isolation of BPyV-like viruses has been reported by several laboratories (reviewed by Schuurman et al., 1991b). In all these cases, the infection of the monkey kidney cell cultures most probably occurred through a BPyV-contaminated batch of calf serum used to supplement the cell culture medium. A high prevalence of antibodies against BPyV has been demonstrated among cattle (Parry et al., 1983; Wognum et al., 1984). In addition, as has recently been reported, BPyV DNA could be amplified from 14 of 20 commercial batches of foetal or newborn calf serum (Schuurman et al., 1991a).

To investigate whether the early gene products of BPyV are capable of transforming primary rodent cells into rapidly growing cells, we transfected the early genes of BPyV, controlled by either the homologous promoter–enhancer sequences or the Rous sarcoma virus (RSV) long terminal repeat (LTR), into primary cell cultures derived from baby rat kidney or mouse embryo. Transformation was scored using the stringent dense focus formation assay. Morphologically transformed cells were subsequently tested for T antigen expression, growth at low serum concentrations, anchorage-independent growth and their tumorigenic potential in vivo.

Methods

Cells and cell culture. Foetal calf serum (FCS) used to supplement cell culture media was tested for BPyV prior to use by using the polymerase chain reaction (PCR; Schuurman et al., 1991a).

Cell cultures of embryonic mouse cells (ME cells) derived from B6/Kh foetuses were cultured in Iacove's modified Dulbecco's medium supplemented with 8% FCS. Kidney cell cultures derived from 4- to 7-day-old baby rats (BRK cells) were cultured in MEM supplemented with Earle's salts and 8% FCS.

Plasmid constructs. Plasmid pXX contained the complete early region of BPyV controlled by the homologous promoter and enhancer sequences. As outlined in Fig. 1, the plasmid was constructed by digesting pBPyV1, encoding the complete genome of BPyV (Schuurman et al., 1990), with XbaI, thereby deleting the major part of the late genes between nucleotides 1007 and 2145. Subsequently, the digested plasmid was religated to create pXX.

Plasmid pRBPy contained the complete early region of BPyV under the transcriptional control of the RSV LTR (Fig. 1). The recombinant was constructed by first digesting pBPyV1 with FnuDII (at nucleotide 16) and XbaI (at nucleotide 2145). Subsequently, the sticky ends of the 2568 bp FnuDII–XbaI fragment containing all the early protein coding sequences of BPyV were filled in using the Klenow fragment of DNA polymerase according to Maniatis et al. (1982). The adoption vector containing an RSV LTR was derived from pRSVneo (Gorman et al., 1983) by digestion with HindIII and BamHI, thereby deleting the complete neomycin resistance gene. Subsequently, the 3393 bp vector fragment was gel-purified and the protruding 5' ends of this fragment were filled in with the Klenow fragment of DNA polymerase.
Thereafter, the fragment was dephosphorylated using calf intestine alkaline phosphatase (CIAP; Boehringer Mannheim) according to Maniatis et al. (1982) and ligated to the blunt-ended FnuDII–XbaI early region fragment of BPyV (Fig. 1).

In pRS1, described as pR-SV40 by de Ronde et al. (1987), the complete early region of simian virus 40 (SV40) under the control of the RSV LTR, was present. In pRB10, originally described as pR-BKV by de Ronde et al. (1987), the complete early region of human polyomavirus BK, under the control of the RSV LTR, was present.

Transformation experiments. Subconfluent BRK or ME cell cultures in 60 mm Petri dishes were transfected with 10 μg of CsCl-purified plasmid DNA (5 μg of pRS1) using the calcium phosphate precipitation assay (Graham & van der Eb, 1973), followed by a 15% glycerol shock 4 h after the addition of the DNA (Frost & Williams, 1978). In the subsequent weeks, microscopic inspection of the cell cultures for the formation of dense foci of morphologically transformed cells was performed periodically. After 8 weeks, the numbers of dense foci formed were counted, and several were picked and expanded individually for further analysis.

Chromosomal DNA isolation. Chromosomal DNA was isolated from embryonic mouse cells by lysing the cells with 1% SDS, 20 mM-EDTA, 50 mM-Tris–HCl pH 8·0 and 100 μg/ml proteinase K overnight at 37 °C. Subsequently, the lysate was extracted once with Tris–EDTA-saturated phenol and ethanol-precipitated. After recovery of the DNA, the samples were treated with RNase A (200 μg/ml) for 1 h at 37 °C prior to extraction with chloroform/isoamyl alcohol (24:1), and ethanol-precipitated.

Southern blotting and hybridization. Chromosomal DNA isolated from cell cultures of either transformed or untransformed embryonic mouse cells was digested with several endonucleases. DNA fragments were separated on 1% agarose gels using the Tris–acetate–EDTA buffer system, followed by transfer of the DNA to nitrocellulose membranes (BAS 85; Schleicher & Schuell). Nitrocellulose filters were hybridized with a pXX probe which had been radioactively labelled with [α-32P]dCTP (3000 Ci/mmol) using a kit for random-primed labelling (Boehringer Mannheim). Overnight hybridization was performed at 65 °C in 6 × SSC, 10% dextran sulphate, 5 μg/ml denatured salmon sperm DNA and 1% SDS. Afterwards, filters were washed three times at 65 °C for 30 min each in 1 × SSC/1% SDS, followed by two washes of 15 min each in 0·1 × SSC/0·5% SDS. Autoradiography in the presence of intensifying screens was performed at −70 °C using Fuji RX films.

Immunocytochemistry. Indirect immunocytochemical detection of large T antigen expression in pRBPy-transformed cells, grown on coverslips and fixed with methanol, was performed using monoclonal antibody (MAb) pAb 416 diluted 1:40 (Oncogene Science); this MAb, directed against the SV40 large T antigen (Harlow et al., 1981), also reacted with the BPyV large T antigen. Peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts), diluted 1:40 in PBS, was used as the second antibody. Colorimetric detection of large T antigen was performed using 3-amino-9-ethylcarbazole (AEC)-peroxidase staining (Graham, 1965). Following incubation of the cells with pAb 416 (60 min at 37 °C), coverslips were washed in PBS and incubated with peroxidase-conjugated rabbit anti-mouse IgG (60 min at 37 °C). After washing in PBS, the cells were incubated for 10 min at room temperature in 50 mM-acetate buffer pH 5·0 containing 0·03% H2O2 and 0·05 volumes of AEC stock solution (4 mg AEC/ml N,N-dimethylformamide; Merck). The staining reaction was stopped by rinsing the coverslips in acetate buffer. Finally, cells were washed in H2O and mounted in PBS-buffered glycerol (1:9 v/v).

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**Fig. 1. Outline of the construction of pXX and pRBPy, as described in Methods.**

**pBPyV1**

- **TRS**
- Early genes
- Late genes

**pXh/XbaI digestion**

**pRSVneo**

- **RSV/LTR**
- neo gene

**Ligation**

**pRBPy**

- **RSV LTR**
- Early genes

**HindlIl/FnuDII**

**Restriction enzyme recognition sequences used for the construction of the recombinants and for the characterization of pRBPy-transformed cells are indicated below these boxes:**

- B, BPyV early genes
- D, BPyV late genes
- [ ], RSV-LTR
- [ ], neomycin resistance gene
- Unshaded open boxes represent vector sequences.

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- Unshaded open boxes represent vector sequences.
Western blotting. Subconfluent cultures of either transformed or untransformed ME cells were washed twice in PBS prior to being lysed in 1 x sample buffer (Laemmli, 1970). After heat denaturation (5 min at 100 °C), cellular extracts were electrophoresed on a 10% polyacrylamide-SDS gel, according to the method of Laemmli (1970). Subsequently the proteins were blotted onto a nitrocellulose membrane (90 min, 35 V), using a sodium carbonate buffer (Dunn et al., 1986). Afterwards, filters were washed in PBS, 0.05% Tween 20 for 30 min. Blocking was performed at 37 °C for 30 min using PBS, 0.05% Tween 20, 8% goat serum. The large T antigens were immunologically detected using MAb pAb 416 diluted 1:100 in PBS/Tween 20/8% goat serum. After 1 h incubation at 37 °C the filters were washed three times for 10 min each in PBS, 0.05% Tween 20, and subsequently incubated for 1 h at 37 °C with a 1:500 dilution of alkaline phosphatase-conjugated rabbit anti-mouse antibody (Dakopatts). After three 10 min washing steps in PBS, 0.05% Tween 20 and finally in substrate buffer (100 mM-Tris–HCl, 100 mM-NaCl, 5 mM-MgCl2, pH 9.5), alkaline phosphatase activity was colorimetrically detected using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt, according to the protocol of the manufacturer (Sigma).

Anchorage-independent growth. Transformed cells were tested in quadruplicate for their potential for anchorage-independent growth by culturing the cells in soft agar (0.3% agarose, type VII, Sigma). For this purpose, 5000 cells were suspended at 38 °C in 2 ml Iscove's medium containing 8% FCS and 0.3% agarose. Subsequently, the suspension was spread on top of a solidified 0.6% agarose/Iscove's medium layer in a 60 mm cell culture dish. About 1 ml of 0.3% agarose/Iscove's medium was added weekly on top of the culture. After 5 weeks of incubation at 37 °C, anchorage-independent growth was scored by counting the number of colonies formed in each of the cell cultures (MacPhearson & Montagnier, 1984).

Tumorigenicity assay. The tumorigenicity of embryonic mouse cells transformed in vitro was tested in immunocompromised newborn rats. Immunosuppression of normal newborn Wistar rats was induced within 48 h of birth by inoculating the animals (subcutaneously in the neck) with 0.1 ml of rat anti-thymocyte globulin (ATG) prepared at the National Institute of Public Health and Environmental Protection, the Netherlands (van Steenis & van Wezel, 1982). On the day ATG treatment was started (day 0), 10⁶ cells to be inoculated were suspended in 0.1 ml PBS and subcutaneously injected in the umbilical region of each of 10 animals. As a positive control, 10⁶ HeLa cells suspended in 0.1 ml PBS were used for inoculation. At days 7 and 14, ATG treatment was repeated. At day 21, the animals were killed and examined macroscopically for the presence of a tumour at the site of inoculation and for metastasis in the peripheral lymph nodes and lungs. Microscopic analysis of these tissues using formalin-fixed, haematoxylin and eosin (HE)-stained paraffin sections was also performed.

Results

To investigate the potential of BPYV to induce the morphological transformation of primary rodent cells, cell cultures derived from embryonic mice and baby rat kidneys were transfected with plasmid constructs encoding the complete early region of BPYV. At 8 weeks after transfection, transformation was scored by dense focus formation. The number of foci scored in each of the transfected cell cultures is shown in Table 1. No foci of morphologically transformed cells were observed upon transfection of ME cells with pXX, the plasmid construct encoding the complete early region of BPYV controlled by its own promoter and enhancer sequences. Neither was transformation observed in experiments with the pXX construct and BRK cells. In contrast, dense foci of morphologically transformed rapidly growing cells were observed after transfection of both BRK and ME cell cultures with pRBPy. This plasmid encodes the complete early region of BPYV under the control of the strong RSV LTR, the promoter and enhancer sequences of which have been shown to be active in many different cell types (Gorman et al., 1982). The frequency of pRBPy-induced cell transformation appeared to be almost equal for each cell type. However, when compared to pRS1, a plasmid encoding the complete early region of SV40 under the control of the strong RSV promoter−enhancer, the frequency of pRBPy-induced dense focus formation was significantly lower and approximately comparable to that observed with pRB10, a plasmid encoding the complete early region of human polyomavirus BK under the control of the RSV promoter−enhancer (Table 1).

Several foci of pRBPy-transformed ME and BRK cells were picked from the initial cell cultures and expanded for further analysis. In subsequent experiments, some properties of three independent colonies of pRBPy-transformed murine cells, designated ME-RBPy-1, -2 and -3 were determined. The selection of these three colonies was based on differences in size and morphology of the cells within the focus.

Characterization of pRBPy-transformed ME cells

After restriction enzyme digestion, electrophoresis and Southern blotting of chromosomal DNA isolated from ME-RBPy cells, the state of BPYV DNA in these cells was investigated by hybridization (Fig. 2). Since no recognition sequence for BamHI is present in the viral genome, integration of BPYV sequences into the host genome could be tested by digesting the chromosomal DNA with this enzyme. In cells derived from all three ME-RBPy colonies, the pRBPy sequences were shown to have integrated into the host genome (Fig. 2, lanes 3, 6 and 12 compared to lane 9). No episomal forms of pRBPy were observed in any of the colonies.

<table>
<thead>
<tr>
<th>DNA</th>
<th>ME cells</th>
<th>BRK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>pXX</td>
<td>0, 0, 0</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0</td>
</tr>
<tr>
<td>pRBPy</td>
<td>0, 1, 1, 2, 1, 1, 2, 2</td>
<td>3, 1, 3, 2, 1, 1, 4, 4</td>
</tr>
<tr>
<td>pRS1</td>
<td>22, 22, 19, 14</td>
<td>19, 14, 19, 18</td>
</tr>
<tr>
<td>pRB10</td>
<td>0, 1, 2, 0</td>
<td>6, 8, 8, 6</td>
</tr>
<tr>
<td>pRSVneo</td>
<td>0, 0, 0, 0</td>
<td>0, 0</td>
</tr>
</tbody>
</table>
An indication of the integrity of the early region of BPyV was obtained by HindIII and BglII digestion. HindIII was expected to generate a 1714 bp BPyV fragment, and BglII a BPyV-specific fragment of 1256 bp, as outlined for pRBPy in Fig. 1. Both fragments were present in each of the cell lines, as shown in Fig. 2. By digesting the chromosomal ME-RBPy DNA with the single cut enzyme EcoRV, it was demonstrated that most probably no more than one copy of pRBPy was present in the genome of each of those cells (results not shown).

Expression of BPyV large T antigen in transformed murine and rat cells was investigated by indirect immunocytochemistry and Western blotting, using MAb pAb 416. This MAb, which was originally directed against the SV40 large T antigen (Harlow et al., 1981), also appeared to recognize the large T antigens of BPyV (Fig. 4) and BK virus. Therefore, the epitope recognized by pAb 416 is likely to be a region of amino acid residues conserved in the large T antigens of SV40, BK virus and BPyV. The epitope for pAb 416 on the SV40 large T antigen is known to be located in the N-terminal region of the unique part of the antigen (Harlow et al., 1981). Since in this region of the large T antigens several stretches of amino acid residues are conserved among the three viruses, the exact epitope cannot be deduced.

Nuclear expression of large T antigen in pRBPy-transformed embryonic mouse cells, as shown in Fig. 3 for ME-RBPy-3 cells, was detected for all three transformed colonies (Table 2). No large T antigen expression was observed in normal mouse cells. Large T antigen expression was observed in almost all ME-RBPy-2 and -3 cells, whereas the proportion of ME-RBPy-1 cells expressing the antigen was about 50% (Table 2).

In addition to the detection of large T antigen expression in ME-RBPy cells, expression of this antigen was also observed by indirect immunocytochemistry in BRK cells that had been transformed by the early region of BPyV (results not shown).

The specificity of pAb 416 in the detection of BPyV large T antigen was further investigated by Western blotting (Fig. 4). As shown, the antibody was capable of detecting a protein with an apparent Mr of about 70K expressed in ME-RBPy-3 cells, but not in untransformed mouse cells or in SV40-transformed murine cells. The size of this protein corresponds very well with the predicted size of BPyV large T antigen, as proposed by Schuurman et al. (1990, 1992).

The in vitro tumorigenic potential of ME-RBPy cells was investigated by testing them for their capacity for anchorage-independent growth (MacPhearson & Montagnier, 1964). All three ME-RBPy colonies tested were capable of such growth, although the relative efficiency appeared to be quite variable (Table 2). The highest percentage of cells forming colonies in soft agar was observed with ME-RBPy-3 cells (11%). These cells were
Table 2. Properties of the various cell lines

<table>
<thead>
<tr>
<th>Property</th>
<th>Cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME-RBPy-1</td>
</tr>
<tr>
<td>T antigen expression*</td>
<td>40 to 50</td>
</tr>
<tr>
<td>Growth in 2% FCS</td>
<td>+</td>
</tr>
<tr>
<td>Anchorage-independent growth‡</td>
<td>4</td>
</tr>
<tr>
<td>Tumorigenic in vivo§</td>
<td>6/10</td>
</tr>
</tbody>
</table>

* Percentage of cells in which nuclear expression of large T antigen was observed.
† NT, Not tested.
‡ Mean percentage of cells forming colonies in soft agar after 5 weeks incubation.
§ Number of rats developing a primary tumour at the inoculation site within 21 days/total inoculated animals.
¶ Each of 10 rats was inoculated with 10⁶ HeLa cells, the standard positive tumorigenicity control; all animals developed a primary tumour within the observation period and metastases developed in both lymph nodes and lung.

Investigated further by growing them in reduced concentrations of FCS. To this end, monolayer cultures of ME-RBPy cells incubated in medium containing 8% FCS were switched to medium containing 2% FCS. As a result, cell division of ME-RBPy-2 cells ceased, whereas ME-RBPy-1 and -3 cells continued to grow efficiently (Table 2).

**In vivo properties of pRBPy-transformed ME cells**

Apart from investigating some of the *in vitro* properties of the pRBPy-transformed murine cells, we also studied the potential of these cells to induce tumours in immunocompromised rats. For this purpose, a tumorigenicity assay was used that is considered suitable for such testing by the WHO (WHO Technical Report Series 745, annex 3, 1987) and has been shown to be extremely sensitive (van Steenis & van Wezel, 1982). On day 0 the immune response of the newborn rats was suppressed by treatment with rat-specific ATG. Subsequently, each of 10 immunocompromised rats was inoculated with 10⁷ transformed or untransformed murine cells.

Twenty-one days after inoculation of the animals with either ME-RBPy-1 or -3 cells, macroscopically visible primary tumours were observed at the site of inoculation (Table 2). Tumours were induced with ME-RBPy-3 cells in all the animals inoculated. In the cell culture obtained by trypsinization of the one ME-RBPy-3 cell-induced tumour tested, expression of large T antigen could clearly be demonstrated by indirect immunocytochemistry using the MAb pAb 416 (data not shown), indicating that the transformed cells used for the inoculation were involved in tumour formation.

Histological examination of HE-stained sections

![Western blot analysis](image-url)
demonstrated mesenchymal-type tumours infiltrating the neighbouring muscle tissues and apparently forming collagen. Therefore, the tumours were classified as fibrosarcomas.

In the assay system employed, tumours have frequently been shown to spread through the body, in particular to the lungs and the peripheral lymph nodes (van Steenis & van Wezel, 1982). Therefore, we examined each of the animals for the presence of metastases in these organs. Metastases of the primary ME-RBPy-3 cell-induced tumours were observed in the peripheral lymph nodes of some of the animals (data not shown). No abnormalities were observed in the lungs.

Macroscopically detectable fibrosarcomas induced by ME-RBPy-1 cells were observed in six of 10 animals. The size of the tumours was significantly smaller than those obtained with ME-RBPy-3 cells, and no abnormalities were observed in either the peripheral lymph nodes or in the lung tissues.

In this experiment, ME-RBPy-2 cells as well as normal ME cells appeared to be non-tumorigenic (Table 2). Large and extensively metastasizing tumours were observed in animals inoculated with $10^6$ HeLa cells, the standard positive control cells used in this assay.

**Discussion**

The results of our experiments demonstrate that when under control of the RSV LTR the early genes of BPyV are capable of inducing dense foci of morphologically transformed cells in rodent cell cultures (Table 1). No foci were obtained upon transfection of these cells with a construct encoding the complete early region of BPyV controlled by the homologous regulatory sequences (pXX). This is probably due to a limited level of BPyV promoter transcriptional activity in rodent cells, resulting in low concentrations of large T antigen. Low concentrations of large T antigen may be insufficient to complex to and titrate out certain cellular proteins involved in cell growth control [e.g. the product of the retinoblastoma tumour suppressor gene (pRB); discussed below]. Efficient expression of genes controlled by the RSV LTR has been described in a variety of eukaryotic cells (Gorman et al., 1982). Therefore, BPyV-mediated transformation of pRBPy-transfected rodent cell cultures is probably achieved by a moderate to high level of expression of the encoded gene products. However, the results listed in Table 2 illustrate that the properties of a transformed cell are not determined exclusively by the amount of large T antigen expressed in the cell, as has been suggested previously (Risser & Pollack, 1974; Jat & Sharp, 1986). ME-RBPy-2 cells were not capable of anchorage-independent growth or of inducing tumours, despite efficient expression of large T antigen by these cells. In contrast, ME-RBPy-1 cells did induce tumours in six of 10 animals, whereas large T antigen expression was observed in a lower percentage of cells when compared to ME-RBPy-2 and -3. These results demonstrate that there is no correlation between the immunological detection of the expression of large T antigen and the tumorigenic properties of pRBPy-transformed cells. In contrast to this, the capacity for anchorage-independent growth as well as efficient growth of the cells in low concentrations of FCS appear to correlate very well with the tumorigenic potential of the cells. This latter correlation is in good agreement with observations made by Shin et al. (1975) using SV40.

As demonstrated by Southern blotting and subsequent hybridization of BamHI-digested chromosomal DNA isolated from ME-RBPy cells, the pRBPy sequences had become integrated into the host cell genome at different locations (compare Fig. 2 lanes 3, 6 and 12). Expression of the viral genes might be influenced by cellular sequences flanking the site of integration or integration of viral sequences into the host genome might alter the expression of the genes at or near the site of integration. Therefore, the site of integration might explain the differences observed between ME-RBPy-1, -2 and -3 cells.

As has been known for many years, several polyomaviruses are capable of morphologically transforming both primary and established mammalian cells cultured in vitro, or even inducing tumours after inoculation of rodents (Salzman, 1986). Induction of morphological cell transformation and tumour formation is accomplished by the early viral antigens (Salzman, 1986). In addition to polyomaviruses, some members of the adenovirus family as well as some members of the papillomavirus family have been shown to be capable of inducing cellular transformation (reviewed in Villarreal, 1989). Essential roles in cell transformation have been demonstrated for the antigens encoded by the E6 and E7 open reading frames of human papillomavirus types 16 and 18, as well as for the E1A and E1B antigens of adenovirus type 5 (Villarreal, 1989).

It has been convincingly demonstrated that the amino acid sequences of the viral antigens involved in cell transformation contain some highly conserved sequence elements (Moran, 1988; Figge et al., 1988). One of these elements, with the minimal consensus core sequence DLXCE, appears to be well conserved among all oncogenic polyoma-, adeno- and papillomaviruses (Moran, 1988). This element has been shown to be involved in complexing with a so-called tumour suppressor gene product, pRB (DeCaprio et al., 1988; Whyte et al., 1988). In SV40 large T antigen, the DLXCE motif is located between amino acid residues 102 and 107; the
amino acid sequences of SV40 large T antigen interacting with pRB have been shown to be located between residues 102 and 115 (Kaelin et al., 1990). Introducing amino acid substitutions between amino acid residues 105 and 115 has demonstrated that the intactness of this domain is essential for the full transformation of both established and primary mouse and rat fibroblasts (Chen & Paucha, 1990). In the amino acid sequence of the putative BPyV large T antigen, the minimal consensus core sequence (DLXCXE) described above has been identified between amino acid residues 59 and 64 (DLHCDE; Schuurman et al., 1990). In addition, significant homology between the SV40 and BPyV large T antigens was observed in the region spanning the consensus core sequence to the C-terminal side. A total of nine of 15 amino acid residues are conserved between SV40 and BPyV, including the DLXCXE motif. The sequence conservation in this part of the BPyV large T antigen might be essential for the putative potential of this virus to complex with pRB and thereby for its potential for cell transformation.

It can be concluded that the early gene products of BPyV are capable of in vitro oncogenic transformation of rodent cells. Therefore, BPyV should be considered as one of the tumorigenic polyomaviruses. Since commercial batches of (foetal) calf serum have been shown to be frequently contaminated with this virus (Schuurman et al., 1991a) it is recommended that cattle sera should be screened for the presence of BPyV prior to use in cell culture.

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References


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