Cloning and Characterization of a Papillomavirus Associated with Papillomas and Carcinomas in the European Harvest Mouse (Micromys minutus)

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Individuals in a colony of European harvest mice (Micromys minutus) were diagnosed with a variety of skin tumors including papillomas, trichoepitheliomas, and sebaceous carcinomas. Papillomavirus group-specific antigens and viruslike particles were detected in the papillomas. A 7.6-kilobase supercoiled circular DNA, which was cleaved once by EcoRI, was visualized in papilloma extracts by low-stringency Southern blot hybridization with a bovine papillomavirus type 2 probe. The molecule was cloned in pUC18, and a restriction map was generated. The molecule was shown to be colinear with the genome of human papillomavirus type 1A by partial sequence analysis. The DNA hybridized to human papillomavirus type 1A, rabbit oral papillomavirus, and the genome of Mastomys natalensis papillomavirus at Tm – 33°C but not to the DNAs of 13 other papillomaviruses. Transformation of NIH 3T3 or C127I cells by tail papilloma extracts or transfected viral DNA was not observed. All 17 tumors examined contained large amounts of viral DNA in a supercoiled, unintegrated form as revealed by Southern blot hybridization. Furthermore, many extracts (25 of 35) from normal organs and skin of individuals with lesions elsewhere on their bodies contained viral DNA. This represents the first reported molecular cloning of a papillomavirus genome from a mouse species.

With the exception of an isolate from an inbred line of the North African multimammate rat (Mastomys natalensis), papillomaviruses have not been isolated from rodent species. Spontaneous papillomas have been infrequently observed in hairless mice (1.2% over a 12-year observation period [28]) and with greater frequency (67% of 30 animals) in mice carrying the repeated epilation gene (20). The multiple papillomas, described as cutaneous horns, in erl+ mice appear to progress to squamous-cell carcinomas. No evidence for viral involvement was forthcoming by electron microscopy, staining for papillomavirus-group-specific antigens, or Southern blot hybridization of DNA extracts with a bovine papillomavirus type 1 (BPV-1) DNA probe (20). Induced papillomas, which may progress to carcinomas, have been observed in mice and rats treated with topical carcinogens and are the basis for the initiator-promotor theory of chemical carcinogenesis (2, 14, 30).

Epithelial proliferations in M. natalensis, classified as exophytic kerototic papillomas, keratinizing squamous epithelial carcinomas, and, most frequently, keratoacanthomas, have been observed in 3% of adult individuals who were members of the inbred line GRA Giessen (5, 31). The keratoacanthomas could be transmitted between animals with cell extracts, and viruslike particles were observed in tumor sections by transmission electron microscopy (33). A virus isolated from these lesions was classified as a papillomavirus based on density, morphology, DNA structure, and protein analysis (24). The viral DNA was cloned and was found to hybridize to DNA from tumor extracts (1). In the same study, viral DNA was detected in normal tissues of unaffected individuals and embryos. It has been hypothesized that the tumors arise from activation of an endogenous papillomavirus genome, since the average copy number of viral DNA in skin cells increases with age or with application of a tumor promoter (12-O-tetradecanoylphorbol-13-acetate), and this increase correlates with tumor formation (1). This phenomenon appears to be unique among characterized mammalian papillomaviruses.

The European harvest mouse (Micromys minutus) is a member of the subfamily Muridae and is one of the smallest rodents. Its natural habitat covers much of the Palearctic region, from Britain across Europe and the Soviet Union to Japan (6, 37). In a colony of harvest mice, individuals were diagnosed with cutaneous acanthomatous hyperplasia, squamous papillomas, epidermal inclusion cysts, trichoepitheliomas, and sebaceous carcinomas. In this paper, we describe the identification, cloning, and characterization of a M. minutus papillomavirus (MmPV) associated with these lesions. This represents the first successful isolation of a papillomavirus from spontaneous lesions in a mouse species.

MATERIALS AND METHODS

Animals and tissues. Lesions were first noted on animals maintained as an exhibit in the small-mammal house at a regional zoo. The first animal arrived fixed in Formalin, and tissues were submitted for histologic evaluation and immunohistological staining. Later specimens were obtained live and biopsied or necropsied to obtain tissues for histologic and molecular studies. Additional animals were obtained from the breeder in Indianapolis, Ind., who had provided the zoo with their specimens and from the University of Illinois, Urbana.

Papillomavirus-group-specific antigens were detected in Formalin-fixed, paraffin-embedded tissues as previously described (39), except that an avidin-biotin complex method (ABC system; Vector Laboratories, Burlingame, Calif.) was used for visualization of the bound antibody.

DNA extraction from lesions and normal tissues. Because of
the small size of most specimens (2 by 2 by 2 mm), total DNA was extracted with 1.5-m1 microfuge tubes as follows. Tissues were finely minced or macerated, digested with proteinase K (50 μg/ml; Sigma Chemical Co., St. Louis, Mo.) for 1 to 3 h at 55°C in 300 μl of 10 mM Tris hydrochloride (pH 8.0)—10 mM EDTA, extracted twice with an equal volume of phenol-chloroform (1:1), extracted once with chloroform alone, and ethanol precipitated. Precipitates were dissolved in 300 μl of 10 mM Tris hydrochloride (pH 8.0)—1 mM EDTA (TE buffer), and RNA was removed by RNase A digestion (20 μg/ml, 1 h at 37°C; Sigma) followed by an additional ethanol precipitation.

DNAs were resuspended in 100 μl of 10 mM Tris hydrochloride (pH 8.0)—1 mM EDTA, and concentrations of suitable dilutions were determined by optical density measurements at 260 nm.

Southern blot hybridizations. After electrophoresis in agarose gels, DNAs were transferred to GeneScreen Plus membranes (New England Nuclear Corp., Boston, Mass.) by the method of Southern (36) as modified by the manufacturer. Hybridizations were carried out for 24 h in different concentrations of formamide at 42°C as previously described (25). Filters were washed in 2× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate at temperatures corresponding to the stringency of hybridization (6, 35). DNA probes of high specific activity (generally 108 cpm/μg) were prepared by nick translation (31) with [α-32P]dCTP (>3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Signals were detected by autoradiography with XAR-5 film (Eastman Kodak Co., Rochester, N.Y.).

When indicated, intensifying screens (Cronex Lightning-Plus, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) were used.

Cloning. Viral DNA was initially detected in Southern blots of electrophoresed papillomavirus DNA extracts following hybridization carried out in 20% formamide with a BPV-2 virion DNA probe. By this method, it was also determined that the viral DNA contained a single EcoRI site. To enrich the extracted total DNA sample for papillomavirus DNA, 45 μg of a papilloma extract known to contain viral DNA was digested with EcoRI and was fractionated by subsequent centrifugation (21). The gradient was prepared by mixing an SW28.1 tube, contained 4 to 40% (wt/vol) sucrose in 1 M NaCl-20 mM Tris hydrochloride (pH 7.5)—5 mM EDTA. The DNA, mixed with 10 μg of lambda phage DNA digested with HindIII, which served as an internal marker, was loaded on top of the gradient in a total volume of 100 μl and centrifuged at 92,500 × g for 2 h at 15°C. Fractions (0.5 ml) were collected with a fractionator (ISCO, Lincoln, Nebr.) and 20 μl of every second fraction was electrophoresed on a 0.8% agarose gel with appropriate size markers in parallel lanes. Fractions containing DNA of about 8 kilobases (kb), as determined by markers, were dialyzed against TE buffer and ethanol precipitated. Various concentrations of the precipitate dissolved in TE buffer were ligated with EcoRI-digested pUC18 DNA. TB-1 cells (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) made competent by CaCl2 treatment (21) were transformed with the ligated DNA, and white colonies from plates containing 5-bromo-4-chloro-3-indolyl-β-d-galactoside were screened for inserts by small-scale alkaline lysis. One clone was obtained, and its identity was confirmed by hybridization with a BPV-2 probe and by using it as a probe against DNA from harvest mouse papillomas in high-stringency Southern blot hybridizations.

Construction of a physical map. Restriction enzyme cleavage sites were determined for the cloned DNA by using single and multiple combinations of enzymes. In addition, to determine the order of cleavage sites for BglII, which cuts the genome in six places, the double-stranded exonuclease Bal31 was used (17). Cloned MmPV DNA (10 μg) was digested with either ClaI or BamHI in a 100-μl volume, and the buffer was then adjusted to 20 mM Tris hydrochloride (pH 8.0)—12 mM MgCl2—12 mM CaCl2—300 mM NaCl. A 15-μl sample, which served as time zero point, was removed, and 1 U of Bal31 (Bethesda Research Laboratories) was added to the mixture. The mixture was incubated at 30°C, and 15-μl samples were removed after 1, 3, 6, 10, 20, and 40 min. Each sample was mixed with 30 μl of 20 mM Tris hydrochloride (pH 7.8)—10 mM MgCl2—1 mM β-mercaptoethanol—25 mM ethylene glycol-bis-(β-aminoethylyther)-N,N,N',N''-tetraacetic acid at 0°C; 5 U of BglII was added and allowed to react for at least 2 h at 37°C. The mixtures were electrophoresed on 1% agarose gels in parallel with a complete BglII-digested DNA, and the locations of sites relative to the initial digestion site (ClaI or BamHI) were determined by the order of disappearance of the bands.

Orientation of the physical map. Several BglII fragments, two fragments surrounding the EcoRI site (EcoRI-SphI and BamHI-EcoRI), and an SphI-ClaI fragment of cloned MmPV were subcloned in M13p18 or M13p19 and were sequenced by the dideoxy termination method of Sanger et al. (34) with a kit obtained from Bethesda Research Laboratories. [35S]dATP and buffer gradient sequencing gels were used (5). Nucleotide similarities between MmPV and human papillomavirus type 1a (HPV-1a) were determined by the ALIGN program of DNASTAR, Madison, Wisc., and these similarities were used to align the two physical maps.

Cross-hybridization analysis. The cloned DNAs of MmPV and 16 other animal and human papillomaviruses were each cleaved with the enzyme used for insertion into their respective vectors, electrophoresed on 1% agarose gels, and transferred to membranes. A radiolabeled MmPV DNA probe (specific activity, 2 × 108 cpm/μg), prepared by nick translation of cloned DNA cleaved with EcoRI and elecrophoresed from an agarose gel, was used for hybridizations of the filters in 25 and 35% formamide. After being washed at an appropriate temperature with ethanol wash, filters were left undigested or digested for 7 days with an intensifying screen. The filter, hybridized in 25% formamide, was washed in 2× SSC—0.1% sodium dodecyl sulfate at 70°C (corresponding to the stringency obtained by hybridization in 50% formamide) and was then reautoradiographed.

Transformation assays. NIH 3T3 and C127I cells were obtained from the American Type Culture Collection, Rockville, Md. Cultures were maintained in 150-cm2 plastic tissue culture flasks (Corning Glass Works, Corning, N.Y.) in minimal essential medium (GIBCO Laboratories, Grand Island, N.Y., and Bethesda Research Laboratories) containing 10% fetal bovine serum (Flow Laboratories, Inc., McLean, Va.) and 100 U of penicillin, 100 U of streptomycin, and 0.25 mg of amphotericin B (Fungizone; Flow Laboratories) per ml in an incubator at 37°C with a 5% CO2 atmosphere. Cells were passaged at a 1:5 ratio approximately every 3 days and were used for assays between passages 6 and 10 as described below.

An extract of ground tail papillomas was prepared in phosphate-buffered saline (pH 7.5) by Dounce homogenization followed by three freeze-thaw cycles and clarification in an Eppendorf centrifuge for 2 min. The extract was filtered through a sterile 0.22-μm-pore-size filter (Schleicher & Schuell, Inc., Keene, N.H.), diluted with minimal essential medium without serum, and used immediately or frozen at
-80°C. This extract was shown to be infectious in harvest mice by tail inoculation. BPV-1 virion particles were prepared by CsCl gradient centrifugation extracts as previously described (25).

Cells about 70% confluent on 60-mm tissue culture plates were covered with 0.5 ml of diluted inoculum (1:5 or 1:25) for 2 h at 37°C. When the cells became confluent (after 1 to 2 days), they were split into five 100-mm culture dishes and were maintained with 10% serum until they became confluent (after 2 to 3 days), at which time the medium was changed to minimal essential medium–2% serum. Cultures were maintained for up to 4 weeks with medium changes every 3 to 4 days. Monolayers were stained periodically with 0.1% crystal violet in 70% ethanol to monitor focus formation.

Samples (10 μg) of MmPV cloned in pUC at the EcoRI site and BPV-1 cloned in pBR322 at the HindIII site were restricted with EcoRI and HindIII, respectively, purified by phenol-chloroform extraction followed by ethanol precipitation, and ligated with T4 DNA ligase. The DNA was reextracted and ethanol precipitated with 60 μg of genomic NIH 3T3 cell DNA, which served as a carrier in subsequent transfection steps (19). Transfection was carried out by the calcium phosphate precipitation method (10) as modified by Groff and Lancaster (11), followed by a 15% glycerol shock (9). After 24 to 48 h, each 60-mm-plate culture was split and plated onto four 100-mm plates, and the cells were maintained as described for infected cells.

Analysis of DNA from lesions and normal tissues. DNAs extracted from 18 lesions from six individuals (1 μg per sample), either digested with EcoRI or undigested, were electrophoresed on 0.8% agarose gels, transferred to GeneScreen Plus membranes, and probed in 50% formamide (Tm – 22°C) with an MmPV-cloned DNA (specific activity, >106 cpm/μg). Additional Southern blots of DNA from tumors and normal tissues were probed in the same way.

To estimate genome copy number in DNA samples, 1 μg of DNA from 17 lesion and 35 normal-tissue DNAs were dot blotted onto nitrocellulose (15) with a Bio-Dot apparatus (Bio-Rad Laboratories, Richmond, Calif.). Nine dilutions of cloned MmPV DNA in 1 μg of carrier DNA, extracted from C127I cells, were also dot blotted onto the filter. The filter was probed with a cloned viral DNA with a specific activity greater than 106 cpm/μg.

RESULTS

Lesions. Adult European harvest mice (M. minutus) of both sexes and at least 18 months of age exhibited firm, raised, usually pigmented masses at the oral and rectal mucocutaneous junctions and on the hair-covered skin of the head, back, limbs, and tail (Fig. 1). In general, multiple lesions at various sites were present, with as many as 11 lesions observed on one individual. The most commonly observed lesions were squamous papillomas, which consisted microscopically of papillary projections of proliferating squamous cells on thin fibrovascular stalks (Fig. 2). Complete necropsy of all animals revealed no internal lesions except in one animal with a solitary sebaceous carcinoma on its head that had multiple gray pulmonary and pleural masses 2 to 3 mm in diameter which were histologically classified as keratinaceous cysts. Complete descriptions of the various lesions found on these animals will be published elsewhere (J. P. Sundberg et al., manuscript in preparation).

Viruslike particles were observed ultrastructurally in nuclei of one squamous papilloma but not in two hyperkeratotic papillomas. Papillomavirus-group-specific antigens were detected in 18 of 28 lesions tested and in no normal tissues or organs.

Cloning of MmPV DNA. Electropherograms of DNA extracted from the first lesions and stained with ethidium bromide did not contain any detectable bands suggestive of viral DNA. However, faint bands were detected when a nick-translated BPV-2 virion DNA was used to probe a Southern blot of these DNAs in 20% formamide (Tm – 44°C). Hybridizations of a BPV-2 DNA probe to restriction enzyme-treated papillomavirus DNA extracts revealed single sites for EcoRI and BamHI.

DNA extracted from a tail papilloma, which contained significant amounts of viral DNA (just visible by ethidium bromide staining), was cleaved with EcoRI, and 6- to 9-kb DNA fragments were isolated by sucrose gradient centrifugation. These fragments were ligated into EcoRI-treated pUC18, transformed into TB-1 cells, and plated with 5-bromo-4-chloro-3-indolyl-p-D-galactoside. White colonies were screened for the appropriate insert by small-scale

FIG. 1. Papillomas on the tail were deeply pigmented and often multiple.

FIG. 2. Squamous papillomas on the tail were characterized by proliferating epithelial projections in a papillary pattern on thin fibrovascular stalks. The sample was stained with hematoxylin and eosin; bar, 250 μm.
judged
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forms based DNA, plasmid was labeled by nick lysis. A alkaline
and HindIII sequences
sequences
Southern mapping of the DNA probe. Fragments of MmPV generated by BgIII digestion were subcloned in M13p18 or M13p19 and were partially sequenced by the technique of Sanger et al. (34). In addition, the small SphI-ClaI, EcoRI-SphI, and BamHI-EcoRI fragments of MmPV were cloned and partially sequenced. With a computer program based on the Wilber and Lipman algorithm (42), several of these short sequences were matched with homologous sequences of the HPV-1a genome (Fig. 4). The

FIG. 3. Physical map of the MmPV genome linearized at the EcoRI site.

FIG. 4. Colinearity of MmPV and HPV-1a DNAs as revealed by sequence analysis. Several small fragments of MmPV DNA generated by BgIII or SphI-ClaI digestion were subcloned in M13p18 or M13p19 and partially sequenced by the method of Sanger et al. (34). Alignment with similar sequences in HPV-1a DNA was determined by the ALIGN program in DNASTAR using a k-tuple size of 5 and a gap penalty of 10. Three sequences, labeled A, B, and C, corresponding to the SphI-ClaI fragment and two BgIII fragments, respectively, showed enough homology with HPV-1a sequences to permit alignment. The homologous areas on each physical map are labeled with the same letter and correspond to the pairs of sequences below the figure. The MmPV sequence is the upper sequence of each pairing, and identical nucleotides are indicated (*). Locations of the HPV-1a sequences are 2,472 to 2,672 (A), 5,940 to 6,140 (B), and 6,142 to 6,242 (C). The percentage of matched bases in each pair of aligned sequences is 49.5% (A), 60% (B), and 59% (C). The genomic organization of HPV-1a is based on the work of Danos et al. (7).
Sphl-ClaI fragment corresponded to part of the E1 open reading frame, and two of the BglII fragments corresponded to parts of the L1 open reading frame. The distances between homologous sequence A and sequences B and C were approximately equal for each respective genome (Fig. 4). The EcoRI-SphI and BamHI-EcoRI sequences were less homologous but showed similarities with the E7 and 5' E1 open reading frames of several HPV's (data not shown).

Cross-homology analysis. MmPV DNA, isolated from its vector by electrolution from agarose gels after restriction digestion, was nick translated and used as a probe in Southern blot hybridizations to other papillomavirus DNAs in 25, 35, and the equivalent of 50% formamide (16). Table 1 shows the results for hybridizations to 16 papillomavirus DNAs. No annealing was observed at the highest stringency ($T_m = 22^\circ$C). In 35% formamide ($T_m = 33^\circ$C), hybridization was limited to HPV-1a and the papillomavirus from M. natalensis (MnPV), and there was very slight hybridization to rabbit oral papillomavirus. MnPV DNA annealed to 10 of the 16 papillomavirus DNAs at $T_m = 40^\circ$C.

Transformation experiments. Foci became visible in BPV-1 virion-infected NIH 3T3 and C127I cells 12 days after infection, but not in cells exposed to dilutions of an infectious MmPV tail papilloma extract. Similarly, BPV-1 DNA-transfected NIH 3T3 and C127I cells showed foci by day 14 (40 foci per µg for NIH 3T3 and 160 foci per µg for C127I cells), while the MmPV DNA-transfected cells showed no foci. The NIH 3T3 cultures were observed for 15 days and the C127I cultures were observed for 4 weeks with no apparent transformation by MnPV. MnPV nucleotide sequences were, however, detected by Southern blot hybridization in Hirt extracts (13) of transfected cells at 4, 9, and 17 days (the last for C127I only) after transfection (data not shown).

Analysis of tumors. Total DNA was extracted from various lesions and analyzed by using a high-specific-activity (>10$^6$)

**TABLE 1. Hybridization of MmPV DNA to the DNAs of other papillomaviruses**

<table>
<thead>
<tr>
<th>Virus*</th>
<th>$T_m = 40^\circ$C</th>
<th>$T_m = 33^\circ$C</th>
<th>$T_m = 22^\circ$C</th>
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<tbody>
<tr>
<td>Equine cutaneous papillomavirus</td>
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<td>Canine oral papillomavirus</td>
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<tr>
<td>Rabbit oral papillomavirus</td>
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<tr>
<td>BPV-1</td>
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<td>BPV-2</td>
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<td>BPV-4</td>
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<td>BPV-5</td>
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<td>BPV-6</td>
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<td>HPV-1a</td>
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<td>HPV-11</td>
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<td>HPV-16</td>
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<td>HPV-18</td>
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<tr>
<td>Deer papillomavirus</td>
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<tr>
<td>European elk papillomavirus</td>
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<td>MnPV</td>
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<tr>
<td>MmPV</td>
<td>+/+</td>
<td>+/+</td>
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* Cloned viral DNAs used in this study were obtained from L. Gissmann and H. zur Hausen (HPV 11, HPV 16, and HPV 18), M. S. Campo (BPV-4, BPV-5, and BPV-6), W. D. Lancaster (HPV-1a), M. Nasseri (CRPV), J. Moreno-Lopez (European elk papillomavirus), and E. Amtmann (MnPV). BPV-1, BPV-2, deer papillomavirus, canine oral papillomavirus, equine cutaneous papillomavirus, rabbit oral papillomavirus, and MmPV were cloned in our laboratory.
cpm/μg). MmPV-cloned DNA probe in Southern blot hybridizations under conditions of high stringency. Figure 5 shows results obtained when 1 μg of undigested DNA from 15 lesions was probed. The three characteristic forms of double-stranded, superhelical circular DNA (circular, linear, and supercoiled forms) were evident in all lesions. The same samples were also digested with EcoRI, and a Southern blot revealed only one band of about 7.6 kb for all samples (data not shown). Undigested DNA and DNA digested by EcoRI, BamHI, and HindIII from three additional lesions (a sebaceous carcinoma and a pulmonary lesion found in one animal and hairless skin from an individual affected with mites) were analyzed by the same method in parallel with DNA from a tail papilloma (data not shown). Viral DNA in the carcinoma and the pulmonary lesion appeared identical to DNA from the papilloma. No evidence of viral DNA was observed in the skin specimen; however, analysis of another lesion of this type in another individual did reveal viral DNA (data not shown). Overexposed autoradiographs of all filters did not contain additional bands, suggesting that viral DNA existed only in unintegrated forms. To confirm this observation, 10 μg of DNA from four papillomas and six lesions judged histologically to be carcinomas were digested with EcoRI and were probed in the same manner. Under no circumstances, including gross overexposure of the filter, were additional bands suggestive of integrated forms detected (data not shown). The sensitivity of these hybridizations was judged to be less than one viral copy per cellular genome, as determined by hybridization to a predetermined range of DNA concentrations on the same filter.

An estimate of viral copy number in each lesion was obtained by dot blotting 1 μg of each DNA and dilutions of cloned MmPV DNA in 1 μg of carrier DNA from C127I cells. The blot was probed with a nick-translated, MmPV-cloned DNA in 50% formamide, and the signals from the DNA in each lesion were compared with those of the standards. Lesions contained from 100 to over 10,000 viral DNA copies per genome equivalent (Fig. 6).

Analysis of normal tissues and organs. Tissues and organs (35 samples) that were normal (by gross and histologic appearance) were obtained during necropsy of six mice with lesions elsewhere on their bodies. Southern blot hybridization of 5 μg of DNA from 27 samples revealed the presence of MmPV-specific DNA in 18 tissues (Fig. 7). Viral DNA was readily detected in the unaffected skin of another individual. Faint bands which developed upon overexposure suggested that viral DNA was also present in six of seven other tissues (data not shown).

Samples (1 μg) of each DNA from 35 normal tissues from six mice were also dot blotted to estimate viral copy number. Results (Fig. 6) indicate that many tissues contained 5 to 50 copies per genome equivalent and that three of four normal skin and one of five lung samples contained more than 100 copies. Of these 35 samples, 18 were judged to have background-level signals; however, the sensitivity of the assay was limited to about five copies per genome equivalent, and Southern blots revealed the presence of viral DNA in some of the samples.

**DISCUSSION**

A colony of European harvest mice was found to have a variety of lesions at cutaneous and mucocutaneous sites. Several distinct morphologic features were noted, including lesions histologically classified as squamous papillomas, acanthomatus hyperplasia, epidermal inclusion cysts, trichoepitheliomas, and sebaceous carcinomas. The morphology of these lesions will be described in detail (Sundberg et al., in preparation). Papillomavirus-group-specific antigens were detected in 18 of 28 lesions, and viral particles were observed in one of three tumors examined. The presence of a papillomavirus was also confirmed by Southern blot hybridization of lesion DNA with a BPV-2 virion DNA probe under conditions of low stringency.

The complete genome of MmPV was cloned at a single EcoRI site in pUC18. A physical map of the 7.6-kb (by gel electrophoresis) molecule was determined by single and multiple digestions with restriction endonucleases and by Bal 31 exonuclease reactions. Colinearity of the genomes of MmPV and HPV-1a was established by sequencing several short regions of MmPV DNA. Comparisons of these sequences to BPV-1, cottontail rabbit papillomavirus (CRPV), and HPV-16 sequences revealed homologies at similar locations; however, since the regions sequenced were short and discontinuous and the sequences were determined for only one strain, detailed interpretation of the observed homologies seemed inappropriate. By Southern blot hybridization studies, MmPV was shown to share relatively more homology with HPV-1a, MmPV, and rabbit oral papillomavirus DNAs than with the DNAs of 13 other papillomaviruses.

Papillomaviruses or papillomavirus DNAs which have been reported to have transforming ability include BPV-1 (4, 18), deer papillomavirus (12), European elk papillomavirus (38), CRPV (40), HPV-1 (41), HPV-5 (41), and dimeric HPV-16 (43). Although there are few reports of failed transformation attempts, it is apparent that many other
papillomaviruses do not readily transform tissue culture cells. Attempts to demonstrate transformation of C127I or NIH 3T3 cells with infectious extracts or transfected DNA of MmPV were unsuccessful. It is possible that the form of the DNA or the limited observation period, particularly with the NIH 3T3 cells, precluded observation of morphologic transformation by MmPV.

Viral DNAs were detected in all lesions, regardless of histologic classification. All DNAs appeared as extrachromosomal monomers in three characteristic forms corresponding to the superhelix, relaxed circular, and linear forms. No additional bands suggestive of integrated DNA were observed even under conditions in which one copy per genome equivalent should have been visible. Viral DNA copy numbers ranged from 100 to well over 5,000. These high copy numbers and the presence of viral antigens indicate that productive infection was occurring in many of these tissues. Although insufficient sample numbers preclude a final conclusion, the observations that no viral antigens were detected in two sebaceous carcinomas and that the viral DNA copy number was low (100 copies) in the one carcinoma examined suggest that productive infection was not occurring in these lesions. In one case, multiple lesions with features of epidermal inclusion cysts were found attached to the lungs and pleura of an animal that also had a solitary sebaceous carcinoma on its forehead. No other internal lesions were noted. The pulmonary lesion, which contained viral DNA, may represent a metastatic focus or may have been a separate lesion associated with MmPV. Pulmonary fibromas containing papillomavirus DNA have been observed in European elk (22), and in severe cases, human laryngeal papillomatosis extends to the lungs (23). These observations indicate that pulmonary epithelial cells are susceptible to papillomavirus infection.

Viral DNAs were also detected in many apparently normal tissues from affected individuals. Molecules appeared not to be integrated and were present in lower copy numbers than in lesions. Based on copy number determination, normal skin generally contained more MmPV DNA than internal organs, with the exception of one lung sample. Normal tissue and organ samples were obtained after lesions had been excised, so the possibility of contamination between affected and unaffected tissues cannot be ruled out. However, precautions were taken to change instruments and gloves between the removal of lesions and of normal tissues, and specimens were placed in separate containers. Furthermore, the detection of viral genomes in more than half of the normal tissues and in at least one sample from every necropsied individual make contamination only a remote possibility for all cases.

The progression from benign to malignant lesions has been well documented for CRPV (32) and for HPVs (26, 27, 29) in humans with epidermodysplasia verruciformis. Progression of lesions to malignancy has also been reported for the papillomavirus affecting M. natalensis (33). The detection of viral genomes in normal tissues of harvest mice bears a striking resemblance to MnPV infection, in which viral DNAs are detected in embryos and normal tissues and increase in copy number in the skin as the animal ages and tumors appear (1). It should be noted that all harvest mice examined in this study were at least 1.5 years old. HPV-6 and HPV-11 DNAs have been found in unaffected tissue near anogenital condylomata. These were deemed latent infections, since recurrence after ablation was correlated with the presence of viral DNA in adjacent normal tissue (8). Whether the detection of viral genomes in normal tissues from M. minutus represents the presence of an endogenous latent papillomavirus or a systemic infection with focal neoplasia remains to be determined. Efforts are in progress to obtain unaffected individuals and embryos for further study.

MmPV is the first papillomavirus isolated from a mouse species. The association of viral DNA with a diverse array of skin tumors, the interesting observation of viral DNA in normal tissues, and the ease of handling small creatures make this a very attractive animal model for studying papillomavirus infection. Attempts to introduce this virus into the common laboratory mouse are in progress. The well-defined histocompatibility antigens of the laboratory mouse and the availability of mutant mice may make it possible to study problems pertaining to organ specificity, susceptibility, and malignant tumor formation with this system.

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