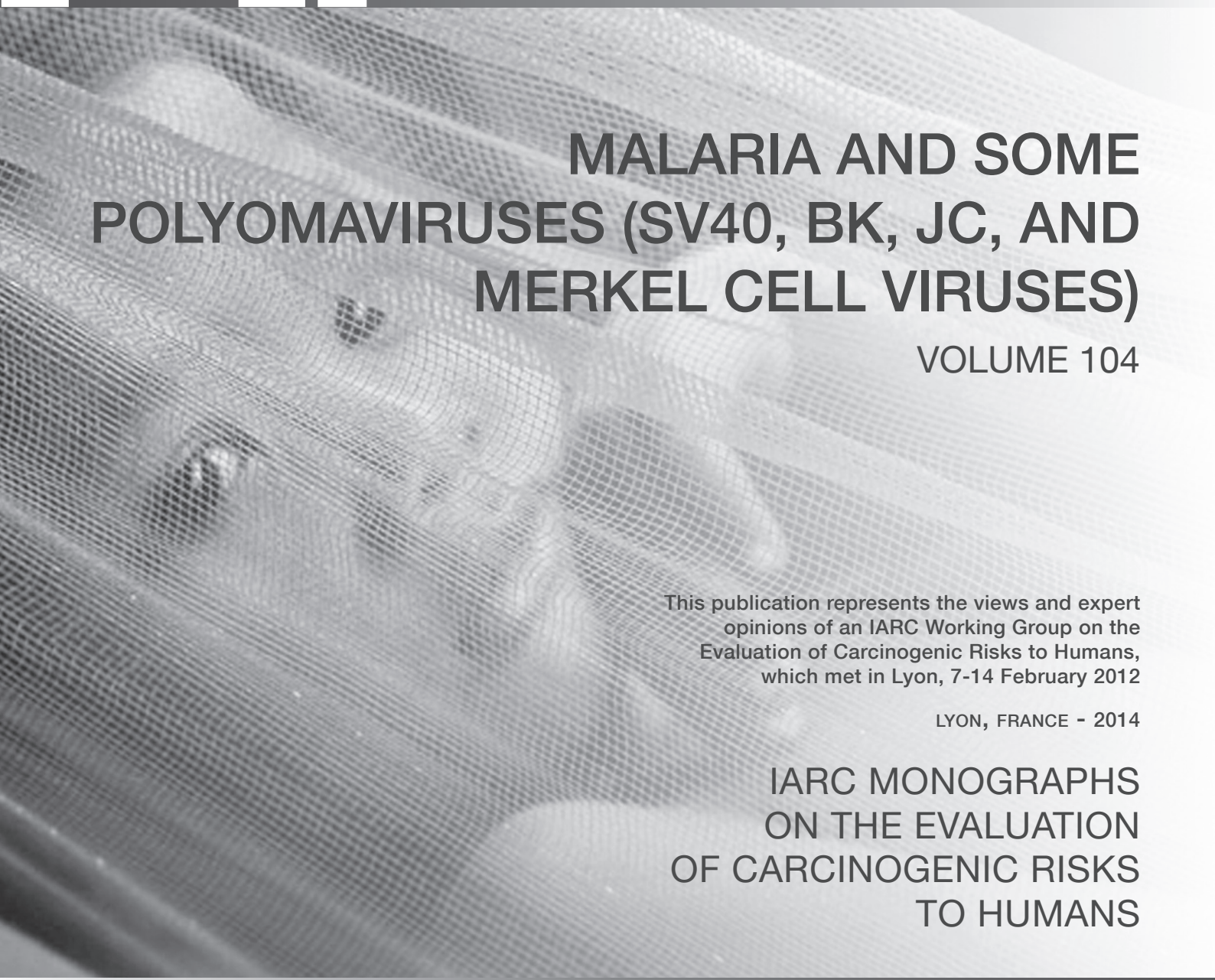
IARC MONOGRAPHS



MALARIA AND SOME POLYOMAVIRUSES (SV40, BK, JC, AND MERKEL CELL VIRUSES)

VOLUME 104

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 7-14 February 2012

LYON, FRANCE - 2014

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

INTRODUCTION TO POLYOMAVIRUSES

1. Discovery

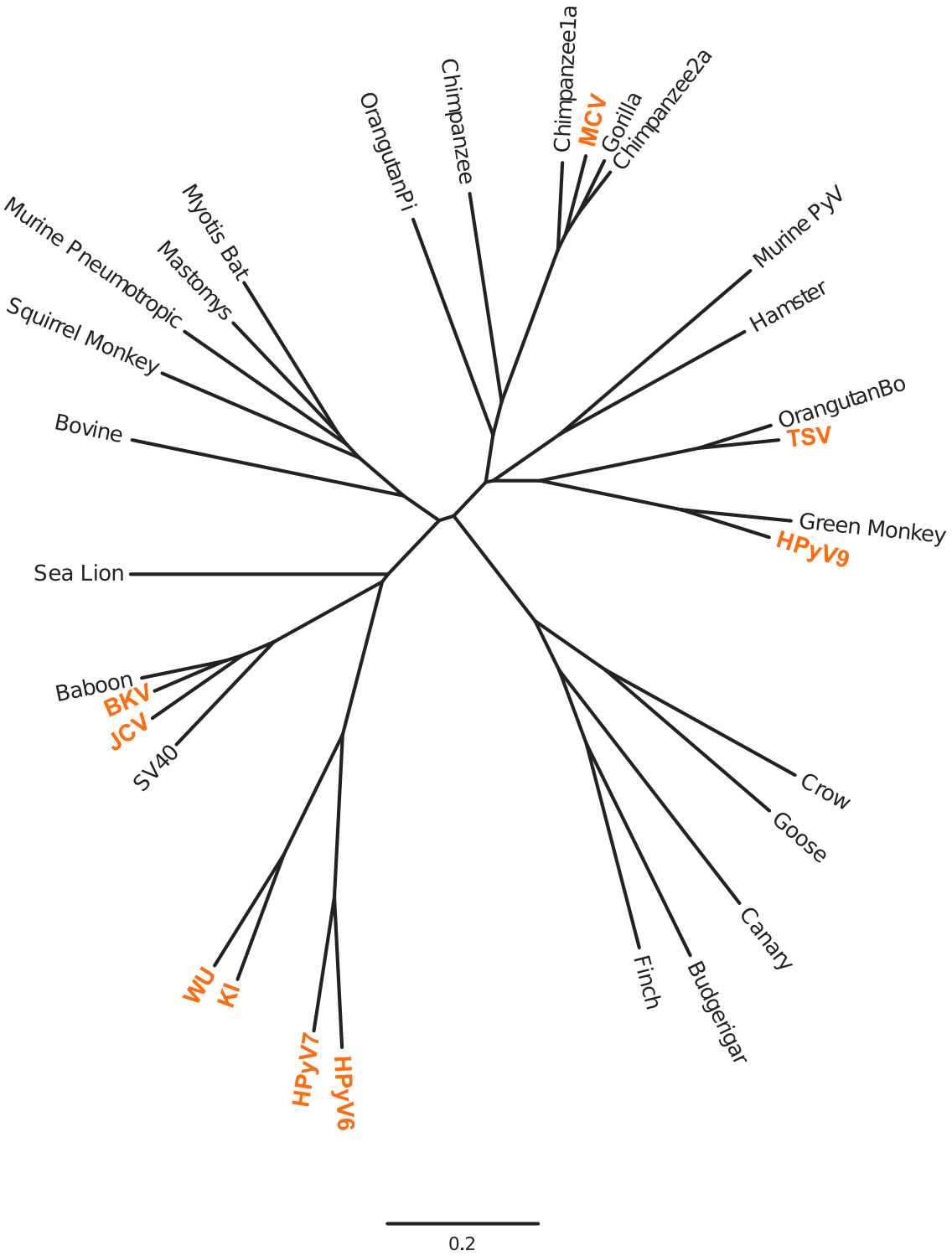
In 1953, Ludwik Gross reported that a filterable infectious agent could cause salivary cancer in laboratory mice (Gross, 1953; Stewart *et al.*, 1957). The cancer-causing agent was found to be a non-enveloped DNA virus that was named murine polyomavirus (from the Greek roots poly-, which means "many," and -oma, which means "tumours"), for its ability to cause tumours in multiple tissues in experimentally infected rodents (reviewed in Sweet & Hilleman, 1960). The discovery spurred renewed interest in the idea that viral infections might be a major cause of cancer in humans.

By the late 1950s, various investigators had succeeded in developing cell culture systems for analysing the transforming activities of murine polyomavirus *in vitro*. This work set the stage for the discovery of the primate polyomavirus simian virus 40 (SV40), which was identified as a contaminant in primary cultures of monkey kidney cells used to produce vaccines against poliovirus (Sweet & Hilleman, 1960; Eddy *et al.*, 1962). Millions of individuals were exposed to infectious SV40 virions present in contaminated polio vaccines administered between 1955 and 1963 (reviewed in Shah & Nathanson, 1976; Dang-Tan *et al.*, 2004).

In 1971, the first two naturally human-tropic polyomaviruses were discovered in specimens from immunocompromised patients (Gardner *et al.*, 1971; Padgett *et al.*, 1971). The two viruses, BK polyomavirus (BKV) and JC polyomavirus (JCV), were eventually found to chronically infect the great majority of humans worldwide (reviewed in Abend *et al.*, 2009; Maginnis & Atwood, 2009). The apparent ubiquity of BKV and JCV makes it difficult to correlate seropositivity for BKV- or JCV-specific antibodies with specific disease states, such as cancer.

Reports in the past four years have revealed the existence of seven more human polyomaviruses. Perhaps the most intriguing of the new species, named Merkel cell polyomavirus (MCV), was discovered through a directed genomic search of an unusual form of skin cancer, Merkel cell carcinoma (MCC) (Feng *et al.*, 2008). Another new polyomavirus, trichodysplasia spinulosa-associated polyomavirus (TSV), was isolated from a rare hyperplastic (but non-neoplastic) trichodysplasia spinulosa skin tumour that can occur in transplant patients (van der Meijden *et al.*, 2010). Little is currently known about the cancer-causing potential of TSV. Five other recently discovered human polyomaviruses – named WU polyomavirus (WUV), KI polyomavirus (KIV), human polyomavirus 6 (HPyV6), HPyV7, and HPyV9 – have not so far been clearly associated with human disease states (Allander *et al.*, 2007; Gaynor *et al.*, 2007; Schowalter *et al.*, 2010; Sauvage *et al.*, 2011; Scuda *et al.*, 2011). The remaining *Monographs* in this Volume will focus on SV40, BKV, JCV, and MCV.

Fig. 1.1 Phylogenetic tree of known polyomavirus species



Human-derived species are in bold type.
Prepared by the Working Group.

2. Taxonomy and phylogeny

The exterior structure of the non-enveloped capsids of members of the viral family Polyomaviridae is strikingly similar to the capsids of a different family of non-enveloped viruses called the Papillomaviridae. Both families carry circular, double-stranded DNA (dsDNA) genomes. Based on these considerations, the two virus groups were originally classified within a single family, the Papovaviridae (a sigla condensation of PApillomavirus POlyomavirus simian VAcuolating). However, sequencing of the genomes of various polyomaviruses and papillomaviruses revealed essentially no detectable sequence homology between the two virus groups and furthermore showed that the two groups had dramatically different genetic organization. Since the sequencing results indicate that polyomaviruses and papillomaviruses probably never shared a common viral ancestor, they were officially split into separate viral families.

Interestingly, several recent reports suggest that polyomaviruses and papillomaviruses may occasionally recombine with one another to produce viable chimeric viruses with mixed genetic characteristics of both families (Woolford *et al.*, 2007). Another recent report documenting a novel viral species that infects eels suggests that polyomaviruses might also recombine with members of other families of DNA viruses to produce viable chimeric progeny (Mizutani *et al.*, 2011). The taxonomy of these apparently chimeric viral species is currently undefined.

A recent proposal that is currently being reviewed by the International Committee on Taxonomy of Viruses suggests that the various members of the family Polyomaviridae be grouped into separate genera (Johne *et al.*, 2011). If approved, this would result in the division of the current sole genus *Polyomavirus* into three genera: *Orthopolyomavirus*, *Wukipolyomavirus*, and *Avipolyomavirus*. The first two genera encompass all the currently known human- and primate-tropic species, while the third genus includes species thought to be tropic only for birds. The four primate polyomavirus species that have been proposed to be potentially implicated in human cancer (SV40, BKV, JCV, and MCV) are all members of the proposed genus *Orthopolyomavirus*.

Although SV40, BKV, and JCV are separate viral species, they are very closely related to one another, sharing about 70–75% identity at the nucleotide level across the entire genome. MCV is only distantly related to the SV40 cluster, sharing less than 35% nucleotide identity across the entire genome (Fig. 1.1). Phylogenetic analyses of BKV and JCV isolates from different populations worldwide have shown that the two virus species exhibit a geographical pattern of genetic drift that closely resembles proposed patterns of prehistoric human migration (reviewed in Yogo *et al.*, 2004, 2009). MCV is somewhat more closely related to murine polyomavirus, with the two species sharing about 50% nucleotide identity across the complete viral genome. Phylogenetic trees based on the nucleotide or amino acid sequences of individual viral gene products give similar results, suggesting that the members of the SV40 cluster diverged from one another relatively recently, while the split between MCV and members of the SV40 cluster occurred in the much more distant past.

3. Structure of the virion

The exterior surface of the polyomavirus virion is a naked protein capsid composed entirely of a single virally encoded protein called capsid viral protein 1 (VP1) (Stehle *et al.*, 1996) (see cover photograph). The virion contains a total of 72 pentameric VP1 capsomers arranged on a $T = 7$d icosahedral lattice (Yan *et al.*, 1996). VP1 subunits are folded into a classic eight-stranded β

jellyroll fold that is shared among a wide variety of viral capsid proteins and the cellular protein nucleoplasmin (Stehle *et al.*, 1994; Carrillo-Tripp *et al.*, 2009). The jellyroll, which forms the core of the pentameric VP1 interface, is arranged perpendicular to the spherical virion, such that the assembled virion has a distinctive knobby appearance. An additional capsid protein, VP2, as well as its N-truncated isoform VP3, associate with the central lumen of each VP1 capsomer (Chen *et al.*, 1998).

In all extensively studied examples, it appears that polyomavirus infectious entry requires interactions between VP1 and one or more cellular glycans that carry at least one sialic acid residue. In several distantly related polyomaviruses, the binding site for the sialylated glycan receptor is formed by a pocket along the outer rim of the apical portion of the VP1 capsomer knob (Stehle and Harrison, 1996; Neu *et al.*, 2008; Neu *et al.*, 2010). The loops that form the receptor binding pocket vary extensively between polyomavirus species, and even among closely related polyomavirus subspecies (Luo *et al.*, 2012). This variation may reflect selective pressure to evade recognition by antibodies that occlude the receptor binding site.

The floor of the canyons between the capsomer knobs is formed primarily by N- and *C*-terminal arms of VP1. The arms are stabilized by disulfide bonds between neighbouring VP1 molecules. The fully mature virion shows a remarkably high degree of stability and can remain fully infectious even after aggressive insults, such as heating at 75 °C for 1 hour (Lelie *et al.*, 1987; Sauerbrei & Wutzler, 2009). This high degree of virion stability raises the possibility that polyomaviruses might be transmitted environmentally or via lightly cooked meat products (reviewed in zur Hausen, 2009, 2012).

Purified VP1 can spontaneously self-assemble into virus-like particles (VLPs) that closely resemble the native virion (Salunke *et al.*, 1986). Such VLPs have been widely used for serological analyses of polyomavirus epidemiology (Hamilton *et al.*, 2000; Carter *et al.*, 2003; de Sanjose *et al.*, 2003; Stolt *et al.*, 2003). Purified polyomavirus VLPs can be immunogenic when administered to laboratory animals, raising serum antibody responses capable of neutralizing native virions *in vitro* (Goldmann *et al.*, 1999; Velupillai *et al.*, 2006; Randhawa *et al.*, 2009). VLPs can even be humorally immunogenic in mice in the absence of functional T-cell immunity (Vlastos *et al.*, 2003), suggesting that VLP-based immunogens might be effective in immunocompromised human subjects who may be at greater risk of polyomavirus-associated disease. This suggests that polyomaviruses might be a suitable target for the development of VLP-based preventive vaccines, similar to the highly successful VLP-based vaccines against hepatitis B virus and human papillomaviruses (HPV).

4. Genomic organization, gene products, and replication

The circular ~5 kb dsDNA polyomavirus genome is roughly divided into two oppositely oriented transcriptional units separated by a non-coding control region (NCCR) (reviewed in Gu *et al.*, 2009) (Fig. 1.2). One transcriptional unit encodes several T-antigens, such as small T-antigen (sT) and large T-antigen (LT). The other transcriptional unit encodes the VP1 and VP2/3 capsid proteins and the agnoprotein. The term T-antigen historically derives from their expression in SV40-induced tumours (Rapp *et al.*, 1964, 1965). Although T-antigen sequences vary among the mammalian polyomavirus species, the overall arrangement of major functional domains of the singly spliced LT gene is broadly conserved and sT is expressed from an unspliced open reading frame encoding a protein of low relative molecular mass (18–20 kDa). The splice

donor used for mRNAs encoding LT is within the sT open reading frame, such that sT and all LT isoforms share a common leader peptide sequence, typically about 80 amino acids long. In addition, many polyomavirus species express multiply spliced transcripts encoding various T-antigen isoforms. Although murine polyomavirus expresses an additional membrane-bound T-antigen protein called middle T, critical for its transforming properties (Fluck & Schaffhausen, 2009), this gene product does not appear to be expressed by the human polyomaviruses.

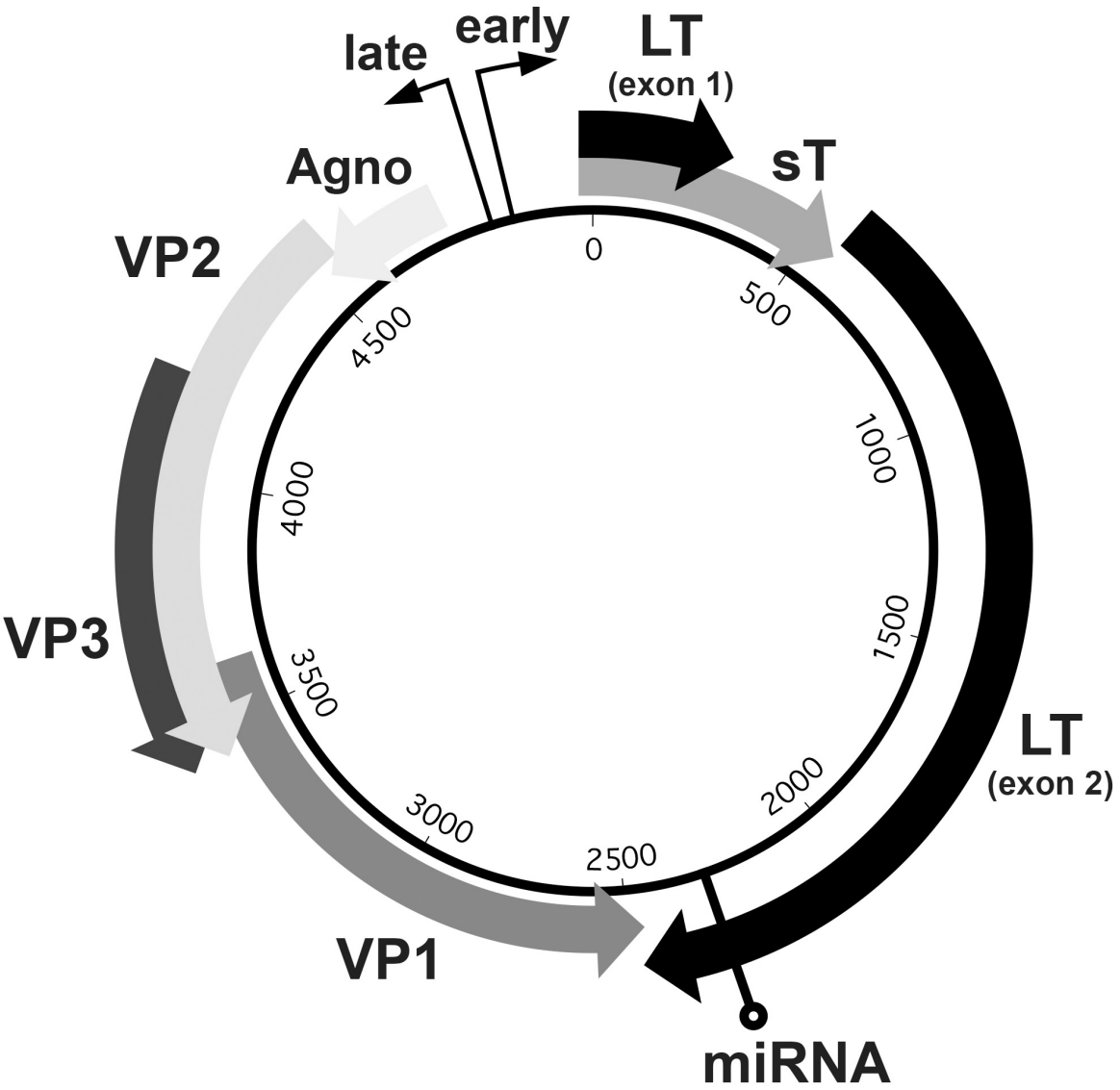
Genes in the T-antigen region are generally expressed at early time points after infectious entry, while the capsid proteins are expressed at high levels only at later time points (Atkin *et al.*, 2009; Feng *et al.*, 2011; Neumann *et al.*, 2011). Early studies of the SV40 NCCR provided some of the first information on the nature of eukaryotic promoters and enhancers, and the SV40 promoter/enhancer system remains one of the most extensively studied examples of eukaryotic transcriptional regulation. The NCCR is highly variable between, and even within, polyomavirus species. Observations indicate that rearrangements in NCCR sequences have important functional consequences for SV40, BKV, and JCV transcriptional regulation, replication, and pathogenesis (reviewed in White *et al.*, 2009a; Yaniv, 2009) (see Section 4 of the *Monographs* on SV40, BKV, and JCV in this Volume).

The 3′ end of the early and late regions is separated by a short segment that contains bi-directional polyadenylation signals. In the case of murine polyomavirus (and likely all other polyomaviruses), termination of transcription is inefficient, particularly for late mRNAs (encoding VP1/2/3). The expression of un-terminated late mRNAs with perfect complementarity to early (T-antigen) mRNAs is thought to play an important role in the regulation of the shift from expression of the T-antigens early after infection to the expression of capsid proteins during the productive late phase of infection (reviewed in Gu *et al.*, 2009). The early-to-late shift may also be partially controlled by expression of microRNAs that appear to be encoded on late strand transcripts transiting the early region (Sullivan *et al.*, 2005; Seo *et al.*, 2008; Seo *et al.*, 2009). These microRNAs may be capable of antagonizing early gene expression and might also exert regulatory effects on the host cell (Bauman *et al.*, 2011).

The polyomavirus NCCR also encompasses the viral origin of replication (Ori). The origin of mammalian polyomaviruses contains multiple pentameric sequences (G(A/G)GGC) that serve as the binding sites for the LT protein. Twelve LT molecules load onto the origin to form a double-hexameric head-to-head ring structure, forming the active helicase for unwinding the origin and recruiting cellular DNA replication machinery (Fanning & Zhao, 2009). Origin unwinding ("melting") occurs adjacent to an A/T-rich sequence. The structure of these conformational changes and DNA binding for LT double hexamers has been solved by crystallography (Li *et al.*, 2003; Gai *et al.*, 2004; Harrison *et al.*, 2011). In addition to its helicase function, LT also serves to recruit various cellular DNA replication factors (Gannon & Lane 1987; Dornreiter *et al.*, 1990; Melendy & Stillman, 1993; Simmons *et al.*, 1996). The species-specific ability of LT to recruit DNA polymerase α-primases of various hosts appears to be a major determinant of polyomavirus host range (see below). Polyomavirus sT proteins also play an unclear, possibly indirect role in facilitating the replication of the viral DNA (Berger & Wintersberger, 1986; Cicala *et al.*, 1994; Kwun *et al.*, 2009).

A small gene product called agnoprotein is encoded 5′ of the VP2 open reading frame in SV40, BKV, and JCV. Proposed roles for agnoproteins include regulation of viral gene expression, virion assembly, cell lysis, and dysregulation of a wide variety of cellular processes (Suzuki *et al.*, 2010; Johannessen *et al.*, 2011; Sariyer *et al.*, 2011; reviewed in Khalili *et al.*, 2005; Moens *et al.*, 2007).

Fig. 1.2 Polyomavirus family genome map



Protein coding sequences are displayed as large arrows. Early transcripts encode small T-antigen (sT) and large T-antigen (LT) proteins. In most polyomaviruses for which transcript maps are available, additional T-antigen isoforms (not shown) are encoded by multiply spliced mRNAs. The late promoter controls the expression of transcripts encoding the VP2, VP3, and VP1 capsid proteins. In SV40, BKV, and JCV, the late transcript also encodes an agnoprotein (Agno). Other polyomaviruses (including MCV) are not known to encode agnoproteins. The origin of replication (Ori, not shown) overlaps with the early promoter. Many polyomaviruses encode a microRNA (miRNA, lollipop) on late region read-through transcripts.
Prepared by the Working Group.

5. Viral life-cycle

Like other non-enveloped virus families, polyomaviruses are believed to breach host-cell membranes after internalization via endocytic pathways. For polyomaviruses whose infectious entry pathways have been extensively studied, a common feature appears to be the engagement of cell-surface glycans that carry at least one sialic acid residue (Tsai *et al.*, 2003; reviewed in Neu *et al.*, 2009). In the cases of murine polyomavirus, SV40, and BKV, the entry receptor is one or more types of sialylated lipids called gangliosides. The target gangliosides appear to be widely distributed on a variety of cell types since these viruses, or reporter vectors based on them, can infect or transduce a wide range of cell lines from various species (Nakanishi *et al.*, 2008). Thus, receptor binding and subsequent steps in the infectious entry process are unlikely to be major determinants of host or tissue tropism. For MCV, there is controversy about whether the sialylated glycans required for infectious entry are displayed in the form of gangliosides or in association with protein (Erickson *et al.*, 2009; Schowalter *et al.*, 2011). In addition to the sialylated glycan co-receptor, MCV also appears to require interactions with a different highly widespread form of cellular glycan called heparan sulfate. Like murine polyomavirus, SV40, and BKV, MCV can bind to and successfully infect (or transduce) a wide range of cell lines from various species (Feng *et al.*, 2011; Neumann *et al.*, 2011; Schowalter *et al.*, 2011). JCV differs from the other polyomavirus species in that it has been proposed to require a serotonin (5-hydroxytryptamine) 2A receptor ($5\text{-HT}_{2A}\text{R}$) in addition to the linear sialylated target LS-tetrasaccharide c (LSTc) (Elphick *et al.*, 2004; Neu *et al.*, 2010). The expression of the unique entry receptors of JCV may dictate its tropism for cells of the central nervous system *in vivo* (see Section 1.1 of the *Monograph* on JCV in this Volume), as well as its relatively restricted cellular entry tropism *in vitro* (Nakanishi *et al.*, 2008).

After engagement of the cognate receptor, polyomaviruses are endocytosed and traffic through the endoplasmic reticulum, where cellular factors facilitate compromise of the cellular lipid bilayer, allowing escape of the viral genome into the cytoplasm. The viral DNA, which may remain at least partially associated with the capsid proteins (Kuksin & Norkin, 2012), then traffics to the nucleus, where transcription factors are recruited to allow early gene expression.

In permissive cells, the early genes target host-cell signalling to drive cell-cycle progression. By promoting cellular expression of DNA replication factors, replication of the episomal viral DNA is facilitated, possibly through a rolling circle replication mechanism (Bjursell, 1978). Expression of the LT antigen can concurrently lead to expression of late gene capsid proteins. Assembly of high levels of encapsidated virus results in active lysis of the host cell.

In many cell types that support the infectious entry of polyomaviruses, the late phase of the viral life-cycle is blocked. Laboratory-adapted BKV and JCV strains with rearranged NCCRs can successfully complete the viral life-cycle in some cultured cell lines. Primary isolates of these viruses with un-rearranged "archetype" NCCRs cannot readily be cultured in conventional cell lines (Broekema & Imperiale, 2012). MCV also appears to replicate very poorly in culture. This may reflect a tendency of these virus species to establish a form of viral latency in many cell types. Under this hypothesis, the viral genome is stably maintained as a low-copy-number episome, expressing few or no viral gene products. In this state, the virus would presumably be resistant to immune clearance, allowing durable long-term maintenance of the infection. This concept is reminiscent of the standard model for the papillomavirus life-cycle, in which the virus is stably maintained in undifferentiated

keratinocytes in the skin and the late phase of the life-cycle is closely tied to the eventual terminal differentiation of the keratinocyte (reviewed in Doorbar, 2005).

6. Host range

Early studies with murine polyomavirus showed that cell lines from non-native hosts, such as Chinese hamsters or Norway rats, do not support high-level replication of the viral genome (reviewed in Atkin *et al.*, 2009). Interestingly, adult hamsters and rats exposed to murine polyomavirus show a greater tendency to develop tumours than do adult mice exposed to murine polyomavirus. SV40 also does not replicate in mouse cells. However, SV40 may pose an exception to the generally narrow host restriction of *Orthopolyomaviruses*, in that it has been shown to replicate in experimentally infected hamsters and can be vertically transmitted to pups *in utero* (Patel *et al.*, 2009). This study also showed that the most robust replication was observed using laboratory-adapted SV40 isolates carrying complex NCCR rearrangements.

Consistent with the apparently limited host range of murine polyomavirus, phylogenetic analysis suggests that members of the proposed *Orthopolyomavirus* genus tend to co-speciate with their host mammals. For example, various chimpanzee and gorilla subspecies each appear to harbour host-specific relatives of MCV (Leendertz *et al.*, 2011). Given the apparently slow rate of genetic divergence of polyomaviruses (Yogo *et al.*, 2009), it appears unlikely that members of this cluster of MCV-related viruses are transmitted between their great ape hosts.

In contrast to *Orthopolyomaviruses*, it appears that at least one member of the proposed genus *Avipolyomavirus* is naturally transmitted among distantly related bird species (Johne *et al.*, 2011). There are currently no comparable examples of productive, naturally occurring transmission of a polyomavirus from one host mammal to another.

References

Abend JR, Jiang M, Imperiale MJ (2009). BK virus and human cancer: innocent until proven guilty. *Semin Cancer Biol*, 19: 252–260. doi:10.1016/j.semcancer.2009.02.004 PMID:19505653

Allander T, Andreasson K, Gupta S *et al.* (2007). Identification of a third human polyomavirus. *J Virol*, 81: 4130–4136. doi:10.1128/JVI.00028-07 PMID:17287263

Atkin SJ, Griffin BE, Dilworth SM (2009). Polyoma virus and simian virus 40 as cancer models: history and perspectives. *Semin Cancer Biol*, 19: 211–217. doi:10.1016/j.semcancer.2009.03.001 PMID:19505648

Bauman Y, Nachmani D, Vitenshtein A *et al.* (2011). An identical miRNA of the human JC and BK polyoma viruses targets the stress-induced ligand ULBP3 to escape immune elimination. *Cell Host Microbe*, 9: 93–102. doi:10.1016/j.chom.2011.01.008 PMID:21320692

Berger H & Wintersberger E (1986). Polyomavirus small T antigen enhances replication of viral genomes in 3T6 mouse fibroblasts. *J Virol*, 60: 768–770. PMID:3022009

Bjursell G (1978). Effects of 2′-deoxy-2′-azidocytidine on polyoma virus DNA replication: evidence for rolling circle-type mechanism. *J Virol*, 26: 136–142. PMID:206720

Broekema NM & Imperiale MJ (2012). Efficient propagation of archetype BK and JC polyomaviruses. *Virology*, 422: 235–241. doi:10.1016/j.virol.2011.10.026 PMID:22099377

Carrillo-Tripp M, Shepherd CM, Borelli IA *et al.* (2009). VIPERdb2: an enhanced and web API enabled relational database for structural virology. *Nucleic Acids Res*, 37: Database issueD436–D442. doi:10.1093/nar/gkn840 PMID:18981051

Carter JJ, Madeleine MM, Wipf GC *et al.* (2003). Lack of serologic evidence for prevalent simian virus 40 infection in humans. *J Natl Cancer Inst*, 95: 1522–1530. doi:10.1093/jnci/djg074 PMID:14559874

Chen XS, Stehle T, Harrison SC (1998). Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry. *EMBO J*, 17: 3233–3240. doi:10.1093/emboj/17.12.3233 PMID:9628860

Cicala C, Avantaggiati ML, Graessmann A *et al.* (1994). Simian virus 40 small-t antigen stimulates viral DNA replication in permissive monkey cells. *J Virol*, 68: 3138–3144. PMID:8151779

Dang-Tan T, Mahmud SM, Puntoni R, Franco EL (2004). Polio vaccines, Simian Virus 40, and human cancer: the epidemiologic evidence for a causal association. *Oncogene*, 23: 6535–6540. doi:10.1038/sj.onc.1207877 PMID:15322523

de Sanjose S, Shah KV, Domingo-Domenech E *et al.* (2003). Lack of serological evidence for an association between simian virus 40 and lymphoma. *Int J Cancer*, 104: 522–524. doi:10.1002/ijc.10993 PMID:12584752

Doorbar J (2005). The papillomavirus life cycle. *J Clin Virol*, 32: Suppl 1S7–S15. doi:10.1016/j.jcv.2004.12.006 PMID:15753007

Dornreiter I, Höss A, Arthur AK, Fanning E (1990). SV40 T antigen binds directly to the large subunit of purified DNA polymerase alpha. *EMBO J*, 9: 3329–3336. PMID:1698613

Eddy BE, Borman GS, Grubbs GE, Young RD (1962). Identification of the oncogenic substance in rhesus monkey kidney cell culture as simian virus 40. *Virology*, 17: 65–75. doi:10.1016/0042-6822(62)90082-X PMID:13889129

Elphick GF, Querbes W, Jordan JA *et al.* (2004). The human polyomavirus, JCV, uses serotonin receptors to infect cells. *Science*, 306: 1380–1383. doi:10.1126/science.1103492 PMID:15550673

Erickson KD, Garcea RL, Tsai B (2009). Ganglioside GT1b is a putative host cell receptor for the Merkel cell polyomavirus. *J Virol*, 83: 10275–10279. doi:10.1128/JVI.00949-09 PMID:19605473

Fanning E & Zhao K (2009). SV40 DNA replication: from the A gene to a nanomachine. *Virology*, 384: 352–359. doi:10.1016/j.virol.2008.11.038 PMID:19101707

Feng H, Kwun HJ, Liu X *et al.* (2011). Cellular and viral factors regulating Merkel cell polyomavirus replication. *PLoS ONE*, 6: e22468 doi:10.1371/journal.pone.0022468 PMID:21799863

Feng H, Shuda M, Chang Y, Moore PS (2008). Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science*, 319: 1096–1100. doi:10.1126/science.1152586 PMID:18202256

Fluck MM & Schaffhausen BS (2009). Lessons in signalling and tumorigenesis from polyomavirus middle T antigen. *Microbiol Mol Biol Rev*, 73: 542–563. doi:10.1128/MMBR.00009-09 PMID:19721090

Gai D, Zhao R, Li D *et al.* (2004). Mechanisms of conformational change for a replicative hexameric helicase of SV40 large tumor antigen. *Cell*, 119: 47–60. doi:10.1016/j.cell.2004.09.017 PMID:15454080

Gannon JV & Lane DP (1987). p53 and DNA polymerase alpha compete for binding to SV40 T antigen. *Nature*, 329: 456–458. doi:10.1038/329456a0 PMID:3309672

Gardner SD, Field AM, Coleman DV, Hulme B (1971). New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet*, 1: 1253–1257. doi:10.1016/S0140-6736(71)91776-4 PMID:4104714

Gaynor AM, Nissen MD, Whiley DM *et al.* (2007). Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog*, 3: e64 doi:10.1371/journal.ppat.0030064 PMID:17480120

Goldmann C, Petry H, Frye S *et al.* (1999). Molecular cloning and expression of major structural protein VP1 of the human polyomavirus JC virus: formation of virus-like particles useful for immunological and therapeutic studies. *J Virol*, 73: 4465–4469. PMID:10196348

Gross L (1953). A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. *Proc Soc Exp Biol Med*, 83: 414–421. PMID:13064287

Gu R, Zhang Z, DeCerbo JN, Carmichael GG (2009). Gene regulation by sense-antisense overlap of polyadenylation signals. *RNA*, 15: 1154–1163. doi:10.1261/rna.1608909 PMID:19390116

Hamilton RS, Gravell M, Major EO (2000). Comparison of antibody titers determined by hemagglutination inhibition and enzyme immunoassay for JC virus and BK virus. *J Clin Microbiol*, 38: 105–109. PMID:10618072

Harrison CJ, Meinke G, Kwun HJ *et al.* (2011). Asymmetric assembly of Merkel cell polyomavirus large T-antigen origin binding domains at the viral origin. *J Mol Biol*, 409: 529–542. doi:10.1016/j.jmb.2011.03.051 PMID:21501625

Johannessen M, Walquist M, Gerits N *et al.* (2011). BKV agnoprotein interacts with α-soluble N-ethylmaleimide-sensitive fusion attachment protein, and negatively influences transport of VSVG-EGFP. *PLoS ONE*, 6: e24489 doi:10.1371/journal.pone.0024489 PMID:21931730

Johne R, Buck CB, Allander T *et al.* (2011). Taxonomical developments in the family *Polyomaviridae*. *Arch Virol*, 156: 1627–1634. doi:10.1007/s00705-011-1008-x PMID:21562881

Khalili K, White MK, Sawa H *et al.* (2005). The agnoprotein of polyomaviruses: a multifunctional auxiliary protein. *J Cell Physiol*, 204: 1–7. doi:10.1002/jcp.20266 PMID:15573377

Kuksin D & Norkin LC (2012). Disassembly of simian virus 40 during passage through the endoplasmic reticulum and in the cytoplasm. *J Virol*, 86: 1555–1562. doi:10.1128/JVI.05753-11 PMID:22090139

Kwun HJ, Guastafierro A, Shuda M *et al.* (2009). The minimum replication origin of Merkel cell polyomavirus has a unique large T-antigen loading architecture and requires small T-antigen expression for optimal replication. *J Virol*, 83: 12118–12128. doi:10.1128/JVI.01336-09 PMID:19759150

Leendertz FH, Scuda N, Cameron KN *et al.* (2011). African great apes are naturally infected with polyomaviruses closely related to Merkel cell polyomavirus. *J Virol*, 85: 916–924. doi:10.1128/JVI.01585-10 PMID:21047967

Lelie PN, Reesink HW, Lucas CJ (1987). Inactivation of 12 viruses by heating steps applied during manufacture

of a hepatitis B vaccine. *J Med Virol*, 23: 297–301. doi:10.1002/jmv.1890230313 PMID:2828525

Li D, Zhao R, Lilyestrom W *et al.* (2003). Structure of the replicative helicase of the oncoprotein SV40 large tumour antigen. *Nature*, 423: 512–518. doi:10.1038/nature01691 PMID:12774115

Luo C, Hirsch HH, Kant J, Randhawa P (2012). VP-1 quasispecies in human infection with polyomavirus BK. *J Med Virol*, 84: 152–161. doi:10.1002/jmv.22147 PMID:22052529

Maginnis MS & Atwood WJ (2009). JC virus: an oncogenic virus in animals and humans? *Semin Cancer Biol*, 19: 261–269. doi:10.1016/j.semcancer.2009.02.013 PMID:19505654

Melendy T & Stillman B (1993). An interaction between replication protein A and SV40 T antigen appears essential for primosome assembly during SV40 DNA replication. *J Biol Chem*, 268: 3389–3395. PMID:8381428

Mizutani T, Sayama Y, Nakanishi A *et al.* (2011). Novel DNA virus isolated from samples showing endothelial cell necrosis in the Japanese eel, *Anguilla japonica*. *Virology*, 412: 179–187. doi:10.1016/j.virol.2010.12.057 PMID:21277610

Moens U, Van Ghelue M, Johannessen M (2007). Oncogenic potentials of the human polyomavirus regulatory proteins. *Cell Mol Life Sci*, 64: 1656–1678. doi:10.1007/s00018-007-7020-3 PMID:17483871

Nakanishi A, Chapellier B, Maekawa N *et al.* (2008). SV40 vectors carrying minimal sequence of viral origin with exchangeable capsids. *Virology*, 379: 110–117. doi:10.1016/j.virol.2008.06.032 PMID:18667220

Neu U, Maginnis MS, Palma AS *et al.* (2010). Structure-function analysis of the human JC polyomavirus establishes the LSTc pentasaccharide as a functional receptor motif. *Cell Host Microbe*, 8: 309–319. doi:10.1016/j.chom.2010.09.004 PMID:20951965

Neu U, Stehle T, Atwood WJ (2009). The Polyomaviridae: contributions of virus structure to our understanding of virus receptors and infectious entry. *Virology*, 384: 389–399. doi:10.1016/j.virol.2008.12.021 PMID:19157478

Neu U, Woellner K, Gauglitz G, Stehle T (2008). Structural basis of GM1 ganglioside recognition by simian virus 40. *Proc Natl Acad Sci USA*, 105: 5219–5224. doi:10.1073/pnas.0710301105 PMID:18353982

Neumann F, Borchert S, Schmidt C *et al.* (2011). Replication, gene expression and particle production by a consensus Merkel Cell Polyomavirus (MCV) genome. *PLoS ONE*, 6: e29112 doi:10.1371/journal.pone.0029112 PMID:22216177

Padgett BL, Walker DL, ZuRhein GM *et al.* (1971). Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet*, 1: 1257–1260. doi:10.1016/S0140-6736(71)91777-6 PMID:4104715

Patel NC, Halvorson SJ, Sroller V *et al.* (2009). Viral regulatory region effects on vertical transmission of polyomavirus SV40 in hamsters. *Virology*, 386: 94–101. doi:10.1016/j.virol.2008.12.040 PMID:19181358

Randhawa P, Viscidi R, Carter JJ *et al.* (2009). Identification of species-specific and cross-reactive epitopes in human polyomavirus capsids using monoclonal antibodies. *J Gen Virol*, 90: 634–639. doi:10.1099/vir.0.008391-0 PMID:19218208

Rapp F, Butel JS, Feldman LA *et al.* (1965). Differential effects of inhibitors on the steps leading to the formation of SV40 tumor and virus antigens. *J Exp Med*, 121: 935–944. doi:10.1084/jem.121.6.935 PMID:14319408

Rapp F, Butel JS, Melnick JL (1964). Virus-induced intranuclear antigen in cells transformed by papovavirus SV40. *Proc Soc Exp Biol Med*, 116:1131–1135.

Salunke DM, Caspar DL, Garcea RL (1986). Self-assembly of purified polyomavirus capsid protein VP1. *Cell*, 46: 895–904. doi:10.1016/0092-8674(86)90071-1 PMID:3019556

Sariyer IK, Saribas AS, White MK, Safak M (2011). Infection by agnoprotein-negative mutants of polyomavirus JC and SV40 results in the release of virions that are mostly deficient in DNA content. *Virol J*, 8: 255 doi:10.1186/1743-422X-8-255 PMID:21609431

Sauerbrei A & Wutzler P (2009). Testing thermal resistance of viruses. *Arch Virol*, 154: 115–119. doi:10.1007/s00705-008-0264-x PMID:19039515

Sauvage V, Foulongne V, Cheval J *et al.* (2011). Human polyomavirus related to African green monkey lymphotropic polyomavirus. *Emerg Infect Dis*, 17: 1364–1370. PMID:21801611

Schowalter RM, Pastrana DV, Buck CB (2011). Glycosaminoglycans and sialylated glycans sequentially facilitate Merkel cell polyomavirus infectious entry. *PLoS Pathog*, 7: e1002161 doi:10.1371/journal.ppat.1002161 PMID:21829355

Schowalter RM, Pastrana DV, Pumphrey KA *et al.* (2010). Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. *Cell Host Microbe*, 7: 509–515. doi:10.1016/j.chom.2010.05.006 PMID:20542254

Scuda N, Hofmann J, Calvignac-Spencer S *et al.* (2011). A novel human polyomavirus closely related to the African green monkey-derived lymphotropic polyomavirus. *J Virol*, 85: 4586–4590. doi:10.1128/JVI.02602-10 PMID:21307194

Seo GJ, Chen CJ, Sullivan CS (2009). Merkel cell polyomavirus encodes a microRNA with the ability to autoregulate viral gene expression. *Virology*, 383: 183–187. doi:10.1016/j.virol.2008.11.001 PMID:19046593

Seo GJ, Fink LH, O'Hara B *et al.* (2008). Evolutionarily conserved function of a viral microRNA. *J Virol*, 82: 9823–9828. doi:10.1128/JVI.01144-08 PMID:18684810

Shah K & Nathanson N (1976). Human exposure to SV40: review and comment. *Am J Epidemiol*, 103: 1–12. PMID:174424

Simmons DT, Melendy T, Usher D, Stillman B (1996). Simian virus 40 large T antigen binds to topoisomerase I. *Virology*, 222: 365–374. doi:10.1006/viro.1996.0433 PMID:8806520

Stehle T, Gamblin SJ, Yan Y, Harrison SC (1996). The structure of simian virus 40 refined at 3.1 A resolution. *Structure*, 4: 165–182. doi:10.1016/S0969-2126(96)00020-2 PMID:8805523

Stehle T & Harrison SC (1996). Crystal structures of murine polyomavirus in complex with straight-chain and branched-chain sialyloligosaccharide receptor fragments. *Structure*, 4: 183–194. doi:10.1016/S0969-2126(96)00021-4 PMID:8805524

Stehle T, Yan Y, Benjamin TL, Harrison SC (1994). Structure of murine polyomavirus complexed with an oligosaccharide receptor fragment. *Nature*, 369: 160–163. doi:10.1038/369160a0 PMID:8177322

Stewart SE, Eddy BE, Gochenour AM *et al.* (1957). The induction of neoplasms with a substance released from mouse tumors by tissue culture. *Virology*, 3: 380–400. doi:10.1016/0042-6822(57)90100-9 PMID:13434017

Stolt A, Sasnauskas K, Koskela P *et al.* (2003). Seroepidemiology of the human polyomaviruses. *J Gen Virol*, 84: 1499–1504. doi:10.1099/vir.0.18842-0 PMID:12771419

Sullivan CS, Grundhoff AT, Tevethia S *et al.* (2005). SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. *Nature*, 435: 682–686. doi:10.1038/nature03576 PMID:15931223

Suzuki T, Orba Y, Okada Y *et al.* (2010). The human polyoma JC virus agnoprotein acts as a viroporin. *PLoS Pathog*, 6: e1000801 doi:10.1371/journal.ppat.1000801 PMID:20300659

Sweet BH & Hilleman MR (1960). The vacuolating virus, S.V. 40. *Proc Soc Exp Biol Med*, 105: 420–427. PMID:13774265

Tsai B, Gilbert JM, Stehle T *et al.* (2003). Gangliosides are receptors for murine polyoma virus and SV40. *EMBO J*, 22: 4346–4355. doi:10.1093/emboj/cdg439 PMID:12941687

van der Meijden E, Janssens RW, Lauber C *et al.* (2010). Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient. *PLoS Pathog*, 6: e1001024 doi:10.1371/journal.ppat.1001024 PMID:20686659

Velupillai P, Garcea RL, Benjamin TL (2006). Polyoma virus-like particles elicit polarized cytokine responses in APCs from tumor-susceptible and -resistant mice. *J Immunol*, 176: 1148–1153. PMID:16394003

Vlastos A, Andreasson K, Tegerstedt K *et al.* (2003). VP1 pseudocapsids, but not a glutathione-S-transferase VP1 fusion protein, prevent polyomavirus infection in a T-cell immune deficient experimental mouse model. *J Med Virol*, 70: 293–300. doi:10.1002/jmv.10394 PMID:12696121

White MK, Safak M, Khalili K (2009a). Regulation of gene expression in primate polyomaviruses. *J Virol*, 83: 10846–10856. doi:10.1128/JVI.00542-09 PMID:19640999

Woolford L, Rector A, Van Ranst M *et al.* (2007). A novel virus detected in papillomas and carcinomas of the endangered western barred bandicoot (*Perameles bougainville*) exhibits genomic features of both the Papillomaviridae and Polyomaviridae. *J Virol*, 81: 13280–13290. doi:10.1128/JVI.01662-07 PMID:17898069

Yan Y, Stehle T, Liddington RC *et al.* (1996). Structure determination of simian virus 40 and murine polyomavirus by a combination of 30-fold and 5-fold electron-density averaging. *Structure*, 4: 157–164. doi:10.1016/S0969-2126(96)00019-6 PMID:8805522

Yaniv M (2009). Small DNA tumour viruses and their contributions to our understanding of transcription control. *Virology*, 384: 369–374. doi:10.1016/j.virol.2008.11.002 PMID:19068262

Yogo Y, Sugimoto C, Zheng HY *et al.* (2004). JC virus genotyping offers a new paradigm in the study of human populations. *Rev Med Virol*, 14: 179–191. doi:10.1002/rmv.428 PMID:15124234

Yogo Y, Sugimoto C, Zhong S, Homma Y (2009). Evolution of the BK polyomavirus: epidemiological, anthropological and clinical implications. *Rev Med Virol*, 19: 185–199. doi:10.1002/rmv.613 PMID:19530118

zur Hausen H (2009). The search for infectious causes of human cancers: where and why. *Virology*, 392: 1–10. doi:10.1016/j.virol.2009.06.001 PMID:19720205

zur Hausen H (2012). Red meat consumption and cancer: reasons to suspect involvement of bovine infectious factors in colorectal cancer. *Int J Cancer*, 130: 2475–2483. doi:10.1002/ijc.27413 PMID:22212999