

Negative Strand RNA Virus Lecture II (Measles, Nipah)

Prolog: VSV and Rhabdoviruses (Continuation)

Wagner and Rose, Chapter 37 of Fields Virology, 3rd Edition.

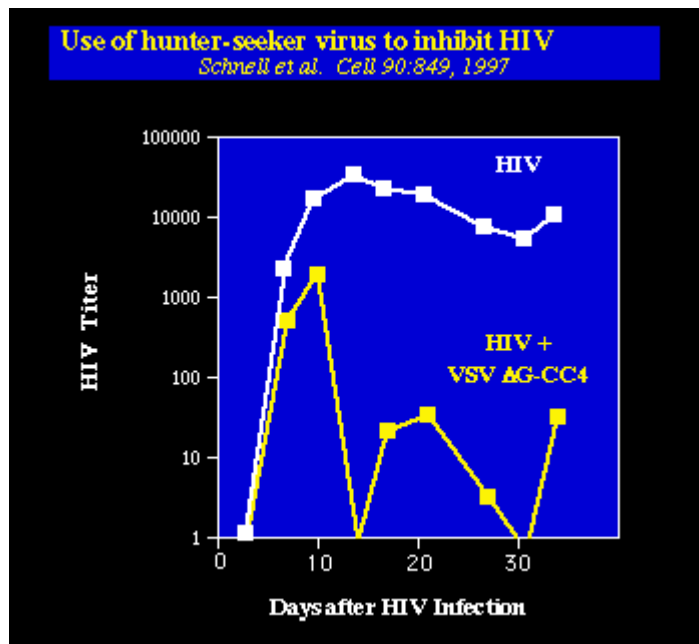
Hunter-Seeker viruses: Nolan, G.P. Cell 90:821, 1997

Hunter-Seeker Viruses

The ability to perform genetic manipulations of the VSV and rabiesvirus genomes has led to the development of sophisticated engineered viruses capable of seeking out and killing specific human cells. The first such hunter-seeker viruses had the ability to seek out and kill cells that express the HIV-1 envelope glycoprotein (*Mebatsion et al. Cell 90:841, 1997; Schnell et al. Cell 90:849, 1997*).

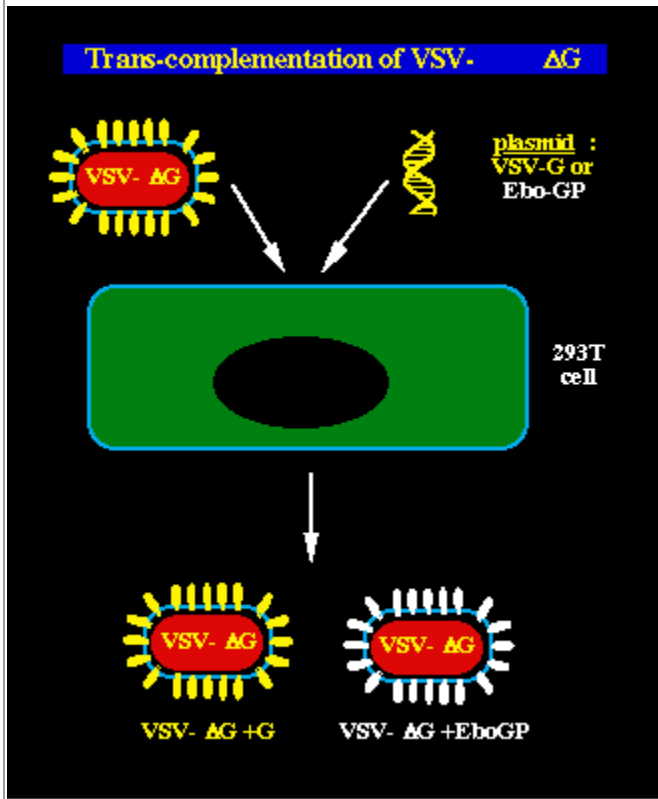
To construct these viruses, John Rose's group at Yale University took a VSV genome, and deleted from it the viral glycoprotein (G) gene. This resulted in the creation of a virus mutant (VSV-ΔG), which was incapable of replication since it lacks coat proteins capable of binding to, and fusing with, target cell membranes. They then inserted into this virus the genes encoding for the human CD4 molecule and the human CXCR4 chemokine receptor. These proteins were incorporated into the coat of the recombinant virus (VSV-ΔG CC4) and gave it the ability to infect cells to which the CD4 and CXCR4 molecules could bind. The only cells which expressed such proteins were those which were infected with HIV-1, since the envelope glycoproteins from certain HIV-1 strains are known to bind to a complex of CD4 and CXCR4.

When the hunter-seeker VSV virus was introduced into HIV-1 infected cell cultures *in vitro*, it was able to dramatically inhibit HIV-1 replication (see below). The hunter-seeker virus not only killed HIV-1 infected cells, but it also acted as a self-replicating watchdog against further HIV-1 spread. As a consequence, minor low level "bursts" of HIV-1 replication which occurred over time in the cell culture were suppressed by the hunter-seeker virus (see below).



The same technology, using G-glycoprotein

VSV trans complementation system



deleted VSV genomes, has been used to study the host cell range of the Ebola virus. For obvious reasons, investigators would prefer not to work with infectious Ebola virus.

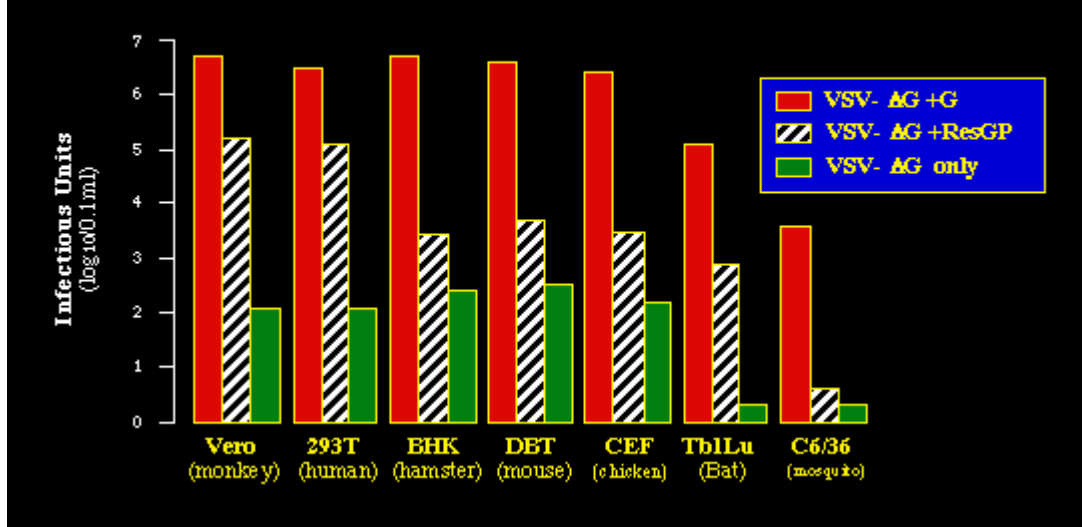
In this case, the envelope coat glycoprotein was added back to the G-deleted VSV (VSV-ΔG) not by its incorporation into the viral genome, but by using a **trans-complementation system**. In this case, cells were transfected with an expression plasmid encoding either the VSV G-protein (VSV-G), or the G-protein from Ebola virus (Ebo-GP), and the cells were then infected with the VSV-ΔG virus (complemented with the G protein; see Figure).

Note that these initial virus stocks (VSV-ΔG +G) were capable of only a single round of infection, since they cannot make G-protein on their own. However, in the transfected cells, the viruses are able to acquire new G-protein, or Ebolavirus-GP glycoprotein, in trans.

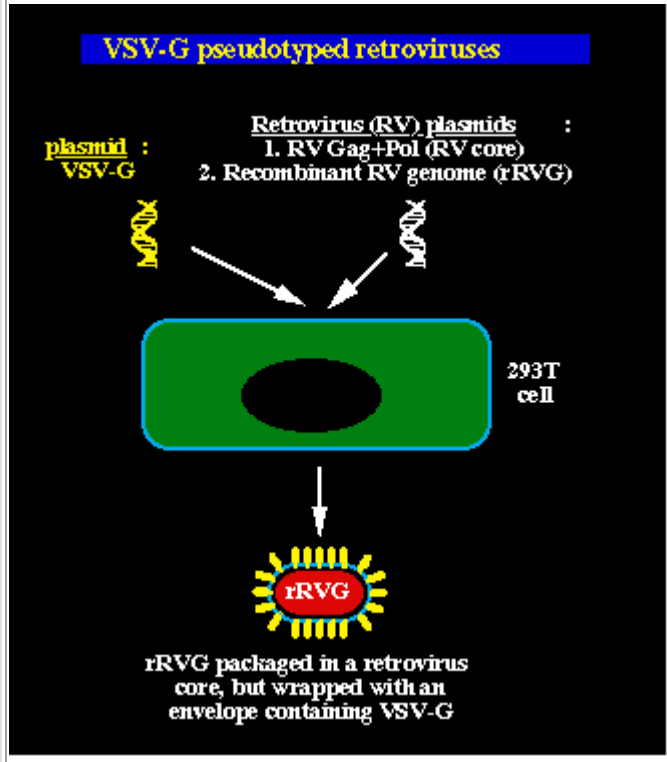
The infectivity and host cell range of the resulting, *trans*-complemented VSV viruses was then examined (see below). The results showed that Ebo-GP binds efficiently to primate cells, but that it is much efficient at binding to cells from other species.

Infectivities of recombinant VSVs for different cell lines

Takada et al. PNAS 94:14764, 1997

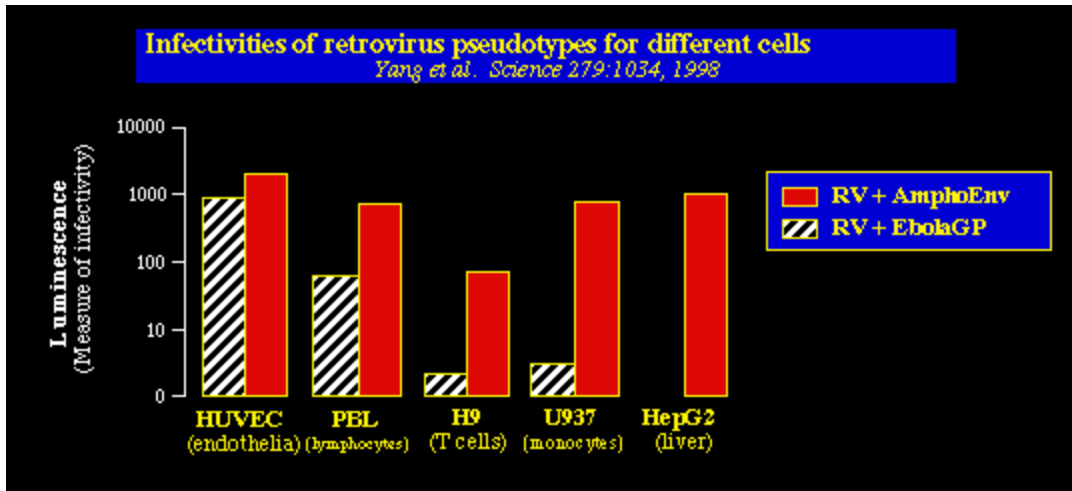


A variation on the theme of *trans*-complementation of G-protein deleted VSV viruses, is the use of VSV G-protein to generate **retrovirus pseudotypes**. Pseudotypes are the result of phenotypic mixing of different viruses, and contain a core (and genome) from one virus, combined with the envelope (and receptor-binding activity) of the second virus.

<p>VSV-G Pseudotyped Retrovirus Vectors</p>  <p>VSV-G pseudotyped retroviruses</p> <p>plasmid : VSV-G</p> <p>Retrovirus (RV) plasmids :</p> <ol style="list-style-type: none"> 1. RV Gag+Pol (RV core) 2. Recombinant RV genome (rRVG) <p>293T cell</p> <p>rRVG packaged in a retrovirus core, but wrapped with an envelope containing VSV-G</p>	<p>In general, pseudotypes can be generated only between fairly closely related viruses (such as VSV and Ebola virus), but exceptions exist. One notable exception are retroviruses. It has long been known that VSV can generate pseudotypes with a variety of retroviruses, including HIV-1.</p> <p>Retrovirus pseudotypes bearing the VSV G-protein in place of the natural retrovirus envelope have several features which make them useful for gene therapy. These include:</p> <ol style="list-style-type: none"> 1. <u>Extended host cell range</u>. The VSV G-protein allows one to deliver the retrovirus "payload" (i.e., the recombinant genome) to a wide array of mammalian & animal cells, including fish. It also allows one to deliver genes to certain human cell types, such as hematopoietic progenitor cells, which are otherwise difficult to target. 2. <u>Increased physical stability</u>. The VSV G-protein is much more stable than the natural retrovirus envelope. This allows one to concentrate the viral particles, and to generate high-titer stocks which are more useful for gene transfer.
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As with the VSV-ΔG system, retrovirus pseudotypes can be used to examine the cell-binding properties of the Ebola virus glycoprotein. Results from one such study are shown below. In this case, the investigators generated retrovirus pseudotypes bearing envelope glycoproteins either from another retrovirus with a broad host range (AmphoEnv) or bearing the Ebolavirus GP (EbolaGP). In both cases, they packaged a recombinant retrovirus genome that contained the gene for firefly luciferase. This allowed them to easily measure the infectivity of the retrovirus pseudotypes for various cell lines, by measuring luciferase production using a luminescence assay.

As shown below, these experiments revealed that the Ebola GP interacts efficiently with a receptor that is present on human vascular endothelial cells (HUVECs), but that this glycoprotein is less efficient at interacting with surface molecules on other human cell types such as T-cells, monocytes and liver cells.



PARAMYXOVIRIDAE

Lamb and Kolakofsky, Fields Virology, 3rd Ed., Chapter 40 (Paramyxoviridae)

Griffin and Bellini, Fields Virology, 3rd Ed., Chapter 43 (Measles virus)

Bhardwaj, N. J. Exp. Med. 186:795, 1997 (MV immune evasion)

Chua et al. Science 288:1432, 2000; Goh et al. N. Engl. J. Med. 342:1229, 2000 (Nipah)

Wang et al. J. Virol. 74:9972, 2000 (Hendra virus)

Subfamily Paramyxo- virinae (Genera: Paramyxo-, Morbilli-, Rubula-)

Respiro-: eg, Parainfluenza viruses 1 and 3; have H and N (single molecule: HN)

Rubula-: eg, mumps virus; have H and N (single molecule: HN); has extra gene (SH)

Morbilli-: eg, measles virus; have H but no N

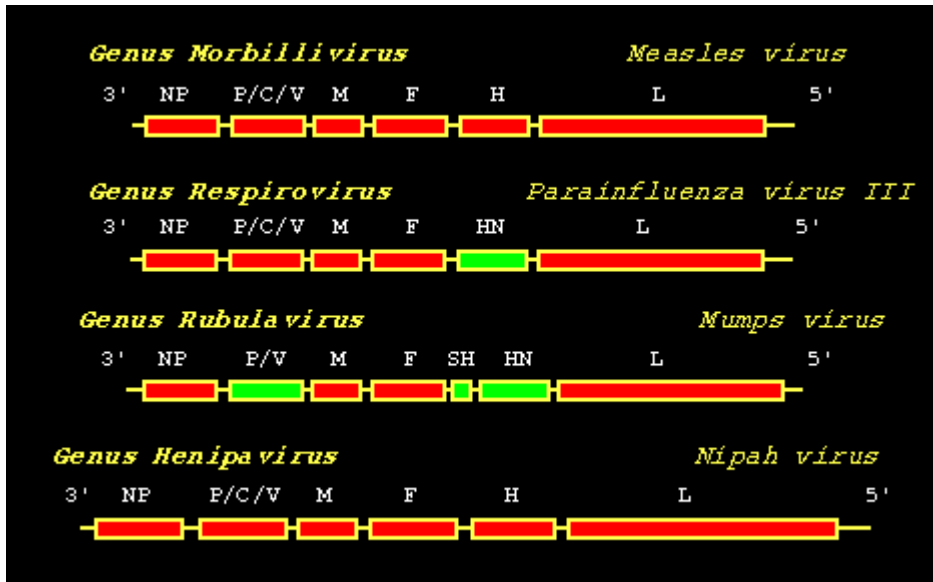
Henipa-: eg, Nipah, Hendra viruses; have H but no N (*this genus has been proposed but is not yet official*)

Subfamily: Pneumo- virinae (Genus: Pneumovirus)

Pneumo-: eg, respiratory syncytial virus (RSV); has neither H nor N; more divergent

Paramyxovirinae: Genomes

Approx. 16 to 18 kb ss RNA genome (negative sense); largest = Henipaviruses (proposed Genus)



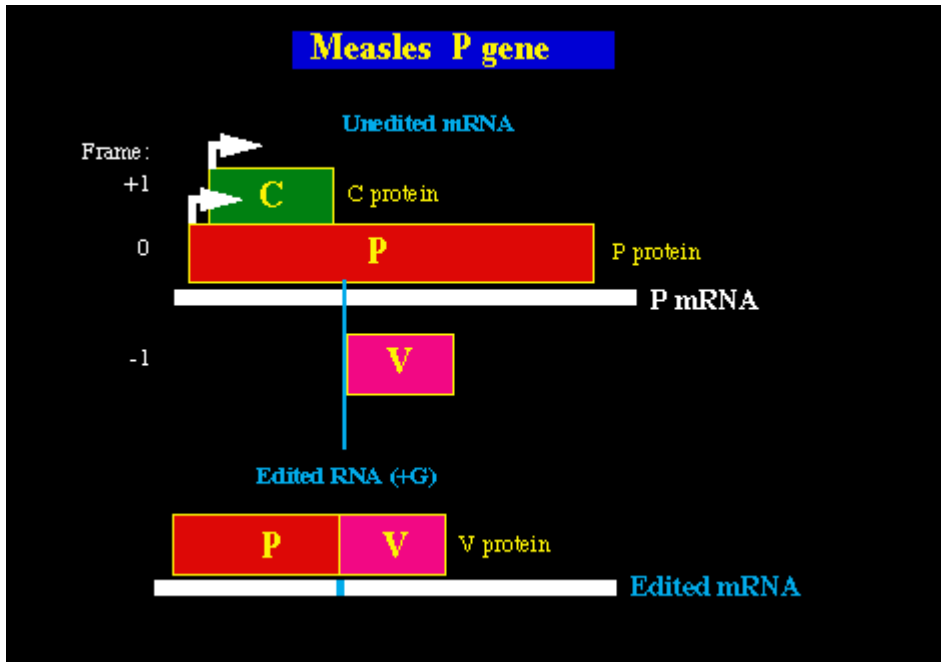
NOTE: C, V proteins are encoded from overlapping reading frames within the P gene region

Measles Virus: Ribonucleoprotein (RNP) proteins

Ribonucleoproteins in Measles virus include the Polymerase, Nucleoprotein and the Phosphoproteins, which are broadly equivalent to their counterparts in the rhabdoviruses. One important difference between Measles and the rhabdoviruses is the fact that the measles virus P gene encodes two other proteins, in addition to the Phosphoprotein. These extra proteins are encoded by the P mRNA but are translated using different initiator methionine codons and overlapping reading frames. The first such product is the **C protein**, which is a small basic protein that is produced from an open reading frame (ORF) that overlaps the N-terminus of the P-gene, in the +1 frame. The C-protein is encoded off P gene transcripts by **internal translational initiation**. *It should be noted that, on a molar basis, the C protein is the major product of the P gene in many paramyxoviruses, and several forms of C protein can be made in some viruses, due to use of several initiator methionine codons.* The function of the C protein appears to be in helping to regulate the switch between mRNA transcription and viral replication. Specifically, the C protein inhibits mRNA synthesis, but it has little effect on genome replication.

The second additional product of the P gene is the **V protein**. The P gene mRNA of Measles virus is cotranscriptionally edited at a specific site downstream of the C ORF. This **RNA editing** event involves the addition of a nontemplated G residue at this position during mRNA synthesis (*this occurs because the RNA polymerase slips or "stutters" when it encounters a run of C residues on the template RNA strand; the reason why this occurs at this particular site and nowhere else may be related to RNA structure*). The result of the addition of this extra G residue to the mRNA is that upon translation, the N-terminal half of the P protein sequences cannot be joined to its normal C-terminus. Instead, it is fused to a conserved cysteine-rich, acidic, domain expressed from the V protein ORF (which is in the -1 ORF relative to P). The fusion protein formed is known as V, and its function is uncertain -- although evidence suggests that it may act to repress genome replication, while having little effect on mRNA transcription.

Why this complexity in the P-gene?? It is probably not an attempt to save space, since the genome is big and could encode small extra proteins. More likely it is a way of co-expressing proteins which perhaps interact or function at a common step in replication.



Measles Virus: Matrix (M) Protein.

This is also functionally analogous to its counterpart in the Rhabdoviruses. The M protein is basic (net + charge at neutral pH) and highly abundant in the virion. The M protein is believed to be the central organizer in **virion morphogenesis**, and it can interact both with itself and with the viral nucleocapsid, as well as with the C-terminal tails (transmembrane anchors) of both the F and H proteins. The M protein is thus important for virus budding and for virus infectivity, and the M gene is often defective (mutated) in persistent MV infections, where budding fails to occur, such as SSPE or subacute sclerosing panencephalitis, a rare and fatal syndrome in which there is persistent MV infection of the brain.

Measles Virus: Envelope proteins

All paramyxoviridae possess two membrane or envelope proteins. One is involved in cell **attachment** and the other mediates **fusion** with the host cell membrane, in a pH-independent manner.

Attachment Protein. The attachment proteins of the paramyxovirinae bind to sialic-acid containing receptors on cells, and these viruses are therefore able to agglutinate red blood cells (hemagglutination). In the case of viruses in the genera respirovirus and rubulavirus, these viral hemagglutinins also possess neuraminidase activity and are thus referred to as **HN proteins** (hemagglutinin-neuraminidase). In the case of viruses in the genus morbillivirus, the hemagglutinin lacks neuraminidase activity (thus, **measles virus encodes an H protein** and not an HN protein).

Role of Neuraminidase. It is believed that neuraminidase prevents aggregation of viral particles to the plasma membrane during viral budding, and thus facilitates virus release from infected cells. This means that neuraminidase must be inhibited during the early steps of virus entry and that it must become activated during the late stages of virus exit. This may occur in part because the activities of hemagglutinin and neuraminidase are regulated by pH and by halide ion concentration. Specifically, the pH and halide ion concentration of the extracellular environment is optimal for hemagglutination, while neuraminidases function best at acidic pH (such as can be found within the Golgi network inside cells).

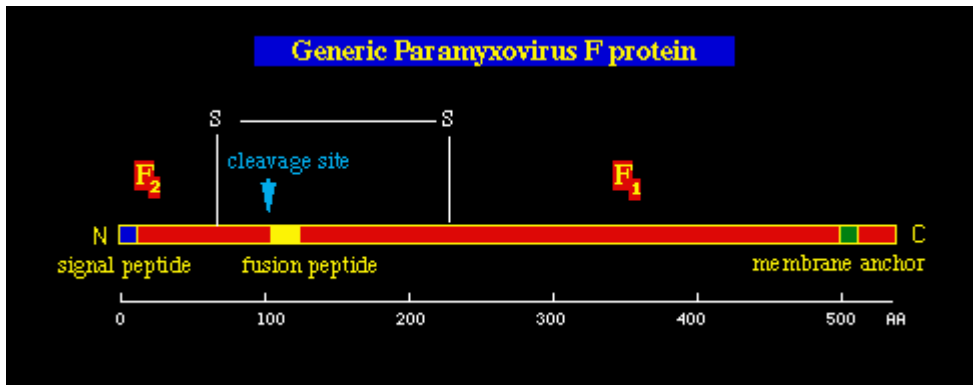
Note that measles virus (MV) does not in fact use sialic acid as its receptor -- presumably because its H protein has a relatively low affinity for sialic acid. As a result, MV does not need a neuraminidase. In stead,

measles virus H protein is thought to bind to a specific receptor, SLAM (*signalling lymphocyte-activation molecule; CDw150*). SLAM is present on some T cells and B cells (Tatsuo et al. *Nature* 406:893, 2000).

The Pneumovirus, respiratory syncytial virus (**RSV**) does not hemagglutinate and its receptor is unknown. In this case, viral attachment to the host cell is mediated by the **G (glycoprotein) protein**.

Note that some highly passaged vaccine strains of MV bind to CD46 -- a molecule which is a member of the immunoglobulin gene superfamily; clinical isolates of MV do not, however, bind CD46.

Fusion protein. Paramyxovirus fusion proteins are initially made as an inactive precursor (F₀). This is cleaved by cellular proteases, to give rise to a short N-terminal fragment (F₂) and a longer C-terminal fragment (F₁). These two fragments remain linked by a disulfide bond. Importantly, the process of proteolysis exposes a hydrophobic and fusogenic domain at the N-terminus of F₁. *Note that proteolytic processing of the protein is absolutely required for viral infectivity.*



The highly hydrophobic region of the F protein is almost certainly shielded from the aqueous environment since exposure would result in aggregation of the protein. Presumably, a conformational change during virus infection results in exposure of the fusion domain, in a manner similar to that described for influenza virus. One difference, however, is that fact that the paramyxoviridae induce fusion at neutral pH (whereas influenza virus requires acidic endosomal pH for fusion). Thus paramyxoviridae can induce "fusion from without". Virions induce rapid cell fusion (prior to viral replication) when added to cultures at high concentration. As a result, Sendai virus envelopes or inactivated Sendai virus particles have been used for many years to promote cell fusion and to derive cell hybrids (eg. for monoclonal antibody production).

Other envelope proteins. The rubulaviruses contain a small gene called **SH** (short hydrophobic protein) which is located between F and HN. This is an integral membrane protein of unknown function.

Pneumovirinae: Genetics and Unique Features



Nonstructural proteins, NS1 and NS2. Pneumovirinae encode two abundant, short nonstructural proteins. Their role is unknown.

SH protein. Also found in rubulaviruses.

22K protein (aka M2 protein). The gene encoding this protein lies between the F and L genes.

P gene products. There is no C- or V- protein produced.

In addition the **genomic organization** of pneumoviruses is unique:

1. The order of the G, F genes is reversed (thus, M2 is adjacent to L)
2. Most importantly, the **M2 (22K), L genes overlap** by 68 nts. Thus, there is no intergenic sequence upstream of L, and so the only way to make the L protein is read-through (ignore!) the transcription termination signal at the end of the M2 (22K) gene. This has the overall result of lowering expression of the L-gene.

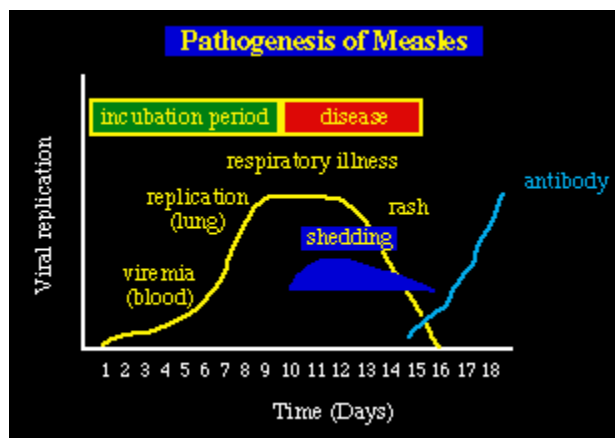
Paramyxovirus replication

In general terms, paramyxovirus replication is broadly similar to that of rhabdoviruses.

One important and unique feature of **measles virus infection**, in particular, is the virus' ability to persistently infect brain cells, which has been implicated in SSPE. Persistent MV infection appears to involve at least two methods for **specific attenuation of M gene expression**. First, specific downregulation of M gene expression can occur as the result of inefficient transcriptional termination and polyadenylation at the upstream ORF (which is expressed normally). Second, biased hypermutation of the M gene region has been described. This is due to the action of a cellular enzyme that converts adenosines to inosines in dsRNA (double-stranded-RNA-adenosine deaminase, dsRAD). *Note that dsRAD is also responsible for site-specific editing of Hepatitis Delta virus RNA.*

Another key feature of paramyxovirus replication is the great **diversity of P gene expression**. This is due both to translational choice (ie, selection of which ATG codon will be used to initiate protein synthesis -- giving rise to P or C proteins) and also to transcriptional choice (ie, insertion of the non-templated G residue in the P gene mRNA, which allows for expression of the V protein).

Pathogenesis of MV infection



MV is a classic emerging pathogen. MV is a relatively new disease of humans which evolved from an animal morbillivirus (MV most closely resembles a pathogen of cattle known as rinderpest virus). Measles was first described in the tenth century. Since it causes a highly contagious acute infection which results in lifelong immunity, and it has no animal reservoir, it requires an urban setting to survive (200,000+ people). Cities this size first emerged ~3000 BC in Egypt and Sumeria. This is likely when measles and mumps emerged. These cities remained isolated until trade began. Epidemics of disease (measles, smallpox) then began around 200-400 AD. Measles was carried to the New World by Europeans and caused many deaths among native Americans, who had not encountered MV previously.

MV is a major killer of the world's children. Worldwide, measles kills about one million children each year, according to the World Health Organization (WHO). The scale of the problem is made even clearer by the fact that a huge and enormously successful measles vaccination campaign in the 1980s and 1990s has resulted in the vaccination of about 80% of the world's children against measles. Were it not for this effort, measles would kill roughly 3 million children each year. Even so, MV still kills roughly 1 million children per year, with a mortality rate of up to 5-15% in some countries.

As a result, WHO and UNICEF are working hard to further raise immunization rates in all villages and cities, including poor areas, and to develop a new measles vaccine that will be more effective in young children (under 2 years of age). This is important because MV infection is more severe in young children, and it is young children who are most at risk for MV infection in the developing world.

In industrialized nations, MV outbreaks still occur despite vaccination. For example, a measles epidemic occurred in the US from 1989 - 1991, resulting in about 55,000 cases of measles and about 130 deaths. This epidemic was traced to the fact that immunization rates for preschool children in many inner-city areas in the US were below 50%. Thus, there was a large population of disease susceptible children.

Ultimately, the WHO and UNICEF intend to eradicate measles completely. This is possible because (1) there is no animal reservoir for MV, (2) there is only one serotype of the virus, (3) most cases are clinically identifiable and (4) an effective vaccine is available (*this is a live, attenuated vaccine which is administered at 9 months in areas where the virus remains prevalent and at 15 months elsewhere; it is administered as part of the MMR vaccination in the US*).

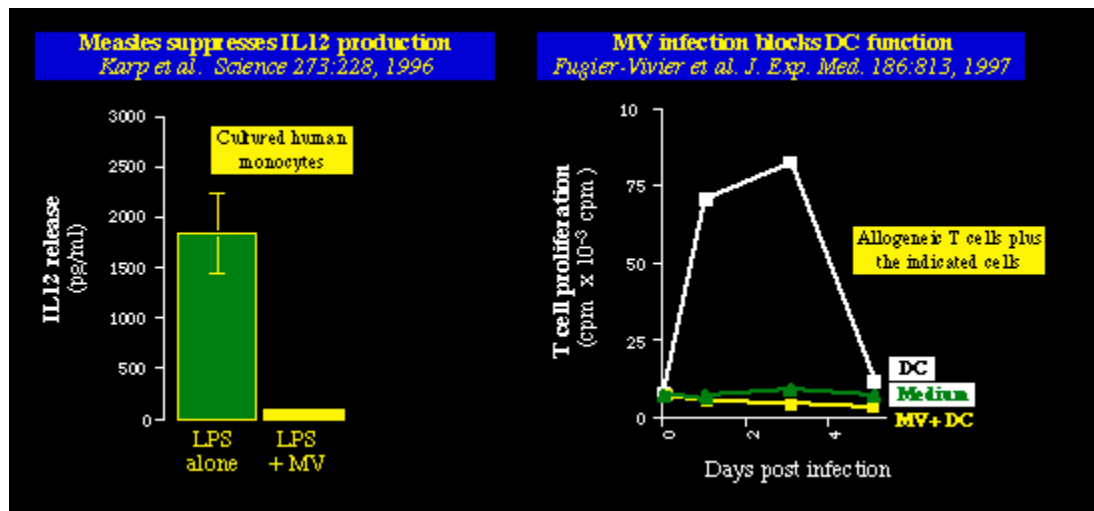
Novel MV vaccines. Efforts to develop MV vaccines that are highly effective in young infants are ongoing. One approach that has shown promise is the use of DNA vaccines encoding the MV hemagglutinin (H) or fusion (F) proteins. These have protected macaque monkeys from challenge with wild-type MV (*Polack et al. Nature Med. 6:776, 2000*).

Pathogenesis. Measles is a childhood infection that is spread by a respiratory route. Following infection, the virus replicates in lymphoid tissues. It then enters the blood (viremia) and spreads through the body, reaching its target tissues (principally, the lungs), where replication occurs. This initial period of infection is asymptomatic and lasts about 10-14 days, at the end of which clinical signs of disease become apparent - notably, fever, cough and conjunctivitis. Roughly 2-3 days after the onset of these symptoms, the characteristic measles rash appears. This coincides with the appearance of an antiviral immune response. Recovery results in lifelong immunity to infection.

Immune responses. Recovery from MV infection is mediated in large part by a cellular immune response to the virus (cytotoxic T cells, CTLs). However, one of the striking features of MV is its ability to cause **immune suppression** *in vivo* and *in vitro*. Production of cellular responses to new antigens is significantly inhibited, which predisposes MV infected children to concurrent infection by other pathogens (such as bacteria, which can cause fatal pneumonia in MV-infected children).

The mechanism(s) by which MV causes immune suppression is believed to involve virus infection of dendritic cells (which are involved in antigen presentation) as well as infection of other immune cells. **MV infection of dendritic cells (DC)** leads to apoptosis, and to inhibition of the ability of DCs to stimulate T cell proliferation (which is important for the generation of immune responses). There is also an inhibition of interleukin (IL)-12 production by monocytes. *IL-12 is crucial for the development of a Th1 response (this is required for generation of cell-mediated immune responses, which provide the primary line of defense against viral infections).*

Autoimmunity. In addition to immune suppression, MV infection can be associated with autoimmunity. Specifically, an autoimmune demyelinating disease, **postinfectious encephalomyelitis (PIE)** is an important complication of measles which occurs within 14-28 days of infection in about 1 in 1000 cases. It is associated with an immune response to myelin basic protein. It is not clear how this autoimmunity is initiated, although it is possible that MV antigens may resemble myelin (molecular mimicry). Thus, an anti-MV immune response may lead to attack on a self antigen -- myelin.



LPS = lipopolysaccharide; an efficient activator of monocytes

DC: dendritic cells. Normally, DC stimulate proliferation of allogeneic T cells; they do not do so if MV-infected

Persistent infection. MV can establish a persistent infection in brain cells *in vitro* and *in vivo*. As noted above, this is often associated with suppression of expression of the viral M protein. Persistent MV infection of the brain can be associated with a very rare disease, Subacute Sclerosing PanEncephalitis (SSPE), which occurs several years after initial MV infection in about 1 in 1,000,000 children.

Clinical features and complications of measles. Serious symptoms of measles include:

- **Respiratory disease.** Pneumonia can be caused by MV itself (giant cell pneumonia), particularly in immune suppressed persons OR (more commonly) by secondary bacterial or viral infections.
- **Gastrointestinal disease.** Diarrhea is a very common complication of measles. This can be a major problem in children who are already malnourished or at risk for malnutrition. Also, the severity of MV infection has been shown to be much worse in children who are deficient for **vitamin A**. *This is one of the major reasons why WHO and UNICEF support vitamin A supplementation programs in the developing world (vitamin A can be administered very cheaply in megadose capsules).*

- **Neurologic disease.** Postinfectious encephalomyelitis (PIE) occurs in about 1 in 1,000 cases, usually within 14-28 days of infection. Slowly progressive neurologic disease can occur in immunosuppressed persons (measles inclusion body encephalitis, or MIBE) and SSPE occurs at a very low frequency in immunologically normal children, usually several years after initial MV infection.
- **Eye disease.** MV is an important cause of blindness due to corneal lesions. Again, vitamin A deficiency has also been implicated in blindness, and may exacerbate MV induced eye damage.
- **Atypical measles.** A severe form of measles, with more prolonged fever, worse skin lesions and more serious pneumonitis, has been observed in individuals who received the inactivated MV vaccine used in the US from 1963 - 1967. This formalin-inactivated vaccine provoked an unbalanced immune response, with high level antibodies to the viral H protein but little reactivity to the F or N proteins.

Other paramyxoviruses

Respiratory syncytial virus (RSV). RSV infects infants from **6 weeks to 6 months of age** and causes 90,000 hospitalizations and **4500 deaths each year in the US**. It usually causes upper respiratory infection, but in 25-40% of cases, lower respiratory symptoms occur. *RSV is the most important viral cause of lower respiratory disease in infants and children.* In the elderly, severe pneumonia can occur. RSV is a major cause of nosocomial (hospital) infections. Aerosolized ribavirin can be helpful, as can passive antibodies (RSV immune globulin). Two antigenic subtypes exist, but there is no effective vaccine, in part because (1) reinfection is common; (2) immunity is incomplete (infection occurs in infants, despite the presence of maternal antibodies).

ParaInfluenza viruses. PIV-1, 2 and 3 are second only to RSV as causes of serious respiratory tract disease in infants and children.

Mumps virus. Mumps usually causes a benign systemic febrile illness with swelling of salivary glands. It can, however, infect the CNS and can cause meningitis, encephalitis, deafness plus orchitis. A live attenuated vaccine has been used in the US since 1967; mumps is now rare.

New and emergent paramyxoviruses

A recent example of an emerging morbillivirus infection occurred in Australia in 1994. 14 horses died as a result of infection with **Hendra virus** in Queensland. Two people who had close contact with these horses also became infected and one developed a fatal respiratory illness. A second fatal case of human Hendra virus infection was subsequently described in another region of Queensland, some 800 kilometers away from the first outbreak. Followup studies have revealed that Hendra virus infection of horses is rare. However, the virus has been found in numerous bats -- suggesting that these animals may be the virus' natural host. *Note that Hendra virus was initially known as equine morbillivirus, but subsequently renamed to reflect its somewhat distant genetic relationship to the morbilliviruses.*

Research into Hendra revealed two previously unknown diseases associated with bats in Australia.

The **Australian bat lyssavirus** was identified in 1996, and is closely related to the rabies virus; it has been associated with at least two human fatalities.

The **Menangle virus** was isolated in 1997 from pigs, and has been associated with a flu-like illness in

humans; it is thought that Menangle is a member of the *Rubulavirus* genus. Like Hendra virus, Menangle virus is also carried by fruit bats.

The isolation of the Hendra virus in 1994, and the Menangle virus in 1997, presaged the identification of Nipah virus in 1999.

Nipah virus was isolated from pigs and from humans with encephalitis in Malaysia; it was spread by a respiratory route through the pig population and caused the death of over 100 Malaysians from encephalitis. All those killed had close contact with pigs (pig farmers and workers) and the outbreak was contained by slaughter of one million pigs. Like Menangle, it is thought that Nipah was transmitted initially by bats.

Pigs are thought to have played a crucial role in the emergence of Nipah, since these mammals are unique in being maintained in very high concentrations, in situations where epidemic disease can easily occur. It has been suggested that Nipah was able to initially establish itself in these pigs, following transmission from fruit bats, and that the virus was able to become adapted to mammals (pigs) over the course of perhaps a couple of years in Malaysian pig farms. Infection of the pigs then gave the virus ready access to humans (pig farmers, pig workers). The linkage of the Nipah virus outbreak to livestock production has important implications, and highlights the potential risks that can be associated with the high intensity farming of hogs and chicken, in particular.

The Nipah and Hendra viruses are genetically more closely related to each other (70-78% nucleotide homology) than to any other member of the family *Paramyxoviridae* (maximum of 49% homology to any other virus in this family). Furthermore, the genomes of Nipah and Hendra viruses are considerably larger than the other *Paramyxoviridae* (> 18 kb, compared to 15-16 kb for the other family members). Nipah and Hendra also share a number of other unique genetic features which mark them as separate members of the *Paramyxoviridae*. As a result, it has been proposed that these viruses be classified into a new genus in the family *Paramyxoviridae*; a proposed new for this group of viruses is the *Henipavirus* Genus (Wang et al. *J. Virol.* 74:9972, 2000).

Nipah and Hendra also share the common feature of being **zoonotic paramyxoviruses** with an expanded host range that includes humans and other animals (horses, pigs); both are thought to be transmitted from a natural reservoir in **fruit bats**.

Phylogenetic analysis of Nipah and Hendra viruses

