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Early steps in rhabdoviral infection

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SUMMARY

The protein G (pG) sequence of rhabdoviruses is well conserved among the same genuses (vesicular stomatitis, rabies, etc.) but unrelated among them. The pG has ~ 500 amino acids (aa), 2-6 glicosylation sites, 12-16 conserved cysteines, 2-3 conserved hydrophobic heptad-repeats, a removed amino terminal signal peptide, a carboxy transmembrane peptide and a carboxy terminal cytoplasmic domain. The pG forms trimers responsible for the binding of these viruses to the host cell receptor(s) and for the low-pH fusion of the viral and host membranes. A few host cellular proteins have been identified as rhabdoviral receptors (acetylcholine receptor, fibronectin, etc.). MAR mutants have been used to map pG regions potentially important for the interactions with the host receptor. Binding of pG to anionic lipids has been also demonstrated and the pG regions implicated in the binding mapped. However, more rhabdoviral receptors are yet to be identified and the relationships among them need to be further investigated. Contrary to many other enveloped viruses, in rhabdoviruses there is no proteolytic processing of the pG to expose a fusion peptide and the conformational changes needed for low-pH fusion are reversible. Fusion defective mutants and low-pH MAR mutants have been used to map regions of pG that might take part in the fusion processes, but there is not yet a mechanism that will explain how fusion does occur. All the above mentioned data make the rhabdoviral fusion an intriging new subject to study viral entry in the host cells.

THE EARLY STEPS IN RHABDOVIRAL INFECTION

Rhabdoviral infections initiate by binding of the virus to specific cellular outer membrane host receptor(s) and are followed by a low pH-dependent fusion of the viral and cellular membranes after being taken up by the cells. The protein G (pG) of the rhabdoviral trimeric spikes is implicated in all the steps.

AVANCES IN THE pG STRUCTURE

General function and structure. One rhabdovirus particle contains around 450 trimers made of pG, each of which forms protruding 84 Å spikes (35, 36). The pG initiates virus attachment to cell receptor(s) (86) and contains the fusion properties of the virus detectable at pH $\leq$ 6 in vesicular stomatitis virus, VSV (43, 78, 79), in rabies virus, RV (35) and in viral haemorrhagic septicaemia virus, VHSV, a rhabdovius infecting fish (27, 48). The pG is also the target for the host neutralizing antibodies (Abs) in VSV (42), VHSV (53), infectious haematopoietic necrosis, IHNV (26) and RV (6, 19).

All the pG of rhabdoviruses have around 500 aa including a signal peptide which is removed in the mature pG, 2-6 potential glycosilation sites, 12-16 conserved cystein residues, 2-3 conserved regions of hydrophobic heptad-repeats (14), a transmembrane highly hydrophobic sequence and an hydrophilic cytoplasmic carboxy-terminal peptide tail with 2 acylated sites (13).

A recent alignment including the pG sequences from several rhabdovirus genuses, VSV, RV, VHSV, hirame rhabdovirus (HRV), IHNV and spring viremia carp virus (SVCV) showed an overall homology of only 18-26% and an identity of less than 5% (25). The pG of Sonchus Yellow Net Virus and Rice Transitory Yellowing Virus, two of the most studied plant rhabdoviruses (accesion numbers L32603 and AB011257), showed also a very low degree of sequence identity with the other rhabdovirus genuses. While we are still waiting for the X-ray structure of the pG of either RV or VSV, some advances in the pG structure might be relevant to the elucidation of the early steps of infection mentioned above (for instance,

hydrophobic heptad repeats, disulphide bonding pattern, etc.).

Hydrophobic heptad repeats. Many enveloped viruses have heptad repeats in the glycoprotein present in their membranes. These heptad repeats are related to viral fusion (73). Heptad repeats form coiled coils as demonstrated from the X-ray structures of some of the proteins were they were found. Heptad repeats forming coiled coils were found as 3-4 contiguous sequences of 7 aa (abcdefg) in which the aa in each position was specified by a probability matrix. The aa most frequently found in positions a-d were F, Y, I, L, V, M, or A (54). However, heptad repeats such as those of Lupas are not present in the pG of rhabdoviruses. Only after considering in positions a-d all the hydrophobic aa (the ones mentioned above plus W, H, T) (70), hydrophobic heptad repeats could be found in the pG of mammalian (VSV and RV), fish (VHSV, IHNV, SVCV) and plant rhabdoviruses (14).

These hydrophobic heptad repeats differ from the ones forming coiled coils (54) not only because of the inclusion of all the hydrophobic aa in the a-d sites, but also because no constrains (probability matrix defined by Lupas) were required for the aa in the rest of the heptad positions. The hydrophobic heptad repeats had low sequence variability among the members of each of the rhabdoviral genus but show absolutely no sequence similarity among the different genuses. It is not known whether these heptad repeats exist in the native pG as alpha helices, coiled coils or any other structure. The presence of numerous helix-breaking proline residues scattered throughout the hydrophobic heptad repeats is a major factor arguing against the existence of these structures as alpha helixes. However, hydrophobic heptad repeat sequences would only mean structures of amphiphatic alpha helixes, thus this paradox will remain elusive until the X-ray structure of some pG could be obtained.

Disulphide bonding. The pG of rhabdoviruses posses 12-16 cysteins (C) depending on the genuses (13). Despite their unrelated aa sequences, the relative positions of most of the C are remarkably conserved throughout all rhabdovirus genuses.

There is only one report on the complete disulphide bonding structure within the extra cellular domain of a rhabdovirus pG, that of VHSV (25). Six disulphide bonds involving 12 C were assigned. Two disulpide bonds formed two long-distance loops between C29-C339 and C44-C295. They bend the lineal sequence bringing together the amino-terminal region and the central region of the pG molecule (Figure 1). Two other disulphide bonds formed short-distance loops located between C inside the 2 long loops. The loop C90-C132 contains the region where MAb C10 MAR mutants map (4) and the loop C195-C265 contains the region where MAb 3F1A12 MAR mutants map (52). The other 2 disulphide bonds (C172-C177 and C231-C236) were highly stable and located between cysteins which were only a few aa of distance from each other.

The existence of at least, a major loop has also been proposed for the pG of VSV (37) and the 3 disulphide bonds already identified in the pG of RV (20) also support the above referred structure. Alignment of the pG from VSV with that from other rhabdoviruses (including RV, VSV and IHNV) (25) indicate that there might be some commom disulphide bonding pattern whithin the rhabdoviruses. However, there were no indications of any alternative disulphide bonding pattern being present in the pG from VHSV as it was proposed before for the pG from RV (20).

BINDING TO THE CELL HOSTS

The cellular receptor(s). The first interaction of the rhabdovirus pG with its cell host depends on the presence of a receptor(s) molecule(s) in the cell host outer membrane. There is little evidence for the specificity of cell receptors as determinants for rhabdoviral cellular susceptibility. Early experiments based on binding assays showed that VSV competed with RV, suggesting the existence of a common receptor for all rhabdoviruses. This common receptor had a chloroform-methanol soluble component(s) that blocked virus binding and infectivity (18, 86). Treatment of susceptible cells with phospholipases but not with trypsin inhibited the binding of VSV or of RV to its host cells (76, 86). Furthermore, phosphatidylserine (PS) was capable of inhibiting both the binding of VSV to the cell host and 90% the VSV plaque formation (65). Both the serine head group and the hydrophobic fatty acid portions of the PS were essential for its inhibitory activity (87). Binding of PS to the pG has been also demonstrated in VHSV (17). The predominant segregation of the PS to the inner part of the cellular membrane (80-90% of the total PS of the membrane), could explain the limited number of the virus attachment sites observed. Despite the apparent role of PS in VHSV or VSV binding (43), it is more probable that it constitutes either the binding to a portion of a larger protein-lipid complex receptor (65, 67) or some binding implicated in subsequent fusion steps (28).

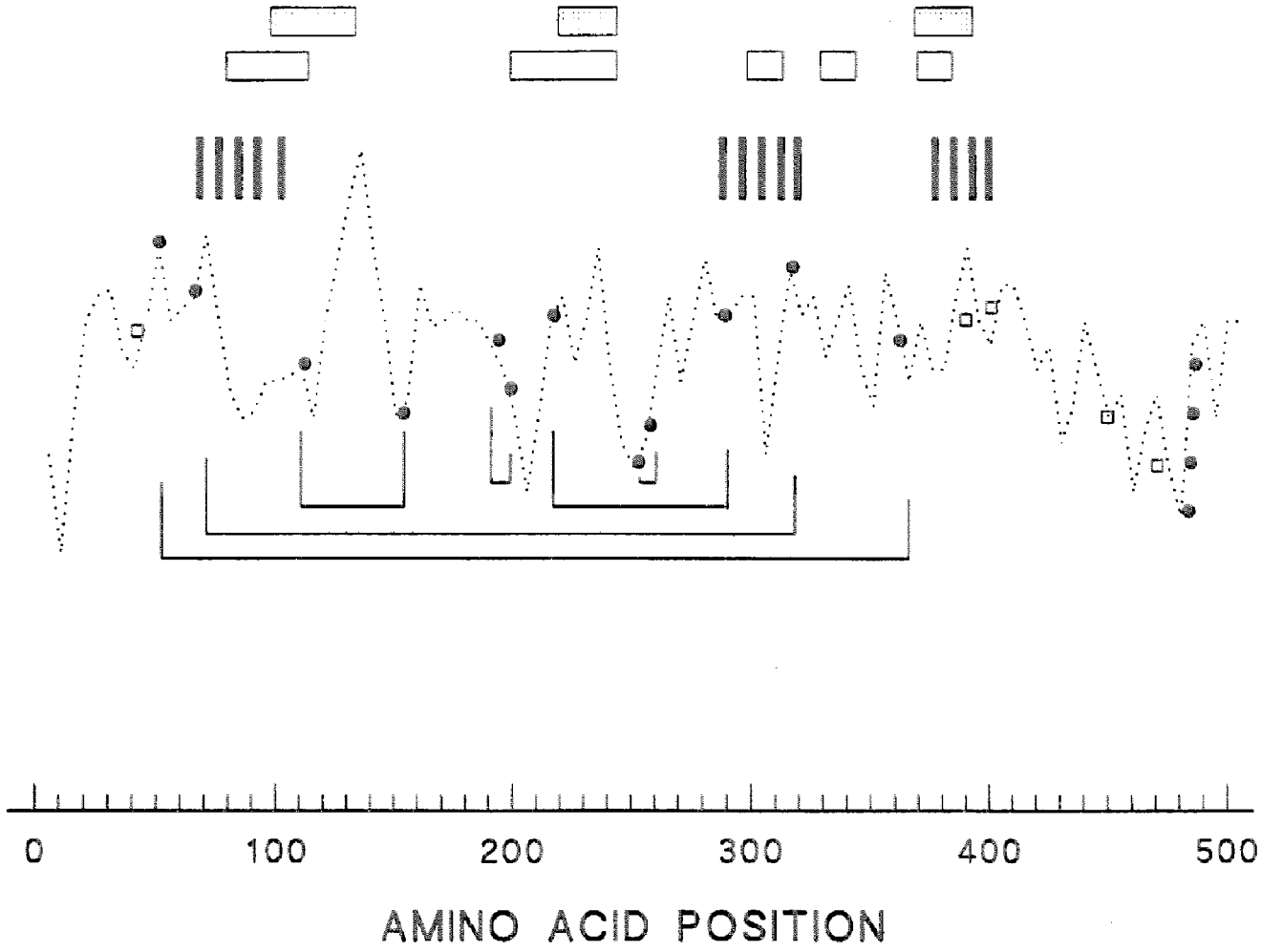

Figure . Map of some motifs in the pG of VHSV. ·····, Hydrophilicity was predicted with the program ANTIGEN (PCGene package). ●, Cysteins. Small □, carbohydrates. | | | |, Hydrophobic heptad repeat regions A, B and C (14). large □, PS phosphatydylserine binding regions (27).⁞⁞⁞, regions binding biotinilated peptides corresponding to the hydrophobic heptad-repeat sequences (30). ————, disulphide bridges (25). The most variable region in the pG sequence has been found between aa 260 to 300 (5).

Different assays were later applied to the problem of identification of receptors for rhabdoviruses. Thus the RV pG sequence from aa 170 to 255 was found to be highly homologous to the regions of the snake venoms that bind to the acetylcholine receptor (AChR) (77). Furthermore, the pG from RV bound to the AChR and behaved as an AChR antagonist (38). Consequently, polyclonal antibodies (PAbs) against RV bound to AChR and more experiments with MAbs confirmed that the AChR is one of the RV receptors (38).

In other recent approach to the same problem, anti-cell-receptor MAbs capable of neutralizing rhabdoviral infectivity were generated. Thus three MAbs against a salmonid fish cell line were selected by their ability to protect those cells from VHSV infection. Immunoprecipitation of the cell membrane proteins with those MAbs identified an heterodimeric complex composed of 200 KDa (later identified as trout fibronectin) and 47 KDa proteins. This complex was most abundant in trout muscle one of the main targets of VHSV. The anti-cell-receptor MAbs were specific

for salmonid cells and blocked the infection of a large pannel of antigenically unrelated fish rhabdoviruses (pikefry, SVCV, eel rhabdovirus, all the serotypes of VHSV and IHNV) (3). Fibronectin, a component of the cell to cell adhesion molecules family, is a 400 KDa homodimer disulphide linked protein found insoluble in fibrillar form or soluble in the plasma (64). A fibronectin complex has been also shown to interact with RV (8). Since other reports have identified the AChR as an initial RV target (7, 33, 49, 50), it is interesting to note that some matrix molecules including fibronectin have been found together with the AChR (21). All these experiments, however, are only beginning to address the question of the complexity of the multiple rhabdovirus receptors and more work needs to be done in this area.

Monoclonal antibody resistant (MAR) mutants. Binding of neutralizing Abs to the pG sites implicated in the interaction of pG with the cell receptors is one of the posible mechanisms for in vitro neutralization of viruses. Thus the pG sequence of neutralizing MAb resistant (MAR) mutants could give an indication of the sites involved in the binding to the cell receptor or in the conformational important sites needed for that interaction.

Extensive analysis of neutralization by MAbs and its MAR induced viral mutant sequences have been carried out only in RV. Few anti-pG MAbs were found which were not neutralizing and/or independent on the pG native conformation (6, 10, 44). Most of the 266 anti-pG RV neutralizing MAbs recognized several overlapping epitopes that make up site II (72.5% of the MAbs) and site III (24.8% of the MAbs), the rest being directed towards sites I and IV (6, 46). MAR mutants to site II had simultaneous aa substitutions located in the aa 34-42 and in the aa 198-200 regions. Site II is, therefore, a discontinous conformational site. In contrast, the MAR mutants to site III had aa substitutions located in aa 330-338 (continous site). By assuming that the aa substitutions of MAR mutants are in the MAb binding regions, we can speculate that this MAb binding regions are related to the early steps of viral infection by means of inhibiting either binding of pG to the cell receptor or some other subsequent step.

Only one biochemical/immunogenic study have been made to map regions of the pG from RV important for neutralization (20). All the cyanogen bromide (CNBr)-cleaved pG peptides induced binding Abs but only peptides aa 20-63, aa 123-198 (highest antigenic peptide) and aa 312-342 induced neutralizing Abs. Peptides aa 20-63/aa 208-255 and aa 208-255/aa 264-310 were bound by disulphide bonds. These results toghether with the MAR mutant results discused above correlate well with the existence of at least 2 different loops in the pG. The first would make a large loop (peptide aa 20-63 bound to aa 208-255) that would contain the highest antigenic peptide (aa 123-198).

FUSION OF VIRAL AND CELLULAR MEMBRANES

pH-dependance of fusion. After the temperature dependent internalization of the cell receptor-bound rhabdovirus, a second step of the rhabdoviral infection cycle is the fusion of the rhabdoviral membrane with the membrane of the host cells. Whereas rhabdoviral binding can occur at 4 °C and pH 7.5, entry of the virus by endocytosis is an energy-dependent even which only occurs at physiological temperature. After endocytosis, the pH is lowered to $\approx$ 5, as suggested by inhibition of fusion with lysosomotropic amines (chloride, ethylenediamine, tetracaine, ammonium chloride, etc.) in VSV (63, 69) and in RV (75).

Confirming the above referred results, pG-phospholipid vesicles fused with acidic phospholipid vesicles only at pH 5 as monitored by electron microscopy or fluorescence energy transfer (24). Hemolysis of human erythrocytes by VSV was also maximal at pH 5 (1) and exposure to pH 5 of infected or transfected cells expressing pG causes cell to cell fusion in VSV (35) as well as in VHSV (48). Although the VSV pG had pH-dependent hemolytic activity (68) apparently requiring its 6 amino terminal aa (66), site-directed mutagenesis (85), demonstrated that other viral pG regions must be implicated in the fusion process (45, 66).

Conformational changes in pG induced by low pH. Exposure of purified VSV virions to pH 5, first induces an accumulation of the pG trimers at the ends of the virions as shown by electron micrographs (9). Exposure of purified pG from RV in the absence of lipids to pH 5 induces a pH-dependent reversible equilibrium between the native fusion-active (83 Å) and the fusion-inactive (113 Å) pG trimers. There is a reversible pH-dependent equilibrium between the native and the low-pH conformation of pG also observed in VSV (22). Exposition of the pG trimers to pH 5.9, resulted either in the inhibition of fusion in the absence of lipids or in the activation of fusion in the presence of lipids. Therefore, activation of the pG trimers for fusion requires a pG conformational change. That change exposes hydrophobic regions of pG at its

surface as demonstrated by alteration of the binding of MAbs to region II and by fluorescence assays (35).

Phospholipid binding. Phospholipids from cellular membranes inhibited attachment and infection of RV (76) and of VSV (2, 18, 55, 69). Phosphatidylserine (PS) was the strongest inhibitor of VSV attachment and infection (65).

A PS binding domain from aa 82-109 (p2) of the pG of VHSV was identified by using pepscan, synthetic peptides, purified recombinant pG and purified VHSV solid-phase phospholipid-binding assays (16, 27, 28). The PS-binding domain contained hydrophobic heptad repeats followed by a short stretch with 2 positively charged aa (13). Hydrophobic heptad repeats followed by positively charged aa (p2-like peptides) were then found in all pG from rhabdoviruses in similar positions with variations in the sequence of aa for each genus, in number of repeats, in the length of the charged aa stretch, and in the kind of charged aa involved (13, 14, 27). All the p2-like synthetic peptides obtained from RV, VSV and IHNV also bound PS (16).

The p2 peptide was able to induce lipid vesicle aggregation, lipid mixing and rapid leakage of lipid vesicle-trapped fluorescent probes in a peptide concentration-dependent manner (58). These observations confirmed previous solid-phase assay results (28) that showed that the PS-p2 binding depends from both hydrophobic and ionic interactions as also shown in other rhabdoviruses (87). Both, fluorescence depolarization or differential scan calorimetric (DSC) studies, demonstrated that the p2 peptide inserted into the hydrophobic core of the anionic phospholipid bilayer maintaining or favouring a ß-sheet conformation. Circular dicroism (CD) studies indicate that, the ß-sheet conformation is practically the only one observed upon interaction with PS and, thus, it should be taken as responsible for the membrane destabilization properties exhibited by the p2 peptide (58).

Low-pH MAR mutants. Neutralizing MAbs which bind to the rhabdovirus only at the low-pH of fusion have been studied in RV. All the MAR mutants derived from 4 different low-pH neutralizing MAbs mapped to regions in aa 29-34, aa 63 and aa 411-415. These studies also confirmed that neutralization required a high number of Ab molecules attached to the virion, irrespective of the region of the protein which was recognized by the Ab (31).

Fusion defective mutants. This kind of mutants have been mostly studied in VSV. A first fusion defective mutant of VSV in aa 117 suggested that the adjacent region (aa 118 to 136) could be involved in the membrane fusion activity. This activity in VSV seems to occur via one or several strech(es) as mapped by fusion-defective mutants generated by aa insertion after aa residues 123, 194, 410 and 415 (51) or in the aa 118-136 region (79). More studies with site-directed mutagenesis, finally identified the sequence of aa 123-137 of the pG of VSV as a putative fusogenic peptide (89). However, more fusion defective mutants were also found in regions between aa 120-150, aa 134-161, aa 190-210, aa 300-360 and aa 409-419 (72). Thus a region between aa 385-418 have been shown to alter the pH at which fusion does occur and a region between aa 443-452 adjacent to the transmembrane domain seems to be also implicated in fusion (72).

Inhibitory synthetic peptides from enveloped viral glycoproteins (gp). Contrary to rhabdoviruses, many enveloped viruses suffer internal cleavage to free the amino-terminal part of the so call fusion peptide and suffer irreversible conformational changes after lowering the pH for fusion. Viral inhibitory peptides have been found among synthetic peptides derived from the external glycoprotein (gp) of most enveloped viruses. For instance, synthetic peptides (DP-107) corresponding to the N-terminal heptad repeats (N-peptides) adjacent to the fusion peptide of the gp of human immunodeficiency virus (HIV) blocked in vitro viral infection and syncytia formation at micromolar concentrations (59, 83). Aa substitutions in the heptad repeats of the N-peptides abrogated its antiviral activity (41, 83). Other synthetic peptides (DP-178) corresponding to the C-terminal (C-peptides) regions adjacent to the transmembrane domain with a strong tendency to form α-helixes, were found with even more potent antiviral activity (nanomolar concentrations) (81, 82). Still other synthetic peptides located within the fusion peptide of the gp of HIV with antiviral activities have been also described (39, 40, 57, 74). Similar viral inhibitory peptides have been found in influenza (47, 88), respiratory syncitial virus (47), paramyxoviruses (61, 62, 71) and measles virus (47, 84).

Because of the ability of C-peptides to associate with N-peptides (12), the antiviral activity of the C-peptides could be due to interference with the function of the N-peptides which are located nearby the fusion peptide (82). The X-ray structure of this part of the gp recently showed that 3 N-peptide α-helices form an interior coiled-coil trimer while three C-peptide helices pack outside (11). This N+C peptide complex folds in

the absence of other viral proteins, is heat stable, mutations in their heptad repeats abolished membrane fusion and peptides derived from the N-peptide or C-peptide regions, inhibited viral infection and syncytia formation. The N-peptides might inhibit fusion by interfering with formation of the interior coiled coil trimer and/or by binding to the outside C-peptide regions. On the other hand, the C-peptides could act by binding to the N-peptide coiled coil trimer (11).

In rhabdoviruses, however, there is not known internal cleavage to release the fusion peptide and the pH-dependent conformational change needed for fusion to occur is reversible. Furthermore, no inhibitory viral peptides have been described yet. The question still remains, is there any inhibitory viral peptides in rhabdoviruses?. If ever found, inhibitory rhabdoviral peptides could clarify some of the aspects and variables involved in the processes of rhabdoviral fusion.

Conclusions.

Indirect studies made with the pG CNBr-cleaved peptides from RV (20) and by MAR mutant sequencing (6) indicated that most of the binding of neutralizing Abs to pG are located into regions aa 34-42/198-200 brought together by disulphide bonding. Binding of enough Ab molecules to these sites (1-2 IgG/3 spikes or 1 IgM/9 spikes) was necessary for in vitro neutralization to occur (31) suggesting some kind of accesibility effect. On the other hand, the RV pG region located between aa 170-255 highly homologous to the snake venon binding region of AchR (77), seems to be implicated at least, in the binding of one of the RV receptors. All these results suggest that receptor binding regions of pG could be situated around aa 170-255 with some participation of more amino-terminal regions although the precise sites remain yet to be identified. There is no other indications or more direct studies made for other rhabdoviruses in this subject.

Many studies have been made to map the fusion peptide of VSV by fusion defective mutants. On the other hand, PS-binding regions of VHSV have been studied by solid-phase binding assays and hydrophobic heptad repeats discovered. The correspondence and requirement of position and structure of the heptad-repeats and cysteins among the pG of mammalian, fish, insect and plant rhabdoviruses despite their different aa sequences suggests its participation in common function(s), most probably related to membrane fusion with host membranes.

Several lines of published evidence suggest that the rhabdoviral PS-binding p2-like regions could be somehow related to membrane fusion with host membranes. For instance, pG regions interacting with phospholipids of the host membrane were identified by hydrophobic photolabeling in aa 74-237 for VSV and in aa 122-198 for RV (23, 34). These photolabeled regions corresponded to hydrophobic heptad repeat regions in both viruses (14). Moreover mapping of fusion defective VSV mutants identified the aa 123-137 stretch (situated immediately before the p2-like peptide) as a putative fusion peptide (32, 51, 79, 89). That the PS-binding to p2 in solid-phase could be relevant to the earlier steps of the infective cycle of VHSV (15), was supported by the capacity of anti-p2 PAbs to recognize both p2 and purified VHSV only at low-pH (28), to inhibit the PS-binding to VHSV in solution only at low pH (27) and to inhibit VHSV-induced cell to cell fusion (29). On the other hand, p2 induced aggregation of lipid vesicles, closed apposition of membranes and destabilization of anionic phospholipid bilayers, most of the steps required for fusion. Furthermore, similar pH dependence profiles were shown by PS-binding to purified VHSV (27) and by pG mediated membrane fusion (48). On the other hand, mutations in the hydrophobic aa of the heptad-repeats of the gp of several paramyxoviruses (61, 62, 71), baculovirus (56), HIV (80) and murine leukemia virus (60), abolished viral fusion, thus suggesting that not only the fusion peptide but also adjacent downstream heptad repeats are important for viral-cellular membrane penetration and destabilization (62). Although there is not yet complete evidence that the interaction PS-p2 is really relevant to virus entry, all the above mentioned findings support the idea that p2-like regions of the pG protein of rhabdoviruses might play an active role in the viral fusogenic processes.

However, not all the regions implicated in fusion are in the amino-terminal part of pG. Thus although regions first defined by fusion defective mutants in VSV are upstream of the amino-terminal heptad repeats, they have been also recently found downstream of the carboxy-terminal heptad repeats (72), suggesting a simetrical structure which could be brought together by disulphide bonding (25). More experiments are needed to clarify the implications of all these regions in fusion. On the other hand, conformational changes induced by the low-pH must be important for fusion to occur. In this respect, computer programs predict an α-helix conformation in the p2 peptide from VHSV, however, biophysical studies have demonstrated a ß structure after insertion in the membrane (58). The p2 peptide could acquire a ß-sheet structure after a change in the conformation of

the pG mediated by the low pH thus enabling it ot interact with the anionic phospholipids. A conformational change in the pG of VHSV when exposed to low-pH seems to be occurring (27, 30) and similar low-pH induced conformational changes in the pG of rabies and VSV have also been reported (35), but we do not know yet how these conformational changes favour the fusion processes.

The fact that complete studies are not yet made in any of the rhabdovirus genus, made these speculations very fragmentary. Rhabdoviruses are original among other enveloped viruses, since there is no proteolytic processing to expose the fusion peptide and the low-pH induced conformational changes needed for fusion are reversible. Thus they remain a new subjet to study some novel mechanisms of viral entry into the host cells.

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References

1. **Bailey, C., D. Miller, and J. Lenard.** 1981. J.Cell Biol. **91:**111-116.

2. **Bailey, C. A., D. K. Miller, and J. Lenard.** 1984. Virology. **133:**111-117.

3. **Bearzotti, M., B. Delmas, A. Lamoureux, A. M. Loustau, S. Chilmonczyk, and M. Bremont.** 1998.Int.Symp.Virus Lower Vertebrates.,Weymouth UK OP37.

4. **Bearzotti, M., A. F. Monnier, P. Vende, J. Grosclaude, P. DeKinkelin, and A. Benmansour.** 1995. Veterinary Research. **26:**413-422.

5. **Benmansour, A., B. Basurco, A. F. Monnier, P. Vende, J. R. Winton, and P. deKinkelin.** 1997. J.Gen.Virol. **78:**2837-2846.

6. **Benmansour, H., H. Leblois, P. Coulon, C. Tuffereau, Y. Gaudin, A. Flamand, and F. Lafay.** 1991. J.Virol. **65:**4198-4203.

7. **Bracci, L., G. Antoni, M. G. Cusi, L. Iozzi, N. Niccolai, S. Petreni, M. Rustici, A. Santucci, P. Soldani, and P. E. Valensin.** 1988. Mol.Immunol. **25:**881-888.

8. **Broughan, J. H., and W. H. Wunner.** 1995. Arch.Virol. **140:**75-93.

9. **Brown, J. C., W. W. Newcomb, and S. Lawrence-Smith.** 1988. Virology. **167:**625-632.

10. **Bunschoten, H., M. Gore, I. J. T. M. Classen, F. G. C. M. Uytdehaag, Wunner.W., and A. D. M. E. Osterhaus.** 1989.J.Gen.Virol. **70:**291-298.

11. **Chan, D. C., D. Fass, J. M. Berger, and P. S. Kim.** 1997. Cell. **89:**263-273.

12. **Chen, T. T., J. K. Lu, M. J. Shamblott, C. M. Cheng, C. M. Lin, J. C. Burns, R. Reimschuessel, N. Chatakondi, and R. A. Dunham.** 1995. Zoological Studies. **34:**215-234.

13. **Coll, J. M.** 1995. Arch.Virol. **140:**827-851.

14. **Coll, J. M.** 1995. Virus Genes. **10:**107-114.

15. **Coll, J. M.** 1996. J.Fish Dis. **18:**519-527.

16. **Coll, J. M.** 1997. Arch.Virol. **142:**2089-2097.

17. **Coll, J. M.** 1995. Virus Res. **26:**399-407

18. **Conti, C., B. Hauttecoeur, M. J. Morelec, B. Bizzini, N. Orsi, and H. Tsiang.** 1988. Arch.Virol. **98:**73-86.

19. **Dietzschold, B., M. Tollis, C. E. Rupprecht, E. Celis, and H. Koprowski.** 1987. J.Infect.Dis. **156:**815-822.

20. **Dietzschold, B., T. J. Wiktor, R. MacFarlan, and A. Varrichio.** 1982. J.Virol. **44:**595-602.

21. **Dimytrenko, G. M., and R. J. Bloch.** 1993. Exp.Cell Res. **206:**323-334.

22. **Doms, R. W., D. S. Keller, A. Helenius, and W. E. Balch.** 1987. J.Cell Virol. **105:**1957-1969.

23. **Durrer, P., Y. Gaudin, R. W. H. Ruigrok, R. Graf, and J. Brunner.** 1995. J.Biol.Chem. **270:**17575-17581.

24. **Eidelman, o., R. Schlegel, T. Tralka, and R. Blumenthal.** 1984. J.Biol.Chem. **259:**4622-4628.

25. **Einer-Jensen, K., T. N. Krogh, P. Roepstorff, and N. Lorenzen.** 1998. J.Virol. **72:**10189-10196.

26. **Engelking, H., and J. C. Leong.** 1989. Virus Res. **13:**213-230.

27. **Estepa, A., and J. M. Coll.** 1996. Virology. **216:**60-70.

28. **Estepa, A., and J. M. Coll.** 1996. J.Virol.Methods. **61:**37-45.

29. **Estepa, A., and J. M. Coll.** 1997. Dis.Aquatic Organ. **28:**185-189.

30. Estepa, A., M. Fernandez-Alonso, and J. M. Coll. 1999. Virus Res. in press.

31. Flamand, A., H. Raux, Y. Gaudin, and R. W. H. Ruigrok. 1993. Virology. 194:302-313.

32. Fredericksen, B. L., and M. A. Whitt. 1995. J.Virol. 69:1435-1443.

33. Gastka, M., J. Horvath, and T. L. Lentz. 1996. J.Gen.Virol. 77:2437-2440.

34. Gaudin, Y., R. W. H. Ruigrok, and J. Brunner. 1995. J.Gen.Virol. 76:1541-1556.

35. Gaudin, Y., R. W. H. Ruigrok, M. Knossow, and A. Flamand. 1993. J.Virol. 67:1365-1372.

36. Gaudin, Y., R. W. H. Ruigrok, C. Tuffereau, M. Knossow, and A. Flamand. 1992. Virology. 187:627-632.

37. Grigera, P. R., W. Keil, and R. R. Wagner. 1992. J.Virol. 66:3749-3757.

38. Hanham, C. A., F. Zhao, and G. H. Tignor. 1993. J.Virol. 67:530-542.

39. Jiang, S., K. Lin, N. Strick, and A. Neurath. 1993. Nature. 365:113.

40. Jiang, S., K. Lin, N. Strick, and A. Neurath. 1993. . Biochem.Biophys.Res.Commun. 195:533-538.

41. Kazmierski, W. M., R. J. Hazen, A. Aulabaugh, and H. StClair. 1996. J. Medical Chemistry. 39:2681-2689.

42. Kelley, J. M., S. U. Emersom, and R. R. Wagner. 1972. J.Virol. 10:1231-1236.

43. Konieczko, E. M., P. A. Whitaker-Dowling, and C. C. Widnell. 1994. Virology. 199:200-211.

44. Kontsekova, E., I. Macikova, M. Novak, L. Dedek, V. Vrzal, and P. Kontsek. 1992. Viral Immunol. 5:213-220.

45. Kotwal, G. J., J. Capône, R. A. Irving, S. H. Rhee, P. Bilan, F. Toneguzzo, T. Hofmann, and H. P. Ghosh. 1983. Virology. 129:1-11.

46. Lafon, M., T. J. Wiktor, and R. I. Macfarlan. 1983. J.Gen.Virol. 64:843-851.

47. Lambert, D. M., S. Barney, A. L. Lambert, K. Guthrie, R. Medinas, D. E. Davis, T. Bucy, J. Erickson, G. Merutka, and S. R. Petteway. 1996. Proc.Natl.Acad.Sci.USA. 93:2186-2191.

48. Lecocq-Xhonneux, F., M. Thiry, I. Dheur, M. Rossius, N. Vanderheijden, J. Martial, and P. DeKinkelin. 1994. . J.Gen.Virol. 75:1579-1587.

49. Lentz, T. L., J. J. Benson, D. Klimowicz, P. T. Wilson, and E. Hawrot. 1986. Molecular Brain Research. 1:211-219.

50. Lentz, T. L., T. G. Burrage, A. L. Smith, J. Crick, and G. H. Tignor. 1982. Science. 215:182-184.

51. Li, Y., C. Drone, E. Sat, and H. P. Ghosh. 1993. J.Virol. 67:4070-4077.

52. Lorenzen, N., K. Einer-Jensen, E. Lorenzen, P. Ahrens, P. Cunningham, and C. Cunningham. 1998. Virus Res. in press.

53. Lorenzen, N., N. J. Olesen, and P. E. Vestergaard-Jorgensen. 1990. J.Gen.Virol. 71:561-567.

54. Lupas, A., M. Van Dyke, and J. Stock. 1991. Science. 252:1162-1169.

55. Mastromarino, P., C. Conti, P. Goldoni, B. Hauttecouer, and N. Orsi. 1987. J.Gen.Virol. 68:2359-2369.

56. Monsma, S. A., and G. W. Blissard. 1995. J.Virol. 69:2583-2595.

57. Nehete, P. N., R. B. Arlinghaus, and K. J. Sastry. 1993. J.Virol. 67:6841-6846.

58. Nuñez, E., A. M. Fernandez, A. Estepa, J. M. Gonzalez-Ros, F. Gavilanes, and J. M. Coll. 1998.Virology 243:322-330.

59. Owens, R. J., C. C. Tanner, M. J. Mulligan, R. V. Srinivas, and R. W. Compas. 1990. AIDS Research Human Retroviruses. 6:1289-1296.

60. Ramsdale, E. E., S. M. Kingsman, and A. J. Kingsman. 1996. Virology. 220:100-108.

61. Rapaport, D., M. Ovadia, and Y. Shai. 1995. EMBO Journal. 14:5524-5531.

62. Reitter, J. N., T. Sergel, and T. G. Morrison. 1995. J.Virol. 69:5995-6004.

63. Rigaut, K. D., D. E. Birk, and J. Lenard. 1991. J.Virol. 65:2622-2628.

64. Sadaghiani, B., B. J. Crawford, and J. R. Vielkind. 1994. Biochemical Cell Biology. 72:343-348.

65. Schlegel, R., T. T. Sue, M. C. Willingham, and I. Pastan. 1983. Cell. 32:639-646.

66. Schlegel, R., and M. Wade. 1985. J.Virol. 53:319-323.

67. Schlegel, R., and M. Wade. 1983. Biochem. Biophys. Res. Commun. 114:774-779.

68. Schlegel, R., and M. Wade. 1984. J.Biol.Chem. 259:4691-4694.

69. Schlegel, R., M. C. Willigan, and I. H. H.Pastan. 1982. J.Virol. 43:871-875.

70. Schulz, G. E., and R. H. Schimer. 1984. ed.Springer-Verlag, N.Y.:2.

71. Sergel-Germano, T., C. McQuain, and T. Morrison. 1994. J.Virol. 68:7654-7658.

72. Shokralla, S., Y. He, E. Wanas, and H. P. Ghosh. 1998. Virology. 242:39-50.

73. Skehel, J. J., and D. C. Wiley. 1998. Cell. 95:871-874.

74. Slepushkin, V. A., G. V. Kornilaeva, S. M. Andreev, M. V. Sidorova, A. O. Petrukhina, G. R. Matsevich, S. V. Raduk, V. B. Grigoriev, T. V. Makarova, V. V. Lukashov, and E. V. Karamov. 1993. Virology. 194:294-301.

75. Superti, F., M. Derer, and H. Tsiang. 1984. J.Gen.Virol. 65:781-789.

76. Superti, J., L. Seganti, H. Tsiang, and N. Orsi. 1984. Arch.Virol. 81:321-328.

77. Tordo, N., H. Bourhy, S. Sather, and R. Ollo. 1993. Virology. 194:59-69.

78. Whitt, M. A., L. Buonocore, C. Prehaud, and J. K. Rose. 1991. . 185:681-688.

79. Whitt, M. A., P. Zagouras, B. Crise, and J. K. Rose. 1990. J.Virol. 64:4907-4913.

80. Wild, C., J. W. Dubay, T. Greenwell, T. Baird, T. G. Oas, C. McDanal, E. Hunter, and T. Matthews. 1994. Proc.Natl.Acad.Sci.USA. 91:12676-12680.

81. Wild, C., T. Greenwell, and T. Matthews. 1993. Aids Res. and Human Reroviruses. 9:1051-1053.

82. Wild, C., T. Greenwell, D. Shugars, L. Rimsky-Clarke, and T. Matthews. 1995.Aids Res. and Human Retroviruses. 11:323-325.

83. Wild, C., C. McDanal, D. Bolognesi, and T. Mathews. 1992. Proc.Natl.Acad.Sci.USA. 77:3273-3277.

84. Wild, T. F., and R. Buckland. 1997. J.Gen.Virol. 78:107-111.

85. Woodgett, C., and J. K. Rose. 1986. J.Virol. 59:486-489.

86. Wunner, W. H., K. J. Reagan, and H. Koprowski. 1984. J.Virol. 50:691-697.

87. Yamada, S., and S. Ohnishi. 1986. Biochemistry. 25:3703-3708.

88. Yao, Q., and R. W. Compans. 1996. Virology. 223:103-112.

89. Zhang, L., and H. P. Ghosh. 1994. J.Virol. 68:2186-2193.