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.
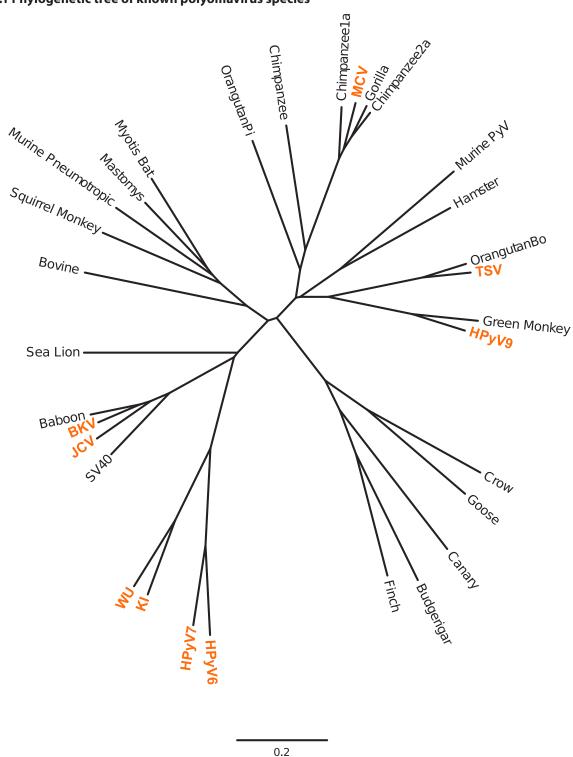
Fig. 1.1 Phylogenetic tree of known polyomavirus species

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 $T = 7d$ 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).
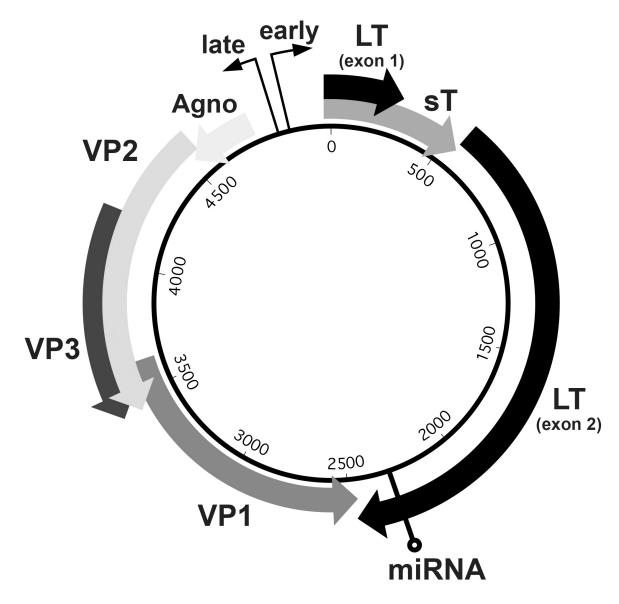
The 3′ end of the early and late regions is separated by a short segment that contains bi-directional polyadenylation signals. In the case of murine polyomavirus (and likely all other polyomaviruses), termination of transcription is inefficient, particularly for late mRNAs (encoding VP1/2/3). The expression 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-HT$_{2A}$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 poly-omavirus 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.

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