Molecular and biological characteristics of avian polyomaviruses: isolates from different species of birds indicate that avian polyomaviruses form a distinct subgenus within the polyomavirus genus

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The isolation and characterization of two avian polyomaviruses, from chicken (BFDV-2) and a parrot (BFDV-3), is reported. Both isolates are closely related to the non-mammalian polyomavirus budgerigar fledgling disease virus (BFDV) isolated from budgerigars (now called BFDV-1), and all three viral genomes are shown to have the same basic size of 4981 bp. A 151 bp insertion was, however, observed in the non-coding region of BFDV-2 which represented an exact duplication of the left half of the non-coding region, including the putative early promoter and amino terminus of the large T antigen. With a further 15 base pairs exchanged elsewhere throughout the three genomes, these viruses have distinct degrees of tropism for various avian species. The production of antibodies directed against a β-galactosidase–large T antigen fusion protein of BFDV-1 is described. These antibodies detected the large T antigen, with an M̄ of approximately 80K, and the small t antigen, with an M̄ of approximately 24K, in cells infected with BFDV isolates. Whereas these antibodies bind with low affinity to the large T antigen of simian virus 40 (SV40), SV40- or mouse polyomavirus-specific antibodies will not bind to the BFDV large T antigen. Antibodies directed against BFDV structural polyptides exhibit broad, reciprocal cross-reactivities with all three structural proteins of mammalian polyomaviruses. The significance of polyomavirus infections in various avian species is discussed. Based on unique structural and biological properties we propose that these viruses should be placed in a distinct subgenus (Avipolyomavirus) within the polyomaviruses.

Introduction

Budgerigar fledgling disease virus (BFDV; Bozeman et al., 1981) represents the first avian virus shown to be a member of the polyomavirus genus in the family Papovaviridae (Müller & Nitschke, 1986; Lehn & Müller, 1986). The complete nucleotide sequence of the BFDV genome has been determined (Rott et al., 1988). Sequence data for the circular dsDNA genome show that BFDV is similar to other well investigated polyomaviruses, such as simian virus 40 (SV40), with which it shares a number of structural and functional characteristics. The early coding region contains open reading frames (ORFs) for two proteins which are synthesized prior to DNA replication, the large T and small t antigens. A middle T antigen as encoded by the rodent polyomaviruses mouse polyomavirus (PyV) and hamster polyomavirus (HaPV) has not been observed. The late coding sequences contain two major ORFs encoding the three structural proteins VP1, VP2 and VP3. As in all polyomaviruses, with the exception of the rodent polyomaviruses mentioned above and monkey B-lymphotropic polyomavirus (LPV), a small ORF designated agnogene is also present in the leader sequence region of the mRNAs coding for the late genes. The non-coding region is expected to contain the origin of replication and the early and late promoter–enhancer sequences.

Despite this overall similarity to SV40, however, remarkable differences from the mammalian polyomaviruses have been observed in the BFDV genome. There is in particular a considerable reduction in size of the large T antigen and, in the non-coding region, fundamental deviations in the replication origin structure as well as the transcriptional control elements (Rott et al., 1988).

Polyomavirus infections of mammals are not associated with clinical disease in the natural non-immunocompromised host. In contrast, and unique for polyo-
maviruses, BFDV has been isolated from fledgling budgerigars (Melopsittacus undulatus) suffering from a contagious acute disease with distinct organ manifestations and mortality rates of up to 100%. There are some indications that BFDV may also be associated with French moul of budgerigars (Taylor, 1969), presumably a milder and more protracted form of a BFDV infection resulting in chronic feathering disorders (Krautwald et al., 1989). In view of these structural and biological characteristics of BFDV, it has been suggested that this virus should be placed in a distinct subgroup within the polyomavirus genus (Rott et al., 1988).

Here we describe the characterization of two additional avian polyomaviruses, BFDV-2 isolated from chickens and BFDV-3 isolated from a parrot. The genomes of these viruses were cloned and sequenced, and their nucleotide sequences were compared with that of the budgerigar isolate described above and now designated BFDV-1. The results of these experiments show that the three independently isolated strains are closely related to each other and that they all exhibit the structural and biological characteristics of avian polyomaviruses. A small number of nucleotide exchanges, evenly distributed throughout the viral genome, however, is observed in this comparison. These deviations are sufficient to enable replication of the isolates with different efficiencies in cultured cells obtained from various avian species. We also describe the preparation of rabbit antibodies directed against the BFDV-1 particles and the preparation of antibodies directed against the BFDV-1 large T antigen by injection into rabbits of a β-galactosidase-T antigen fusion protein overexpressed in Escherichia coli. Authentic early and late proteins could be demonstrated in infected cultured cells with these antibodies. Cross-reactivities as observed among the late structural polyepitides of mammalian and avian polyomaviruses were not found among large T and small t antigens. It is proposed that the avian polyomaviruses be placed in a distinct subgroup within the polyomavirus genus of the family Papovaviridae; by analogy with adenoviruses of avian origin (Aviadenovirus; Francki et al., 1991), the designation Avipolyomavirus is suggested.

**Methods**

**Cells and viruses.** Chicken embryo (CE) cells prepared as described previously (Müller & Nitschke, 1986) were cultured at 38 °C in Dulbecco’s modified Eagle’s medium supplemented with 5% heat-inactivated foetal calf serum. Muscovy duck embryo (MDE) cells were prepared analogously from commercially obtained fertilized Muscovy duck (Cairina moschata) eggs after incubation at 38 °C for 16 days.

The isolation of polyomavirus (BFDV-1) from liver and spleen of acutely diseased budgerigars in CE cells has been described (Kaleta et al., 1984; Müller & Nitschke, 1986). Virus isolate 1 8879, now designated BFDV-2, had been isolated from the drinking water and faeces in a replacement layer farm that had suffered from three cycles of severe outbreaks of Gumboro disease. Virus isolate 1 8869, now designated BFDV-3, was from the liver of a hand-fed blue and yellow macaw (ARA ararauna), 5 to 6 months old, with ascesis and subcutaneous haemorrhages. In both cases, virus was isolated by inoculating 7-day-old specific pathogen-free (SPF) chicken embryos with 0.2 ml of virus-containing material via the yolk sac. SV40 was propagated in TC-7 cells at 38 °C.

**Growth and purification of viruses.** Viruses were grown on monolayers of CE cells (BFDV-1, BFDV-2) or MDE cells (BFDV-3) until extensive cytopathic changes became visible after incubation at 38 °C for 6 to 7 days. Virus particles were purified and concentrated by repeated centrifugation in CsCl gradients essentially as described by Müller & Nitschke (1986).

**Preparation and analysis of viral DNA.** DNA was released from virus particles by treatment with 50 μg/ml proteinase K in 10 mM-Tris-HCl pH 7.5, 0.5% SDS at 37 °C for 30 min. Samples were extracted once with phenol/chloroform and twice with chloroform, followed by precipitation using ethanol without carrier.

**Construction of clones, DNA sequencing and sequence data analysis.** Based on restriction enzyme mapping data obtained for the new isolates and for BFDV-1 (Rott et al., 1988), DNA released from virus particles was double-digested at the unique BamHI (position 2308) and EcoRI (4820) restriction sites. Each of the fragments was cloned into the BamHI/EcoRI site of the plasmid pUC931 (Stratagene). Competent XL1-Blue cells (Stratagene) were used for transformation. The cloned fragments were subjected to a series of single and double restriction enzyme digestions using AhaIII, EagI, EcoRV, HindIII, KpnI, PstI, Sall and SacII under conditions outlined by the suppliers (Boehringer Mannheim, New England Biolabs). The resulting BFDV DNA fragments in a size range from 201 to 953 bp were cloned into either pUC931 or pBluescript II KS+ (Stratagene) and used for DNA sequencing. To demonstrate biological activity for the genome fragments employed for subcloning and sequencing, plasmid DNAs were recombined and a Sal fragment corresponding to the complete genome of BFDV was recircularized and transfected (Cellphic Transfection Kit, Pharmacia) into CE or MDE cells. After 4 days of incubation, transfected cells were frozen and thawed three times, cell debris was removed by centrifugation and the supernatant was used to infect fresh CE or MDE cells. Replication of virus was evident from cytopathic changes; early and late gene products were demonstrated by Western blotting.

DNA sequencing was performed with the dideoxynucleotide chain termination method (Sanger et al., 1977), using either M13 primers for pUC931 or SK/KS and T3/T7 primers for insert-carrying pBluescript II KS+ DNAs. DNA reaction products were separated on 6% polyacrylamide gels containing 8 M-urea at 55 °C.

For the preparation of a β-galactosidase–large T antigen fusion protein, a synthetic oligonucleotide containing BamHI, NcoI, AsuII, PstI, AffI, SpII and PstI restriction sites was cloned into the multiple cloning site of plasmid pSS920* (Scholtissek & Grosse, 1988), resulting in plasmid pHL594. An 1817 bp NcoI/SphI fragment of the BFDV genome, expected to encode large T antigen and from which a predicted intron sequence (Rott et al., 1988) had been removed, was cloned into the NcoI/SphI sites to yield pHL620. Finally the lacZ promoter of pSS20* was replaced by the stronger proh4 promoter from pBR2 (Rihs & Peters, 1989) via Clal/HindIII sites to yield pHL623 which was transfected into competent JM101 cells.

**Preparation of antisera against virus-specific polypeptides.** β-Galactosidase–large T antigen fusion protein expressed in E. coli was purified by gel filtration chromatography (Sephacryl-S-300, Pharmacia), mixed with Freund’s complete adjuvant (Difco), and used to immunize rabbits by a total of three injections.
Antisera against BFDV-1 structural polypeptides were produced in rabbits by three intramuscular injections of purified virus emulsified in Freund's incomplete adjuvant (Difco) at 3 week intervals. Antibodies elicited against CE cellular components were removed by passing the sera through a column of Sepharose 6B/CL (Pharmacia) to which extracts of uninfected CE cells had been conjugated after CNBr activation as described by Müller & Becht (1982).

**SDS–PAGE and immunoblotting.** These were carried out as described by Becht et al. (1988). Proteins were separated by SDS–PAGE in 12.5% gels using a discontinuous buffer system (Laemmli, 1970). In Western blotting experiments, proteins were transferred from the gels to blotting membranes (filter type GV; pore size 0.22 µm; Millipore). Membranes were blocked either with 10% horse serum or with 1% non-fat dry milk. Primary antibodies were used at a dilution of 1:2000, biotinylated secondary antibody at a dilution of 1:500.

Radiolabelling and immunoprecipitation of viral polypeptides. Radiolabelling of polypeptides in CE or TC-7 cells and immunoprecipitation were carried out essentially as described by Müller & Becht (1982). Briefly, after a pulse with [35S]methionine (specific activity 80 mCi/mmol; NEN-DuPont) for the times indicated in the legends to the figures, cells were washed with PBS and resuspended in dissociating radioimmunoprecipitation assay (RIPA) buffer [10 mM-phosphate buffer pH 7.2, containing 10 mM-EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS and 2% Trasylol (Bayer)]. Lysates were clarified by centrifugation and after the addition of antibodies and Protein A (Staphylococcus aureus), antigen–antibody complexes were washed with washing buffer (10 mM-phosphate buffer pH 7.0, containing 1 M-NaCl, 10 mM-EDTA, 40 mM-NaF and 0.2% Triton X-100) twice, and once with water. Bound material was then eluted with SDS-PAGE sample buffer at 100 °C for 5 min, and samples were subjected to SDS-PAGE and autoradiography.

**Results**

**Propagation of BFDV-2 and BFDV-3 in cell culture and purification of virus particles**

Cytopathic agents isolated from chickens (BFDV-2) or a parrot (BFDV-3) were inoculated onto 60% confluent CE and MDE cells. After 48 h at 38 °C, cytopathic changes as described for BFDV-1 (Müller & Nitschke, 1986) were observed in CE cells infected with BFDV-2 and in MDE cells infected with BFDV-2 or BFDV-3, respectively. Virus particles purified from infected cell cultures by Freon 113 treatment and CsCl gradient centrifugation had a buoyant density of 1.34 g/ml. Electron microscopic examination revealed non-enveloped icosahedral particles with diameters of about 46 to 48 nm and an overall appearance identical to the avian polyomavirus BFDV-1 (Müller & Nitschke, 1986).

**Restriction enzyme analysis of viral DNAs**

The genomic DNAs of BFDV-2 and BFDV-3 released from purified virus particles were subjected to agarose
Table 1. Comparative nucleotide analyses in the genomes of BFDV-1, BFDV-2 and BFDV-3*

<table>
<thead>
<tr>
<th>Nucleotide number</th>
<th>Region in genome</th>
<th>Nucleotide exchange (amino acid encoded)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BFDV-1</td>
</tr>
<tr>
<td>34</td>
<td>Non-coding</td>
<td>A (-)</td>
</tr>
<tr>
<td>199</td>
<td>Non-coding</td>
<td>C (-)</td>
</tr>
<tr>
<td>230/235</td>
<td>Non-coding</td>
<td>(415) bp inserted</td>
</tr>
<tr>
<td>(416)†</td>
<td>Non-coding</td>
<td>C (-)</td>
</tr>
<tr>
<td>584</td>
<td>Non-coding</td>
<td>T (-)</td>
</tr>
<tr>
<td>1330</td>
<td>VP2/VP3: 118</td>
<td>A (Ile)</td>
</tr>
<tr>
<td>1601</td>
<td>VP2/VP3: 209</td>
<td>A (Ser)</td>
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<tr>
<td>1638</td>
<td>VP2/VP3: 221</td>
<td>T (Val)</td>
</tr>
<tr>
<td>2289</td>
<td>VP1: 131</td>
<td>T (Phe)</td>
</tr>
<tr>
<td>3224</td>
<td>T: 521</td>
<td>T (His)</td>
</tr>
<tr>
<td>3382</td>
<td>T: 485</td>
<td>C (His)</td>
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<tr>
<td>3468</td>
<td>T: 440</td>
<td>T (Phe)</td>
</tr>
<tr>
<td>4123</td>
<td>T: 221</td>
<td>T (Leu)</td>
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<tr>
<td>(4306)‡</td>
<td>T: 161</td>
<td>C (Met)</td>
</tr>
<tr>
<td>(4307)‡</td>
<td>T: 60</td>
<td>G (Leu)</td>
</tr>
<tr>
<td>4433</td>
<td>T: 18</td>
<td>T (Asp)</td>
</tr>
<tr>
<td>4504</td>
<td>T: 95</td>
<td>C (Pro)</td>
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<tr>
<td>4758</td>
<td>T/t: 75</td>
<td>A (Glu)</td>
</tr>
<tr>
<td>4948</td>
<td>T/t: 12</td>
<td>T (Cys)</td>
</tr>
</tbody>
</table>

* Tabulation of nucleotide exchanges in the coding upper (late region) or lower (early region) strands, and indication of amino acids translated from respective codons.
† In BFDV-1, BFDV-2 and BFDV-3, an additional C has been determined at position 416, thus expanding the BFDV DNA sequence published by Rott et al. (1988) to a total of 4981 bp.
‡ In this position, GC as published by Rott et al. (1988) was re-determined to read CG in BFDV-1, BFDV-2 and BFDV-3, which also results in an amino acid sequence Met-Leu instead of Ile-Val.

gel electrophoresis. Three bands were visible which migrated to similar positions as DNA released from BFDV-1 particles (Müller & Nitschke, 1986). Restriction enzyme digestions with BamHI or EcoRI, which had been shown to cut the BFDV-1 genome only once (Lehn & Müller, 1986; Rott et al., 1988), resulted in linear DNA molecules which migrated in positions corresponding to about 5000 bp (Fig. 1a).

To obtain a preliminary indication of the genetic similarities between these three viruses, a comparative restriction enzyme analysis was performed. The genomes of BFDV-2 and BFDV-3 were cut into two half-size fragments by double digestion with BamHI and EcoRI, which had been shown to cut the BFDV-1 genome only once (Lehn & Müller, 1986; Rott et al., 1988), resulted in linear DNA molecules which migrated in positions corresponding to about 5000 bp (Fig. 1a).

A comparative evaluation of the three BFDV DNA sequences revealed a total number of 15 base pair exchanges, only four of which are identical in BFDV-2 and BFDV-3 DNA, but did not cleave BFDV-1 DNA (Fig. 1b). Also, PstI digestion revealed heterogeneity in fragment size, since one of the BFDV-2 fragments migrated more slowly than the corresponding fragment of BFDV-1 or BFDV-3 indicating that the genome of BFDV-2 was about 150 bp larger.

Nucleotide sequence comparison

The availability of BFDVs isolated from different avian species with clear differences in species specificity (see below) and the deviations observed in restriction enzyme mapping prompted us to determine the complete nucleotide sequences of BFDV-2 and BFDV-3, which were then compared with the known sequence of BFDV-1.

In the course of this analysis three instead of two Cs at position 416/417 were determined for BFDV-2 and BFDV-3 DNA. Re-examination of the BFDV-1 sequence, however, revealed the presence of an extra C in this sequence, thus giving rise to a correction of the published total number of bp in BFDV-1 (Rott et al., 1988) to 4981 bp. Re-examination of the large T antigen-encoding sequence also showed that at positions 4306/4307 the sequence reads GC instead of CG, also resulting in an NdeI restriction site at this position [G(CATATG)].

A comparative evaluation of the three BFDV DNA sequences revealed a total number of 15 base pair exchanges, only four of which are identical in BFDV-2 and BFDV-3; nine of the base pair exchanges will also result in an exchange of amino acids. The respective nucleotide as well as amino acid exchanges are listed in Table 1 with reference to their location in the BFDV...
Characteristics of avian polyomaviruses

Fig. 2. Schematic representation of a 151 bp insertion in the non-coding region of BFDV-2 DNA, consisting of a duplication of nucleotides 4962 to 131; the sequence GCTC present in the genomes of BFDV-1 and BFDV-3 at position 231 to 234 is deleted. Dark boxes mark the large T antigen/small t antigen coding region, hatched boxes represent sequence repetitions of 45 bp in the viral non-coding regions (Rott et al., 1988) and flags indicate the putative promoter for early gene transcription (TATAAAT at positions 53 to 47).

Fig. 3. Characterization of BFDV-1 early (a) and late (b) gene products. [35S]Methionine-labelled mock-infected (lane 1) or BFDV-1-infected (lane 2) CE cells were lysed at 16 h post-inoculation (p.i.) (a) or 32 h p.i. (b) and analysed by radiolmmunoprecipitation, followed by SDS-PAGE on 15% gels and autoradiography. Antibodies directed against the ß-galactosidase-large T antigen fusion protein (lanes 1, 2), SV40 large T antigen (lanes 3, 4) and sera collected from rats bearing PyV-induced tumours (lanes 5, 6), respectively. (b) Polypeptides in labelled mock-infected (lane 1) or BFDV-1-infected (lane 2) CE cells, and mock-infected (lane 3) or SV40-infected (lane 4) TC-7 cells were precipitated using antibodies directed against SV40 large T antigen. Precipitates were washed and analysed by SDS-PAGE, followed by autoradiography. Besides full size SV40 large T antigen, several protein bands due to proteolytic degradation are visible in (a), lane 4.

Fig. 4. Determination of relationships among BFDV-1 and SV40 early gene products by RIPA. (a) [35S]Methionine-labelled mock-infected (lanes 1, 3, 5) and SV40-infected (lanes 2, 4, 6) TC-7 cells were lysed at 20 h p.i. Labelled polypeptides were precipitated using polyclonal antibodies directed against the ß-galactosidase-large T antigen fusion protein (lanes 1, 2), SV40 large T antigen (lanes 3, 4) and sera collected from rats bearing PyV-induced tumours (lanes 5, 6), respectively. (b) Polypeptides in labelled mock-infected (lane 1) or BFDV-1-infected (lane 2) CE cells, and mock-infected (lane 3) or SV40-infected (lane 4) TC-7 cells were precipitated using antibodies directed against SV40 large T antigen. Precipitates were washed and analysed by SDS-PAGE, followed by autoradiography. Besides full size SV40 large T antigen, several protein bands due to proteolytic degradation are visible in (a), lane 4.

Expression of BFDV large T antigen in E.coli and preparation of rabbit antisera

E. coli cells containing plasmid pHL623, in which a ß-galactosidase-large T antigen fusion protein gene is inserted under the control of the inducible p<sub>pae</sub> promoter were induced by the addition of IPTG (1 mM final concentration). After 4 h at 37 °C the cells were collected...
and lysed with double-strength Laemmli sample buffer. Following centrifugation at 15,000 r.p.m. for 30 min, the supernatant was subjected to gel filtration and eluted fractions were analysed by SDS-PAGE (not shown). A fusion protein with an apparent $M_r$ of about 170K, in a size range as calculated from the inserted fusion gene sequence, eluted in the first few fractions. These fractions were collected, dialysed extensively against PBS and used to immunize rabbits. High titre polyclonal antibodies recognizing both BFDV large T and small t antigen of all three virus strains (see below) were elicited.

Characterization of BFDV gene products

BFDV early and late gene products in virus-infected cells were characterized by RIPA and Western blotting, using rabbit antibodies prepared as described above. Antibodies directed against a β-galactosidase-large T antigen fusion protein precipitated two polypeptides of 80K and 24K from BFDV-1-infected, $[^{35}S]$methionine-labelled CE cells, but not from similarly treated mock-infected cells (Fig. 3a). As shown by Western blotting, similar polypeptides were also present in CE or MDE cells infected with BFDV-2 or BFDV-3, respectively (Fig. 5a). Antibodies elicited with BFDV-1 virus particles precipitated three proteins of approximately 42K, 39K and 30K from radiolabelled BFDV-1-infected CE cells (Fig. 3b); similar polypeptides were present in BFDV-2 or BFDV-3 infected cells as shown by Western blotting (Fig. 5b).

To determine the relationships among early and late gene products of avian and mammalian polyomaviruses at the serological level, cross-reaction experiments were performed. It is evident from Fig. 4(a) that large T and small t antigens from SV40-infected TC-7 cells were precipitated by antibodies directed against BFDV-1 large T antigen to a slight, but significant extent; a similar reaction was obtained using sera from rats in which tumours had been induced with PyV (Fig. 4a). A corresponding reaction pattern could also be demonstrated by Western blotting (not shown). In no case, however, could any reaction be recognized with virus-specific polypeptides in BFDV-infected cells when mono- or polyclonal antibodies prepared against SV40 large T antigen, or the PyV rat antibodies described above, were used for precipitation (Fig. 4b). On the other hand,
antibodies directed against BFDV-1 (late) structural polypeptides also precipitated virus-specific polypeptides from SV40-infected TC-7 cells (not shown).

Replication of BFDV isolates in CE and MDE cells

When BFDV isolates were inoculated onto subconfluent monolayers of CE cells, cytopathic changes became evident in cultures infected with BFDV-1, originally isolated from fledging budgerigars, and in cultures infected with BFDV-2 which had been isolated from faeces and drinking water in poultry houses. Cytopathic changes were not visible in CE cells infected with the isolate from the liver of a parrot (BFDV-3), even after repeated passages and various incubation temperatures. However, when MDE cells were inoculated, cytopathic changes became visible in the cultures infected with each of the three BFDV isolates.

Western blotting experiments were performed to test whether early and late gene products were present in BFDV-3-infected CE or MDE cells. Results of these experiments presented in Fig. 5 show that neither early (a) nor late (b) gene products are present in BFDV-3-infected CE cells, whereas prominent protein bands are visible in BFDV-3-infected MDE cells. Since similar cell numbers had been used in these experiments, it is evident from these data that BFDV-1 and BFDV-2 replicate in CE cells, whereas BFDV-3 does not. In contrast, BFDV-3 is able to replicate to a similar extent in MDE cells, whereas replication of the other two strains in this cell type is significantly reduced.

Discussion

In this communication we have described the isolation of two viruses, from chicken and a parrot, which, with regard to morphological and physicochemical criteria as well as tissue culture properties, revealed the characteristics of polyomaviruses. Analysis of their DNA sequences confirmed that both genomes were very similar to each other and also to that of BFDV (now called BFDV-1) isolated from budgerigars (Kaleta et al., 1984; Krautwald et al., 1989) and found to be the first polyomavirus of non-mammalian origin (Müller & Nitschke, 1986; Lehn & Müller, 1986). Disregarding an insertion/deletion observed in BFDV-2, the basic size of all three viral genomes was determined to be 4981 bp. Fifteen base pair exchanges in the three isolates were found distributed throughout the genomes. One of the exchanges in BFDV-3 was localized in a known conserved region (cr1) or functional domain at position 4948 (Pipas, 1992) of the large T antigen. The transition of T to G changed the coding sequence at amino acid 12 from cysteine to glycine.

The 151 bp insertion in BFDV-2 represents an exact reduplication of the left half of the viral non-coding region, including one of the two large T antigen-binding sites and the putative early promoter plus 19 bp of the amino-terminal region of large T antigen; these sequences are found inserted immediately upstream of one of the putative late promoters (D. Luo, H. Müller & G. Hobom, unpublished). Whether this duplication is of functional significance remains to be determined. Duplications and rearrangements in viral genomes have been observed after only a few undiluted passages of SV40 in tissue culture (Brockman et al., 1973; Norkin et al., 1981). A remarkable degree of heterogeneity, including similar duplications in the transcriptional control region, has frequently been observed for BK virus in tissue culture, and may affect the biological characteristics of the isolates (Rubinstein et al., 1987; Sundsfjord et al., 1990).

In order to obtain large T antigen-specific antibodies, rabbits were immunized with a β-galactosidase–large T antigen fusion protein prepared in E. coli. These antibodies demonstrated authentic large T and small t antigen in BFDV-infected cells. It became evident that the $M_\text{r}$ of large T antigen was considerably higher than that predicted from the nucleotide sequence (Rott et al., 1988), even if various post-translational modifications were taken into account. This is in accordance with our observation that an intron of only 195 nucleotides is removed from BFDV early pre-mRNA (D. Luo, H. Müller & G. Hobom, unpublished), so that large T antigen consists of 599 instead of 554 codons as predicted from the DNA sequence. Small t antigen revealed a size similar to that predicted.

The early genes of mammalian and avian polyomaviruses show only limited homology (Rott et al., 1988; Pipas, 1992), also reflected by the results of our serological investigations showing only a slight asymmetric reactivity of antibodies directed against BFDV-1 large T antigen with large T antigen in SV40-infected cells, but no reaction of SV40 large T antigen-specific antibodies with BFDV-1 large T antigen. Serological cross-reactions have been reported within the SV40 class (Pipas, 1992) of large T antigens (Take moto et al., 1982; Brade et al., 1983). Results of immunoprecipitation experiments as described here show that there exists some antigenic relationship between PyV, as the prototype member of the PyV class (Pipas, 1992), and SV40 large T antigens, but not with BFDV early proteins. Nucleotide sequence comparisons reveal broad homologies between the late genes of avian and mammalian polyomaviruses (Rott et al., 1988); these homologies are also evident from serological cross-reaction among late gene products as observed previously (Lehn & Müller, 1986) and described in the present study.
BFDV-1 has been isolated from nestling budgerigars suffering from an acute multisystemic disease with clear organ manifestations and high mortality rates (see Müller & Nitschke, 1986 and Lehn & Müller, 1986 for references). Intranuclear inclusion bodies have been observed (Davis et al., 1981) and viral DNA could be demonstrated by Southern blotting (H. Lehn & H. Müller, unpublished) in numerous organs of affected birds. Whereas most of the mammalian polyomaviruses have restricted tissue- and cell-type tropisms (Shah, 1990), avian polyomaviruses are similar to PyV which has been shown to replicate in various organs of infected mice (Wirth et al., 1992; Rochford et al., 1992). A clear difference in organ specificity, however, is reflected by the observation that BFDV-1 is able to replicate efficiently in the brain (Davis et al., 1981; H. Lehn & H. Müller, unpublished), whereas PyV is not (Wirth et al., 1992).

Replication of BFDV-1 in the brain of infected birds may cause the high mortality rates observed in BFDV-1-infected budgerigar aviaries. Serological investigations (Wainright et al., 1987) and polymerase chain reaction (PCR) assays (Phalen et al., 1991) indicate that polyomaviruses appear to affect a wide range of psittacine and other species of pet birds. Accordingly the macaw from which BFDV-3 had been isolated exhibited signs typical of a budgerigar fledgling disease. Results of PCR assays also suggest the possibility of persistent infections in adult birds, which may result in chronic disorders similar to French moult. The significance of polyomavirus infections in chickens remains to be determined. The demonstration of BFDV-specific antibodies by Western blotting in sera collected from clinically healthy broiler flocks (H. Müller, unpublished observations) are indicative of persistent polyomavirus infections in Central Europe. BFDV-2 had been isolated on layer farms suffering from infections with infectious bursal disease (IBD) virus. Susceptible chickens infected with IBD virus at an age of less than 3 weeks do not exhibit clinical signs, but have a subclinical infection characterized by destruction of the lymphoid cells in the bursa of Fabricius and immunosuppression (Gumboro disease; see Becht, 1980 and Kibenge et al., 1988 for reviews). It may be speculated that a persistent BFDV-2 infection has been re-activated due to immunosuppression, resulting in virus replication and excretion, a situation similar to BK and JC virus infections in man (Shah, 1990).

Formally it cannot be excluded that BFDV-1, BFDV-2 and BFDV-3 represent variants or subtypes of a single virus. However, the following arguments favour the notion that these viruses are indeed individual BFDV strains: they have been isolated from both Psittaciformes (BFDV-1, BFDV-3) and Phasianiformes (BFDV-2), which are only distantly related to each other; both BFDV-1 and BFDV-2, but not BFDV-3 replicate in CE cells, and the three viruses replicate with distinctly different efficiencies in MDE cells. This is indicative of the biological significance of the nucleotides exchanged in their genomic sequences, and proves that these isolates cannot be regarded as identical.

There are common and unique features among mammalian and avian polyomaviruses. Unique features of avian polyomaviruses consist of structural as well as biological characteristics. The large T antigens of avian polyomaviruses show the least structural similarity (about 15%; Pipas, 1992) to other members of the group. Since it can be assumed that similar biochemical activities have to be carried out by this multifunctional regulatory protein from both avian and mammalian viruses, it may be expected that functionally equivalent domains reveal considerable structural deviations. The non-coding regions of the avian polyomaviruses investigated so far show extensive deviations from those of the mammalian polyomaviruses. In particular, the indicative pentanucleotide G(A/G)GGC sequence repetition near the origin of replication which is highly conserved in mammalian polyomaviruses is missing in BFDV. Unique biological characteristics refer to the low cell type- and tissue-tropism, as well as the obvious broad host range of these viruses. Since they were isolated from species other than budgerigars, the original designation BFDV (Bozem et al., 1981) may be misleading, but should be retained because of the similarities to BFDV-1. We propose that these viruses should be placed in a distinct subgroup within the polyomavirus genus of the family Papovaviridae. By analogy with adenoviruses of avian origin (Aviadenovirus; Franke et al., 1991), the designation Avipolyomavirus is suggested for this subgroup.

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References


Characteristics of avian polyomaviruses


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