Interfacial Domains in Sindbis Virus 6K Protein

DETECTION AND FUNCTIONAL CHARACTERIZATION*

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Alphaherpesviruses are enveloped animal viruses with single-stranded positive RNA tightly packaged by the capsid. The structural proteins are synthesized from a subgenomic mRNA encoding a polyprotein that is proteolytically processed (for review, see Refs. 1–3). Capsid (C)1 protein is first synthesized and detaches from the rest of the polyprotein by autocatalytic proteolysis. Once C protein has been liberated into the cytoplasm, polyprotein synthesis continues to be associated with endoplasmic reticulum (ER) membranes. The exposed amino terminus contains a signal sequence that interacts with ER membranes and directs the glycoprotein precursor (E3-E2-6K-E1) into the lumen of the ER. The precursor is associated with the ER membrane, spanning the lipid bilayer six times. Soon after synthesis, this precursor is cleaved at both ends of the 6K protein by a cellular protease present in the ER, generating the products PE2 (E3 + E2), 6K, and E1. PE2 and E1 then associate to form dimers that travel with 6K through the vesicular system to the plasma membrane. As a final activation step, PE2 is cleaved by a furin-like protease present in a post-Golgi compartment, giving rise to glycoproteins E3 and E2. The glycoproteins transported to the plasma membrane expose their amino terminus ectodomains to the external medium, whereas the carboxyl domains remain facing the cytoplasm. The virus genomes replicated in the cytoplasm interact with the C protein to form nucleocapsids. The assembled nucleocapsids subsequently interact with the carboxyl domain of E2. This interaction provokes the wrapping of the capsid with the lipid envelope, concomitant with the budding of virus particles (1–3).

6K is a small hydrophobic polypeptide acylated with fatty acids (4, 5). Despite the association of the 6K protein with the plasma membrane and its interaction with E1-E2, very little 6K is incorporated into the budded virus particles (4, 5). Although 6K protein provides the cleavage sites in the glycoprotein precursor for signalase activity, a Semliki forest virus variant lacking the entire 6K is processed between E2 and E1 (6, 7). This virus mutant is not defective in synthesis and transport of glycoproteins or in nucleocapsid formation; its major defects concern the budding process. Another function assigned to Semliki forest virus 6K protein is to provide the signal sequence for E1 translocation to the lumen of the ER (8). Notably, E1 is properly translocated in the 6K-deleted Semliki forest virus mutant (6, 7). Similarly, Sindbis virus (SV) variants with single or multiple amino acid substitutions in the 6K have defects in virion release, leading to the formation of multinucleated virus particles (4, 9–11). Proper proteolytic processing of the virus glycoproteins is hampered in an SV variant bearing an insertion of 15 amino acids in the 6K protein. This variant exhibits a transdominant phenotype, but virus particles display a morphology similar to that of wt virus.
by which 6K protein enhances virion release remains unknown. Though the budding process was impaired. Together, these virus was able to cleave the glycoprotein precursors proteolytically and transport them into the plasma membrane, although the budding process was impaired. Together, these observations suggest a function for 6K in the release of virions from infected cells. However, the exact molecular mechanism by which 6K protein enhances virion release remains unknown.

This article reports an unexplored feature of SV 6K that might be related to the promotion of the virion-release process (i.e., the affinity of 6K for the membrane interface). It was found that the amino-terminal 6K ectodomain contains two hydrophobic-at-interface segments that can mediate association of this sequence with the external membrane monolayer. Conservation of the interfacial 6K segments among dengue virus members of the Alphavirus genus suggests a functional role for these motifs. Accordingly, SV 6K variants containing substitutions that interfere with the capacity of the amino terminus to partition into membranes without affecting overall 6K hydrophobicity were obtained and characterized. The data support the hypothesis that 6K participates in virus release through direct interaction with membranes that compromise the permeability barrier. A model is proposed that accounts for the involvement of 6K in this phenomenon.

EXPERIMENTAL PROCEDURES

Cells, Viruses, and Plasmids—Baby hamster kidney (BHK-21) cells were grown at 37 °C in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum and nonessential amino acids. SV was derived from the cDNA clones described below after transfection of the RNAs synthesized in vitro.

Full-length SV cDNA Clones—A full-length cDNA clone of Sindbis virus pT7SVwt (13) was used as the wt parental clone to generate mutantized SV variants. pT7SVYLW/AAA, pT7SVFW/AAA, and pT75VYLW/AAAFWV/AAA carry mutations in the 6K gene: 9YLW11xAAA, 18FWV20xAAA, and both 9YLW11xAAA and 18FWV20xAAA, respectively. These replicons were made in two steps. First, pT7SVwt was digested with ApaI sites in the above plasmid. The replicon coding only for the sequence coding for the proteins E3 and E2 was deleted. The open reading frame was not broken, and the remaining sequence coded for the 45 amino-terminal amino acids of E3 fused to 31 carboxyl-terminal amino acids of E2 plus 6K and E1. In a second step, the different 6K sequences were introduced at the same time as the E1 sequence was codified. Digestion products replaced the sequences located between the BssHI and Spfl fragment in pT7SVwt by PCR-amplified and subsequently digested fragments incorporating the 6K substitutions.

SV Replicas—Several SV replicons were obtained by deleting glycoprotein genes: C + 6Kwt, C + 6KYLW/AAAA, C + 6K FWV/AAA, and C + 6KYLW/AAA, FWV/AAA. These replicons were made in two steps. First, pT7SVwt was digested with BssHI and Stul and the recessed ends filled in with DNAApo I, Large (Klenow fragment). Thus, part of the sequence coding for the proteins E3 and E2 was deleted. The open reading frame was not broken, and the remaining sequence coded for the 45 amino-terminal amino acids of E3 fused to 31 carboxyl-terminal amino acids of E2 plus 6K and E1. In a second step, the different 6K sequences were introduced at the same time as the E1 sequence was deleted, using the unique restriction sites BssHI, upstream of 6K, and Apol, downstream of E1. Sequences with the different 6Ks were amplified by PCR and oligonucleotides that incorporated the restriction sites BssHI and Apol and a stop codon were ligated into the BssHI/Apol sites in the above plasmid. The replicon coding only for the capsid protein was obtained by replacing the pT7SVwt sequence between the AartIII and Apol sites by a PCR-amplified fragment encoding the capsid sequence from the AartIII site to the end and a stop codon in sites BssHI and Apol and a stop codon placed after the 6K sequence. Digestion products replaced the sequences located between the BssHI and Apol sites in the above plasmid. The replicon coding only for the capsid protein was obtained by replacing the pT7SVwt sequence between the AartIII and Apol sites by a PCR-amplified fragment encoding the capsid sequence from the AartIII site to the end and a stop codon. 6K protein expression from the extra subgenomic promoter placed in the same genome did not produce appreciable reversion. In addition, the functions of the 6K protein cannot be rescued by the corresponding counterparts from related virus species. Thus, the substitution of the SV 6K gene by the 6K counterpart from Ross River virus leads to the small plaque phenotype and reduced formation of infectious virus (14). This SV variant with the 6K gene from Ross River virus was able to cleave the glycoprotein precursors proteolytically and transport them into the plasma membrane, although the budding process was impaired. Together, these observations suggest a function for 6K in the release of virions from infected cells. However, the exact molecular mechanism by which 6K protein enhances virion release remains unknown.

Transfection of BHK Cells—Subconfluent BHK cells were harvested, washed with ice-cold phosphate-buffered saline, and resuspended in phosphate-buffered saline at a density of about 2.5 × 10⁶ cells/ml. A 25-μl aliquot of T7 RNA polymerase transcription mixture with about 10 μg RNA from the different cDNA constructs was added to 0.4 ml of cells, and the mixture was transferred to a 2-mm electroporation cuvette. Electroporation was performed at room temperature by generating two consecutive 1.5-kV, 25-microFarad pulses using a Gene Pulser apparatus (Bio-Rad) as described by Liljestrom et al. (6). The cells were then diluted in growth medium and seeded onto culture plates.

Electron Microscopy—Transfected cells were processed for electron microscopy as follows. At 16 h.p.e. (hours post-electroporation), cells were fixed with 2% glutaraldehyde in 0.2 M HEPES buffer, pH 7.4, for 1 h at room temperature and immediately scraped off the plate. These were then washed twice and resuspended in 0.2 M HEPES buffer, pH 7.4. After fixation, they were dehydrated and infiltrated with Epon. Thin sections were obtained and stained with uranyl acetate and lead citrate.

6K Expression and Membrane Permeabilization in E. coli BL21(DE3)—Permeability changes to the antibiotic hygromycin B (HB) induced by the expression of 6K proteins were measured in E. coli cells as described previously (16, 17). Briefly, E. coli BL21(DE3) cells transformed with the described pET11 recombinant plasmids were grown in M-9 medium (18) and induced with 1 mM isopropyl-1-thio-β-D-galacto-pyranoside (IPTG). At 30 and 60 min after induction, proteins were pulse-labeled for 15 min with [35S]Met-Cys in the presence or absence of 0.5 mM HB. Cells were subsequently harvested and the radiolabeled products analyzed by SDS-PAGE, fluorography, and autoradiography. Membrane association of the 6K proteins in these experiments was analyzed as follows. Radioactively labeled cells were harvested by centrifugation, resuspended in buffer A (50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM EDTA, pH 8, 1 mM dithiothreitol) lyzed by sonication and centrifuged at 10,000 rpm for 15 min. The supernatant fraction obtained was further subjected to ultracentrifugation at 100,000 rpm for 1 h. The pellet fraction was resuspended in buffer B (10 mM Tris-HCl, pH 9.3, 1 mM β-mercaptoethanol). Three aliquots of the pellet membrane fraction were processed as follows: 1) untreated as a control; 2) treated with 8 M urea, and 3) treated with 0.5% SDS. After further ultracentrifugation at 100,000 rpm for 1 h, equivalent amounts of supernatant and pellet fractions were analyzed by SDS-PAGE and fluorography.

RESULTS

Hydroporphic Analysis of SV 6K—The sequence alignments for representative 6K products derived from dengue alphaviruses (1) are shown in Fig. 1A. Trp-11 and Trp-19 are completely conserved at the amino terminus of these sequences (Fig. 2A, bold characters). When average interfacial hydrophobicity was plotted for several of these representative sequences (Fig. 1B), the hydrophaphy plots detected two consecutive peaks at the short amino-terminal 6K ectodomain, immediately preceding the transmembrane domain (TMD). The average hydrophaphy plots in Fig. 1B were calculated according to the so-called Wilimy-White(WW)hydrophophcy-at-interface scale (19, 20). In contrast with classical hydrophobicity scales, this is a whole-residue scale (i.e. it includes contributions from peptide bonds as well as amino acid side-chains) based on the water-to-membrane interface transfer-free energies for each amino acid. Consequently, the average interfacial hydrophobicity of an arbitrary sequence directly reflects the tendency of such a sequence to partition from water into membrane interfaces or, in other words, to promote the first step of the protein-membrane interaction process leading to integration (21). The
positive 6K peaks indicate that the conserved amino-terminal stretches have a propensity to interact with and to remain immersed in the interfacial region of the membrane. Because this side of 6K faces the external side of the membrane, the presence of these segments confers on the 6K ectodomain the ability to interact differentially with the external membrane monolayer.

**Generation of 6K Interfacial Mutants**—To assess the role of the interfacial domains in 6K protein, several variants were generated (Fig. 2). Because the goal was to explore the functional importance of the hydrophobic-at-interface character of the sequences, substitutions specifically affecting this parameter that did not, however, induce appreciable changes in the overall sequence hydrophobicity, were selected. The hydropathy plots in Fig. 2 illustrate this strategy. Kyte-Doolittle plots (22), based on relative side-chain hydrophobicities, accurately predict transmembrane regions in constitutive integral membrane proteins (translocated with energy cost during biogenesis). Positive peaks are consistent with translocated TMD regions remaining stably inserted in membranes. Accordingly, the Kyte-Doolittle plots in Fig. 2 detect the TMD region of 6K as a positive peak. None of the 6K mutations generated altered this hydrophobicity pattern (Fig. 2).

In contrast with Kyte-Doolittle, the WW algorithm is based on the water-to-membrane interface transfer-free energies for each amino acid (19). The membrane interfaces are distinct regions of the bilayer, characterized by their chemical heterogeneity and sharp polarity changes with distance. Wimley and White (19) found unexpected differences in the relative hydrophobicities of the amino acid residues when these residues partition from water into interfaces of phospholipid bilayers. In particular, hydrophobic sequences rich in Trp residues show the greatest tendency to partition spontaneously from the aqueous phase into the membrane. The different substitutions made in SV 6K in the present work resulted in abolition of the first, second, or both amino interfacial subdomains (Fig. 2).

**Analysis of Sindbis Viruses with 6K Interfacial Variants**—To test the effect of the 6K mutations on different aspects of the virus life cycle, the different SV variants in 6K were first reconstituted. BHK cells were transfected with RNA transcribed from these SV variants. The proteins synthesized at 16 h.p.e. were analyzed by SDS-PAGE. Fig. 3A shows that the
three SV 6K mutants synthesized viral proteins as efficiently as wt SV. Thus, the levels of synthesized protein and host translation inhibition were similar for all viruses assayed. In addition, no defects were found in the processing of the different viral structural proteins. These results indicate that the cleavage sites in the viral polyprotein contributed by the 6K variants were able to undertake proteolytic processing adequately. Moreover, no alterations were noted in the migration of the viral glycoproteins, suggesting that their post-translational processing was not hampered by the mutations in 6K.

Previous analyses have indicated that one of the major defects of alphaviruses with a mutated 6K appears at a very late step in the virus life cycle: the process of virus budding. Therefore, the exit of viruses from the infected BHK cells was investigated by electron microscopy. Fig. 3B shows a number of SV particles exiting from wtSV-infected cells. The morphology of these virus particles was normal. In contrast, a clear defect was observed with the three 6K variants analyzed. In all three cases, virus particles accumulated at the plasma membrane. Although their morphology showed no anomaly, these particles were unable to detach efficiently from cells. It is concluded that the three mutations introduced in the 6K sequence confer defects in virus budding, such that the pinching-off of the assembled virus particles is inefficient. In turn, this defect leads to the formation of virus plaques of smaller size (data not shown).

Enhanced Membrane Permeability Provoked by 6K and Its Variants in E. coli Cells.—The clearest activity that 6K shows when individually expressed in cells is the capacity to increase membrane permeability to a number of solutes (17). To assess the participation of the interfacial region of 6K in this process, wt 6K and the three 6K gene variants were cloned and expressed in E. coli cells using an inducible system of gene expression (Fig. 4). Membrane permeability was analyzed by the HB test (16, 17). As shown in Fig. 4A, expression of the wt 6K gene not only permeabilized prokaryotic cells to the antibiotic HB, but also induced rapid cell lysis, such that the synthesis of 6K lasted only a few minutes upon 6K induction. By comparison, the three 6K variants showed a reduced permeabilization capacity and lower toxicity after induction. In particular, the variant with both mutated tripeptides (i.e. 9YLW11xAAA/
18FWV20xAAA) maintained the same expression level 60 min after induction, and membrane permeability to HB did not appreciably change.

To assess further whether mutations introduced in the interfacial sequence affected the capacity of 6K to interact with membranes, the experiment shown in Fig. 4B was performed. After protein labeling, the cells were broken, and the membrane-containing fraction was obtained. The wt 6K protein seemed associated with the membrane fraction even after urea treatment. However, incubation of this membrane fraction with SDS detergent led to the release of 6K from membranes. The data in Fig. 4B demonstrate that the 9YLW11xAAA/18FWV20xAAA variant behaves in a way akin to wt 6K. Together, these results suggest that both wt 6K and its 9YLW11xAAA/18FWV20xAAA 6K variant are integral membrane proteins. Therefore, the double modification of the interfacial sequence does not hamper the ability of 6K to become an integral membrane protein, although it does affect its ability to perturb the membrane permeability barrier.

Membrane Permeability Induced by 6K Variants in BHK Cells—The potent permeabilizing capacity of 6K in prokaryotic cells was recognized several years ago (17), but the action of this protein in mammalian cells remains unknown. To address this matter, a system was designed based on SV replicons that facilitate sequence of 6K is crucial for the correct activity of this protein with respect to enhancing membrane permeability (Figs. 4 and 5) and virus budding (Fig. 3). Some instability of this protein in mammalian cells.

FIG. 4. Membrane permeability in E. coli cells that synthesize 6K proteins. A, cultures of E. coli (BL21(DE3)) transformed with pETT1–6K (wt, YLW/AAA, FWV/AAA, and YLW/FWV/AAA/AAA) plasmids were induced to express the different 6K protein with 1 mm isopropyl-1-thio-β-D-galactopyranoside. 30 or 60 min after induction, cells were labeled for 15 min with [35S]Met-Cys in the presence (+) or absence (−) of 0.5 mM HB, and then processed for gel electrophoresis analyses as indicated in Fig. 3A. B, interaction of 6K proteins with membranes: the cultures indicated in the figure were induced, labeled with [35S]Met-Cys, and processed to analyze the membrane fraction, as indicated under "Experimental Procedures." Lane 1, supernatant fraction after 100,000 rpm centrifugation; lane 2, pellet fraction after 100,000 rpm centrifugation; lane 3, supernatant fraction of urea-treated sample 2; lane 4, pellet fraction of the urea-treated sample 2; lane 5, supernatant fraction of the SDS-treated sample 2; lane 6, pellet fraction of the SDS-treated sample 2.

FIG. 5. Membrane permeabilization in BHK cells that synthesize 6K proteins. A, schematic representation of the full-length SVwt cDNA clone and the different SV-derived replicons ("rep") used in this experiment. B, protein synthesis in BHK cells electroporated with the different RNAs. BHK cells electroporated with in vitro-transcribed RNA from the different constructs indicated in the figure were labeled at 16 h.p.e. with [35S]Met-Cys in the absence (−) or presence of 0.5 or 1 mM HB. Mock cells, as well as cells electroporated with RNA from "rep" C, were used as negative controls. Cells electroporated with RNA from pT7SVwt were used as a positive control of permeabilization to HB. C, percentage inhibition of protein synthesis exerted by HB in cells that expressed the different 6K genes. Densitometric analyses of protein C were used to calculate the inhibition of protein synthesis by HB into cells transfected with RNA encoding only C protein (Fig. 5B). Notably, HB readily entered into BHK cells transfected with full-length RNA from wt SV, leading to a profound inhibition of viral translation. The expression of wt 6K enhanced membrane permeability to HB, whereas this effect was not as strong with the 6K variants. Thus, with respect to synthesis-inhibition levels, it may be inferred that 9YLW11xAAA, 18FWV20xAAA, and 9YLW11xAAA/18FWV20xAAA substitutions provoked a reduction in the capacity of wt 6K to induce HB entry by 25, 66, and 94%, respectively. These findings are consistent with the hypothesis that the integrity of the interfacial sequence of 6K is crucial for the correct activity of this protein with respect to enhancing membrane permeability (Figs. 4 and 5) and virus budding (Fig. 3). Some instability of the double 6K mutant was also observed, perhaps indicating that the mutated region plays a part in governing protein degradation in mammalian cells.

DISCUSSION

There are a number of virus-encoded proteins capable of enhancing cell membrane permeability. These proteins can be classified into two broad groups. One comprises viral glycopro-
teins with a particular architecture in their transmembrane regions and the sequences proximal to that domain (23–26). Oligomerization of these proteins may lead to pore formation and membrane disturbance (27). The other is a group of viral polypeptides that destabilize membranes and includes a number of small and very hydrophobic proteins. These integral membrane polypeptides are also able to oligomerize, promoting pore formation in biological membranes. Consequently, they are known as viroporins (16, 17, 28–32). The alphavirus 6K protein is a typical viroporin that contains a transmembrane region that anchors it to membranes during protein synthesis. Despite its relative structural simplicity, 6K plays several roles during the virus life cycle. Apart from its participation in polyprotein processing, the major function of 6K stems from its requirement for efficient virus budding from infected cells (6, 7, 4, 9–14). The exact molecular mechanisms of virus budding are not yet fully understood. The fact that 6K enhances membrane permeability led to the proposal that this activity facilitated the exit of viruses from cells. Perhaps the destabilization of the plasma membrane by 6K promotes the release of virus particles to the medium. This may rely not only on the physical modification of the membrane but also on the local dissipation of ionic gradients by the pore-formation capacity of 6K.

The 6K transmembrane region is thought to allow the early formation of a hairpin structure embedded in the ER membrane. This structure positions the polypeptide amino and carboxyl termini in the ER lumen (8) (Fig. 6A). Little is known about the stability of this predicted structure or the eventual topology adopted by 6K embedded in the plasma membrane.

Prediction of membrane protein structure and stability has been recently implemented with the introduction of the WW hydrophobicity-at-interface scale (20). The present work analyzed the interfacial hydrophobicity of alphavirus 6K. The predication of a given sequence for membrane interfaces over bulk apolar or polar phases seems to be dictated by the presence of aromatic residues. WW analysis shows the presence of two stretches containing invariant Trp residues within the 6K protein ectodomain, with a high tendency to partition into membrane interfaces. The preservation of these interfacial sequences is crucial for enhanced membrane permeability by 6K protein. The 6K variants mutated in the pretransmembrane region were unable to permeabilize membranes even though they seemed to be membrane-integral products. Thus, the integration of 6K into the membrane is insufficient for membrane destabilization, but this 6K amino-terminal region is also necessary to permeabilize membranes and for efficient virus budding.

We have previously reported (13) the isolation of an SV variant, Del6K-revQ21L, with a partially deleted 6K that displays an almost genuine interfacial domain (Figs. 2B and 6B). This variant shows correct proteolytic processing and transport but still exhibits defects in virus budding comparable with those observed when the 6K gene is completely obliterated. Therefore, expression of the isolated interfacial region does not restore 6K function either. It would seem that a sequence immersed in the membrane interface followed by the transmembrane anchor represents the minimal structure required for functional 6K to induce membrane alterations (Fig. 6C).

Exposure to different lipid environments during protein trafficking might affect 6K structure and membrane topology. In particular, it has been proposed that cells rely on membrane thickness to sort proteins destined for the plasma membrane from the Golgi apparatus (33). Accordingly, the TMDs of plasma membrane proteins are longer than those of Golgi proteins (34). It is therefore possible that differences in membrane thickness, such as those that occur along the secretory pathway, might play a role in regulating 6K topology and structure. Computation of the free energy according to values determined by Wimley and White (19, 20) indicates that the inside-outside TMD (number 2 in model 6A) would not remain stably immersed in the membrane core (data not shown). It is therefore conceivable that a topology such as that shown in Fig. 6C, with the interfacial region (gray cylinder) followed by a single transmembrane helix (white cylinder) would be more favorably adopted by the secreted versions of 6K (see also the predictions in Fig. 1).

Several forms of peptide-chain embedding within the external membrane monolayer interface might influence 6K-induced membrane perturbation and permeabilization. In principle, the interfacial sequence might be required to disrupt interactions between lipid molecules and might be directly involved in the destabilization of membrane integrity (Fig. 6C). A similar mechanism has previously been proposed for poliovirus 3A protein (35). The differential surface increase of the external membrane monolayer might also contribute to membrane deformation and bending (positive curvature) at the points of virus budding (4, 7). The tendency to minimize energetically those perturbations might be exploited to assemble oligomeric transmembrane pores that actually induce the rupture of the permeability barrier. Moreover, the interfacial region might participate in the regulation of the opening of those transmembrane pores.

**Fig. 6. Schematic representation of 6K structure and membrane topology.** A, model for 6K protein inserted into the ER membrane. According to the model proposed by Liljestrom and Garoff (8), 6K is predicted to fold as a membrane-embedded hairpin constituted by: 1) an outside-inside transmembrane domain (thick cylinder); 2) an inside-outside transmembrane domain (narrow cylinder); and 3) a Cys-rich loop that reverses chain direction. One of the interfacial segments (gray ellipsoids) might contribute to the outside-inside TMD in this state. The carboxyl- and amino-terminal ends of the peptide chain would face the ER lumen and be cleaved therein. B, the Del6K-revQ21L product consists mainly of both interfacial segments that are not transferred into the membrane core. C, model for 6K secreted to the plasma membrane (PM). In this version, 6K contains an interfacial helix (gray cylinder) followed by a single transmembrane helix (white cylinder). The interfacial domain might disrupt phospholipid cohesion at the external membrane monolayer (see also models for poliovirus 3A protein interacting with membranes in Ref. 35).
Finally, it should be noted that the presence of complex helical transmembrane regions might be of functional importance to viral proteins that induce membrane perturbations required for fusion or permeabilization. Thus, an interfacial region similar to the 6K sequence described here has been reported to occur in the region proximal to the transmembrane anchor of human immunodeficiency virus gp41 fusion protein (24). A long interfacial sequence preceding the transmembrane anchor seems to be a common structural motif in fusogenic proteins belonging to several virus families. Our initial analyses indicate that interfacial helices adjacent to membrane-spanning domains might represent a motif conspicuously present also among members of the viroporin protein family. A comparative study is under way in an effort to establish the extent to which interfacial sequences contribute to the pore-forming activity of this family of viral proteins.

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