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Review

Genomics and structure/function studies of *Rhabdoviridae* proteins involved in replication and transcription

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ABSTRACT

Some mammalian rhabdoviruses may infect humans, and also infect invertebrates, dogs, and bats, which may act as vectors transmitting viruses among different host species. The VIZIER programme, an EU-funded FP6 program, has characterized viruses that belong to the *Vesiculovirus*, *Ephemerovirus* and *Lyssavirus* genera of the *Rhabdoviridae* family to perform ground-breaking research on the identification of potential new drug targets against these RNA viruses through comprehensive structural characterization of the replicative machinery. The contribution of VIZIER programme was of several orders. First, it contributed substantially to research aimed at understanding the origin, evolution and diversity of rhabdoviruses. This diversity was then used to obtain further structural information on the proteins involved in replication. Two strategies were used to produce recombinant proteins by expression of both full length or domain constructs in either *E. coli* or insect cells, using the baculovirus system. In both cases, parallel cloning and expression screening at small-scale of multiple constructs based on different viruses including the addition of fusion tags, was key to the rapid generation of expression data. As a result, some progress has been made in the VIZIER programme towards dissecting the multi-functional L protein into components suitable for structural and functional studies. However, the phosphoprotein polymerase co-factor and the structural matrix protein, which play a number of roles during viral replication and drives viral assembly, have both proved much more amenable to structural biology. Applying the multi-construct/multi-virus approach central to protein production processes in VIZIER has yielded new structural information which may ultimately be exploitable in the derivation of novel ways of intervening in viral replication.

Contents

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1. Introduction

1.1. The VIZIER programme

The aim of the VIZIER programme, an EU-funded FP6 program, VIZIER, was to characterize RNA viruses, including strains of medical interest and the core enzymes/proteins of their replication machinery (Coutard et al., 2008). Among other RNA viruses, this program focused on rhabdoviruses. The final aim of the program was to allow identification of potential new drug targets through comprehensive structural characterization of the replicative machinery. In this paper we review recent developments in that topic, with special attention to results obtained within the framework of the VIZIER programme.

1.2. Taxonomy of the Rhabdoviridae

The order *Mononegavirales* was the first created in the virus taxonomy. It formally recognized remarkable relationships that were documented over the years among three families of negative-stranded RNA viruses, including the *Rhabdoviridae*, *Paramyxoviridae* and *Filoviridae* (Pringle, 1997). Subsequently, the order has been expanded (and is still growing) to include a fourth family, *Bornaviridae*, and accommodate a number of newly described viruses that prototype deeply separated phylogenetic lineages mostly in the founding families (Fig. 1).

Several dozen species unevenly populate the major lineages of the Mononegavirales. Two most profoundly separated lineages of the *Paramyxoviridae* were recognized as subfamilies, the *Paramyxovirinae* and *Pneumovirinae* including, respectively, five and two genera. The *Rhabdoviridae* family seems to include the largest diversity of viruses that was partitioned into six genera. In contrast, the *Bornaviridae* and *Filoviridae* families are very compact and include one and two genera, respectively. Most recently, two closely related viruses were described that prototype a separate lineage most closely related to bornaviruses and is provisionally dubbed the genus *Nyavirus* (Mihindukulasuriya et al., 2009). Mammals, and in many cases humans, can be infected by viruses of all major mononegavirus lineages excluding three Rhabdovirus genera, *Nucleorhabdovirus* and *Cytorhabdovirus* that infect plants, and *Novirhabdovirus* infecting fish. Some mammalian rhabdoviruses may also infect invertebrates which may serve as vectors transmitting viruses among different host species (Bourhy et al., 2008b). The VIZIER programme has characterized viruses that belong to *Vesiculovirus*, *Ephemerovirus* and *Lyssavirus* genera of the *Rhabdoviridae* family.

Rhabdoviruses infect a wide range of mammals, including man, and transmission is commonly vector-borne, particularly by haematophagous insects. The relationships among many of these unassigned rhabdoviruses have been determined based on serological cross-reactions and, more recently, according to phylogenetic analysis performed on partial sequences of the polymerase and nucleoprotein genes. Six serogroups (Hart Park, Le Dantec, Bahia Grande, Timbo, Sawgrass and Kern Canyon) have been distinguished, three of them being supported as independent clades by the phylogenetic analysis. Furthermore, based on phylogeny, four other groups can be proposed: Almpiwar, Tibrogargan, Mount Elgon bat and Kolongo and Sandjimba (Fig. 2) (Bourhy et al., 2005). It is proposed to name all vesiculoviruses, ephemeroviruses and several of the proposed groups infecting mammals and mosquitoes which share common phylogenetic relationships as the Dimarhabdovirus supergroup (Bourhy et al., 2005). There is a constantly growing list of rhabdoviruses (presently 85), isolated from a variety of vertebrate or invertebrate hosts, that are partially characterized and are still waiting for definitive species assignment (Bourhy et al., 2008a).

Currently, there are seven recognized genotypes (GT) or species of lyssavirus defined on the basis of their genetic similarity: rabies virus (RABV, GT1) responsible for classical rabies in terrestrial mammals globally and in bats on the American continent, as well as the cause of most rabies-related human deaths worldwide; Lagos bat virus (LBV, GT2); Mokola virus (MOKV, GT3); Duvenhage virus (DUVV, GT4); European bat lyssavirus type 1 (EBLV-1, GT5); European bat lyssavirus type 2 (EBLV-2, GT 6); and Australian bat lyssavirus (ABLV, GT7). Additionally, four new lyssavirus genotypes that infect bats in central and southeast Asia have been proposed: Aravan virus, Khujand virus, Irkut virus and West Caucasian Bat virus (Arai et al., 2003; Botvinkin et al., 2003; Kuzmin et al., 2005).

1.3. Genome organization

All mononegaviruses possess a monopartite genome whose size varies twofold, from ~9 kb (Bornaviridae) to 19 kb (Filoviridae), except for one recently characterized plant virus, the orchid fleck virus, which exhibits a bipartite genome (Kondo et al., 2006). The antigenome replica of the Mononegavirales encodes several positionally conserved genes located in separate open reading frames (ORFs) that may be interspersed by genes unique to distinct lineages. The order of the backbone genes from the 3′- to 5′-ends of the genome is N–P–M–G–L, where N – nucleoprotein, P – phosphoprotein, M – matrix protein, G – glycoprotein and L – the large protein or polymerase. The products of the first four genes produce major proteins forming enveloped virions and they may be known under other names in some viruses (see below). Additional ORFs are located between the phosphoprotein and the matrix protein genes and between the glycoprotein and polymerase genes (Fig. 3). The

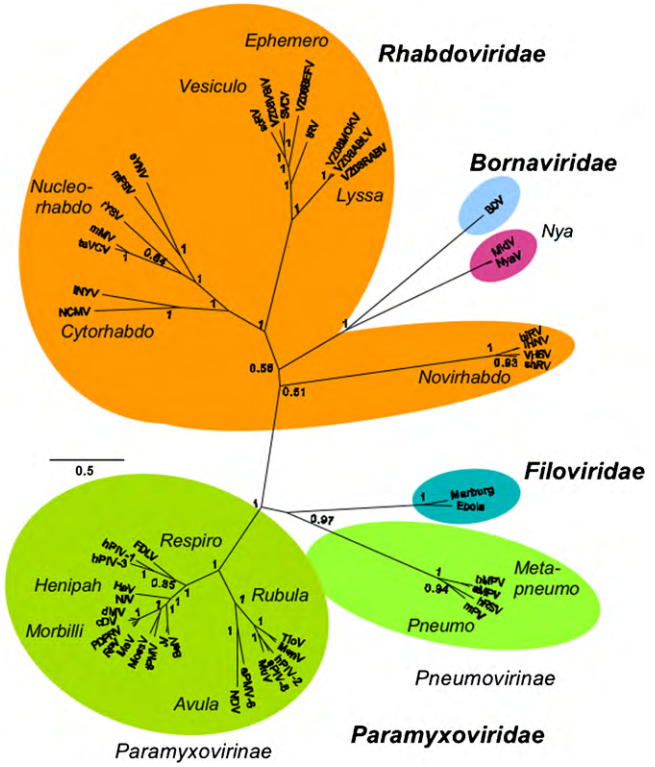

Fig. 1. Phylogeny of the order *Mononegavirales*. A multiple alignment of the L proteins region flanked by Block I (Poch et al., 1990), from the N-terminus, and ending in the middle of Block IV from a representative set of the Mononegavirales was produced using muscle (Edgar, 2004) and ClustalX (Thompson et al., 1997) programs with manual adjustment. This alignment was used to construct a Bayesian posterior probability tree. Only informative blocks representing most conserved alignment regions (I.V. Antonov, A.M. Leontovich and A.E. Gorbalenya, in preparation) were included in the analysis (428 positions in total). The alignment construction and processing was assisted by Viralis software (Gorbalenya et al., 2010). Two independent Bayesian MCMC chains (4 million steps, 10% burn-in) were run under the WAG amino acid substitution model (Whelan and Goldman, 2001) and rate heterogeneity among sites (gamma distribution with 4 categories) as implemented in BEAST software (Drummond and Rambaut, 2007). The uncorrelated relaxed molecular clock approach (lognormal distribution) (Drummond et al., 2006) with the exponential growth model was applied. Convergence of runs was verified using Tracer (Rambaut and Drummond, 2007). Numbers at branching points indicate posterior support values (values ~1.0 of bifurcations leading to extremely closely related viruses are not shown). The scale bar represents 0.5 amino acid substitutions per site on average. The following viruses were included in the analysis: NiV, Nipah virus (NC_002728); HeV, Hendra virus (NC_001906); PDPRV, Peste-des-petits-ruminants virus (NC_006383); MeV, Measles virus (NC_001498); RPV, Rinderpest virus strain Kabete O (NC_006296); dMV, Dolphin morbillivirus (NC_005283); cDV, Canine distemper virus (NC_001921); tPMV, Tupaia paramyxovirus (NC_002199); MossV, Mossman virus (NC_005339); JV, J-virus (NC_007454); BeiV, Beilong virus (NC_007803); hPIV-3, Human parainfluenza virus 3 (NC_001796); hPIV-1, Human parainfluenza virus 1 (NC_003461); FDLV, Fer-de-lance virus (NC_005084); NDV, Newcastle disease virus (NC_002617); aPMV-6, Avian paramyxovirus 6 (NC_003043); MuV, Mumps virus (NC_002200); sPIV-5, Simian parainfluenza virus 5 (NC_006430); hPIV-2, Human parainfluenza virus 2 (NC_003443); MenV, Menangle virus (NC_007620); TioV, Tioman virus (NC_004074); hMPV, Human metapneumovirus (NC_004148); aMPV Avian metapneumovirus (NC_007652); mPV, Pneumonia virus of mice J3666 (NC_006579); hRSV, Human respiratory syncytial virus (NC_001781); Ebola, Zaire ebolavirus (NC_002549); Marburg, Lake Victoria marburgvirus (NC_001608); scRV, Siniperca chuatsi rhabdovirus (NC_008514); SVCV, Spring viremia of carp virus (NC_002803); tRV, Tupaia rhabdovirus (NC_007020); sYNV, Sonchus yellow net virus (NC_001615); mFSV, Maize fine streak virus (NC_005974); rYSV, Rice yellow stunt virus (NC_003746); taVCV, Taro vein chlorosis virus (NC_006942); mMV, Maize mosaic virus (NC_005975); NCMV, Northern cereal mosaic virus (NC_002251); lNYV, Lettuce necrotic yellows virus (NC_007642); BDV, Borna disease virus (NC_001607); MidV, Midway virus (FJ554525); NyaV, Nyamanini virus (FJ554526); VHSV, Viral hemorrhagic septicemia virus (NC_000855); shRV, Snakehead rhabdovirus (NC_000903); IHNV, Infectious hematopoietic necrosis virus (NC_001652); hiRV, Hirame rhabdovirus (NC_005093); VZ08MOKV, Mokola virus; VZ08RABV, Rabies virus; VZ08ABLV, Australian bat lyssavirus; VZ08BEFV, Bovine ephemeral fever virus; VZ08VSIV, Vesicular stomatitis Indiana virus. VIZIER sequences are indicated by names starting with VZ*xx* where *xx* represents the number of the project that contributed the sequence. Virus names were extracted from GenBank/Refseq annotations using SNAD (Sidorov et al., 2009) http://veb.lumc.nl/SNAD/. Families, subfamilies and genera are indicated; for genera, the virus suffix was excluded from the names. Nyavirus is a provisional floating genus in the order (Mihindukulasuriya et al., 2009).

L gene encodes a multidomain protein, including a putative RNA-dependent RNA polymerase, that is virion-associated and mediates replication and expression of the virus genome.

1.4. Virion structure

Rhabdoviruses, like other Mononegavirales, are enveloped viruses with a lipid bilayer envelope generated from the plasma membrane of the infected host cell. Rhabdoviruses virions are rod- or bullet-shaped particles approximately 100–430 nm long and 45–100 nm in diameter.

Extending from the surface of the membrane is one glycoprotein (G) inserted into the envelope. Under and associated to the membrane by hydrophobic and electrostatic interactions is a layer formed by the matrix protein (M) which shares conserved tertiary structure within Mononegavirales families but not between them (Dessen et al., 2000; Graham et al., 2008; Money et al., 2009; Ruigrok et al., 2000; Timmins et al., 2004). The M protein condenses the nucleocapsid and gives the lyssavirus virion its bullet-shaped appearance. In addition to interacting with nucleocapsid, M protein also associates with the lipid bilayer (Dancho et al., 2009; Manie et al., 2000; Solon et al., 2005) and the glycoprotein (Mebatsion et al., 1999) suggesting that the M protein could form a link between the nucleocapsid and glycoproteins in the viral envelope. Inside the particle is the helical ribonucleoproteic core consisting of the negative-sense, single-stranded RNA wrapped by the nucleoprotein (N). In rhabdoviruses, the N protein is composed of two main domains and two smaller extra domains extending from the N- and C-termini. The RNA wraps around the main N-terminal part of the N protein, with each N protomer making contact with 9 nucleotides. The bound RNA is totally enclosed by the N with its C-terminal main domain covering the bound RNA. Additional contacts between neighboring N protomers participate in the polymerization of N, leading to formation of the nucleocapsid (Albertini et al., 2006).

The nucleocapsid is associated with smaller amounts of two other proteins: the phosphoprotein (P) and the large protein (L). These two proteins are responsible for the RNA polymerase activity associated with viral particles. L proteins carry all the enzymatic RNA polymerase activities, while P links L to the N–RNA complex (Bourhis et al., 2006; Gerard et al., 2009).

The P protein has a modular organization with three structured domains alternating with two disordered domains (Gerard et al., 2009). The N-terminal ordered domain contains a site of interaction with single N molecules, the first disordered region contains phosphorylation sites that may be involved in regulation of transcription, the central oligomerization domain, a second disordered region that (at least in rabies virus) is able to interact with cellular partners, and finally the C-terminal ordered region that interacts with N–RNA complexes.

The L protein is poorly characterized. It is thought to perform RNA synthesis as well as mRNA capping and polyadenylation activities (Grdzelishvili et al., 2005; Li et al., 2006; Ogino et al., 2005). Bioinformatics studies show that the rhabdovirus L protein is about 220–250 kDa and has six highly conserved domains proposed to be individually responsible for each of the multiple L functions (Poch et al., 1990; Svenda et al., 1997). Expression and purification of L have so far been elusive, and consequently very little information is available about its enzyme activities or structural organization.

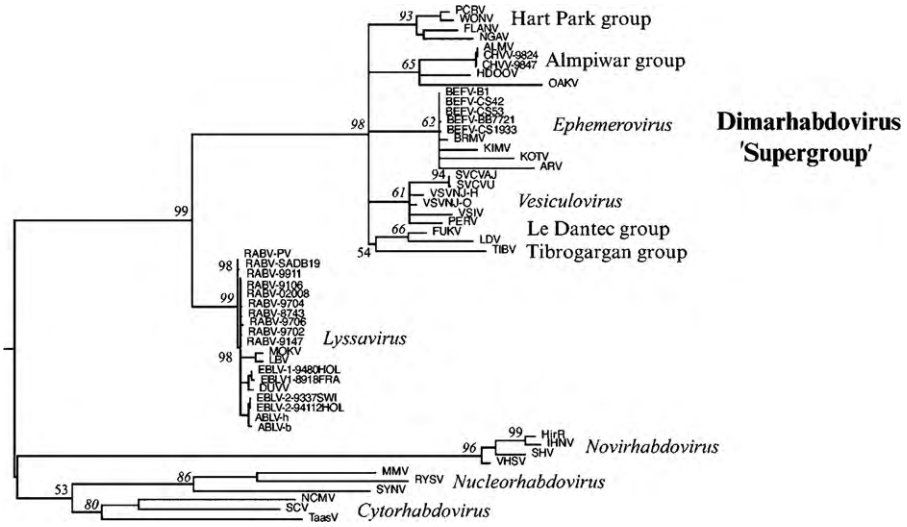

Fig. 2. Phylogenetic relationships of the *Rhabdoviridae* based on a maximum likelihood analysis of a 158 residue alignment of the L polymerase region (Bourhy et al., 2005). The established rhabdovirus genera, as well as new groups proposed here, are indicated. Horizontal branches are drawn to scale and quartet puzzling frequencies are shown for key nodes (values in italics are for genera, groups and supergroups, while all other quartet puzzling frequencies are shown in normal font). The tree is mid-point rooted for purposes of clarity only and all potential outgroup sequences were deemed too divergent to include in the analysis.

2. Replication cycle

The replication cycle of all rhabdoviruses involves the same general steps. After the first steps of attachment, penetration and uncoating, the nucleocapsid and all the components necessary for early transcription are released into the cytoplasm of the infected cell. The surface glycoprotein mediates the attachment of the virion. After attachment, viral particles are endocytosed. The decreasing pH along the endocytosis pathway induces a change in the conformation of the G protein, which becomes a fusion protein (Roche et al., 2006, 2007). In its fusion conformation, G mediates the mixing of the endosomal and viral membranes that allows the release of the nucleocapsid into the cytoplasm. Membrane fusion may occur with an internal vesicle of the multivesicular body. The release of the nucleocapsid into the cytoplasm then involves a back-fusion step between the internal vesicle and the membrane that surrounds the multivesicular body (Luyet et al., 2008). The negative-sense ribonucleoproteic complex is then released into the cytoplasm and used as a template for primary transcription.

For transcription, the viral polymerase uses a stop–start mechanism with a single entry point at the 3′ extremity of the genome. The polymerase produces first the short leader RNA that is not capped or polyadenylated. It then restarts with transcription of the first gene on the genome i.e. the N gene. This transcript is capped and polyadenylated by the viral polymerase, as are all the other viral transcripts. At the end of the N gene the viral polymerase starts again and so on until the end of the last gene (Fig. 4). There is a gradient in the quantity of each transcript depending on its order and its distance from the 3′ end of the genome. Indeed, at each stop a fraction of the polymerase dissociates from the RNA template; in this way regulating the relative abundance of each viral protein. The necessity for efficient infection to regulate the relative abundance of viral proteins and the conservation of transcription mechanism

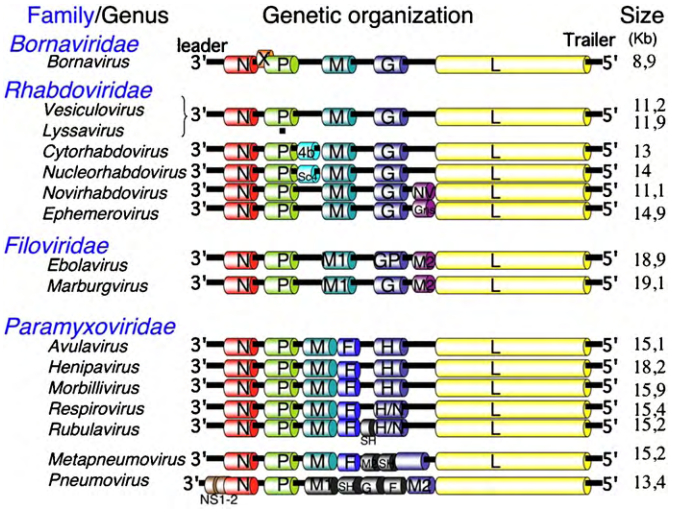

Fig. 3. Genetic organization of Mononegavirales. Mononegavirales possess a monopartite genome whose size varies twofold, from ~9 kb (Bornaviridae) to 19 kb (Filoviridae). The order of the backbone genes from the 3′- to 5′-ends of the genome is N–P–M–G–L, where N – nucleoprotein, P – phosphoprotein, M – matrix protein, G – glycoprotein and L – the large protein or polymerase. Additional ORFs are located between the phosphoprotein and the matrix protein genes and between the glycoprotein and polymerase genes. Large open reading frames are indicated by the colored boxes (adapted from Bourhy et al., 2008a). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

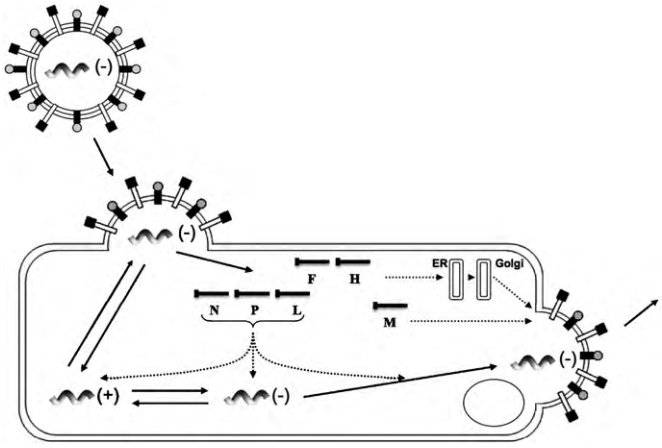

Fig. 4. Replication cycle of rhabdoviruses. The replication cycle of all rhabdoviruses involves the same general steps: attachment, penetration, uncoating, transcription of the different genes, translation of the different viral proteins (N, P, M, G and L), viral genome replication, encapsidation and budding.

among the Mononegavirales probably explains the conservation of the basic gene order (Fig. 3).

After accumulation of neo-synthesized viral proteins, the RdRp switches to a processive mode and ignores gene junctions to synthesize a full-length positive, complementary copy called the antigenome. The level of the N protein mediates the switch from transcription to replication. When the amount of N protein is sufficiently high to allow encapsidation of the nascent RNA chain, the polymerase switches to the replication mode (Arnheiter et al., 1985; Fearns et al., 1997; Plumet et al., 2005; Vidal and Kolakofsky, 1989). The stochiometry of L and P proteins in the viral polymerase complex is modified (Gupta et al., 2003) consistent with the fact that the viral polymerase does not respond to the same signals during transcription and replication. The amounts of rhabdovirus genomes and antigenomes are equivalent and both can be encapsidated. By a mechanism similar to that of positive-strand synthesis, the antigenome is used as a template to direct synthesis of encapsidated minus-strand genome (Leppert et al., 1979). This latter strand can be used as a template for synthesis of both new mRNAs (in a process referred to as secondary transcription) and antigenomic RNA. Genomic RNA can also be incorporated into progeny virions during the budding process.

After mRNA translation, cellular chaperones are used to transport the M protein to the plasma membrane and glycoproteins from the ER to the golgi apparatus to the plasma membrane. Like other enveloped viruses, the budding process involves cooperation of viral and cellular proteins to form virions. The viral components are assembled near the plasma membrane where new virus vesicles bud from sites on the host cell membrane.

3. Pathogenesis

3.1. Introduction

RNA viruses of the family *Rhabdoviridae* comprise arthropod-borne agents that infect plants, fish and mammals, as well as a variety of non-vector-borne mammalian viruses. The *Rhabdoviridae* family presently comprises six genera, and members of three of these genera – *Vesiculovirus*, *Lyssavirus* and *Ephemerovirus* – have been obtained from a variety of animal hosts and vectors, including mammals, fish and invertebrates. The remaining three rhabdovirus genera are more taxon-specific in their host preference. Novirhabdoviruses infect numerous species of fish, while cytorhabdoviruses and nucleorhabdoviruses are arthropod-borne and infect plants (Bourhy et al., 2005, 2008a). Although vesiculoviruses have often been used as a model to study replication and transcription mechanisms in rhabdoviruses, we will focus on the lyssaviruses, which are the only etiological agents of human disease in the rhabdovirus family.

3.2. Lyssaviruses

Lyssaviruses cause rabies, an acute encephalomyelitis transmitted to humans by rabid animals. Although effective and economical control measures are available, and although the World Health Organization (WHO) and the World Health Organization for animals both consider it a high priority zoonosis, rabies unfortunately remains a neglected disease in a large part of the world, especially in Africa and Asia (Knobel et al., 2005). Rabies is transmitted by a bite, scratch or lick on damaged skin or by projection of infectious material (i.e. saliva or lacrymal liquid) on mucosae. Except in some rare cases of transmission by organ or tissue transplantation, human-to-human transmission has never been described. Rabies virus is highly neurotropic, and after inoculation it is retrogradely transported by peripheral neurons before being passed on to second- and higher-order neurons without being taken up by glia (Kelly and Strick, 2000). The glycoprotein G is the only protein component of the viral envelope that mediates viral entry into host cells (Etessami et al., 2000). Several receptors have been shown to play a role in cell attachement and entry: NCAM (Thoulouze et al., 1998), P75NTR (Tuffereau et al., 1998, 2001) and acetylcholine receptor (Lentz et al., 1982). However other mechanisms are probably responsible for the tropism of the virus for neuronal cells (Lafon, 2005; Langevin et al., 2002; Tuffereau et al., 2007). The infection is responsible for severe neurological disorders and is invariably fatal in the absence of timely administration of post-exposure prophylaxis consisting of several doses of vaccine and immune globulin (Anonymous, 2005). However, when the symptoms appear post-exposure, prophylaxis is no longer effective, and no specific therapy is available (Jackson, 2009). Each year, at least 15 million people receive treatment after being exposed to suspected rabid animals (Bourhy et al., 2009); however, 55,000 people still die in Asia and Africa, based on a WHO estimate (Knobel et al., 2005). The main reservoir of the disease is the dog but several other species of the orders *Carnivora* and *Chiroptera* act also as reservoir of different species (or genotypes) of lyssaviruses.

4. Molecular epidemiology

4.1. Introduction

Rapid evolution, and consequently high diversity and fitness, are the major driving forces of virus virulence, tropism, host range and transmission, and thus the perpetuation of the Mononegavirales. An important determinant of this genetic variability is the error-prone nature of the RNA-dependent RNA polymerase, which during virus replication may result in high rates of mutation, sequence deletion, insertion, and recombination between genomes. Due to the absence of efficient proofreading and post-replicative repair activities associated with RNA replicases, mutation rates of RNA viruses have been estimated to vary between approximately 10^{-3} and 10^{-5} substitutions per nucleotide copy. As a result, viral population are constantly shaped by evolutionarily driven forces, and genetic diversification is a common process in virus evolution. In the long term, this leads to the segregation of new variants, species or genus. The VIZIER programme contributed substantially to research aiming at understanding the origin, evolution and diversity of lyssaviruses.

4.2. Origin and evolution of the lyssaviruses

Phylogenetic analyses of the lyssaviruses have revealed the existence of seven genotypes or species, although this number is likely to increase with more intensive sampling (Bourhy et al., 1993; Gould et al., 1998; Kuzmin et al., 2005). Genomic and evolutionary studies have most often utilized partial genome sequences, particularly of the nucleoprotein and glycoprotein genes, with little consideration of genome-scale evolution.

Collaborative work obtained in the framework of VIZIER reported the first genomic and evolutionary analysis using complete genome sequences of all recognized lyssavirus genotypes, including 14 new complete genomes of field isolates from six genotypes and one genotype that was completely sequenced for the first time (Fig. 5) (Delmas et al., 2008). This analysis revealed that all lyssaviruses have the same genomic organization (Fig. 3). It also identified strong geographical structuring, with the greatest genetic diversity in Africa, and an independent origin for the two known genotypes that infect European bats. It also suggested that multiple genotypes exist within the diversity of viruses currently classified as 'Lagos Bat'. This rigorous phylogenetic analysis, based

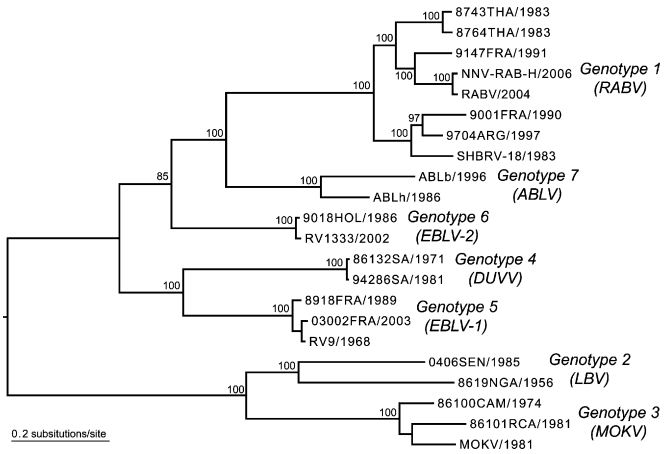

Fig. 5. Phylogenetic relationships of 22 complete coding regions of lyssavirus genomes representatives of the seven genotypes (Delmas et al., 2008). The phylogeny was inferred using an ML procedure, and all horizontal branches are scaled according to the number of substitutions per site. Boot strap values (>95%) are shown for key nodes. The tree is mid-point rooted for purposes of clarity only.

on full-length genome sequences, provides the best discriminatory power for genotype classification within the lyssaviruses.

Of the mammalian RABV, those that circulate in dogs (*Canis lupus familiaris*) are responsible for more than 99% of human cases worldwide (Knobel et al., 2005). However, despite its role as a vector for human disease, the extent and structure of viral biodiversity in this key vector species, as well as the mode and time scale of its evolution, have only been studied on a limited geographical range. To address these questions on a global scale, a comparative analysis of RABV gene sequence data was performed in the framework of VIZIER. From this, we identified six clades of RABV in non-flying mammals, each of which has a distinct geographical distribution, most likely reflecting major physical barriers to gene flow. Indeed, a detailed analysis of phylogeographic structure revealed slow and only limited viral movement among continents, countries and geographical localities (Bourhy et al., 2009). The distribution of the different clades and their relationships are shown in Fig. 6. Using Bayesian coalescent methods, this analysis revealed that the sampled lineages of canid RABV derived from a common ancestor that originated within the past 1500 years probably from the Indian subcontinent (Fig. 6). Additionally, no evidence was found for either positive selection or widespread population bottlenecks during the global expansion of canid RABV. Further supporting the concept that the stochastic processes of genetic drift and population

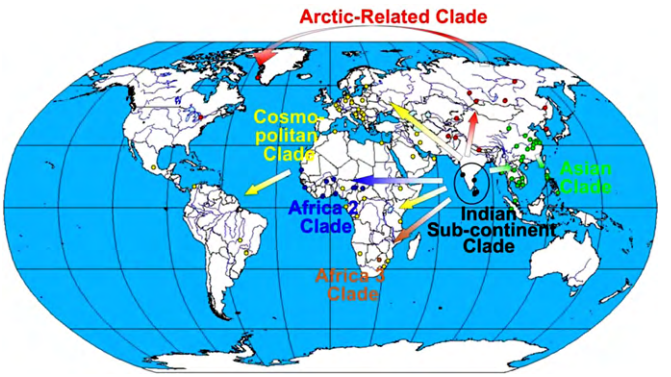

Fig. 6. Origin and evolution of dog rabies virus. Reconstruction of the spatial dynamics of dog rabies virus, based on ML phylogeny of 151 sequences from the N-coding region (Bourhy et al., 2008b). The major clades with bootstrap support values (>90%) of rabies virus are indicated, in different colors. The arrows indicate migration events, as determined in Bourhy et al. (2008b).

subdivision are the most important factors shaping the global phylogeography of canid RABV.

4.3. Emergence of new variants adapted to new hosts

Determining the genetic basis of the traits that govern cross-species transmission also clearly represents a major goal for future research on the rhabdoviruses. However, it is important to note that patterns of cross-species transmission may also be in part determined by the ecological factors that shape host contact rates.

During virus replication, high mutation frequencies result in the generation of virus populations in which the majority of genomes differ by more than a single nucleotide and can therefore be defined as a spectrum of mutants derived from dominant parent copies. The word quasispecies (also sometimes referred to as population polymorphism or intra-host variation) was introduced to describe the resulting heterogeneous population structure generated by RNA viruses (Eigen and Schuster, 1979). This intra-host variation provides the virus with the capacity to adapt immediately to changing environmental circumstances, including the changing of hosts as demonstrated for lyssaviruses (Benmansour et al., 1992; Bourhy et al., 1999; Morimoto et al., 1998).

Rabies viruses are able to establish productive infections in dogs but also in many other host species (Nadin-Davis et al., 1994). Rabies viruses sampled from other species of the *Canidae* family, such as foxes and raccoon dogs, as well as hosts belonging to other families within the *Carnivora* order – the *Herpestidae* in southern Africa and the *Mephitidae* (skunks) in America – are interspersed within the phylogenetic diversity of dog RABV. While we found no significant evidence for adaptive evolution, our observations strongly suggest that the dog has served as the main vector for inter-species RABV transmission, generating viral lineages that then spread to other taxa (Bourhy et al., 2009).

An important example of such a successful host switch involved the transfer of the virus from dogs to the red fox (*Vulpes vulpes*) in Northeast Europe during the 1930s (Bourhy et al., 1999). After the initial cross-species transmission event, rabies virus was able to spread rapidly westward and southward through European red fox populations in the subsequent 60 years.

5. Progress towards control using antivirals

5.1. Introduction

Considering the rapid evolution, periodic emergence, capacity of spread and change of host and tropism of *Mononegavirales*, and in particular of rhabdoviruses, there is a critical need for broad-spectrum antiviral molecules. We will here review the molecules that have shown some effects, together with the step of the viral multiplication that they target, our aim being to focus more on the structure-based design of small-molecule inhibitors of viral replication.

5.2. Current state of the art of antivirals against Mononegavirales

Concerning rabies and lyssaviruses, several regimens of post-exposure prophylaxis (PEP) have been developed and validated by the WHO. They mainly consist of several injections of vaccine associated with passive immunotherapy in the case of the most severe wounds (Anonymous, 2005). Passive immunotherapy is based on human or equine immunoglobulins or F(ab′)2 fragments. A recently developed cocktail of human monoclonal antibodies is under study (Bakker et al., 2005, 2008; de Kruif et al., 2007) and will probably be on the market in 2013.

Considering the availability of efficacious biologicals to fight against rabies, the reader might be surprised by the fact that rabies

is re-emerging in many countries, and that the global incidence in humans remains high. Besides reasons of infrastructure and organization that will not be detailed here, several explanations may be discussed (Wilde, 2007). First, there is a global lack of accessibility to these products to populations at risk in developing countries. Second, PEP is also becoming less and less effective as the delay in days between exposure and the start of the PEP is increasing. Third, PEP is no longer effective after the onset of the symptoms. Therefore, a lot of efforts have been made to develop a curative treatment that will be effective in rabid patients, but so far none been found, despite some proposed protocols (Willoughby, 2007; Willoughby et al., 2005) which subsequently became highly controversial (Hemachudha et al., 2006; Jackson et al., 2008).

One other main reason for the re-emergence of rabies is probably that very few molecules have been so far reported to exert activity against the virus. Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a broad-spectrum RNA virus inhibitor that was discovered almost four decades ago. However, a lack of activity is reported so far for rhabdoviruses. Ammonium-5-tungsto-2-antimoniate (HPA23) and analogues were shown to produce a protective effect in rabies-infected mice; HPA23 was even able to delay mortality in rabies-infected foxes (Bussereau et al., 1988). The mechanism by which these compounds exert their anti-rabies activity has not been unravelled. Ketamine was one of the therapeutic agents used as a therapy for a human rabies survivor who did not receive rabies vaccine (Willoughby, 2007; Willoughby et al., 2005). Unfortunately, when re-examined in infected mouse primary neuron cultures and in adult ICR mice using the rabies challenge virus, ketamine given intraperitoneally did not lead to any beneficial effects (Jackson et al., 2008).

SAH hydrolase inhibitors are endowed with broad-spectrum antiviral activity against (−)ssRNA viruses, including the rhabdoviruses (De Clercq, 2005). The antiviral activity spectrum of SAH hydrolase inhibitors against RNA viruses includes among other rhabdoviruses. Among the most potent SAH hydrolase inhibitors and antiviral agents rank carbocyclic 3-deazaadenosine, neplanocin A and 3-deazaneplanocin A. Some of these compounds are particularly active against the vesicular stomatitis virus (a possible surrogate for rabies virus) and have proven to be effective against a filovirus, the Ebola virus (Huggins, 1989). Even when administered as a single dose of 1 mg/kg on the first or second day after an Ebola Zaire virus infection in mice, 3-deazaneplanocin A reduced peak viremia by more than 1000-fold compared with mock-treated controls, and most or all of the animals survived (Bray et al., 2000). This protective effect was likely not the result of a direct antiviral activity but probably mediated by the production of high concentration of IFN-α in virus-infected mice (Bray et al., 2002). A possible explanation for such mechanism may be the blocking of the 5′-capping of the nascent viral (+)RNA strands, of which the maturation is dependent on methylation. Hence these molecules would prevent the dissociation of these strands from the viral (−)RNA genome and thus lead to an accumulation of replicative intermediates containing double-stranded RNA stretches, that may in turn result in the high level production of interferon.

Besides the SAH hydrolase, various other enzymes, in particular involved in host cell nucleoside/nucleotide pathways have been reported to be good targets for inhibition of RNA virus replication. The OMP decarboxylase inhibitor pyrazofurin [3-(β-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide, as prototype of the OMP decarboxylase inhibitors, is active against several Mononegavirales including rhabdoviruses (vesicular stomatitis virus) (Andrei and De Clercq, 1993; Georges-Courbot et al., 2006). Carbocyclic analogues of the normal nucleoside cytidine, such as cyclopentylcytosine (C-Cyd, carbodine) and cyclopentenylcytosine (Ce-Cyd) inhibit a variety of viruses including paramyxoviruses and rhabdoviruses (vesicular stomatitis virus) (De Clercq et al., 1990). The putative target is CTP synthetase, the enzyme that catalyses the conversion of UTP to CTP, which is the final step in the *de novo* pyrimidine biosynthetic pathway. Although these compounds exert often potent *in vitro* antiviral activity, the fact that they target a cellular function renders them not very selective, despite the fact that this function is crucial for efficient viral replication. As outlined in this review, rhabdoviruses offer many potential targets for inhibition by selective antiviral agents. The development of potent and selective antiviral agents against these viruses should be eminently feasible.

5.3. Structural analysis of proteins involved in rhabdovirus replication

5.3.1. Introduction

The structure-based design of small-molecule inhibitors of viral replication has a strong track record of success in the development of novel antiviral drugs. The starting point of any such drug discovery programme is the determination of the structure of key components of the replication machinery and in particular the RNA-dependent RNA polymerase. However, the proteins involved in replication of *Rhabdoviridae* have largely proven refractory to recombinant expression, and structural data on individual components are therefore very limited.

To date the crystal structure of N (Albertini et al., 2006; Green et al., 2006) and two crystal structures and one NMR structure for parts of the P protein (Ding et al., 2006; Mavrakis et al., 2004; Ribeiro et al., 2008) have been determined for two rhabdoviruses, VSV and RABV, whereas no high-resolution structural data is available for the L protein. Although not an integral component of the replicative machinery, the M protein plays a number of key roles during the viral replication cycle (Connor et al., 2006; Finke and Conzelmann, 2003). However, before the start of the VIZIER project, the only structural information available on rhabdovirus M proteins was the structure of a thermolysin-stable M core (M^{th}) of VSV Indiana (VSV_{Ind}), which lacked approximately 50 residues from the N-terminus and the surface-exposed hydrophobic loop between residues 121 and 124 (Gaudier et al., 2002). In this section, the approaches made in the VIZIER project to obtain further structural information on the rhabdoviral replication proteins will be reviewed.

5.3.2. Experimental methods

Two strategies were used to produce recombinant proteins by expression of both full length or domain constructs in either *E. coli* or insect cells, using the baculovirus system. In both cases parallel cloning and expression screening at small-scale of multiple constructs, including the addition of fusion tags, was key to the rapid generation of expression data. DNAs encoding either full-length genes or domains designed from a combination of published analyses (Poch et al., 1990) and further work carried out in the VIZIER programme were amplified by PCR from cDNAs generated by reverse transcription of viral RNA templates extracted from infected cells (Delmas et al., 2008). Gene sequences were inserted into expression vectors by ligation-independent cloning (Berrow et al., 2007; Walhout et al., 2000) to generate multiple constructs targeted at L, M and P proteins. All constructs incorporated a hexahistidine tag at either the N- or C-terminus to enable detection and purification of expressed genes. The protein production platforms operated by OPPF (Berrow et al., 2007) and the AFMB (Vincentelli et al., 2003, 2005) were used to screen for soluble protein expression and subsequent purification and crystallization.

5.3.3. Results and discussion

5.3.3.1. L proteins. Based on sequence analysis, the L protein of non-segmented, negative-sense RNA viruses carries six highly conserved domains (Poch et al., 1990). The constructs that were designed for the L protein initially relied on these data to target a domain. Among the different activities thought to be carried out by the L protein of non-segmented negative RNA viruses, the capping enzymes are very attractive for structural analysis, because an original capping process based on GDP rather than GMP transfer is catalysed by the VSV L protein (Ogino and Banerjee, 2007). This unusual guanylyltransferase activity requires a non-canonical G*xx*T[n]HR amino acids motif on Domain V of the VSV L protein (Li et al., 2008). Complementary to this process, the methyltransferase activity is carried by the Domain VI (Galloway et al., 2008; Grdzelishvili et al., 2005; Li et al., 2005). It was thus decided to focus on Domains V and VI.

To that aim, three constructs encompassing either (i) Domain VI alone (amino acids numbering 1675–1871 according to the ABLV L protein), (ii) Domain VI with the C-terminus part of the L protein (amino acids 1668–2128 ABLV L protein numbering), or (iii) the combination of Domains V, VI and the C-terminus part of the L (amino acids 1087–2128, ABLV L protein numbering). The equivalent three constructs were made for the L proteins of four rhabdoviruses, Australian bat lyssavirus (ABLV), mokola virus (MOKV), VSV Indiana (VSV_{Ind}) and rabies virus (SADB) and evaluated for expression in *E. coli*. Low-level soluble expression was observed for only two constructs of ABLV (Table 1). These were scaled up, but the data suggested that they did not give homogenous samples following an IMAC purification step, because of low expression. Small changes to the amino acid start and end points of recombinant protein constructs can have a major effect on soluble expression as shown by Graslund et al. (2008). Therefore a series of 84 further constructs were designed and tested in which the boundaries of the Domains V and VI of ABLV were systematically varied by 5–10 residues. Disappointingly none of these constructs showed any improvement in the level of soluble expression suggesting that either *E. coli* is not the appropriate expression system for L protein C-terminal domains or that the rational domain design did not target the correct boundaries of the domains to be expressed.

In parallel with the work on expression of the C-terminal region of the L protein in *E. coli*, the use of insect cells using the baculovirus expression system was evaluated for the N-terminal region of L, which encompasses the RNA-dependent RNA polymerase (RdRp). Baculoviruses have been used previously to produce the whole L protein of VSV for functional studies only (Chen et al., 2006). From a series of N-terminal constructs of the L protein of the MOKV we identified a number of large fragments which produced soluble proteins in insect cells (Fig. 7). Interestingly, soluble expression was enhanced by both the presence of the native N-terminal region and by fusion to maltose binding protein (MBP) (Alexandrov et al., 2001). In a pilot experiment, production of the construct encompassing amino acids 1–880 fused to MBP was scaled up to 100 mL and the yield of pure, soluble protein following removal of the tag was 100 μg, sufficient for initial crystallization trials in fluidigm chips (Hansen and Quake, 2003; Hansen et al., 2002). Small crystals have been obtained indicating that the protein is of sufficient quality for crystallization, but further work is required to improve crystal growth. These findings have not yet undergone peer review.

Low protein expression remains a challenge, though from this preliminary work it appears that expression in insect cells will be the way forward to obtaining structural information for these complex and difficult to express proteins.In another approach synthesised DNA has been used in an attempt to avoid potential problems associated with rare codons and transcription termination due to unwanted secondary structure formation, but a series

Table 1
Summary of Rhabdovirus L, M, and P expression.

Virus	Protein	Construct (aa)	Soluble expression – Coomassie		
			N-His	C-His	N-SUMO
ABL	L	1675–1710	+	N/A	N/A
ABL	L	1668–2128	–	N/A	N/A
ABL	L	1087–2128	+	N/A	N/A
SAD	L	1674–1870	–	N/A	N/A
SAD	L	1667–2127	–	N/A	N/A
SAD	L	1086–2127	–	N/A	N/A
VSV	L	1640–1836	–	N/A	N/A
VSV	L	1633–2109	–	N/A	N/A
VSV	L	1072–2109	–	N/A	N/A
Mokola	L	1673–1869	–	N/A	N/A
Mokola	L	1666–2126	+	N/A	N/A
Mokola	L	1673–2126	–	N/A	N/A
Mokola	Matrix	1-end	–	–	++
Mokola	Matrix	46-end	+++	–	++
Mokola	Matrix	46–110	–	–	Tag only
Mokola	Matrix	111–202	–	–	–
Mokola	Matrix	46–130	–	–	N/A
Mokola	Matrix	131-end	–	–	N/A
Lagos	Matrix	1-end	–	–	++
Lagos	Matrix	46-end	+++	–	++
Lagos	Matrix	46–110	–	–	Tag only
Lagos	Matrix	111–202	–	–	–
Lagos	Matrix	46–130	–	–	N/A
Lagos	Matrix	131-end	–	–	N/A
Thailand	Matrix	1-end	–	–	++
Thailand	Matrix	46-end	+++	–	+
Thailand	Matrix	46–110	–	–	Tag only
Thailand	Matrix	111–202	–	–	–
Thailand	Matrix	46–130	–	–	N/A
Thailand	Matrix	131-end	–	–	N/A
VSV	PHOSPHO	Full length	++	+	N/A
SAD	PHOSPHO	Full length	+	+	N/A
ABL	PHOSPHO	Full length	++	++	N/A
9147	PHOSPHO	Full length	+	+++	N/A
THAI	PHOSPHO	Full length	+	++	N/A
LAG	PHOSPHO	Full length	+	++	N/A
MOK	PHOSPHO	Full length	–	++	N/A

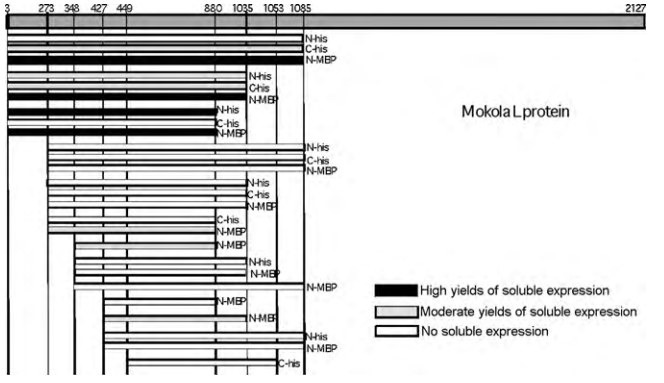

Fig. 7. Small-scale insect expression screen of L-domain constructs of a lyssavirus (MOKV). Domain boundaries were determined using VaZyMolO (Ferron et al., 2005) and cloned into the appropriate pOPIN vectors using In-fusion (Berrow et al., 2007). Recombinant baculoviruses were prepared using the method developed by Jones and co-workers (Zhao et al., 2003). Transfections and infections were all performed in 24-well culture dishes, using 500 μl Sf9 cells and serum-free media in each well. Following one round of virus amplification, expression was tested by infecting Sf9 cells with different amounts of viruses and harvesting at different time points. Expression screening was performed by detergent lysis of the cells while still attached to the plate, followed by robotic Ni-NTA protein extraction of the soluble fraction (obtained by centrifugation). Expression was analysed using SDS-PAGE followed by Coomassie blue staining. The figure shows a summary of the best results obtained indicating that two factors appeared to improve the amount of soluble protein obtained: (1) the presence of the N-terminus of MOKV L and (2) the use of the MBP fusion tag.

of VSV N-terminal His-tagged constructs only produced insoluble polypeptide when expressed in *E. coli*.

5.3.3.2. P proteins. The P protein fulfils a crucial role during viral RNA synthesis, as it is an essential cofactor for the RNA-dependent RNA polymerase activity of L. A recent model proposes that during replication, the L protein forms a complex with P, which in turn binds to the N–RNA polymer and acts as a bridge to allow access of L to the RNA. This model also suggests that P may feed the newly synthesised viral RNA strand with free N for immediate encapsidation (Albertini et al., 2006, 2008). Expression of P proteins cloned from a number of different viruses was evaluated in both *E. coli* and insect cells. The results showed that full-length P can be expressed in both systems consistent with previous reports (Mavrakis et al., 2004; Schoehn et al., 2001).

For ease of use, *E. coli* was selected for the production of protein for crystallization experiments (Table 1). Trials were set up for four different rhabdovirus P proteins from which crystals were obtained for MOKV P, but only of a stable C-terminal domain. This was presumably due to proteolysis of the full-length protein during crystallization as observed for RABV P (Mavrakis et al., 2004) and reflects the modular organization of the P protein. The C-terminal N–RNA binding domain forms an independently folded proteolytically stable fragment which follows a region of unstructured sequence interrupted by a putative dimerisation domain (Gerard et al., 2009). Using both site-directed mutagenesis and yeast two-hybrid experiments to measure P–N interaction in lyssaviruses, the relative roles of key amino acids involved in the interaction of P to N and located in this C-terminal domain of P were precisely determined, completing and for some of them confirming previous studies (Jacob et al., 2001). Four residues K212, K215, L225 and R261 were shown to be involved in P–N binding (Assenberg et al., 2010). The high degree of conservation of these positions despite the high variability of this region of the P further supports their importance. Disrupting this interaction may offer a novel way of blocking viral replication.

At the N-terminus of the protein there is a hydrophobic region which is associated with N^0 binding (N^0 refers to the RNA-free form of N) (Mavrakis et al., 2006). Reflecting a high level of amino acid sequence conservation (68%), the structure of the MOKV P is essentially identical to that of RABV P (Mavrakis et al., 2004), both in terms of conformation and electrostatic properties (Assenberg et al., 2010). By contrast, the structure of the N–RNA binding domain of VSV P (Ribeiro et al., 2008) shows significant differences from the lyssavirus structures. In particular, it lacks the equivalent of RABV/MOKV P α3 and α6 and has a shorter α1, making it a much more compact structure (Fig. 8). Although the precise location of the residues involved in binding N–RNA are at topologically distinct sites, it is clear that the C-terminal domains of rhabdovirus P proteins share a common fold (Assenberg et al., 2010). More largely, this study revealed that the N–RNA binding domains of the P proteins of the *Rhabdoviridae* and the *Paramyxoviridae* exhibit structural relationships, and are probably derived from a common ancestor, despite very limited primary sequence conservation.

5.3.3.3. M proteins. The matrix (M) proteins of rhabdoviruses are multi-functional proteins essential for virus maturation and budding (Bieniasz, 2006; Harty et al., 1999) that also regulate the expression of viral and host proteins. M has also been implicated in controlling the balance between transcription and replication of the viral genome (Connor et al., 2006; Finke and Conzelmann, 2003) and in modulating transcription of host cell (Komarova et al., 2007) and translation (von Kobbe et al., 2000). To date, no structure of a full-length rhabdovirus matrix protein has been reported. The problem appears to be producing the protein in a non-aggregated form suitable for crystallization.

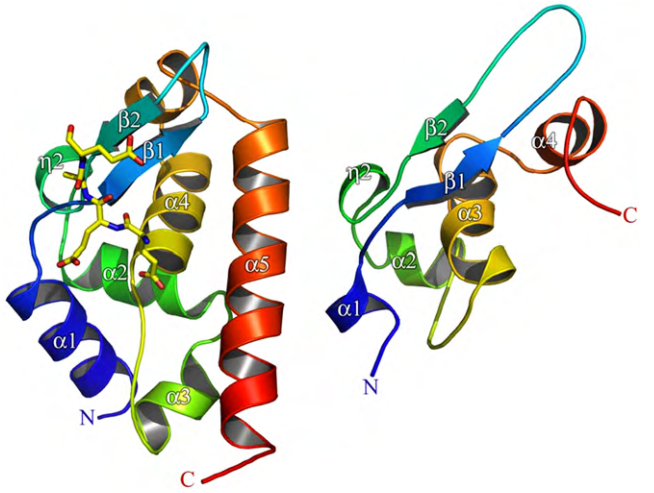

Fig. 8. Side by side comparison of the structures of MOKV (left) and VSV (right, PDB. Id 2K47) phosphoprotein. For MOKV P, the crystal is shown in an interaction between a positively charged pocket implicated previously in N–RNA binding (Assenberg et al., 2010; Jacob et al., 2001), and the last 4 C-terminal residues of a neighboring MOKV P molecule in the crystal (shown in yellow in the left figure). The latter consists of 3 acidic amino acids which is therefore highly similar to the acidic region immediately following S389 in N, known to be important in the interaction with P. This raises the possibility that the interaction seen in the crystal mimicks (at least part of) the interaction between N–P. This contrasts with VSV P, for which the C-terminal ~10 amino acids (including VSV P alpha 5) have been implicated in binding N instead (Das et al., 1997; Takacs and Banerjee, 1995) suggesting that the N-binding sites on P are at topologically distinct sites between MOKV and VSVP. Both molecules are rainbow ramped from blue (N-terminus) to red (C-terminus). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

To address this issue, we have screened three lyssavirus M proteins for soluble expression in *E. coli* making use of different tags, including fusion to the ubiquitin-like protein SUMO (Butt et al., 1989; Malakhov et al., 2004). Of the constructs produced, only the SUMO fusions proved to be soluble (Table 1) and enabled the subsequent crystallization and structure determination of the Lagos bat virus (LBV) and VSV serotype New Jersey (VSV_{NJ}) M proteins (Assenberg et al., 2008; Graham et al., 2008). The structures of M of LBV and VSV_{NJ} and VSV_{ind} (Gaudier et al., 2002) are remarkably similar, despite sharing less that 10% sequence identity, when structurally aligned (138 residues are aligned out of 202 with an alpha carbon root mean square deviation of 3.1 Å). However, comparing the structures of the matrix proteins of *Rhabdoviridae* with those of Ebola virus (Dessen et al., 2000) and Borna disease virus (Dessen et al., 2000; Neumann et al., 2009), the only other Mononegavirales whose M protein structures have been determined, reveals the remarkable structural diversity of matrix proteins among *Rhabdoviridae*, *Bornaviridae* and *Filoviridae* (Fig. 9). As proposed by Timmins et al. (2004), this strongly suggests that matrix proteins of different viruses have nothing in common, but rather have arisen independently to carry out a very similar structural role in the assembly of certain enveloped viruses.

The structures of the rhabdovirus M proteins reveal that they share a similar overall fold and that oligomerize via a stretch of amino acids (in the otherwise-disordered N-terminus) that binds to a similar region on the globular domain. Although the binding region is similar in both structures, the molecular details of the interaction interface differ dramatically between VSV_{NJ} and LBV M. In the case of VSV M, this involves docking of a conserved phenylalanine residue in the N-terminal region into a deep hydrophobic pocket, while association in the LBV M depends upon the binding of a type-II polyproline helix into a shallow groove on the surface of the protein (Fig. 10). This inter-molecular interaction provides a plausible mechanism for the self-assembly of M, lead-

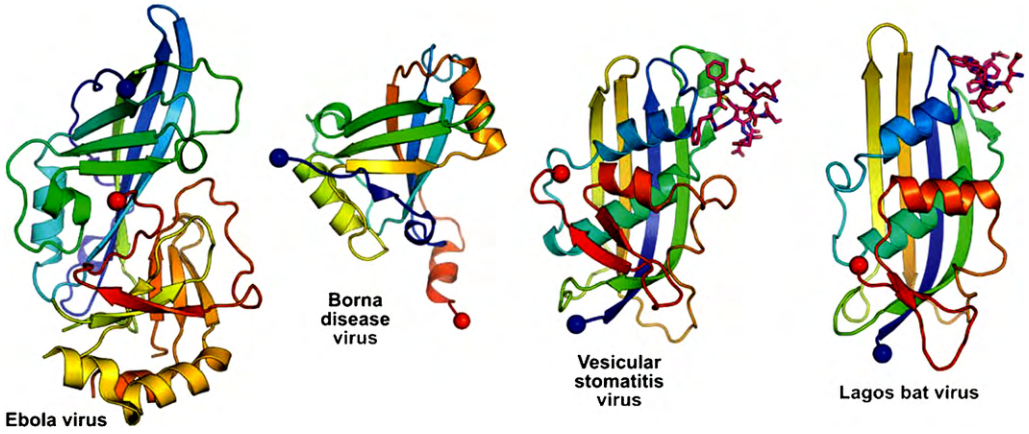

Fig. 9. Mononegalevirales matrix protein structures. The matrix proteins of Ebola virus (VP40), Borna disease virus, vesicular stomatitis virus and Lagos bat virus are shown as cartoons coloured from blue (N-terminus) to red (C-terminus). While the Borna disease virus matrix protein resembles the N-terminal domain of Ebola virus VP40, there is no structural similarity between these proteins and the matrix proteins of vesicular stomatitis virus and Lagos bat virus. For vesicular stomatitis virus and Lagos bat virus the stretch of residues from the otherwise-disordered N-terminus that bind to the main globular domain of the protein are shown as sticks (Dessen et al., 2000; Graham et al., 2008; Neumann et al., 2009). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ing to enhanced affinity for membranes. Disrupting this interaction may offer a novel way of blocking viral assembly. Further, the differences in the interaction between VSV and LBV may provide a structural framework for understanding the distinct cytopathic effects of vesiculoviruses and lyssaviruses.

6. What is next for antiviral drug design against the Mononegavirales?

The list of rhabdoviruses, and more largely of the Mononegavirales, is not complete, and more viruses will certainly emerge and be characterized in the near future, illustrating the large genetic diversity of these viruses and their impact on human health. However, antiviral drug discovery against Mononegavirales has been lagging behind that for other RNA viruses, in particular the (+)RNA viruses. There are multiple reasons behind this observation, and it is certainly not the lack of important human pathogens that has failed to ignite clinical and scientific interest. Perhaps the most advocated reason for the failure to discover interesting antiviral molecules is the virus architecture itself. First, the protection of the replication/transcription machinery within the RNP may provide intrinsic resistance to the access of small inhibitors to active sites known to represent interesting targets. This is also reflected in the crystallization success rate in the VIZIER project, which was much lower for (−)RNA viruses than (+)RNA viruses. Hence, inhibitor screening produces notoriously low "hit" rates compared to those directed against other RNA viruses, an observation correlated with the low abundance of publications describing the discovery of small molecule inhibitors against the Mononegavirales. Second, the vaccine field has shown impressive success against historic viruses, such as rabies, and current viruses such as measles that are robustly attacked by ongoing efficient vaccination campaigns.

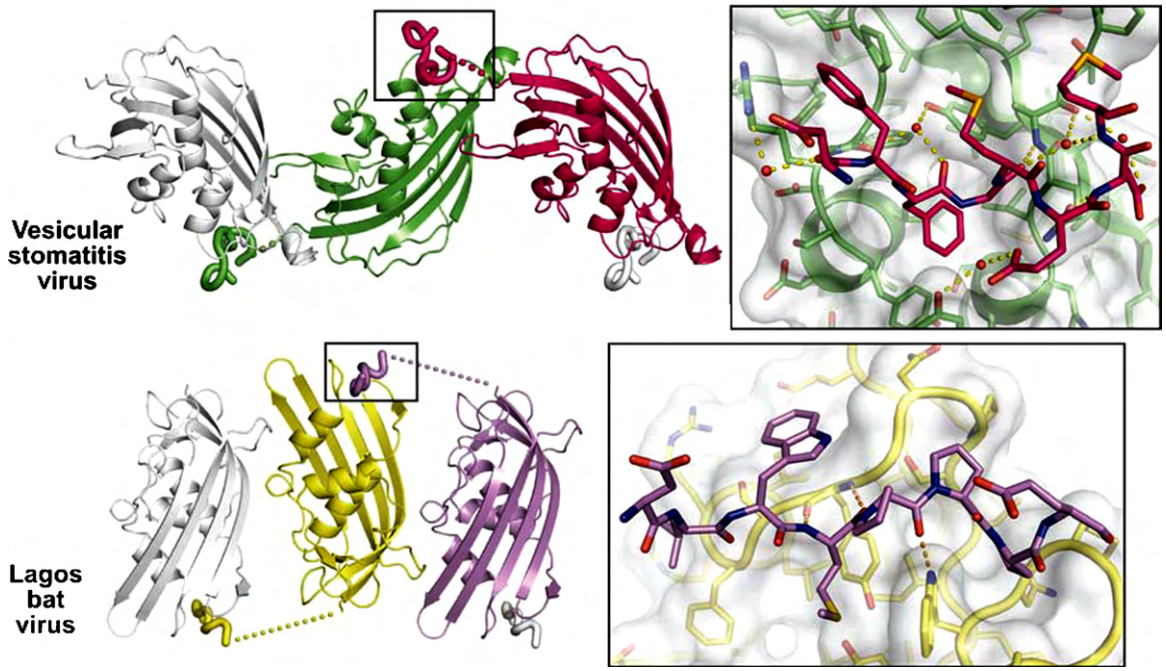

Fig. 10. Comparison of M protein structures. Self-association of rhabdovirus matrix proteins. Residues from the mostly-disordered N-terminal segment of rhabdovirus M proteins are observed binding to grooves on the surface of adjacent M proteins, thereby forming non-covalent linear polymers of M in the crystals. Insets highlight the different molecular details of the interactions between the N-terminal segments and the globular domain (Graham et al., 2008).

Targeting the enzyme activities involved in the replication of the Mononegavirales, and in particular rhabdoviruses, using a structure-based design approach remains a major goal of antiviral research. Some progress has been made in the VIZIER programme towards dissecting the multi-functional L protein into components suitable for structural and functional studies. The phosphoprotein polymerase co-factor (P) and the structural matrix protein (M), which play a number of roles during viral replication and drive viral assembly, have both proved much more amenable to structural biology than L. Applying the multi-construct/multi-virus approach deduced from the studies on rhabdovirus diversity central to the protein production processes in VIZIER has yielded new structural information which may ultimately be exploitable in the derivation of novel ways of intervening in viral replication.

There are also several observations that soon may stimulate these investigations. First, the plasticity and rapid rate of evolution of the Mononegavirales, as exemplified by Henipah outbreaks, have shown that the emergence of new highly lethal agents is no fiction, reinforcing the necessity for preparedness against this virus order. Second, pharmaceutical feasibility has combined with a growing medical need for effective therapies against respiratory syncytial virus, another member of the Mononegavirales. This will undoubtedly foster scientific collaborative effort that will stimulate the field as a whole. And third, influenza research has recently shown, through successful structural genomic approaches, that it is timely to launch novel drug discovery programs on extremely interesting targets, such as the influenza polymerase. Indeed, the polymerase was considered until now a very difficult challenge in structural biology, much as for the Mononegavirales. Recent crystal structures of the influenza polymerase subunits (Dias et al., 2009; Guilligay et al., 2008; He et al., 2008; Kuzuhara et al., 2009; Obayashi et al., 2008; Tarendeau et al., 2007, 2008; Yuan et al., 2009) have shown that novel avenues are now offered to the structure-based drug design field, pointing to better conserved targets that are less prone to genetic variation than the neuraminidase. It is tempting to speculate that the time is ripe for similar significant advances in the Mononegavirales field, in which vaccination and antiviral approaches will combine against this vast reservoir of human pathogens.

References

Albertini, A.A., Wernimont, A.K., Muziol, T., Ravelli, R.B., Clapier, C.R., Schoehn, G., Weissenhorn, W., Ruigrok, R.W., 2006. Crystal structure of the rabies virus nucleoprotein–RNA complex. Science 313, 360–363.

Albertini, A.A., Schoehn, G., Weissenhorn, W., Ruigrok, R.W., 2008. Structural aspects of rabies virus replication. Cell. Mol. Life Sci. 65, 282–294.

Alexandrov, A., Dutta, K., Pascal, S.M., 2001. MBP fusion protein with a viral protease cleavage site: one-step cleavage/purification of insoluble proteins. Biotechniques 30, 1194–1198.

Andrei, G., De Clercq, E., 1993. Molecular approaches for the treatment of hemorrhagic fever virus infections. Antiviral Res. 22, 45–75.

Anonymous, 2005. WHO expert consultation on rabies. In: World Health Organ Tech. Rep. Ser. WHO, Geneva, pp. 1–88.

Arai, Y.T., Kuzmin, I.V., Kameoka, Y., Botvinkin, A.D., 2003. New lyssavirus genotype from the lesser mouse-eared bat (*Myotis blythi*), Kyrghyzstan. Emerg. Infect. Dis. 9, 333–337.

Arnheiter, H., Davis, N.L., Wertz, G., Schubert, M., Lazzarini, R.A., 1985. Role of the nucleocapsid protein in regulating vesicular stomatitis virus RNA synthesis. Cell 41, 259–267.

Assenberg, R., Delmas, O., Graham, S.C., Verma, A., Berrow, N., Stuart, D.I., Owens, R.J., Bourhy, H., Grimes, J.M., 2008. Expression, purification and crystallization of a lyssavirus matrix (M) protein. Acta Crystallogr. Sect. F: Struct. Biol. Cryst. Commun. 64, 258–262.

Assenberg, R., Delmas, O., Ren, J., Vidalain, P.O., Verma, A., Larrous, F., Graham, S.C., Tangy, F., Grimes, J.M., Bourhy, H., 2010. The structure of the N–RNA binding domain of the Mokola virus phosphoprotein. J. Virol. 84, 1089–1096.

Bakker, A.B., Marissen, W.E., Kramer, R.A., Rice, A.B., Weldon, W.C., Niezgoda, M., Hanlon, C.A., Thijsse, S., Backus, H.H., de Kruif, J., Dietzschold, B., Rupprecht, C.E., Goudsmit, J., 2005. Novel human monoclonal antibody combination effectively neutralizing natural rabies virus variants and individual *in vitro* escape mutants. J. Virol. 79, 9062–9068.

Bakker, A.B., Python, C., Kissling, C.J., Pandya, P., Marissen, W.E., Brink, M.F., Lagerwerf, F., Worst, S., van Corven, E., Kostense, S., Hartmann, K., Weverling, G.J., Uytdehaag, F., Herzog, C., Briggs, D.J., Rupprecht, C.E., Grimaldi, R., Goudsmit, J., 2008. First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. Vaccine 26, 5922–5927.

Benmansour, A., Brahimi, M., Tuffereau, C., Coulon, P., Lafay, F., Flamand, A., 1992. Rapid sequence evolution of street rabies glycoprotein is related to the highly heterogeneous nature of the viral population. Virology 187, 33–45.

Berrow, N.S., Alderton, D., Sainsbury, S., Nettleship, J., Assenberg, R., Rahman, N., Stuart, D.I., Owens, R.J., 2007. A versatile ligation-independent cloning method suitable for high-throughput expression screening applications. Nucleic Acids Res. 35, e45.

Bieniasz, P.D., 2006. Late budding domains and host proteins in enveloped virus release. Virology 344, 55–63.

Botvinkin, A.D., Poleschuk, E.M., Kuzmin, I.V., Borisova, T.I., Gazaryan, S.V., Yager, P., Rupprecht, C.E., 2003. Novel lyssaviruses isolated from bats in Russia. Emerg. Infect. Dis. 9, 1623–1625.

Bourhis, J.M., Canard, B., Longhi, S., 2006. Structural disorder within the replicative complex of measles virus: functional implications. Virology 344, 94–110.

Bourhy, H., Kissi, B., Tordo, N., 1993. Molecular diversity of the Lyssavirus genus. Virology 194, 70–81.

Bourhy, H., Kissi, B., Audry, L., Smreczak, M., Sadkowska-Todys, M., Kulonen, K., Tordo, N., Zmudzinski, J.F., Holmes, E.C., 1999. Ecology and evolution of rabies virus in Europe. J. Gen. Virol. 80 (Pt. 10), 2545–2557.

Bourhy, H., Cowley, J.A., Larrous, F., Holmes, E.C., Walker, P.J., 2005. Phylogenetic relationships among rhabdoviruses inferred using the L polymerase gene. J. Gen. Virol. 86, 2849–2858.

Bourhy, H., Gubala, A.J., Weir, R.P., Boyle, D.B., 2008a. Animal rhabdoviruses. In: Mahy, B.W.J.V.R., Van Regenmortel, M.H.V. (Eds.), Encyclopedia of Virology, 3rd edn, pp. 111–121.

Bourhy, H., Reynes, J.M., Dunham, E.J., Dacheux, L., Larrous, F., Huong, V.T., Xu, G., Yan, J., Miranda, M.E., Holmes, E.C., 2008b. The origin and phylogeography of dog rabies virus. J. Gen. Virol. 89, 2673–2681.

Bourhy, H., Goudal, M., Mailles, A., Sadkowska-Todys, M., Dacheux, L., Zeller, H., 2009. Is there a need for anti-rabies vaccine and immunoglobulins rationing in Europe? Euro Surveill., 14.

Bray, M., Driscoll, J., Huggins, J.W., 2000. Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor. Antiviral Res. 45, 135–147.

Bray, M., Raymond, J.L., Geisbert, T., Baker, R.O., 2002. 3-Deazaneplanocin A induces massively increased interferon-alpha production in Ebola virus-infected mice. Antiviral Res. 55, 151–159.

Bussereau, F., Picard, M., Blancou, J., Sureau, P., 1988. Treatment of rabies in mice and foxes with antiviral compounds. Acta Virol. 32, 33–49.

Butt, T.R., Jonnalagadda, S., Monia, B.P., Sternberg, E.J., Marsh, J.A., Stadel, J.M., Ecker, D.J., Crooke, S.T., 1989. Ubiquitin fusion augments the yield of cloned gene products in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 86, 2540–2544.

Chen, M., Ogino, T., Banerjee, A.K., 2006. Mapping and functional role of the self-association domain of vesicular stomatitis virus phosphoprotein. J. Virol. 80, 9511–9518.

Connor, J.H., McKenzie, M.O., Lyles, D.S., 2006. Role of residues 121 to 124 of vesicular stomatitis virus matrix protein in virus assembly and virus–host interaction. J. Virol. 80, 3701–3711.

Coutard, B., Gorbalenya, A.E., Snijder, E.J., Leontovich, A.M., Poupon, A., De Lamballerie, X., Charrel, R., Gould, E.A., Gunther, S., Norder, H., Klempa, B., Bourhy, H., Rohayem, J., L'Hermite, E., Nordlund, P., Stuart, D.I., Owens, R.J., Grimes, J.M., Tucker, P.A., Bolognesi, M., Mattevi, A., Coll, M., Jones, T.A., Aqvist, J., Unge, T., Hilgenfeld, R., Bricogne, G., Neyts, J., La Colla, P., Puerstinger, G., Gonzalez, J.P., Leroy, E., Cambillau, C., Romette, J.L., Canard, B., 2008. The VIZIER project: preparedness against pathogenic RNA viruses. Antiviral Res. 78, 37–46.

Dancho, B., McKenzie, M.O., Connor, J.H., Lyles, D.S., 2009. Vesicular stomatitis virus matrix protein mutations that affect association with host membranes and viral nucleocapsids. J. Biol. Chem. 284, 4500–4509.

Das, T., Pattnaik, A.K., Takacs, A.M., Li, T., Hwang, L.N., Banerjee, A.K., 1997. Basic amino acid residues at the carboxy-terminal eleven amino acid region of the phosphoprotein (P) are required for transcription but not for replication of vesicular stomatitis virus genome RNA. Virology 238, 103–114.

De Clercq, E., 2005. Antiviral drug discovery and development: where chemistry meets with biomedicine. Antiviral Res. 67, 56–75.

De Clercq, E., Bernaerts, R., Shealy, Y.F., Montgomery, J.A., 1990. Broad-spectrum antiviral activity of carbodine, the carbocyclic analogue of cytidine. Biochem. Pharmacol. 39, 319–325.

de Kruif, J., Bakker, A.B., Marissen, W.E., Kramer, R.A., Throsby, M., Rupprecht, C.E., Goudsmit, J., 2007. A human monoclonal antibody cocktail as a novel component of rabies postexposure prophylaxis. Annu. Rev. Med. 58, 359–368.

Delmas, O., Holmes, E.C., Talbi, C., Larrous, F., Dacheux, L., Bouchier, C., Bourhy, H., 2008. Genomic diversity and evolution of the lyssaviruses. PLoS One 3, e2057.

Dessen, A., Volchkov, V., Dolnik, O., Klenk, H.D., Weissenhorn, W., 2000. Crystal structure of the matrix protein VP40 from Ebola virus. EMBO J. 19, 4228–4236.

Dias, A., Bouvier, D., Crepin, T., McCarthy, A.A., Hart, D.J., Baudin, F., Cusack, S., Ruigrok, R.W., 2009. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. Nature 458, 914–918.

Ding, H., Green, T.J., Lu, S., Luo, M., 2006. Crystal structure of the oligomerization domain of the phosphoprotein of vesicular stomatitis virus. J. Virol. 80, 2808–2814.

Drummond, A.J., Rambaut, A., 2007. BEAST: bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Drummond, A.J., Ho, S.Y., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.
Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinform. 5, 113.
Eigen, M., Schuster, P., 1979. The Hypercycle. A Principle of Natural Self-Association. Springer-Verlag, Berlin.
Etessami, R., Conzelmann, K.K., Fadai-Ghotbi, B., Natelson, B., Tsiang, H., Ceccaldi, P.E., 2000. Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an *in vitro* and in vivo study. J. Gen. Virol. 81, 2147–2153.
Fearns, R., Peeples, M.E., Collins, P.L., 1997. Increased expression of the N protein of respiratory syncytial virus stimulates minigenome replication but does not alter the balance between the synthesis of mRNA and antigenome. Virology 236, 188–201.
Ferron, F., Rancurel, C., Longhi, S., Cambillau, C., Henrissat, B., Canard, B., 2005. VaZyMolO: a tool to define and classify modularity in viral proteins. J. Gen. Virol. 86, 743–749.
Finke, S., Conzelmann, K.K., 2003. Dissociation of rabies virus matrix protein functions in regulation of viral RNA synthesis and virus assembly. J. Virol. 77, 12074–12082.
Galloway, S.E., Richardson, P.E., Wertz, G.W., 2008. Analysis of a structural homology model of the 2′-O-ribose methyltransferase domain within the vesicular stomatitis virus L protein. Virology 382, 69–82.
Gaudier, M., Gaudin, Y., Knossow, M., 2002. Crystal structure of vesicular stomatitis virus matrix protein. EMBO J. 21, 2886–2892.
Georges-Courbot, M.C., Contamin, H., Faure, C., Loth, P., Baize, S., Leyssen, P., Neyts, J., Deubel, V., 2006. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob. Agents Chemother. 50, 1768–1772.
Gerard, F.C., Ribeiro Ede Jr., A., Leyrat, C., Ivanov, I., Blondel, D., Longhi, S., Ruigrok, R.W., Jamin, M., 2009. Modular organization of rabies virus phosphoprotein. J. Mol. Biol. 388, 978–996.
Gorbalenya, A.E., Lieutaud, P., Harris, M., Coutard, B., Kleywegt, G.K., Kravchenko, A.A., Samborskiy, D.V., Sidorov, I.A., Leontovich, A.M., Jones, T.A., 2010. Practical application of bioinformatics and informatics by the multidisciplinary VIZIER consortium. Antiviral Res..
Gould, A.R., Hyatt, A.D., Lunt, R., Kattenbelt, J.A., Hengstberger, S., Blacksell, S.D., 1998. Characterisation of a novel lyssavirus isolated from Pteropid bats in Australia. Virus Res. 54, 165–187.
Graham, S.C., Assenberg, R., Delmas, O., Verma, A., Gholami, A., Talbi, C., Owens, R.J., Stuart, D.I., Grimes, J.M., Bourhy, H., 2008. Rhabdovirus matrix protein structures reveal a novel mode of self-association. PLoS Pathog. 4, e1000251.
Graslund, S., Sagemark, J., Berglund, H., Dahlgren, L.G., Flores, A., Hammarstrom, M., Johansson, I., Kotenyova, T., Nilsson, M., Nordlund, P., Weigelt, J., 2008. The use of systematic N- and C-terminal deletions to promote production and structural studies of recombinant proteins. Protein Exp. Purif. 58, 210–221.
Grdzelishvili, V.Z., Smallwood, S., Tower, D., Hall, R.L., Hunt, D.M., Moyer, S.A., 2005. A single amino acid change in the L-polymerase protein of vesicular stomatitis virus completely abolishes viral mRNA cap methylation. J. Virol. 79, 7327–7337.
Green, T.J., Zhang, X., Wertz, G.W., Luo, M., 2006. Structure of the vesicular stomatitis virus nucleoprotein–RNA complex. Science 313, 357–360.
Guilligay, D., Tarendeau, F., Resa-Infante, P., Coloma, R., Crepin, T., Sehr, P., Lewis, J., Ruigrok, R.W., Ortin, J., Hart, D.J., Cusack, S., 2008. The structural basis for cap binding by influenza virus polymerase subunit PB2. Nat. Struct. Mol. Biol. 15, 500–506.
Gupta, A.K., Shaji, D., Banerjee, A.K., 2003. Identification of a novel tripartite complex involved in replication of vesicular stomatitis virus genome RNA. J. Virol. 77, 732–738.
Hansen, C., Quake, S.R., 2003. Microfluidics in structural biology: smaller, faster em leader better. Curr. Opin. Struct. Biol. 13, 538–544.
Hansen, C.L., Skordalakes, E., Berger, J.M., Quake, S.R., 2002. A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion. Proc. Natl. Acad. Sci. U.S.A. 99, 16531–16536.
Harty, R.N., Paragas, J., Sudol, M., Palese, P., 1999. A proline-rich motif within the matrix protein of vesicular stomatitis virus and rabies virus interacts with WW domains of cellular proteins: implications for viral budding. J. Virol. 73, 2921–2929.
He, X., Zhou, J., Bartlam, M., Zhang, R., Ma, J., Lou, Z., Li, X., Li, J., Joachimiak, A., Zeng, Z., Ge, R., Rao, Z., Liu, Y., 2008. Crystal structure of the polymerase PA(C)–PB1(N) complex from an avian influenza H5N1 virus. Nature 454, 1123–1126.
Hemachudha, T., Sunsaneewitayaku, l.B., Desudchit, T., Suankratay, C., Sittipunt, C., Wacharapluesadee, S., Khawplod, P., Wilde, H., Jackson, A.C., 2006. Failure of therapeutic coma and ketamine for therapy of human rabies. J. Neurovirol. 12, 407–409.
Huggins, J.W., 1989. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. Rev. Infect. Dis. 11 (Suppl. 4), S750–S761.
Jackson, A.C., 2009. Update on rabies diagnosis and treatment. Curr. Infect. Dis. Rep. 11, 296–301.
Jackson, A.C., Scott, C.A., Owen, J., Weli, S.C., Rossiter, J.P., 2008. Human rabies therapy: lessons learned from experimental studies in mouse models. Dev. Biol. (Basel) 131, 377–385.
Jacob, Y., Real, E., Tordo, N., 2001. Functional interaction map of lyssavirus phosphoprotein: identification of the minimal transcription domains. J. Virol. 75, 9613–9622.
Kelly, R.M., Strick, P.L., 2000. Rabies as a transneuronal tracer of circuits in the central nervous system. J. Neurosci. Methods 103, 63–71.
Knobel, D.L., Cleaveland, S., Coleman, P.G., Fevre, E.M., Meltzer, M.I., Miranda, M.E., Shaw, A., Zinsstag, J., Meslin, F.X., 2005. Re-evaluating the burden of rabies in Africa and Asia. Bull. World Health Organ. 83, 360–368.
Komarova, A.V., Real, E., Borman, A.M., Brocard, M., England, P., Tordo, N., Hershey, J.W., Kean, K.M., Jacob, Y., 2007. Rabies virus matrix protein interplay with eIF3, new insights into rabies virus pathogenesis. Nucleic Acids Res. 35, 1522–1532.
Kondo, H., Maeda, T., Shirako, Y., Tamada, T., 2006. Orchid fleck virus is a rhabdovirus with an unusual bipartite genome. J. Gen. Virol. 87, 2413–2421.
Kuzmin, I.V., Hughes, G.J., Botvinkin, A.D., Orciari, L.A., Rupprecht, C.E., 2005. Phylogenetic relationships of Irkut and West Caucasian Bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. Virus Res. 111, 28–43.
Kuzuhara, T., Kise, D., Yoshida, H., Horita, T., Murazaki, Y., Nishimura, A., Echigo, N., Utsunomiya, H., Tsuge, H., 2009. Structural basis of the influenza A virus RNA polymerase PB2 RNA-binding domain containing the pathogenicity-determinant lysine 627 residue. J. Biol. Chem. 284, 6855–6860.
Lafon, M., 2005. Rabies virus receptors. J. Neurovirol. 11, 82–87.
Langevin, C., Jaaro, H., Bressanelli, S., Fainzilber, M., Tuffereau, C., 2002. Rabies virus glycoprotein (RVG) is a trimeric ligand for the N-terminal cysteine-rich domain of the mammalian p75 neurotrophin receptor. J. Biol. Chem. 277, 37655–37662.
Lentz, T.L., Burrage, T.G., Smith, A.L., Crick, J., Tignor, G.H., 1982. Is the acetylcholine receptor a rabies virus receptor? Science 215, 182–184.
Leppert, M., Rittenhouse, L., Perrault, J., Summers, D.F., Kolakofsky, D., 1979. Plus and minus strand leader RNAs in negative strand virus-infected cells. Cell 18, 735–747.
Li, J., Fontaine-Rodriguez, E.C., Whelan, S.P., 2005. Amino acid residues within conserved domain VI of the vesicular stomatitis virus large polymerase protein essential for mRNA cap methyltransferase activity. J. Virol. 79, 13373–13384.
Li, J., Wang, J.T., Whelan, S.P., 2006. A unique strategy for mRNA cap methylation used by vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. 103, 8493–8498.
Li, J., Rahmeh, A., Morelli, M., Whelan, S.P., 2008. A conserved motif in region v of the large polymerase proteins of nonsegmented negative-sense RNA viruses that is essential for mRNA capping. J. Virol. 82, 775–784.
Luyet, P.P., Falguieres, T., Pons, V., Pattnaik, A.K., Gruenberg, J., 2008. The ESCRT-I subunit TSG101 controls endosome-to-cytosol release of viral RNA. Traffic 9, 2279–2290.
Malakhov, M.P., Mattern, M.R., Malakhova, O.A., Drinker, M., Weeks, S.D., Butt, T.R., 2004. SUMO fusions and SUMO-specific protease for efficient expression and purification of proteins. J. Struct. Funct. Genomics 5, 75–86.
Manie, S.N., de Breyne, S., Vincent, S., Gerlier, D., 2000. Measles virus structural components are enriched into lipid raft microdomains: a potential cellular location for virus assembly. J. Virol. 74, 305–311.
Mavrakis, M., McCarthy, A.A., Roche, S., Blondel, D., Ruigrok, R.W., 2004. Structure and function of the C-terminal domain of the polymerase cofactor of rabies virus. J. Mol. Biol. 343, 819–831.
Mavrakis, M., Mehouas, S., Real, E., Iseni, F., Blondel, D., Tordo, N., Ruigrok, R.W., 2006. Rabies virus chaperone: identification of the phosphoprotein peptide that keeps nucleoprotein soluble and free from non-specific RNA. Virology 349, 422–429.
Mebatsion, T., Weiland, F., Conzelmann, K.K., 1999. Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. J. Virol. 73, 242–250.
Mihindukulasuriya, K.A., Nguyen, N.L., Wu, G., Huang, H.V., da Rosa, A.P., Popov, V.L., Tesh, R.B., Wang, D., 2009. Nyamanini and midway viruses define a novel taxon of RNA viruses in the order Mononegavirales. J. Virol. 83, 5109–5116.
Money, V.A., McPhee, H.K., Mosely, J.A., Sanderson, J.M., Yeo, R.P., 2009. Surface features of a Mononegavirales matrix protein indicate sites of membrane interaction. Proc. Natl. Acad. Sci. U.S.A. 106, 4441–4446.
Morimoto, K., Hooper, D.C., Carbaugh, H., Fu, Z.F., Koprowski, H., Dietzschold, B., 1998. Rabies virus quasispecies: implications for pathogenesis. Proc. Natl. Acad. Sci. U.S.A. 95, 3152–3156.
Nadin-Davis, S.A., Casey, G.A., Wandeler, A.I., 1994. A molecular epidemiological study of rabies virus in central Ontario and western Quebec. J. Gen. Virol. 75 (Pt. 10), 2575–2583.
Neumann, P., Lieber, D., Meyer, S., Dautel, P., Kerth, A., Kraus, I., Garten, W., Stubbs, M.T., 2009. Crystal structure of the Borna disease virus matrix protein (BDV-M) reveals ssRNA binding properties. Proc. Natl. Acad. Sci. U.S.A. 106, 3710–3715.
Obayashi, E., Yoshida, H., Kawai, F., Shibayama, N., Kawaguchi, A., Nagata, K., Tame, J.R., Park, S.Y., 2008. The structural basis for an essential subunit interaction in influenza virus RNA polymerase. Nature 454, 1127–1131.
Ogino, T., Banerjee, A.K., 2007. Unconventional mechanism of mRNA capping by the RNA-dependent RNA polymerase of vesicular stomatitis virus. Mol. Cell 25, 85–97.
Ogino, T., Kobayashi, M., Iwama, M., Mizumoto, K., 2005. Sendai virus RNA-dependent RNA polymerase L protein catalyzes cap methylation of virus-specific mRNA. J. Biol. Chem. 280, 4429–4435.
Plumet, S., Duprex, W.P., Gerlier, D., 2005. Dynamics of viral RNA synthesis during measles virus infection. J. Virol. 79, 6900–6908.
Poch, O., Blumberg, B.M., Bougueleret, L., Tordo, N., 1990. Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. J. Gen. Virol. 71 (Pt. 5), 1153–1162.
Pringle, C.R., 1997. The order Mononegavirales – current status. Arch. Virol. 142, 2321–2326.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.4.

Ribeiro Jr., E.A., Favier, A., Gerard, F.C., Leyrat, C., Brutscher, B., Blondel, D., Ruigrok, R.W., Blackledge, M., Jamin, M., 2008. Solution structure of the C-terminal nucleoprotein–RNA binding domain of the vesicular stomatitis virus phosphoprotein. J. Mol. Biol. 382, 525–538.
Roche, S., Bressanelli, S., Rey, F.A., Gaudin, Y., 2006. Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein G. Science 313, 187–191.
Roche, S., Rey, F.A., Gaudin, Y., Bressanelli, S., 2007. Structure of the prefusion form of the vesicular stomatitis virus glycoprotein G. Science 315, 843–848.
Ruigrok, R.W., Schoehn, G., Dessen, A., Forest, E., Volchkov, V., Dolnik, O., Klenk, H.D., Weissenhorn, W., 2000. Structural characterization and membrane binding properties of the matrix protein VP40 of Ebola virus. J. Mol. Biol. 300, 103–112.
Schoehn, G., Iseni, F., Mavrakis, M., Blondel, D., Ruigrok, R.W., 2001. Structure of recombinant rabies virus nucleoprotein–RNA complex and identification of the phosphoprotein binding site. J. Virol. 75, 490–498.
Sidorov, I.A., Reshetov, D.A., Gorbalenya, A.E., 2009. SNAD: sequence name annotation-based designer. BMC Bioinform. 10, 251.
Solon, J., Gareil, O., Bassereau, P., Gaudin, Y., 2005. Membrane deformations induced by the matrix protein of vesicular stomatitis virus in a minimal system. J. Gen. Virol. 86, 3357–3363.
Svenda, M., Berg, M., Moreno-Lopez, J., Linne, T., 1997. Analysis of the large (L) protein gene of the porcine rubulavirus LPMV: identification of possible functional domains. Virus Res. 48, 57–70.
Takacs, A.M., Banerjee, A.K., 1995. Efficient interaction of the vesicular stomatitis virus P protein with the L protein or the N protein in cells expressing the recombinant proteins. Virology 208, 821–826.
Tarendeau, F., Boudet, J., Guilligay, D., Mas, P.J., Bougault, C.M., Boulo, S., Baudin, F., Ruigrok, R.W., Daigle, N., Ellenberg, J., Cusack, S., Simorre, J.P., Hart, D.J., 2007. Structure and nuclear import function of the C-terminal domain of influenza virus polymerase PB2 subunit. Nat. Struct. Mol. Biol. 14, 229–233.
Tarendeau, F., Crepin, T., Guilligay, D., Ruigrok, R.W., Cusack, S., Hart, D.J., 2008. Host determinant residue lysine 627 lies on the surface of a discrete, folded domain of influenza virus polymerase PB2 subunit. PLoS Pathog. 4, e1000136.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Thoulouze, M.I., Lafage, M., Schachner, M., Hartmann, U., Cremer, H., Lafon, M., 1998. The neural cell adhesion molecule is a receptor for rabies virus. J. Virol. 72, 7181–7190.
Timmins, J., Ruigrok, R.W., Weissenhorn, W., 2004. Structural studies on the Ebola virus matrix protein VP40 indicate that matrix proteins of enveloped RNA viruses are analogues but not homologues. FEMS Microbiol. Lett. 233, 179–186.
Tuffereau, C., Benejean, J., Blondel, D., Kieffer, B., Flamand, A., 1998. Low-affinity nerve-growth factor receptor (P75NTR) can serve as a receptor for rabies virus. EMBO J. 17, 7250–7259.
Tuffereau, C., Desmezieres, E., Benejean, J., Jallet, C., Flamand, A., Tordo, N., Perrin, P., 2001. Interaction of lyssaviruses with the low-affinity nerve-growth factor receptor p75NTR. J. Gen. Virol. 82, 2861–2867.
Tuffereau, C., Schmidt, K., Langevin, C., Lafay, F., Dechant, G., Koltzenburg, M., 2007. The rabies virus glycoprotein receptor p75NTR is not essential for rabies virus infection. J. Virol. 81, 13622–13630.
Vidal, S., Kolakofsky, D., 1989. Modified model for the switch from Sendai virus transcription to replication. J. Virol. 63, 1951–1958.
Vincentelli, R., Bignon, C., Gruez, A., Canaan, S., Sulzenbacher, G., Tegoni, M., Campanacci, V., Cambillau, C., 2003. Medium-scale structural genomics: strategies for protein expression and crystallization. Acc. Chem. Res. 36, 165–172.
Vincentelli, R., Canaan, S., Offant, J., Cambillau, C., Bignon, C., 2005. Automated expression and solubility screening of His-tagged proteins in 96-well format. Anal. Biochem. 346, 77–84.
von Kobbe, C., van Deursen, J.M., Rodrigues, J.P., Sitterlin, D., Bachi, A., Wu, X., Wilm, M., Carmo-Fonseca, M., Izaurralde, E., 2000. Vesicular stomatitis virus matrix protein inhibits host cell gene expression by targeting the nucleoporin Nup98. Mol. Cell 6, 1243–1252.
Walhout, A.J., Temple, G.F., Brasch, M.A., Hartley, J.L., Lorson, M.A., van den Heuvel, S., Vidal, M., 2000. GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. Methods Enzymol. 328, 575–592.
Whelan, S., Goldman, N., 2001. A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol. Biol. Evol. 18, 691–699.
Wilde, H., 2007. Failures of post-exposure rabies prophylaxis. Vaccine 25, 7605–7609.
Willoughby Jr., R.E., 2007. A cure for a rabies? Sci. Am. 296, 88–95.
Willoughby Jr., R.E., Tieves, K.S., Hoffman, G.M., Ghanayem, N.S., Amlie-Lefond, C.M., Schwabe, M.J., Chusid, M.J., Rupprecht, C.E., 2005. Survival after treatment of rabies with induction of coma. N. Engl. J. Med. 352, 2508–2514.
Yuan, P., Bartlam, M., Lou, Z., Chen, S., Zhou, J., He, X., Lv, Z., Ge, R., Li, X., Deng, T., Fodor, E., Rao, Z., Liu, Y., 2009. Crystal structure of an avian influenza polymerase PA(N) reveals an endonuclease active site. Nature 458, 909–913.
Zhao, Y., Chapman, D.A., Jones, I.M., 2003. Improving baculovirus recombination. Nucleic Acids Res. 31, E6-6.