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Review

Virion-associated protein kinases

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Abstract. Many purified virions, particularly enveloped virus particles (such as retrovirus, hepadnavirus, herpesvirus, orthomyxovirus, and paramyxovirus), contain protein kinase (PK) activity. This type of PK has been called virion-associated protein kinase (VAPK). Even though some VAPKs are identified either as a cellular PK or viral-encoded kinase, many remain to be identified. Although the roles of VAPKs are not yet well characterized, there is ample evidence to suggest their importance in viral infectivity, uncoating, transcription, and replication.

Key words. Virion-associated protein kinase; protein kinase; virus.

Introduction

During the past decade, the relationship between viral pathogenesis and host cellular signalling has attracted considerable attention because of its fundamental importance and clinical significance. Protein kinase (PK) and the phosphorylation reaction indeed play an important role within a virus life cycle. Some PKs, interestingly, are localized within the purified virion. Certain PKs that are associated with the purified virion (virus particle), therefore, are called virion-associated or virus-associated protein kinases (VAPKs). VAPKs were first reported in some enveloped viruses as early as 30 years ago [1]. Since then, many viruses from different families have been shown to contain VAPKs. These studies focused on (i) biochemical characterization of the kinase activity (i.e., substrate specificity, ion dependence, and cofactor stimulation), (ii) architectural localization of the kinase within the virion (such as nucleocapsid or tegument), (iii) identification of the origin of the VAPK (i.e., cellular origin or viral gene product), and (iv) VAPK functional. Evidence has now accumulated to suggest that VAPKs are important in the virus life cycle, including steps in viral infectivity, uncoating, transcription, and replication. In many cases, however, the role of phosphorylation by the VAPK is unknown. Although several reviews have dealt with virus-associated enzymes or viruses encoding PK [2–5] the current review will on recent advances in our understanding of VAPKs.

Retroviridae-associated kinases

A well-studied case of VAPK is the human immunodeficiency virus (HIV)-associated kinase. An impermeabilized envelope of purified HIV can be removed by buffer containing Nondiet P-40 (NP-40). With addition of [γ-^{32}P]ATP and exogenous substrate [such as histone or myelin basic protein (MBP)] to these permeabilized HIV particles, PK activity has been shown. This indicates that the compartmentation of the kinase is located in the paranucleoid region (the lumen space between the envelope and nucleocapsid; fig. 1A). Phosphoamino acid analysis (PAA), 'in-gel kinase assay,' and immunoblot experiments demonstrated that HIV-1 particles incorporated two PKs. One is in an active (i.e., phosphorylated) form, as an extracellular signal-regulated protein kinase 2/mitogen-activated protein kinase (ERK2/MAPK; 43

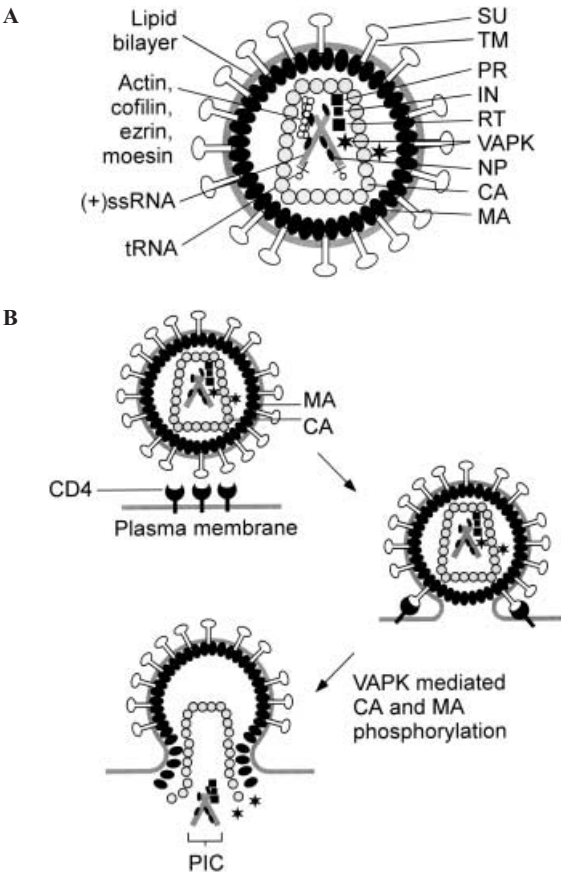

Figure 1. (*A*) HIV is a (+)ssRNA virus with particles approximately 110 nm in diameter. Capsid protein (CA) forms the HIV nucleocapsid, which is generally ovocylindrical in shape. The nucleocapsid contains two identical ssRNA genomes (~9.2 kb) and tRNA molecules, nucleocapsid protein (NP), protease (PR), integrase (IN), reverse transcriptase (RT), Vif, Vpr, Nef, p6, and cytoskeleton proteins. The HIV-associated kinase (VAPK) is inside the nucelocapsid. Surrounding the nucleocapsid is the membranous lipid envelope with matrix protein (MA), major surface glycoprotein (SU, gp120), and transmembrane protein (TM, gp41). (*B*) The HIV-associated PK may be involved in the step of virus infection and uncoating (PIC, preintegration complex).

kDa), while the other is an unidentified 53-kDa PK [6–8]. A similar observation was also obtained from the purified human T-cell leukemia virus type 1 (HTLV-1) virion. The HIV-associated kinases can phosphorylate the viral structural proteins such as the matrix protein (MA) and capsid protein (CA) [6–9].

Phosphorylation on MA facilitates the dissociation of MA from the membrane envelope and prevents reassociation of the viral preintegration complex (PIC) with the cytosolic membrane, thereby allowing the PIC to translocate into the nucleus [6, 8; reviewed in ref. 10]. Hence, phosphorylation by the HIV-associated kinase has biological significance in viral uncoating. Moreover, the inactive form or inhibitor-treated ERK2 which is packaged in an HIV virion can impair the infectivity of the virion [8]. The phosphorylation event within HIV, therefore, is also important for virus infectivity. Although CA is inaccessible by ERK2/MAPK in the virion [7–9], the reverse transcription process is unable to complete in CA mutant viruses with mutated phosphoacceptor sites [9]. Therefore, the HIV-associated kinases for CA phosphorylation play a role in virus genome maturation. In addition, phosphorylation of CA is required immediately after virus entry into the target cell [9]. Phosphorylation of CA in the viral capsid structure could generate some repulsion powers which destabilize the capsid (fig. 1B).

Recently, incorporation of Nef-associated serine/threonine kinase activity into the simian immunodeficiency virus (SIV) neurovirulent recombinant virions was demonstrated by the in vitro kinase assay of Nef immunoprecipitates prepared from isolated virions [11, 12]. Since Nef is located in the nucleocapsid, the Nef-associated PK should be located in the interior of the nucleocapsid (fig. 1A). Nef can form a kinase complex in the cytoplasm called the Nef-associated kinase complex (NAKC) [reviewed in refs 13, 14]. Although the Nef-associated kinase has not yet been identified, some data suggest that it belongs to the p21-activated kinase (PAK) family [15]. Nef has been shown to phosphorylate MA [16] and enhance virion infectivity [12, 17]. Although the contribution of phosphorylation to MA is unknown, this phosphorylation event is believed to play a role in viral infectivity.

Strand and August [1] also described PK activity in Rauscher murine leukemia virus (R-MLV) and avian myeloblastosis virus (AMV). Several viral proteins in the R-MLV virion are phosphorylated by VAPK. The kinase activity in R-MLV and AMV contains two distinct phosphorylation activities on exogenous basic and acidic substrates [1, 18, 19]. Two PKs of molecular weight 42–46 and 60–64 kDa have been further identified from AMV. They can be distinguished based on chromatographic properties, sedimentation behavior, and phosphoacceptor protein specificities [20]. In murine sarcoma virus (MSV), a low molecular-weight (LMW) serine/threonine PK (16.5 kDa) has been purified by gel filtration and conventional chromatography methods [21–23]. Moreover, mouse mammary tumor virus (MMTV), feline leukemia virus (FeLV), and Rous sarcoma virus (RSV) have also been demonstrated to contain a kinase inside the virion [24].

Hepadnavirus-associated kinases

Beside retroviruses, PK activity has also been detected in another member of the reverse-transcribing virus family, hepatitis B virus (HBV; fig. 2A).

PK activity has been shown in the HBV virion and nucleocapsids purified from plasma of hepatitis virus-infected

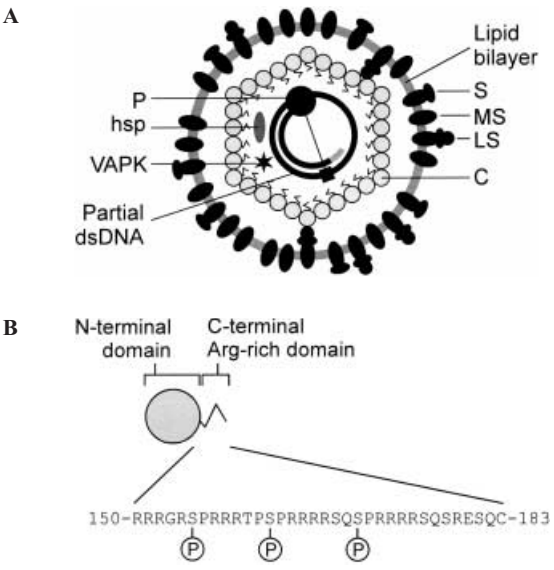

Figure 2. (*A*) HBV contains a circular, partially dsDNA virus (~3.2 kb). The infectious Dane particle is on average 42 nm in diameter. The envelope, derived from endoplasmic reticulum, is embedded in many surface proteins – large (LS), middle (MS) and major (or small; S) surface proteins. The nucleocapsid is arranged by core protein (C) in a $T = 3$ or 4 icosahedral lattice. Inside the nucleocapsid, the dsDNA genome (with an RNA primer), viral-encoded polymerase (P), host heat shock protein (hsp, or cheapron), and HBV-associated kinase (VAPK) are shown. (*B*) The amino acid sequence of the HBV core protein C terminus is shown (subtype *adr* or *ayw*). Phosphorylation sites are indicated.

patients [25]. It has also been shown in the hepatitis virus nucleocapsids isolated from the livers of hepadnavirus-infected ground squirrels [26, 27], woodchucks [28], and ducks [29–31]. Although the enveloped HBV virion can be permeabilized by NP-40, the inner nucleocapsid shell is very stable and strong enough to maintain an intact structure under detergent treatment [32, 33]. Moreover, the protein shell of the nucleocapsid is penetrated by holes between core proteins, which can be observed by cryoelectron microscopy [34, 35]. These holes are unique in that [^{32}P]ATP can diffuse readily through them and act as a phosphate donor for the kinase located inside the nucleocapsid. The HBV-associated kinase, which is located inside the interior of the immunoprecipitated nucleocapsid, can therefore phosphorylate the HBV core proteins in vitro [36–38].

PAA has identified serine residues of the core protein that were phosphorylated in vitro by PK in HBV [27, 39]. In mutagenesis studies, three serine residues have been identified in the C-terminal arginine-rich domain of the HBV core protein as the phosphorylation sites (fig. 2B) [40, 41]. The core protein of duck hepatitis B virus (DHBV) has one threonine and three serines phosphorylation sites within the 28-amino acid C-terminal sequence [42, 43], and the different phosphorylation locations within the C-terminus were linked with the functions of viral DNA synthesis and virus maturation [43]. Since the phosphorylation and dephosphorylation processes occur within a C-terminal region which is important for HBV pregenomic RNA (pgRNA) encapsidation, conversion of pgRNA into minus-strand DNA (reverse transcription), and synthesis of plus-strand DNA [reviewed in ref. 44], we can speculate that the kinase may have essential functions in viral genome packaging and maturation. The relevance of core protein phosphorylation to the hepatitis virus life cycle is under investigation, because the reported data have been controversial [41–43, 45, 46].

A reconstitution experiment demonstrated that the nucleocapsid is able to package both protein kinase C (PKC) or protein kinase A (PKA) into nucleocapsids. In contrast, the data from PK inhibitor or immunoblot experiments indicated the packaging of PKC, but not PKA or cdc kinases [45, 47]. Another cellular kinase, called ribosome-associated protein (RAP) had been identified in the HBV virion and is therefore to be termed core-associated kinase (CAK; 46 kDa) [48, 49]. PK activity of glyceraldehyde-3-phosphate dehydrogenase (GAPD-PK) has also been demonstrated inside the HBV virion [50].

Herpesviridae-associated kinases

VAPK is also detected in dsDNA viruses such as herpes simplex virus (HSV; fig. 3) [51–55]. The substrates of this HSV-associated kinase are virion polypeptides. From the 'peel off' experiment using NP-40 or urea, the PK is known to be located in the tegument region [52, 54, 56]. At least two genes of HSV-1 encode PK, which is associated with purified virion. US3 (*US3* gene product) and VP18.8 (*UL13* gene product) are present in the HSV virion [57–61]. Both gene products can phosphorylate serine and threonine sites, rather than tyrosine residues. These PKs are located in the tegument region. Although tegument proteins in purified HSV virions are not phosphorylated [62–64], exogenous cellular kinases such as

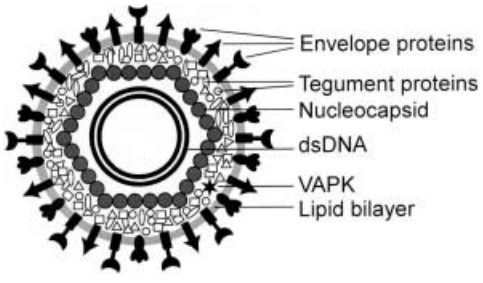

Figure 3. HSV-1 is the best characterized herpesvirus. Key features of the HSV virions are the linear dsDNA (150 kb)-containing core ($T = 16$ icosahedral capsid), the layer of proteinaceous material tegument between the capsid outer surface and envelope inner surface, and the membranous envelope (supplied from the inner nuclear membrane) containing numerous viral glycoproteins.

PKA, PKC, or casein kinase 2 (CK2) and VAPK can phosphorylate tegument proteins in vitro after the tegument release assay (in 1% Triton X-100) [65]. The dissociation of major tegument proteins from HSV virions in infected cells may be initiated by phosphorylation events mediated by VAPK and/or cellular kinases.

Moreover, the HSV encodes its own ribonucleotide reductase (RR; designated ICP6 for HSV-1 and ICP10 for HSV-2) which has been assigned serine/threonine PK activity [66–69]. This enzyme consists of a large subunit, RR1 (136–140 kDa), and a small subunit, RR2 (38 kDa). The PK activity of RR is Mn^{2+} dependent [67, 70]. From the results of iodination experiments and the nonionic detergent Triton X-100 solubility test, HSV-2 RR (ICP10) has been localized to within the tegument region [71]. HSV-associated kinases (US3, VP18.8, and RR) are possibly incorporated into the tegument when the nucleocapsids bud from the nuclear membrane during virion biogenesis.

In the study of human herpesvirus varicella zoster virus (VZV), open reading frame (ORF) 62 encodes a viral structural protein IE62 (175 kDa), which can be autophosphorylated [72]. In centrifugation and trypsin-resistant assays, IE62 was found to be most likely present in the tegument of the VZV virion. In addition, IE62 can be phosphorylated inside the VZV virion. This may reflect the ability of IE62 to autophosphorylated in the tegument structure of the VZV virion [72].

The presence of VAPK in the herpes group has also been reported in human cytomegalovirus (HCMV) after disrupting of the envelope [73–75]. The kinase is divalent cation (Mg^{2+} or Mn^{2+}) dependent and a cyclic nucleotide-independent serine/threonine PK [76, 77].

Pseudorabies virus (PRV)-associated PK (PRV-PK; 38 kDa) was identified by chromatographic and immunoblot analysis [56, 59]. Chromatographic analysis on diethylaminoethyl (DEAE)-cellulose and phosphocellulose (PC)-cellulose showed that PRV contains more than one kinase which are responsible for the phosphorylation of viral components inside the virion [56]. These kinases in PRV are likely to be located in the tegument of the virus, similar to HSV [56]. Moreover, the amino acid sequence of the VAPK catalytic domain among HSV, VZV, and PRV (an alphaherpesvirus) is conserved [59, 78, 79].

Rhabdoviridae-associated PK

Kinase activity has been detected in the single-stranded RNA (ssRNA) virus, vesicular stomatitis virus (VSV; fig. 4) [1, 80]. The presence of PK has been shown in immunoprecipitated VSV virion [81, 82]. The VSV-associated kinase is not located on the surface of the virus particles, since it becomes unmasked only after solubilization of the envelope lipids and glycoprotein by a nonionic detergent [83]. VSV-associated PK in the virion is capable of phosphorylating viral polymerase L proteins, P proteins (formerly called NS transcription factor), and matrix (M) proteins [84–91].

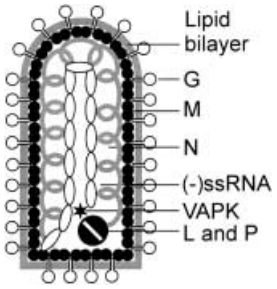

Figure 4. The bullet-shaped VSV particle, approximately 180 nm in length and 65 nm wide, contains (–)ssRNA (~6.4 kb) which is tightly encased in the major nucleocapsid protein (N) and the minor L (large) polymerase and P proteins. On the VSV membrane, integral glycoprotein (G) and peripheral matrix (M) proteins line the outer and inner surface of the virion membrane, respectively.

The ability to phosphorylate viral substrates was earlier proposed to be among the many diverse functions of the polymerase L protein. This L-associated kinase activity, however, could be separated by an additional purification step. Based on the biochemical studies [91–93], three different PKs are associated with the VSV virion. One activity is specific for M protein phosphorylation and the other two for P protein phosphorylation. At least two of these PK activities are associated with the viral ribonucleoprotein (RNP) and so-called RNP-associated PK (RNP-PK). One is manifested by a CK2 [94] and the other seems to be the PK activity of L polymerase [95, 96]. L polymerase of VSV has a PK activity which can specifically phosphorylate the virion P protein [95]. Since phosphorylation is a requirement for VSV transcription *in vitro* and the release of transcriptase complex components (L, P, and N) [97, 98], the kinase reaction suggested the possibility of conformational change in RNP structure during transcription. Moreover, the phosphorylation of the M protein in VSV might disrupt the virion envelope and thus be required for viral uncoating when it enters its host cell [92, 99].

In the family Rhabdoviridae, VSV, rabies virus, and Kern Canyon virus all contain VAPK [100].

Orthomyxoviridae- or Paramyxoviridae-associated PK

PK activity has been reported in influenza virus [24, 101–103], which is a segmented, negative-stranded RNA virus (fig. 5). This kinase activity was observed in type A, B, and C influenza viruses [103]. From biochemical and immunoblot data, the influenza virus-associated kinase is known to be the host cell CK2 [103]. Viral nucleoprotein (NP) undergoes changes in phosphorylation during the course of infection [101, 104–106]. The phosphorylation

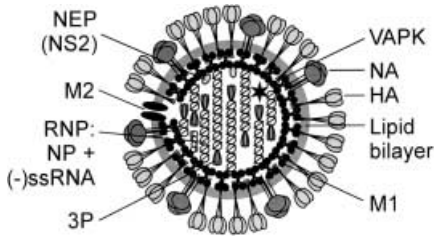

Figure 5. Influenza A virus particles have a fairly regular appearance, and are 80–120 nm in diameter. Three types of integral membrane protein, hemagglutinin (HA), neuraminidase (NA), and ion channel protein (M2), insert through the lipid bilayer of the viral membrane. The matrix proteins (M1) underlie the lipid bilayer. Within the envelope, eight segments of (–)ssRNAs (ranging from 890 to 2341 nucleotides) interact with nucleoproteins (NP) to form the helical ribonucleocapsid (RNP). The viral polymerase complexes (3P), consisting of three polymerase proteins (PB1, PB2, and PA), associate with the RNPs. The viral protein nuclear export proteins [NEP; also known as nonstructural protein 2 (NS2)] are also found in the virion.

patterns of NP were influenced by PKC inhibitor [105]. Further studies indicated that the PA protein in the influenza virus polymerase complex (3P; complex of PA, PB1, and PB2 proteins) is phosphorylated in vivo and is a substrate of CK2 in vitro [107, 108]. Because the site of influenza virus replication and transcription is located in the infected cell nucleus, and CK2 is a ubiquitous PK present in nucleus, cytoplasm, and plasma membrane [reviewed in refs 109, 110], distinguishing the role of virus-associated and host cellular CK2 in PA phosphorylation is difficult.

Sendai virus is a nonsegmented, negative-stranded, enveloped RNA virus. The presence of a low concentration of NP-40 enhanced the phosphorylation reaction in the virus [111, 112]. The optimum pH specificity, temperature, and cofactor requirements for Sendai virus-associated kinase have been characterized [112]. The phosphothreonine from the phosphorylated product of Sendai virus has been identified by thin-layer electrophoresis [112]. By immunoblot and the cofactor requirement test, PKCζ has been identified in the Sendai virion [113]. Moreover, the viral transcriptional activity assay indicated that PKCζ remains tightly associated with the viral RNP in the virion [113]. The substrates of PKCζ are nucleocapsid proteins and viral phosphoproteins (P) [113]. Phosphorylation plays an important role in the primary transcription of Sendai viral mRNAs [113].

The kinase inhibitor and immunoblot data indicated that PKCζ, and not PKCα, β, δ, and CK2, is packaged in the virion of human parainfluenza virus type 3 (HPIV3) [114]. The substrate of HPIV3-associated PK is viral P protein [114]. The inhibition of HIVP3 replication by PKCζ-specific pseudosubstrate inhibitor peptide indicated that the replication of HPIV3 required PKCζ function [114].

Picornaviridae- and Togaviridae-associated kinase

So far, all the discussed viruses have been enveloped viruses. Foot-and-mouth disease virus (FMDV) is a nonenveloped (naked) virus which belongs to the picornavirus family. A trypsin-resistance assay indicated that the FMDV-associated kinase is located within the particle [115]. Therefore, FMDV is a nonenveloped RNA virus that also has VAPK. The FMDV-associated kinase phosphorylates the serine residues of viral structural proteins VP2 and VP3 [115]. Sucrose gradient-purified FMDV was disrupted by dithiothreitol and Triton X-100 and three peaks of kinase activity (P2, D1, and D2) were identified by ion-exchange chromatography on DEAE- and PC-cellulose columns [116]. Coxsackievirus B4 (CVB4), another picornavirus, also contained a VAPK [117].

Members of the togavirus family (e.g., Sindbis virus and Semliki forest virus) have also been reported to contain VAPK [118, 119].

Poxviridae- and Adenoviridae-associated VAPK

The vaccinia virus (fig. 6) core structure has been shown to contain a high level of PK activity [53, 80, 119–123]. Biochemical studies showed that vaccinia *B1R* and *F10L* genes encode vaccinia protein kinase 1 (VPK1, 34 kDa) and vaccinia protein kinase 2 (VPK2, 52 kDa), respectively [124, 125]. VPK1 phosphorylates the 40S ribosomal subunits Sa and S2, and viral H5R protein [126, 127]. VPK2 can be autophosphorylated and is able to phosphorylate a casein kinase 1 (CK1) substrate peptide [128]. Temperature-sensitive virus studies implied that VPK2 plays an essential role in the early stage of virion assembly [129, 130].

Milker's nodule virus (MNV; or pseudocowpox), which belongs to the family *Poxviridae,* has also been demonstrated to contain a kinase inside the virion [131].

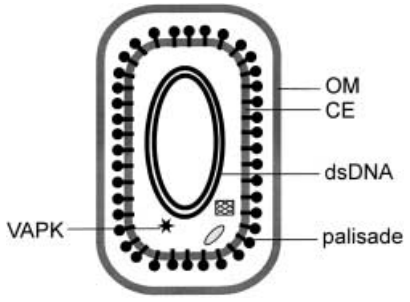

Figure 6. The vaccinia extracellular enveloped virion contains both an outer membrane (OM) and internal core envelope (CE). The CE is composed of a smooth membrane with regularly arranged spires (palisade). Beneath the CE are the dsNDA genome, RNA polymerase (hatched box), poly(A) polymerase (shadowed oval), transcription factors, and VAPK.

A PK activity has been demonstrated in sucrose gradient-purified adenovirus type 2 (Ad2) [132, 133]. The adenovirus-associated kinase can phosphorylate polypeptide IIIa of the adenovirus particle [132].

Amphibian virus-associated PK

The purified frog virus 3 (FV3; formerly called frog polyhedral cytoplasmic deoxyribovirus) contains a 50 to 55-kDa VAPK [53, 134, 135]. This FV3-associated kinase requires Mg^{2+} or Mn^{2+}, but not Ca^{2+}. It has a broad pH optimum between 7.0–8.5 [136, 137]. From immunological and genetic studies, the FV3-associated kinase is known to be a viral gene product [138]. The role of PK in FV3 replication is not known, but virion core proteins are phosphorylated in vivo [139].

Insect virus-associated kinase

Granulosis virus (granulovirus; GV) proteinaceous matrix (called granulin) and envelope can be removed by alkaline conditions and NP-40, respectively. The DNA-protein core is released using the chelating agent ethylenediaminetetraacetic acid in the presence of high salt. The kinase activity associated with the capsid fraction can be recovered from a glycerol gradient [140]. The divalent cation-dependent kinase activity present in GV is tightly associated within the capsid structure. The GV basic core protein VP12 (a DNA-binding protein) was found to be the substrate of GV-associated kinase [140]. By using PAA and nuclear magnetic resonance, the phosphoserine and phosphoarginine residues have been identified [140]. The GV-associated kinase may function in the release of viral DNA from the nucleocapsid (uncoating) and decondensation of the viral DNA due to phosphorylation of VP12 [141].
VAPK is also present in *Autographa californica* nuclear polyhedrosis virus (AcMNPV). A serine/threonine protein kinase has been identified in both the occluded forms (occluded virus) and in the extracellular nonoccluded virus of AcMNPV [142].
Chilo iridescent virus-associated kinase can phosphorylate some viral proteins and exogenous basic proteins [137].

Plant virus-associated kinase

VAPK is present in plant as well as animal viruses. Cauliflower mosaic virus (CaMV) is one of the most studied of the caulimoviruses, a group of dsDNA viruses which infect plant. Since the overlapping translation frames and replication manner of CaMV, HBV, and most retroviruses are similar, these three types of virus have been classified in the same family [143]. Therefore, some investigators were interested in studying whether a VAPK was present in the CaMV virion. A cyclic nucleotide-independent serine/threonine PK activity has been found associated with purified CaMV particles [144–146]. Capsid protein (42 kDa) and its precursor (58- and 110-kDa proteins) have been phosphorylated in vivo by this kinase [145]. This is the first and only plant virus that, so far, has been indicated to contain a VAPK.

Conclusion

Viral pathogenesis is a widespread and serious problem. Many aspects of virus molecular biology are understood in detail. However, recent research has emphasized that

Table 1. Virion-associated protein kinases.

Family	Genus	Virus	PK(s)	Location	Substrate(s)	Possible function(s)
The DNA and RNA reverse-transcribing viruses						
Retroviridae	Lentivirus (Primate lentivirus)	HIV-1	ERK2/MAPK [7], 53-kDa kinase [7, 8], Nef-associated PK [11, 12]	paranucleoid region and nucleocapsid	CA, MA [6–9, 16]	virus infectivity, reverse transcription complex establishment, viral genome maturation, uncoating after infection
		HTLV-1	ERK2 [7]	paranucleoid region and nucleocapsid	CA, MA [7]	
	ALV-releated viruses	AMV	42 to 46 and 60 to 64-kdA PK [1, 18, 19]	inside virion	10 to 25-kDa viral protein [19]	?
	MLV-related viruses (mammalian type C)	R-MLV	42 to 46 and 60 to 64-kDa PK [1, 18, 19]	inside virion	?	?
	MLV-related viruses (mammalian type C)	MSV	16.5-kDa Ser/Thr PK [21–23]	inside virion	?	?
	B-type virus	MMTV	unknown PK [24]	inside virion	?	
	Spumavirus	FeLV	unknown PK [24]	inside virion	?	?
	Avian-leukosis-sarcoma	RSV	unknown PK [24]	inside virion	?	?

Table 1 (continued)

Family	Genus	Virus	PK(s)	Location	Substrate(s)	Possible function(s)
Hepadna-viridae	Orthohepadnavirus	HBV	PKC [45–47], CAK (RAP) [48, 49], GAPD-PK [50]	nucleocapsid	core protein [26, 27, 39–41]	viral pgRNA packaging and genome maturation
	Orthohepadnavirus	GSHV	unknown Ser/Thr PK [26, 27]	nucleocapsid	core protein [26, 27]	?
	Orthohepadnavirus	WHV	unknown Ser/Thr PK [28]	nucleocapsid	core protein	?
	Orthohepadnavirus	DHBV	unknown Ser/Thr PK [29–31]	nucleocapsid	core protein [29, 42, 43]	viral genome maturation
Caulimo-viridae	Caulimovirus	CaMV	unknown Ser/Thr PK [144–146]	inside virion	capsid protein and its precusor [145]	?
The dsDNA viruses						
Herpes-viridae	Alphaherpesvirinae (Simplexvirus)	HSV	viral US3 [58], viral VP18.8 [60, 61], viral RR-PK [66–69], cellular PK (?)	tegument	tegument proteins [65]	viral uncoating
	Alphaherpesvirinae (Varicellavirus)	VZV	IE62 [72]	tegument	IE62 [72], tegument proteins (?)	viral uncoating (?)
	Alphaherpesvirinae (Suid herpesvirus 1)	PRV	38 kDa viral product [55, 58]	tegument	tegument proteins [56]	viral uncoating (?)
	Cytomegalovirinae (Cytomegalovirus)	HCMV	unknown PK [73–77]	inside virion	tegument proteins (?)	?
Iridoviridae	Chiloiridovirus	CIV	unknown PK [137]	inside virion	viral proteins [137]	? ?
	Ranovirus	FV3	50- to 55-kDa [53, 134, 135]	inside virion	core protein [139]	?
Poxviridae	Orthopoxvirus	vaccinia virus	VPK1 and VPK2 [124, 125]	inside virion	viral H5R protein and host ribosomal subunit [126, 127], VPK2 [128]	virus assembly
	Parapoxvirus	MNV	unknown PK [131]	inside virion	?	?
Adenoviridae	Mastadenovirus	adeno-virus	unknown PK [132,133]	inside virion	viral protein IIIa [132]	?
Baculoviridae	Granulovirus	GV	unknown Ser/Arg PK [140]	inside virion	?	viral uncoating
		AcMNPV	unknown PK [142]	inside virion	?	?
The ssDNA viruses (No reported VAPK yet)						
The negative-sense ssRNA viruses						
Orthomyxo-viridae	Influenzavirus	influenza virus	CK2 [103]	inside virion	viral PA protein (?) [107, 108]	viral genome maturation (?)
Paramyxo-viridae	Paramyxovirus virus	Sendai	PKCζ [113]	inside virion	nucleoprotein and P protein [113]	viral transcription
	Paramyxovirus	HPIV3	PKCζ [114]	inside virion	P protein [114]	viral eplication
Rhabdo-viridae	Vesiculovirus	VSV	CK2 [94]	inside virion	L, P, and M proteins [84–91]	viral transcription and uncoating
	Lyssavirus	rabies virus	unknown PK [100]	inside virion	?	?
	Lyssavirus	KCV	unknown PK [100]	inside virion	?	?
The positive sense ssRNA viruses						
Picorna-viridae	Aphthovirus	FMDV	host Ser/Thr PK [116]	inside virion	VP2, VP3 [115]	?
	Enterovirus	CVB4	unknown PK [117]	inside virion	?	?
Togaviridae	Alphavirus	Sindbis virus	unknown PK [118]	inside virion	?	?
	Alphavirus	SFV	unknown PK [118]	inside virion	?	?
The dsRNA viruses (No reported VAPK yet)						

the dependence of the viral life cycle on cellular factors is far greater than previously anticipated. More and more interactions between viral and cellular components are being discovered. Most likely, individual steps of viral replication will involve several host factors. A prominent example is the virus and PK.

From the 1970s through the 1980s, many publications reported the presence of PK within purified virus. Due to knowledge about PKs in signal transduction, identification of VAPKs flourished from the 1990s, supported by improved biochemical techniques. To date VAPKs have been found in all families except dsRNA and ssDNA viruses (table 1), and VAPKs are present in both oncogenic and nononcogenic virus classes. In some cases, the cellular PK was identified in the purified virion (virus-associated host cell PK), while in others, specific viral proteins have been shown to have enzymatic activity (virus-encoded VAPKs), but some still remain unknown (table 1).

An important consideration is the function of the VAPK in the virus particle. Although VAPK could be a minor structural component of the viral architecture, it may play an important role in the virus life cycle. Usually, electrostatic-repulsion and conformational-change models have been proposed for PK activity in viral capsid structure phosphorylation (addition of negatively charged phosphate groups). Therefore, one of the possible functions of VAPKs is modification of virion structural proteins leading to uncoating (such as VAPK in HIV-1, HSV, VSV, and GV). Virus assembly (such as VAPK in vaccinia virus) and regulation of viral nucleic acid replication and transcription (such as VAPK in HIV, HBV, Sendai virus, and VSV) are also possible functions of VAPKs. However, most VAPK function(s) in the virion remain unclear.

Thus, an interesting question emerges from the studies of VAPKs with regard to their necessity in the virus life cycle: are VAPKs required for virus growth? Studies on both HSV-associated PK gene *US3* or *UL13* deletion mutants may give us some hints. Although the spread of *US3*-deficient mutants is highly restricted in mice [147], virus yield from Vero cells and expression of viral glycoprotein B were similar to wild-type HSV virus [148]. The results indicate that *US3* is not required for virus growth in culture. The *UL13*-deficient mutant, however, was low in both virulence and virus yield [149]. The results suggest that *UL13* may contribute to virus virulence and growth. In another case, temperature-sensitive mutant studies, VPK1 and VPK2 in vaccinia virus were essential for virus growth [129, 130]. Thus different viruses apparently utilize different systems.

The present of PK in the virion also raises the question whether VAPKs are specifically incorporated into the virion or are just a part of the cellular contents that become nonspecifically incorporated into the virion. Since ERK2/MAPK and VP18.8 (the *UL13* gene product) in HIV and HSV, respectively, play a role in virus infectivity and virulence [8, 149], the viruses seem to require VAPK for the complete life cycle. The specificity of PK incorporation could be important. But the US3 protein in HSV is not required for virus growth [148]. In addition, although CK2 in influenza virus requires polymerase subunit PA phosphorylation [107, 108], CK2 is a ubiquitous PK present in relatively high levels, and the host cell nucleus contains CK2 which could virion-associated CK2. Thus, some VAPKs are apparently not essential for the virus life cycle. The specificity of PK incorporation may not be important, and is difficult to elucidate. From the perspective of enzyme kinetics, PK should interact with the binding motif(s) and then phosphorylate the phosphate acceptor site(s). So far, however, studies on VAPKs have focused on the substrate(s) of the associated PK. This question still remains unclear.

After millions of years of virus and host molecular coevolution, some cellular kinases are believed to replace the viral synthesis kinase, some small-genome viruses can encapsidate the cellular kinase to replace their lack of kinase gene. For example, the mononegavirales (a group of nonsegmented, single-stranded, negative-sense RNA viruses such as rhabdoviruses and paramyxoviruses) all are relatively small and simple viruses, and possess no more than 10–12 genes. They contain kinases of cellular origin. HSV, however, is a species of the subfamily Alphaherpesviridae and encodes at least 84 proteins from its large DNA genome. Two of the proteins, encoded by the viral genes *UL13* and *US3*, are PKs.

Findings about VAPKs might provide some new insights into the mechanism of the viral life cycle. A detailed study of VAPKs would provide insight into the molecular basis of the virus-host interaction. Identifying these host factors and characterizing their relationship with the viral life cycle will certainly reveal novel targets for specific antiviral strategies [150].

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