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**Entry, infection, replication and egress of human
Polyomaviruses: an update.**

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Abstract

Polyomaviruses (PyVs), belonging to the family *Polyomaviridae*, are a group of small, non-enveloped, double-stranded, circular DNA viruses, widely distributed in the vertebrates. Polyomaviruses cause no apparent disease in adult laboratory mice, but cause a wide variety of tumors when artificially inoculated into neonates or semi-permissive animals. A few human polyomaviruses, such as BK, JC, Merkel cell polyomavirus, have been unequivocally linked to pathogenesis under conditions of immunosuppression. Infection is thought to occur early in life which persists for the lifespan of the host. Over evolutionary time scales, it appears that polyomaviruses have slowly co-evolved with specific host animal lineages. Host cell surface glycoproteins and glycolipids seem to play a decisive role in the infectious entry, and in channeling virions to specific intracellular membrane-bound compartments and ultimately to the nucleus, where the genomes are replicated and packaged for release. Targeting of the infecting virion or viral genome to this multiplication site is therefore an essential process in the productive viral infection as well as in latent infection and transformation. This review summarizes the major findings related to the characterization of the nature of PyV-host protein interactions and their outcomes in host cell invasion.

Key Words: Polyomavirus, infection, receptor, co-receptor

Introduction

Polyomaviruses (PyVs) are non-enveloped, icosahedral, double-stranded small DNA viruses that were among the first mammalian viruses to be analyzed molecularly. Comparison of viral genomes, especially the viral origin of replication, indicates that after evolving from a common ancestor this group may have diverged with their natural hosts (Soeda et al. 1980; Buck et al. 2016). Thirteen human polyomaviruses have been identified to date. BK virus (BKPyV) was the first and JC virus (JCPyV) was the second in the group to be identified and were named after the initials of the patients from whom they were first isolated (Gardner et al. 1971; Padgett et al. 1971). Human polyomavirus KI (KIPyV) and WU (WUPyV) were named after institutions where they were first reported (Allander et al. 2007; Gaynor et al. 2007). Merkel cell polyomavirus (MCPyV) and trichodysplasia spinulosa (TS) polyomavirus (TSPyV) were named based on their discovery in Merkel cell carcinoma (MCC, a rare form of skin cancer) (Feng et al. 2008) and in TS, a rare skin condition (Meijden et al. 2010). HPyV6 and HPyV7 were detected in human skin (Schowalter et al. 2010) and HPyV9 in both serum and skin (Scuda et al. 2011), and were named according to their order of discovery. Malawi polyomavirus (MWPyV) (Siebrasse et al. 2012), also known as HPyV10 (Buck et al. 2012) or Mexico polyomavirus (Yu et al. 2012), was detected in stool and skin samples roughly simultaneous. Saint Louis polyomavirus (STLPyV) was detected in the clinical stool specimen (Lim et al. 2013), HPyV12 in human liver tissue (Korup et al. 2013), and New Jersey Polyomavirus (NJPyV) in endothelium of a muscle biopsy from a pancreatic transplant recipient (Mishra et al. 2014).

Classical viral infection in the host cell is initiated by interactions between viral capsid or envelope proteins and cell surface receptors. Internalization of the virion occurs either by fusion of the envelope and the plasma membrane or via endocytic pathways that lead to escape of the

virion from an endocytic or other vesicular compartment into the cytosol (Smith and Helenius 2004; Marsh and Helenius 2006). Attachment to cellular receptors can directly trigger conformational changes in virus surface structures or can activate specific signaling pathways that facilitate viral entry. Replication of the viral genome, viral protein synthesis, assembly of the viral particles and their release from the cell use the host cellular machinery. The release of the virion occurs via budding followed by exocytosis or cell lysis (Marsh and Helenius 2006). The release of non-enveloped viruses usually occurs through cell lysis, but in some cases, they may escape either by some secretory mechanisms (Altenburg et al. 1980), or may use cellular autophagy pathways for exit (Jackson et al. 2005). Recent evidences indicated that non-enveloped viruses can employ additional escape route as a viral quasispecies population, via membrane-bound vesicles, as found in several enteroviruses, including hepatitis A virus, hepatitis E virus, bluetongue virus, coxsackievirus, rhinovirus, and poliovirus (Altan-Bonnet and Chen 2015).

Seroepidemiological surveys, conducted so far throughout the world show that seroprevalence of BKPyV/JCPyV, SV40, KIPyV, WUPyV and MCPyV is high in those populations (Brown et al. 1975; Stolt et al. 2003; Kean et al. 2009). Antibodies to both BKPyV and JCPyV are present in 70-80% of the adult population. The viruses appear to be ubiquitous, with almost no region of the world being free of anti-PyV antibodies, except in some remote populations (Brown et al. 1975). Primary infections with BKPyV and JCPyV viruses probably occur in childhood and are presumed to be subclinical (Padgett and Walker 1973; Shah et al. 1973). The viruses presumably harbor peripheral blood lymphocytes or can remain latent in the kidney [Salzman (ed.), 1986; Dorries et al. 1994; Greenlee, 1997]. It has been hypothesized that the human PyVs probably persist indefinitely in the kidney and are reactivated and excreted in

the urine during immunological impairment (Heritage et al. 1981; Arthur et al. 1986). Immunosuppression or other viral infections may also lead to reactivation of PyVs. This model of infection may be true to all human PyVs including WUPyV and KIPyV (Heritage et al. 1981; Arthur et al. 1986; Gaynor et al. 2007; Allander et al. 2007).

There has been a resurgence of interest in the study of occurrence, genotype and pathogenic associations of human PyVs in the recent years, especially after the discovery of MCPyV which has been implicated strongly in the etiology of MCC. With the growing incidences of acquired and induced immunosuppression, a thorough knowledge in PyV-host interactions may be beneficial in their control from the standpoint of developing antiviral formulations. This review encompasses the current knowledge on interactions of PyV proteins with cellular components during virion entry, infection, replication and egress from the infected cells.

Human Polyomavirus and Disease

The name 'polyoma' is derived from the Greek terms: *poly*, meaning many, and *oma*, meaning tumors and were named so because they were shown to cause a wide variety of tumors when artificially inoculated into neonatal rodents (Primrose and Dimmock 1980). Polyomaviruse particles are of about 500 Å diameter, having a sedimentation coefficient of 240 S. The capsid is made up of 360 copies of the major capsid protein VP1 arranged in 72 pentamers that are connected to the internal viral protein VP2 or VP3, the minor constituents of the viral capsid. The genome is divided into two major regions, an 'early' region, expressed prior to the initiation of viral DNA replication, and encodes three to four viral tumor antigens (T/t/mT- antigens; LTA_g/STAg/MTAg); and a 'late' region that typically encodes three viral capsid proteins (VP1,

VP2 and VP3). However, MCPyV virions, having native viral regulatory sequences, do not seem to express detectable amounts of VP3 capsid protein in cell culture system (Schowalter and Buck 2013). A small nonstructural protein, agnoprotein or Leader Protein 1 (LP1), is also encoded from the late leader region in few PyVs (SV40, BKPyV and JCPyV), however, other characterized human PyVs do not seem to express this protein. Agnoprotein seems to have roles in viral gene expressions, replication and virus release (Gerits and Moens, 2012). SV40 VP4, a newly identified late gene product, expressed very late during cell lysis, has been found to interact with experimental biological membranes and facilitate their disruption (Daniels et al. 2007; Raghava et al. 2011). A non-coding regulatory region or Non-Coding Control Region (NCCR), comprising of the viral origin of replication, is present between the 'early' and 'late' coding regions. The NCCR is the most variable genomic region that contains regulatory elements and usually undergoes sequence rearrangements during reactivation, thus largely influencing the outcome of the infection. Diverse deletion/duplications within the NCCRs from immunocompromized patients have been shown to augment early gene expression and rate of replication in BKPyV and JCPyV (Gosert et al. 2008; Gosert et al. 2010).

The major capsid protein VP1 (~ 45 kDa) is required in the capsid assembly and the minor capsid proteins – VP2 (~ 38 kDa) and VP3 (~ 27 kDa) help in the uncoating process when the viruses penetrate host cells. Polyomavirus infection in permissive cells is initiated by the binding of the virion to a receptor on the outer cell membrane. The tumor antigens (T/t-antigens) are known to alter the cellular control systems and have a key role in replication, transcription of late viral genes and assembly of virions (Brodsky and Pipas 1998).

BKPyV: The reactivation of BKPyV in immunocompromised host has been associated with haemorrhagic cystitis, BKV-nephropathy, and ureteral stenosis. BKPyV associated post-

transplant nephropathy is a prominent cause of renal transplant dysfunction and has become a clinical problem in allograft recipients (Hirsch 2002; Trofe et al. 2002). The typical renal histological finding of BKPyV-associated nephritis refers to scattered interstitial inflammatory cell infiltration, lysis, atrophy, necrosis of the tubular epithelium, and fibrosis of the interstitium (Drachenberg et al. 2005). Human renal proximal epithelial cells (HRPTEC) are considered to be one of the principal natural targets of BKPyV. Persistence of PyVs in patients with renal allografts may be the important cause of progressive graft dysfunction and graft loss. BKPyV persists in the urinary tract of the individual and are shed in the urine asymptotically. BKPyV exists in four genotypes (I – IV) and subtypes and are distinguishable by neutralization serology (Pastrana et al. 2013). Subtype Ia is thought to be the most prevalent subtype and is prevalent worldwide and subtype IV is less common and its prevalence appears to vary in different world regions (Jin et al. 1993; Jin 1993; Takasaka et al. 2004).

JCPyV: JCPyV was isolated from the brain tissue of a 38 year-old man whose initials were 'JC' (Padgett et al. 1971). JCPyV, a human neurotropic polyomavirus, is associated with neurological diseases such as PML (Progressive Multifocal Leukoencephalopathy). PML, a rare fatal demyelinating disease of the central nervous system, occurs in individuals with immunosuppression involving both humoral and T-cell mediated immune response and is mainly observed in 3%–5% HIV-infected individuals (Major 2010). It has also been observed as a late complication in patients with chronic lymphocytic leukemia, Hodgkin's disease, lymphosarcoma, sarcoidosis (Gardner et al. 1984; Greenlee 1997). The main symptoms of the disease include cognitive impairment, visual deficit and motor dysfunction, although it may vary based on the location and size of the lesions. The role of JCPyV in malignant transformation is controversial. JCPyV virus was thought to exist in five to ten or more genotypes and Type 6 from West Africa

represents the ancestral JCPyV genotype (Pavesi 2003). Several Asian subtypes have been identified and the Indian subtype have been designated as Type 2D (Cui et al. 2004). However, recent viral metagenomic analyses by Buck and others suggest that distantly related PyVs may recombine to produce new chimeric lineages and that PyVs may have slowly co-evolved with individual host animal lineages through an established mechanism known as intra-host divergence (Buck et al. 2016). This led the group to propound a modified classification scheme of the Polyomaviruses that account for these intra-host diversifications.

MCPyV: MC polyomavirus (MCPyV) was detected in Merkel Cell Carcinoma (MCC) (Feng et al. 2008) and has been found to be present in 80-85% of MCC tumor specimen (Dalianis and Hirsch 2013, and references therein). MCPyV has been associated with cellular transformation in highly aggressive primary cutaneous neuroendocrine skin neoplasm termed as MCC and is considered the only human polyomavirus to date to cause tumors in its natural host (Arora et al. 2012; Spurgeon and Lambert 2013). During detection of the virus the LTA_g was found to be truncated before the helicase domain in 80% of the cases (Duncavage et al. 2009), however maintaining pRb-binding ability, and the VP1 region to be deleted (Sharp et al. 2009). MCPyV DNA has been detected mostly in non-melanoma skin cancers such as in squamous cell carcinoma, basal cell carcinoma and Bowens disease in immunosuppressed individuals (Kassem et al. 2009). Apart from detection in MCC and other disease, MCPyV has been detected in upper (Bialasiewicz et al., 2009; Goh et al., 2009) and lower respiratory tract infections (Babakir-Mina et al. 2010).

KIPyV and WUPyV: Both KIPyV and WUPyV were discovered in the year 2007 in broncho-alveolar lavage and nasopharyngeal aspirates (Allander et al. 2007; Gaynor et al. 2007). KIPyV and WUPyV have been detected worldwide in respiratory specimens and clinical samples

(Babakir-Mina et al. 2008; Babakir-Mina et al. 2009; Kiasari et al. 2008; Bialasiewicz et al. 2008; Foulongne et al. 2008; Payungporn et al. 2008; Abed et al. 2007; Han et al. 2007; Le et al. 2007) suggesting a widespread infection but their involvement in human disease or tumor development is not clear (Giraud et al. 2008; Giraud et al. 2009). Reactivation and excretion of KIPyV/WUPyV have also been reported in the gastrointestinal tract of immunosuppressed transplanted patients (Siebrasse et al. 2016) and in HIV-patients (Sharp et al., 2009).

HPyV6 and HPyV7: Human polyomavirus 6 and Human polyomavirus 7 were first found in the year 2010 during a study that involved isolation of full-length wild-type MCV DNA from the skin of healthy volunteers (Schowalter et al. 2010). Both HPyV6 and HPyV7 have been detected in urine, faces and nasopharyngeal swabs in transplant recipients (Siebrasse et al. 2012). Their relationships with a disease in immunosuppression or in malignant transformations have recently been indicated in few studies (Schrama et al. 2014; Ho et al. 2015). A novel HPyV7 strain has recently been associated with HIV/AIDS-associated pruritic rash (Nguyen et al. 2016).

TSPyV: The intracellular presence of virus particles in a patient with trichodysplasia spinulosa was first demonstrated in 1999 (Haycox et al. 1999). Identification of the virus was not achieved until much later in plucked facial spines of a heart transplant patient with trichodysplasia spinulosa (Meijden et al. 2010). Trichodysplasia spinulosa (TS) is a rare skin disease in immunocompromised hosts and is characterised by the development of facial follicular papules and keratotic protrusions (spicules or spines) with alopecia of the eyelashes and brows (Haycox et al. 1999; Heaphy et al. 2004; Osswald et al. 2007; Sadler et al. 2007; Lee et al. 2008; Schwieger-Briel et al. 2010). The presence and high seroprevalence of TSPyV in healthy individuals suggest a subclinical latent infection. The development of tumor with TSPyV remains to be investigated.

HPyV9: Human polyomavirus 9 was first discovered in the serum of a kidney transplant patient (Scuda et al. 2011) and has not been associated with any disease till date. The phylogenetic analysis of HPyV9 genome showed high similarity with the genome of the African Green Monkey-derived lymphotropic polyomavirus (LPV) than with other PyVs.

MWPyV: MWPyV was isolated from a stool sample of a healthy child from Malawi (Siebrasse et al. 2012). In another study done by Buck et al. a virus was identified in the specimen of a patient with warts with hypoglobulinemia, infections, and myeloathexis syndrome and was named HPyV10 that has 95% to 99% similarity with MWPyV, suggesting that they were the same virus (Buck et al. 2012). More recently, a human polyomavirus named MXPpyV was detected in stools of children (Yu et al. 2012), which was found to be closely related to MWPyV/HPyV10. The association of the virus with infection in the gastrointestinal system or any other disease remains to be investigated.

STLPyV, HPyV12 and NJPyV: STL polyomavirus (STLPyV) was recovered from the fecal microbiota of a child in Malawi. However, its association with diarrhea is not significant. Sequence comparison with other polyomaviruses revealed that the virus shares maximum similarity with the MWPyV (Lim et al. 2013). HPyV12 was detected in the liver as well as colon, rectum, and feces of a single individual but its relation to the disease in human remains to be investigated (Korup et al. 2013). The most recently discovered species, NJ polyomavirus (NJPyV), was isolated from the muscle biopsy of a 33-year-old pancreatic transplant recipient (Mishra et al. 2014). The name New Jersey polyomavirus was after the place from where the patient belonged.

Viral Entry

The mechanism of virus entry in non-enveloped viruses can involve various mechanisms such as disruption of endosome at low internal pH in Adenovirus, pore formation at the plasma membrane or endosomes in Poliovirus or entry via caveolae in SV40 (Marsh and Helenius 2006; Mudhakar and Harashima 2009).

Receptors/Coreceptors: A number of cell surface receptors have been identified of which, few act as single molecular species and few as multiple-receptor set for viruses. Some other viruses depend on a more complex receptor which involves interaction with at least two distinct plasma membrane components. Virus-host cell receptor interactions are specific and use distinct receptors and entry mechanisms to infect cells (Table 1). Glycoproteins are proteins containing oligosaccharide chains covalently attached to polypeptide side-chains and glycolipids are lipids attached to the carbohydrate by a glycosidic bond. Gangliosides (GD1a, GD1b, GT1b, and GM1) are glycolipids i.e. glycosphingolipids with one or more sialic acids (e.g. n-acetylneuraminic acid, NANA) linked differentially to the sugar chain. More than 60 gangliosides are known that differ from each other mainly by the position and number of NANA residues. Sialic acid is N- or O-substituted derivative of neuraminic acid, a monosaccharide with a 9-carbon backbone and is found mostly in glycoproteins and gangliosides. Glycosaminoglycans (GAGs) or mucopolysaccharides are long unbranched polysaccharides that consist of a repeating disaccharide unit consisting of an amino sugar along with uronic sugar or galactose except for keratan.

High resolution crystal structures of truncated murine PyV (mPyV) VP1 protein, complexed with branched disialylated hexasaccharide receptor fragments, were instrumental in

revealing the roles played by VP1 pentamers in contacting cellular sugar moieties. Each subunit of the VP1 pentamer has two antiparallel β -sheets with jelly-roll topology, and some of the loops that connect the β -strands are extensive, containing additional secondary structure elements (a small three-stranded anti-parallel β -sheet, one α -helix, and four 3_{10} -helices) (Stehle and Harrison 1996; Stehle and Harrison 1997). These studies showed that at least three surface grooves (pockets 1, 2 and 3) of mPyV VP1 are important in recognizing α 2,3-linked α -5-N-acetylneuraminic acid (sialic acid, SA or NANA) on the surface of susceptible cells. In addition to the usual α 2,3-linked sialic acid, some mPyV strains also bind to branched oligosaccharides that carry a second α 2,6-linked sialic acid. Binding of low tumorigenicity strains to the branched-chain receptors depends on the presence of glycine at position 91 on the outer surface of VP1 (Fried et al. 1981; Cahan et al. 1983; Stehle et al. 1994; Stehle and Harrison 1997). PyV VP1 residues Tyr-72, Arg-77, Gly-78, His-298, and Asn-93 were identified as essential components of pockets 1 and 2. Single mutations in these residues resulted in the complete loss of infectivity (Stehle et al. 1994; Stehle and Harrison 1997). Based on binding and flotation assays Tsai et al (2003) later demonstrated that specific gangliosides, GD1a and GT1b for mPyV and GM1 for SV40, can serve as plasma membrane receptors. Their model, based on crystal structure of VP1-oligosaccharide complex, indicated that the sialic acid- α 2,3-Gal moiety on the left branch of GD1a can interact with VP1, as proposed by others (Stehle et al. 1994; Stehle and Harrison 1996; Stehle and Harrison 1997) (Figure 1). GT1b ganglioside, which has an additional attached sialic acid (residue 4R) can also bind VP1, and therefore, suggests binding of different mPyV strains equally to both GD1a and GT1b (Tsai et al. 2003). Structurally and biologically similar SV40 has also been shown to interact with GM1 gangliosides in flotation and immunoblotting assays (Tsai et al. 2003).

To investigate the linkage of sialic acid in BKPyV infection, Vero cells were treated with *Vibrio cholerae* neuraminidase (NA) or *Streptococcus pneumoniae* sialidase S (SAS) and then challenged with the virus (Dugan et al. 2005). Both NA and SAS inhibited infections, that suggested a role for α 2,3-linked sialic acid in BKPyV infections. The investigators also examined the role of specific linkages of sialic acids in infection and to determine whether glycoproteins or gangliosides were involved in infection through complete removal of sialic acid from Vero cells. The results suggested that α 2,3-linked sialic acid on an N-linked glycoprotein is sufficient to mediate infection (Dugan et al. 2005). Low and coworkers used ganglioside-containing liposomes in a binding assay with purified BKPyV and further proved that terminal α 2-8-linked disialic acid motif present in the GD1b and GT1b gangliosides plays an important role in these interactions (Low et al. 2006). Studies based on VLP-based neutralizing assays indicated that VP1 capsid proteins of each of the major BKPyV genotypes can reciprocally escape from neutralization by antibodies raised against the other types. These serotype-defining differences between the genotypes can be related to a few key amino acid differences within the VP1-BC2 surface loop (Pastrana et al. 2013). Based on cell-based transduction assays, this study also indicated that different BKPyV genotypes and sub-types may have altered attachment requirements and virulence potential *in vivo*, reflecting the changes in amino acids within their VP1 capsomere knob (Pastrana et al. 2013). Komagome and co-workers found through screening that, the virus-like particles (VLP) containing the JCPyV major viral capsid protein VP1 had the highest affinity for neoglycoproteins containing the terminal α 2-6-linked sialic acid (Komagome et al. 2002). While it was clearly established that PyV infections depend on an interaction between VP1 and sialic acids, a glycan-array screening and infectivity assay further established that JCPyV interacts with a linear sialylated penta-saccharide with the sequence NeuNAc- α 2,6-

Gal- β 1,4-GlcNAc- β 1,3-Gal- β 1,4Glc (Lactoseries tetrasaccharide c or LSTc) on host glycoproteins and glycolipids (Neu et al. 2010). Conflicting results are now available with regard to the cellular entry of JCPyV. One set of experiments suggests that JCPyV internalization and cellular entry depends on the expression of serotonin receptor 5-hydroxytryptamine (5-HT) receptor 5-HT_{2A}R in the susceptible cells after attaching to sialylated sugars (Elphick et al. 2004; Assetta et al. 2013). While *in vitro* studies of Chapagain and co-workers suggested that JCPyV infection in the human brain microvascular endothelial cells is independent of 5HT_{2A}R receptor (Chapagain et al. 2007). A subsequent study has shown that there could be a sialylated glycan-independent infectious entry pathway for both JC and BKPyV, as found in heparin-like glycan-dependent MCPyV infection (Schowalter et al. 2011). The study reports complete inhibition of cellular entry of PML-mutant JCPyVs and a sialic acid non-binding mutant BKPyV, when blocked by heparin or a heparan sulfate-blocking antibody (C. B. Buck, personal communication; manuscript in preparation) (Figure 2).

High resolution crystal structures of HPyV6 and HPyV7 VP1 revealed the presence of elongated loops that cover the bulk of the outer virion surfaces and obstruct a groove that binds sialylated glycan receptors as in other PyVs (Stroh et al. 2014). This study could not detect interactions of VP1 with α 2,3- and α 2,6-linked sialic acids by nuclear magnetic resonance spectroscopy, hence HPyV6 and HPyV7 probably engage non-sialylated cellular receptors for host cell recognitions. Liposome floatation assay of Erickson et al. (2009) proved that the sialic acids on both the arms of GT1b are necessary for binding MCPyV VP1, but GD1a, having α 2,3-linked monosialic acid modification on the right arm, and GD1b, having no sialic acid on the left arm, do not bind to MCPyV VP1. However, sialylated glycans are not required for the initial interaction of MCPyV with cultured human cell line but may be required as co-receptors in the

entry process. Glycosaminoglycans (GAGs), such as heparan sulfate and chondroitin sulfate, serve as primary receptors during the entry process (Schowalter et al. 2011; Schowalter et al. 2012).

Systems virology has been instrumental in the global understanding of hitherto unknown virus–host interactions and especially of the dynamic nature of host responses against viruses (Bhattacharjee 2015). Therefore, this approach can discover new mutations in PyV VP1 genes, their implications in tissue tropism, potential for cross-species infectivity, and also in the development of host-based anti-viral drugs (Kilcher and Mercer, 2014). Interestingly, in a recent study, an intra-patient evolution of BKPyV has been shown to produce neutralization-escape VP1 variants that may have altered tissue tropism through binding to a different spectrum of cell-surface sialylated glycan receptors compared to the wild type viruses (C. B. Buck, personal communication; manuscript in preparation).

Receptor Mediated Endocytosis: All PyVs, except JCPyV, generally enter the host cells through structures called ‘caveolae’ present in the plasma membrane of the host (Anderson et al. 1996; Richterova et al. 2001; Pelkmans and Helenius 2003; Damm et al. 2005; Dugan et al. 2006; Parton and Simons 2007). However, SV40 can enter and infect cells by a clathrin-, caveolae- and dynamin II-independent pathways that deliver the virus via non-endosomal, cytosolic organelles to the ER. This work was based on detection of LTA_g in caveolin-1 deficient post-infected mouse embryonic fibroblast and human hepatoma cell line (Damm et al. 2005).

Growing body of evidences indicates that viruses, including nonenveloped viruses, can use specific membranes microdomains or ‘membrane rafts’ to penetrate the host cell. Caveolae

are lipid-raft rich, flask-shaped, 50-100 nm invaginations present in the plasma membranes that are stabilized by VIP-21 or caveolins (e.g., caveolin-1 or cav-1), containing mostly cholesterol and sphingolipid (Rothberg et al. 1992). Caveolin-2 is often associated with caveolin-1 in the same cell or tissue types, whereas expression of caveolin-3 is found in skeletal and cardiac muscles. Caveolae are present at high concentrations on several cell types such as adipocytes, muscle cells, endothelial cells, but few or none in hepatocytes, neuronal cells, and lymphocytes. Most cell surface caveolae show limited motility and dynamics, anchored by actin cytoskeleton that play a key role in controlling their activity and keeping them in place (Thomsen et al. 2002).

SV40 first binds to the major histocompatibility complex class I (MHC-I) antigens on the cell surface and is then trapped in the caveolae, which pinches off as vesicle that contains caveolin-1 (Breau et al. 1992; Pelkmans et al. 2001). Subsequent internalization of the virus requires activation of tyrosine kinases and dynamin II (GTPase) driven GTP hydrolysis, that result in a signaling cascade leading to slow but efficient internalization (Oh et al. 1998; Pelkmans et al. 2002; Parton and Richards 2003). After internalization, some of the caveolar ligands are then sorted to endosomes and the Golgi complex or transported to cav-1-positive, pH-neutral caveosomes (Pelkmans et al. 2001; Parton and Richards 2003). From the caveosomes, SV40 is transported by a microtubule-mediated process to the ER (Pelkmans et al. 2001), and then is released into the cytosol. From the cytosol, the virus particles enter the nucleus through nuclear pore complexes (Kasamatsu and Nakanishi 1998).

Early studies indicated that the binding of poliovirus and papillomaviruses to the cell receptors promotes conformational changes in the major capsid proteins VP1, which are then transmitted to the other capsid proteins (Magnuson et al. 2005; Schelhaas et al. 2007). This conformational change results in the exposure of hydrophobic sequences at the viral capsid

surface that may participate in membrane penetration. These conformational changes in the viral capsid proteins could be detected by changes in their sensitivity to proteases or in the antigenic properties of the viral proteins (Fricks and Hogles 1990; Selinka et al. 2003). The recognition of sialic acid moieties on host cell membrane proteins induces conformational changes in both major and minor capsid proteins, as evidenced by resistance to protease (e.g., trypsin) digestion by the VP1-SA complexes, which is probably required for binding to entry receptors and internalization, thus is crucial for productive infection in mPyV (Cavaldesi et al. 2004).

Kinetics and mechanisms of BKPyV entry have been studied in monkey kidney cell line Vero to investigate the roles of clathrin-dependent or caveola-dependent endocytosis (Eash et al. 2004; Eash and Atwood 2005). Several important findings emanated from these studies: virus internalization is slow and occurs between 2 and 4 hours post-infection, is independent of clathrin-coated-pit assembly, dependent on an intact caveolin-1 scaffolding domain and microtubule network, sensitive to tyrosine kinase inhibition, and requires membrane cholesterol. Similar results were observed in the study of BKPyV entry into human renal proximal tubular epithelial cells HRPTEC (Moriyama et al. 2007). The JCPyV virus enters into the target cells via clathrin-mediated endocytosis, dependent on actin polymerization and proper assembly of clathrin-coated pits, and is comparatively faster than the entry kinetics of SV40 (Pho et al. 2000). After entry into the cells, the virus localizes to the early and late endosomes and is hydrolyzed within the lysosome and therefore, unlike SV40, a low pH environment within the lysosome is critical for the productive JCPyV infection (Ashok and Atwood 2003). Cytoplasmic transport of JCPyV in glial cells depends on microtubule and intermediate filament, as evidenced by the sensitivity of productive infection to cytochalasin D, nocodazole, and acrylamide (Ashok and Atwood 2003). Their data suggested that microtubules may be involved either directly with the

viral proteins or through a dynactin-independent dynein family member, or microtubules may be required for transport of intermediate filament-associated proteins and hence proper assembly of intermediate filaments facilitating JCPyV transport to the nucleus (Ashok and Atwood 2003). While it was known that the engagement of SV40 to cell surface receptors can induce tyrosine kinase-mediated phosphorylation of caveolin-1 (Dangoria et al. 1996), JCPyV binding to human glial cells induces intracellular signal that triggers downstream phosphorylation or activation of the MAPKs ERK1 and ERK2, and the viral entry is dependent on involvement of the endocytic protein eps15 (Querbes et al. 2004).

MCPyV is a member of a divergent clade of polyomaviruses that lack the conserved VP3 N-terminal motif. Schowalter and Buck (2013) also showed that VP3 is neither detectable in MCPyV-infected cells nor found in native MCPyV virions. Experimental mutation of possible alternative VP3-initiating methionine codons did not significantly affect MCPyV infectivity in culture and VP2 knockout resulted in a 100-fold decrease in native MCPyV infectivity. Their pseudovirus-based experiments confirmed that VP2 plays an essential role in infection of some cell but not in all. Their work also showed that myristoylation of N-terminus of VP2 was important for its function.

ER mediated viral trafficking: Complex membranous network within the host cell such as plasma, endolysosomal, and endoplasmic reticulum (ER) membranes, owing to its specialized ER-to-cytosol retro-translocation machinery, have an important role in the initiation and progression of viral infections (Inoue and Tsai, 2013). Polyomaviruses are the only viruses known to penetrate the ER membrane during entry into the host (Tsai et al. 2003; Inoue and Tsai, 2013). Earlier electron microscopic studies revealed that the virus enters into host cells as small

vesicles and are then targeted to the ER (Mattern et al. 1966; Mackay and Consigli 1976; Kartenbeck et al. 1989). Upon arrival on to ER, SV40 is released into the ER lumen from GM1, presumably through a release process involving ER-resident proteins (Inoue and Tsai 2011).

The ER-localized SV40 is large and intact, contains the minor capsid proteins VP2, VP3, and the genome. The intact viral particle then penetrates the ER membrane to reach the cytosol where it disassembles (Inoue and Tsai 2011). During penetration of the ER, the virus first undergoes host-triggered conformational changes which then cross the ER membrane, and then finally is released into the cytosol (Inoue and Tsai 2013). Conformational changes in PyV capsid, the disassembly process and transport of its genetic material from ER to the cytosol require the participation of several putative ER-resident proteins. ER-resident redox-active protein disulfide isomerase family members (PDI, ERp57, and ERp72) can isomerize or reduce the virus disulfide bonds and can play an important role in destabilizing PyV-VP1 disulphide bond network (Inoue and Tsai 2013). However, the precise combination of PDI members engaging a specific PyV family member may differ because of subtle differences in the viral disulfide bond arrangements. In some PyVs, a dimeric redox-inactive PDI protein (ERp29) untangles the VP1 carboxy-terminal arms exposing hydrophobic VP2 and VP3 to generate a hydrophobic viral particle (Magnuson et al. 2005; Rainey-Barger et al. 2007). The hydrophobic virus is probably maintained in a soluble state by binding to the Hsp70 chaperone BiP in a reaction regulated by the J-domain containing co-chaperone ERdj3 (Goodwin et al. 2011).

In the second step, the hydrophobic virus integrates into the ER membrane to initiate membrane penetration, where ER-associated degradation (ERAD) membrane components (Derlin-1, Derlin-2, Sel1L, RMA1, BAP29/BAP31) have been proposed to facilitate PyV's exit, at least indirectly, to the cytosol (Inoue and Tsai 2013 and references therein). Although not

well-understood, the release of virions into the cytosol may depend on the ER membrane J proteins (DNAJ B12, B14, and C18) that stimulate the binding between the virus and cytosolic Hsp70 (Inoue and Tsai 2013). The SV40 capsid proteins VP2/VP3 have been shown to play an important role in the ER escape (putatively through viroporin) and are regulated by their interaction with VP1 major capsid protein (Daniels et al. 2006a; Daniels et al. 2006b). ERp29, structurally related to PDI protein (Mkrtchian et al. 1998), acts as a factor in inducing a conformational change in PyV particles by exposing the C-terminus of VP1 protein without the complete disintegration of the virion structure (Magnuson et al. 2005). Portions of VP2 and VP3 are also exposed that mediate the binding to the ER membrane, although only VP2 can perforate the experimental model membranes (Rainey-Barger et al. 2007).

The ERAD pathway during BKPyV intracellular trafficking has been studied in renal proximal tubule epithelial (RPTE) cells using proteasome and ERAD inhibitors (Jiang et al. 2009; Bennett et al. 2013). Transfection of dominant-negative ER-transmembrane proteins Derlin-1 and Derlin-2 in RPTE cells and GST pull-down assays revealed the involvement of Derlin-1, but not Derlin-2, in BKPyV escape from ER to the cytosol (Jiang et al. 2009; Bennett et al. 2013). Proteasome function was also indicated in successful BKPyV trafficking, like SV40, in proteasome-inhibition-assays (Bennett et al. 2013).

Nuclear Entry and Replication

Nuclear entry: Nuclear transport of molecules less than 9 nm in diameter or proteins smaller than 40 kDa pass through the aqueous channels of nuclear pore complex (NPC) by passive diffusion but transport of larger molecules (>45 kDa) is mediated by facilitated nuclear transport

that requires signals on both, the molecule to be transported and on the cytoplasmic receptors (Pante and Kann 2002 and references therein; Wentz and Rout 2010).

Nucleocytoplasmic transport of cargo >45 kDa depends on transport factors belonging to the family of karyopherin β proteins (e.g. importin β /importin α or IMP β /IMP α). The β -karyopherins that mediate nuclear import are generally known as importins (IMPs), whereas those mediating nuclear export are known as exportins (EXPs). The IMP α s are adaptors that function as heterodimers with IMP β 1 in nuclear import, while IMP β s can mediate transport in either direction through the NPC (Fulcher and Jans 2011). Importins associate with their macromolecular cargo in the cytoplasm, either directly or indirectly via adaptor proteins. They dock to components of the nucleoporins, translocate to the opposite side of the nuclear envelope to release their cargo. The cargo release is achieved by association of the importins with the GTPase Ran in the GTP-bound form (RanGTP). In nuclear export, cargo recognition occurs in the nucleus in the presence of RanGTP and the complex dissociates in the cytoplasm upon GTP hydrolysis. A variety of strategies has been employed by viruses for the transport of their genomic cargo that includes disassembly at the NPC, disruption of the NE or may involve both of them (Fay and Pante, 2015). The nuclear entry/exit of cargo protein involves NPC on the nuclear membrane, recognition of nuclear localization sequences (NLSs) on the proteins to be imported by specific IMPs/EXPs (Lange et al. 2010; Wentz and Rout 2010; Marfori et al. 2011; Fulcher and Jans 2011).

SV40 must expose NLSs that are internal in the virion architecture in order to enter the nucleus via interaction with the host's nuclear import machinery. SV40 may escape from the ER to the cytoplasm and then enter the nucleus through the NPC, or it may enter the nucleus directly from the ER by penetrating the inner nuclear membrane (INM); the available data favors the

former possibility (Cohen et al. 2011). SV40 particles undergo a partial disassembly in the ER which exposes internal capsid proteins VP2 and VP3. The partially disassembled SV40 particles retain some of the SV40 capsid proteins (VP1, VP2, and VP3) in addition to the viral genome (Norkin et al. 2002). The best characterized nucleotransport signal is the classical NLS (cNLS) for nuclear protein import, which consists of either one (monopartite) or two (bipartite) stretches of basic amino acids. Monopartite cNLSs are exemplified by the SV40 LTA_g-NLS (¹²⁶PKKKRRV¹³²) (reviewed in Lange et al. 2007). Accessibility and enhancement of nuclear transport depend on phosphorylation-mediated modulation of NLS/NES of the protein cargo. In SV40 LTA_g, protein kinase CK2 phosphorylation increases the affinity of recognition of the NLS by IMP α / β 1, thereby accelerating the nuclear import rate, which can be further enhanced by phosphorylation by double-stranded DNA-dependent protein kinase (dsDNA-PK) at specific sites (Fulcher and Jans 2011). The SV40 virion may enter the nucleus through the NPC through the interactions between NLSs at the common C-terminal basic amino acids of VP2 and VP3 (VP1/VP2-NLS) and the canonical nuclear import machinery (Nakanishi et al. 2002; Nakanishi et al. 2007). Anti-IMP α /IMP β antibody-based co-immunoprecipitation assays with SV40 DNA post infection in permissive cells indicated that minor capsid proteins must associate with virion DNA for nuclear entry (Nakanishi et al. 2002; Nakanishi et al. 2007). Only a small fraction of virions that expose an NLS deliver their genomes into the nucleus, however, a complete dissociation of the virion within the cell cytoplasm prevented the nuclear entry of the viral DNA (Nakanishi et al. 2002; Nakanishi et al. 2007). Kuksin and Norkin (2012) reported that VP2/VP3-NLS may function only in targeting SV40 disassembly intermediates to the nucleus but SV40 genomes dissociate from VP2/3 before or at the point of nuclear entry and thus enter the nucleus devoid of these viral proteins. However, contrasting views on nuclear entry of SV40

have been provided by a study which indicated that viral infection leads to a breakdown of the nuclear lamina and disruption of the nuclear envelope, allowing the virus to pass directly from the ER lumen to the nucleus (Butin-Israeli et al. 2011).

Since BKPyV-VP2/VP3 share homology with that of SV40 at the basic C-terminal amino acids important for nuclear localization, elegant site-directed BKPyV VP2/VP3-mutation and drug-based inhibition of IMP β in RPTE cells have been designed to ascertain the commonality of nuclear entry mechanism (Bennett et al. 2015). This study could show that specific lysines in VP2/VP3 are important for localization of BKPyV to the nucleus and that VP2K319T and VP3K200T mutants were defective in nuclear localization. The importance of the major capsid protein VP1 was reiterated, as in SV40, by showing that expression of this protein in transfected cells enabled nuclear transportation of mutated minor proteins (VP2K319T and VP3K200T). It was also shown in ivermectin-based inhibition assay that the inhibition of IMP α/β largely affected early gene expression of BKPyV in RPTE cells. In an earlier study by Qu et al. (2004), nuclear entry mechanism in JCPyV was studied that used FITC-labeled JCV-VLP in infection of HeLa (non-permissive) and SVG (permissive) cells. Post-infection fluorescence study showed that the nuclear entry of NLS-deleted-VLPs was markedly inhibited in HeLa/SVG cells, highlighting the importance of VP1-NLS in the nuclear translocation of VLPs. This study also indicated the importance of both IMP α and β in the nuclear entry by JCPyV-VLPs (Figure 3). Nuclear sequestration of human endogenous hVam6p (involved in cytoplasmic lysosomal trafficking) by MCPyV-LTag has been reported which may help in MCPyV replication and egress (Liu et al. 2011).

DNA Replication: The replication of PyV minichromosomes has been historically used as a model system to study the mechanisms of cellular DNA replication (Kelly et al. 1988; Challberg and Kelly 1989; Stillman 1989; Hurwitz et al. 1990). There are controversial views with regard to the resemblance of *in vitro* replication of PyV and the host DNA replication (Fanning et al. 2009). Recent findings indicate that PyV minichromosomes undergo multiple rounds of replication and there is a dispensability of DNA polymerase ϵ (epsilon) (Fanning et al. 2009). Additionally, it was found that at least SV40 and mPyV induce an ATM-mediated DNA damage response in infected cells, which might in the contrary arrest normal cell cycle. The present state of knowledge indicates that simian SV40 and mPyV manipulate host DNA damage signaling and repair pathways, in a largely unknown manner, to control viral minichromosome replication (Fanning et al. 2009). In the MCPyV infected cells, components of the ATM- and ATR-mediated DNA damage response (DDR) pathways were found to accumulate in the MCPyV LTag-positive nuclear foci, suggesting an important role of DDR in viral DNA replication (Tsang et al. 2014).

The *in vitro* study of SV40 replication has been instrumental in establishing fundamental processes of initiation of eukaryotic DNA replication (Bullock 1997). The replication of SV40 or mPyV DNA requires the viral origin of DNA replication and only one virus-encoded protein, the large T-antigen (LTag) to replicate the viral mini chromosome. The other necessary 40 proteins, a set of ten human cellular proteins, are supplied by the host cells (Waga and Stillman 1994; Bruckner et al. 1995; Bullock 1997). In JCPyV, Hsp70 interacts with VP2/VP3 and LTag; and accumulates heavily in the nucleus of the infected cells. An association between the VP2/VP3 and LTag through their DNA binding domains was also observed, that enhances LT-Ag DNA binding to Ori resulting in the induction of viral DNA replication (Saribas et al. 2014).

The SV40 origin of replication is a 64 base pair segment of the viral genome (extends between nucleotides 5211 and 31) which contains all the sequence elements required for *in vivo* and *in vitro* DNA replication (Deb et al. 1986; Deb et al. 1987; Challberg and Kelly 1989; Bullock 1997). The arrangement of the SV40 and mPyV core origin is very similar to the core origin domains of BKPv/JCPv. The SV40 core origin contains three functional regions: (1) an early palindrome (EP); (2) four GAGGC pentanucleotide sequences, collectively referred to as the pentanucleotide palindrome (PEN) or Binding Site; and (3) 17-bp AT-rich domain. These three domains coordinate the binding, melting, and unwinding activities of LTA_g (Borowiec et al. 1990; Bullock 1997). The 71 base pair MCPyV replication origin was shown to include a poly(T)-rich tract and 8 variably oriented, GAGGC-like pentanucleotide sequences, of which 4 are required for the interaction of MCPyV-LTA_g (Kwun et al. 2009). The entire origin of MCPyV contains 10 potential LTA_g binding sequences (PS1 to PS10) and the optimal initiation of replication requires both the viral STA_g expression and intact cellular Hsc70 binding to support efficient viral DNA replication (Kwun et al. 2009).

The SV40 LTA_g plays a central role in the initiation of viral DNA replication by means of binding to four pentamer repeats (PEN) of core origin. Once bound to the origin, the LTA_g assembles into a head-to-head double hexamer in the presence of ATP. Binding of LTA_g causes a significant change in the local DNA structure at the viral replication origin (Kelly et al. 1988; Challberg and Kelly 1989; Stillman 1989; Borowiec et al. 1990; Bullock, 1997). Initially, two LTA_g DNA-binding domains (OBD; amino acids 131 to -259) bind to each half of the palindromic sites of the origin and then promote assembly of eight additional LTA_g monomers with the concomitant binding of ATP/ADP with the LTA_g AAA⁺ (ATP associated/ATPbinding) domain. Then the duplex DNA at the origin melts through its interaction with β -hairpin motifs of

the AAA+ domain of LTA_g (Gai et al. 2004; Reese et al. 2006). The remaining four OBDs of the LTA_g hexamer interact with the already formed two OBD-PEN complex to form an open spiral and ultimately each hexamer surrounds one strand and displaces the other strand of the origin DNA leading to helicase structure (Reese et al. 2006; Fanning et al. 2009). The role of J domain of viral LTA_g, either in a directly or indirectly, seems to be a requisite in viral DNA replication, at least *in vivo* (Bullock 1997; Sullivan and Pipas 2002). The ATPase activity of LTA_g is functionally related to its helicase activity and both the activities are present in the same molecule (Kelly et al. 1988). However, LTA_g-mediated unwinding reaction requires the presence of additional cellular proteins, such as single strand DNA binding protein or replication protein A (RPA) and Topoisomerase I and II (Jiang et al. 2006).

Both LTA_g and STA_g antigens are required for MCPyV-positive MCC cell survival and proliferation (Houben et al. 2012). In enhancing the LTA_g-dependent replication, the MCPyV-STA_g targets the cellular SCF (complex of Skp1, Cul1 and F-Box protein) ubiquitin ligase protein complex, SCF^{Fbw7}, where, Fbw7 has been shown to be dysregulated/mutated in several cancers and to be important in diverse signaling pathways regulating cell proliferation and tumor suppression (reviewed in Kwun et al. 2013). MCPyV-STA_g enhances genome replication by promoting accumulation of the LTA_g through the inhibition of Fbw7 (Kwun et al. 2013). This targeting of SCF^{Fbw7} is mediated through an LT-stabilization domain (LSD) of STA_g. Mutation in STA_g-LSD abolishes stabilization of LTA_g, eliminates cell transformation, inhibits viral replication and induction of several cellular oncoproteins, including c-Myc and cyclin E.

In the next step, DNA polymerase α -primase (pol-prim) synthesizes RNA primers at the origin of DNA replication (Stillman 1989; Bruckner et al. 1995; Fanning et al. 2009). Experimental evidence indicates that at least two subunits of pol-prim form specific complex

with helicase domain of each SV40-LTAg hexamer (Challberg and Kelly 1989; Stillman 1989; Huang et al. 1998). The synthesis of nascent RNA primer for DNA synthesis seems to be dependent on the concomitant binding of LTAg to both pol-prim and RPA (Fanning et al. 2009). This event probably leads to compact binding of RPA with the single-stranded DNA which allows pol-prim to gain access to the DNA template for primer synthesis. Similar RPA-ssDNA binding remodeling by LTAg may lead to primer extension by pol-prim and also the switch to DNA polymerase δ /RFC/PCNA (Fanning et al. 2009).

Agnoprotein enhances the DNA binding activity of LTAg to the viral origin (Ori). Phenylalanine residues are known to have critical roles in protein-protein interaction, protein folding, and stability. Mutation of all the three phenylalanine residues present in the agnoprotein resulted in inefficient replication of mutant virus (Saribas et al. 2012).

Viral Assembly and Egress

Assembly of virions occurs either in the cytosol or in the nucleus of a host cell that involves polymerization of the capsid protein-like viral minichromosomes (Garber et al. 1980). The release of non-enveloped virus usually occurs through cell lysis. But in some cases, they may escape either by some secretory mechanisms (Altenburg et al. 1980) or use cellular autophagy pathways for exit (Jackson et al. 2005). During maturation, changes in viral chromatin composition can be observed that may include an increase in the level of H3 and H4 histone, acetylation in pre-virion and mature virions, degradation of H1 histones, and association of non-histone proteins with mature extracellular virion chromatin complex [Salzman (ed.) 1986].

The SV40 late genes encode viral structural proteins which remain silent during the early phase of infection. Viral structural proteins (VPs) are gradually added to the viral DNA-histone

complex to form immature virions which subsequently mature to give rise to extracellular mature virions [Fanning and Baumgartner, 1980; Salzman (ed.) 1986]. *In vitro* studies with expressed VP1 protein of mPyV, JCPyV and BKPyV revealed that it has the capability of self-assembly into virus-like particles (VLPs), and are composed of 72 capsomers that include only pentamers arranged in a T = 7 surface lattice (Montross et al. 1991; Chang et al. 1997; Li et al. 2002). These VLPs had the same antigenicity like that of natural viral empty capsids (Li et al. 2002). Heterologous *in vivo* expression studies showed that the VP1 capsid protein expressed in *Saccharomyces cerevisiae* and cultured insect cells, retained native antigenic as well as sialic acid-binding capacities (Hale et al. 2002). Disulfide bonds are also found to have an important role in maintaining the integrity of the human polyomavirus VP1 structure by protecting calcium ions from chelation (Chen et al. 2001). DNA binding capacity of JCPyV-VP2 has been studied which showed that the last 13 amino acids, most importantly Lys (332) and Lys (336) within the DNA binding domain of VP2, were essential for this binding (Huang et al. 2001). The host chaperone system, including Hsp70, has an important role in the viral assembly process as it binds to VP1 post-translation. Chromy and co-workers showed that Hsp70 chaperones interact with the C-terminal domain of VP1 to inhibit calcium-mediated capsid assembly *in vitro* and both prokaryotic and eukaryotic Hsp70 chaperones can assemble VP1 capsomeres *in vitro* into uniform capsids through an energy-dependent reaction (Chromy et al. 2003). Sucrose gradient sedimentation and cesium chloride gradient ultracentrifugation analyses of the mature virions of agnoprotein-negative JCPyV revealed that they could assemble into irregularly sized virions and that agnoprotein alters the efficiency of formation of VP1-VLPs. Further analyses showed that some fractions of agnoprotein colocalize with VP1 in the nucleus suggesting its role in virion assembly (Suzuki et al. 2012). Some of the viruses assemble at specific intracellular locations,

often termed ‘virus factories’ that represent sub-cellular scaffolds, where genomes and capsid proteins are brought together for assembly into virion particles (Erickson et al. 2012). The site for nuclear virus assembly factories is promyelocytic leukemia nuclear bodies (PML-NBs). In JCPyV, the major and minor capsid proteins cooperatively accumulate in the nuclear domain 10 (ND10), also known as PML-NBs, where they assemble in to virion particles (Shishido-Hara et al. 2004).

The process of release of PyV from the host cell has been studied earlier by Clayson et al. (1989), where the workers have observed that the escape mechanism of SV40 does not involve cell lysis and may be released from the apical surfaces of the infected cells. Similarl non-lytic release mechanism has recently been observed in BKPyV (Evans et al. 2015). It was also demonstrated by the group that ~1% of total infectious virus progeny is released into the media of cultured primary renal epithelial (RPTE) cells by 48 hour post-infection and that this egress route can be inhibited by an anion channel blocker (DIDS) known to affect cellular secretion pathways. However, recent evidences indicate that late agnoprotein (JCPyV) or VP4 (SV40) can act as ‘viroporin’ and facilitate plasma membrane permeablization/disruption and thus enhancing the viral release (Suzuki et al., 2010; Raghava et al. 2011)

Summary and Concluding Remarks

In the last few decades, extensive research on the functions of the individual viral proteins in the host cell and that on their interactions with cellular signaling pathways has been instrumental in discovering hitherto unknown virus–host interactions and especially of the dynamic nature of host responses in viral pathogenesis. Initial interaction between a virus and a host cell is mediated by attachment of the viral surface glycoproteins to the host cell surface attachment

factors, which can be either a glycolipid or a glycoprotein. While mPyV and simian SV40 recognize gangliosides GD1a/GT1b and GM1 respectively, BKPyV utilizes α 2,3-linked sialic acid of a glycoprotein and/or α 2-8-linked disialic acid motif of the GD1b/GT1b gangliosides (glycolipid) for interacting with host cell plasmamembrane. JCPyV recognizes a linear sialylated pentasaccharide (LSTc) on host cell glycoproteins and glycolipids or α 2-6-linked sialic acid on glycoprotein for its attachment to host cells. JCPyV has also been shown to bind additionally to a 5-HT₂ family of serotonin receptors. However, sialylated glycans (glycolipid and glycoprotein) are not required for initial attachment of MCPyV virions to susceptible cells but may be required as co-receptors in the entry process. Glycosaminoglycans, such as heparan sulfate and chondroitin sulfate, may serve as initial attachment receptors during MCPyV infectious entry to keratinocytes or melanocytes.

Internalization of the virion occurs either by fusion of the envelope and the plasma membrane or via an endocytic pathway which then escapes the endocytic compartment (ER) and enters the cytosol. Caveolae-mediated internalization occurs in all human PyVs except in JCPyV, which employs clathrin-mediated endocytosis. Cellular transport of the virus-cargo to the ER depends on microtubule network. Upon arrival on to ER, viruses are released into the ER lumen, presumably through a release process involving ER-resident proteins. The unique environment within the ER directs specific structural rearrangements in the viral capsid proteins like VP2/VP3, by a process that involves PDI/ERp57 (BKPyV, JCPyV and SV40) or ERp29 (mPyV). The structurally modified virus then engages different retrotranslocons depending on Derlin-1/Sel1L (BKPyV, JCPyV and SV40) or Derlin-2 (mPyV) for their escape to the cytosol. This is followed by further capsid rearrangements and subsequent engagement of nuclear pore complex for delivering the genome to the nucleus. Nucleo-cytoplasmic transport of the viral

cargo depends on IMP β /IMP α . Importins associate with their macromolecular cargo in the cytoplasm via the mediation of adaptor proteins. They dock to components of the nucleoporins, translocate to the opposite side of the nuclear envelope to release their cargo for viral genome replication and packaging. Subsequent to DNA replication and chromatin maturation within the host cell nucleus, virus capsid assembly is initiated. The host chaperone system, including Hsp70, has an important role in the viral assembly process that involves an important role played by agnoprotein, at least in JCPyV. The release of PyVs (e.g., BKPyV) from cells has been reported to be non-lytic in nature and may bud-off from apical surfaces of the infected cells. Viroporin-mediated lytic release of JCPyV is also indicated by few studies.

Polyomavirus VP1 surface-loop variations and key mutations at specific sites appear to be the determinants in initial interactions of this capsid protein in the infectious entry process. Intra-patient mutational variations can also be speculated to be key events in modifying tissue-troism, virulence and subsequent infections. Genetic and protein-level variations existing among the different genus of this virus group, and their interactions with key proteins of the host cell, can be used in designing a framework for a treatment regime, especially in viruses that have proven pathogenic potential in human. A thorough knowledge regarding virus-host interactions may be instrumental in developing host-centric drug targets, thus avoiding viral resistance.

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Table 1: Human polyomaviruses, disease associations, cellular receptor/co-receptors and entry mechanism

Sl. No.	Virus Name	Abbreviations	Disease associations	Receptor(s)/ Co-receptor(s) (entry mechanism)	References*
1	BK polyomavirus	BKPyV	Hemorrhagic cystitis and Nephropathy	Gangliosides GD1b, GT1b (caveolin-mediated endocytosis)	Dugan et al (2005); Low et al (2006)
2	JC polyomavirus	JCPyV	Progressive Multifocal Leukoencephalopathy (PML)	LSTc/5-HT ₂ (clathrin-mediated endocytosis)	Liu et al (1998); Assetta et al (2013)
3	KI polyomavirus	KIPyV	Respiratory symptoms (?)	Not Known	-
4	WU polyomavirus	WUPyV	Respiratory symptoms (?)	Not Known	-
5	Merkel cell polyomavirus	MCPyV	Merkel cell carcinoma	Heparan sulfate/Sialylated glycan	Schewalter et al (2011); Neu et al (2012)
6	Human polyomavirus 6	HPyV6	Squamous cell carcinoma (?), Keratoacanthoma (?)	Not Known	-
7	Human polyomavirus 7	HPyV7	Pruritic rash (?), thymoma (?)	Not Known	-
8	Trichodysplasia Spinulosa-associated polyomavirus	TSPyV	Trichodysplasia Spinulosa	Not Known	-
9	Human polyomavirus 9	HPyV9	Not Known	Not Known	-
10	MW polyomavirus	MWPyV	Not Known	Not Known	-
11	STL polyomavirus	STLPyV	Not Known	Not Known	-
12	Human polyomavirus 12	HPyV12	Not Known	Not Known	-
13	New Jersey polyomavirus	NJPyV	Vascular myopathy (?)	Not Known	-

a. References reporting receptors and co-receptors

Figure Captions

Figure 1. Gangliosides involved in polyomavirus binding. The general structural organization is depicted at the left. GM1, mono-sialotetrahexosylganglioside 1.

Figure 2. Receptor-mediated endocytosis and intracellular trafficking to endoplasmic reticulum (ER) in polyomaviruses. Sialylated glycans, MHC I and gangliosides on cell surfaces bind to and facilitate entry of SV40, mPyV, BKPyV, whereas JCPyV require LSTc and 5-HT₂ for its attachment and entry respectively. MCPyV require primarily glycosaminoglycans and also gangliosides. SV40, mPyV and BKPyV are internalized through caveosomes that traffic through the cytoplasm, using microtubule network, to late endosome and finally delivered to ER. JCPyV is internalized through clathrin-coated vesicles. Within the ER lumen SV40, BK/JC PyVs interact with ERp57 and PDI, a process required for the partial disassembly of viral capsids, and are then retrotranslocated to the cytoplasm via ER transmembrane proteins Derlin1 and Sell1. Murine mPyV interact with ERp29 and require Derlin2 for its exit. LSTc, sialyllacto-N-tetraose c; GAG, glycosaminoglycan; 5-HT₂, 5-hydroxytryptamine

Figure 3. Mechanism of nuclear entry in SV40/BKPyV/JCPyV. ONM, Outer nuclear membrane; INM, Inner nuclear membrane; NPC, Nuclear pore complex; IMP, Importin.

Table I: Human polyomaviruses, disease associations, cellular receptor/co-receptors and entry mechanism

Sl. No.	Virus Name	Abbreviations	Disease associations	Receptor(s)/Co-receptor(s) (entry mechanism)	References ^a
1	BK polyomavirus	BKPyV	Hemorrhagic cystitis and Nephropathy	Gangliosides GD1b, GT1b (caveolin-mediated endocytosis)	Dugan et al (2005) Low et al (2006)
2	JC polyomavirus	JCPyV	Progressive Multifocal Leukoencephalopathy (PML)	LSTc/5-HT ₂ (clathrin-mediated endocytosis)	Liu et al (1998) Assetta et al (2013)
3	KI polyomavirus	KIPyV	Respiratory symptoms (?)	Not Known	-
4	WU polyomavirus	WUPyV	Respiratory symptoms (?)	Not Known	-
5	Merkel cell polyomavirus	MCPyV	Merkel cell carcinoma	Heparan sulfate/Sialyted glycan	Schowalter et al (2011) Neu et al (2012)
6	Human polyomavirus 6	HPyV6	Squamous cell carcinoma (?) Keratoacanthoma (?)	Not Known	-
7	Human polyomavirus 7	HPyV7	Pruritic rash (?), thymoma (?)	Not Known	-
8	Trichodysplasia Spinulosa-associated polyomavirus	TSPyV	Trichodysplasia Spinulosa	Not Known	-
9	Human polyomavirus 9	HPyV9	Not Known	Not Known	-
10	MW polyomavirus	MWPyV	Not Known	Not Known	-
11	STL polyomavirus	STLPyV	Not Known	Not Known	-
12	Human polyomavirus 12	HPyV12	Not Known	Not Known	-
13	New Jersey polyomavirus	NJPyV	Vascular myopathy (?)	Not Known	-

a. References reporting receptors and co-receptors

635x343mm (96 x 96 DPI)

Figure 2. Receptor-mediated endocytosis and intracellular trafficking to endoplasmic reticulum (ER) in polyomaviruses. Sialylated glycans, MHC I and gangliosides on cell surfaces bind to and facilitate entry of SV40, mPyV, BKPvV, whereas JCPvV require LSTc and 5-HT₂ for its attachment and entry respectively. MCPvV require primarily glycosaminoglycans and also gangliosides. SV40, mPyV and BKPvV are internalized through caveosomes that traffic through the cytoplasm, using microtubule network, to late endosome and finally delivered to ER. JCPvV is internalized through clathrin-coated vesicles. Within the ER lumen SV40, BK/JC PyVs interact with ERp57 and PDI, a process required for the partial disassembly of viral capsids, and are then retrotranslocated to the cytoplasm via ER transmembrane proteins Derlin1 and Sel1L. Murine mPyV interact with ERp29 and require Derlin2 for its exit.† LSTc, sialyllacto-N-tetraose c; GAG, glycosaminoglycan; 5-HT₂, 5-hydroxytryptamine

317x222mm (96 x 96 DPI)

Figure 3. Mechanism of nuclear entry in SV40/BKPyV/JCPyV. ONM, Outer nuclear membrane; INM, Inner nuclear membrane; NPC, Nuclear pore complex; IMP, Importin.

476x387mm (96 x 96 DPI)