The molecular basis of mengovirus hemagglutination
by Linda Margaret Mann

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Microbiology
Montana State University
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Abstract:
Mengovirus is a small neurotropic picornavirus that may also cause myocarditis and diabetes. The ability of the virus to bind and enter particular cell types is the major determinant of tissue tropism and depends'on surface features of the virus capsid and the presence of suitable cell receptors. Hemagglutination, the ability of the virus capsid to agglutinate erythrocytes, is a model system for cell-virus interactions. The goal of this thesis is to determine the molecular basis of mengovirus hemagglutination.

Mengovirus 37A is a heat stable, hemagglutination-positive variant which is virulent in mice. Two mutants 280 and 205, isolated from 37A by acriflavine mutagenesis, are unable to agglutinate erythrocytes, possess a small plaque phenotype, and are avirulent in mice. Two revertants of 205 have regained the hemagglutination-positive phenotype of 37A but retain the small plaque size and avirulence of mutant 205.

The molecular basis of mengovirus hemagglutination was determined by comparison of the nucleotide sequences determined by cDNA and consensus RNA sequencing of the capsid coding region of 37A, mutants 280 and 205, and revertants 205-A7 and 205-D2.

Two nucleotide differences were found in the 1D capsid between the hemagglutination-positive mengovirus (37A, 205-A7 and 205-D2) and the hemagglutination-negative mutants 280 and 205. These base changes result in the replacement of arginine 231 and proline 232 with lysine and serine residues in the mutants. A computer model of the mengovirus model based on the atomic structure of the M variant of mengovirus predicts that the change from proline to serine at residue 232 will alter the position of residue 231 on the capsid surface.

Two discrepancies were found between the cDNA sequence and the consensus RNA sequence of 205 indicating that sequencing a single cDNA clone is not sufficient to determine a true consensus sequence.

Consensus RNA sequencing was extended into the 5'-noncoding region of the genomic RNAs. A single base difference was found between 37A and 280 and 205, 205-A7, and 205-D2.

Eleven coding changes were identified in the 1B, 1C, and 1D capsid coding regions between 37A and the M variant. Several of these changes are in positions which may affect capsid stability.

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HEMAGGLUTINATION

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Doctor of Philosophy

in

Microbiology

MONTANA STATE UNIVERSITY
Bozeman, Montana

June 1990

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APPROVAL

of a thesis submitted by
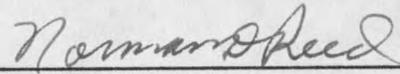
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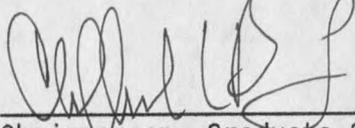
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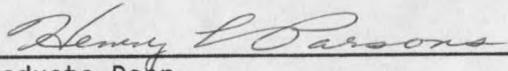Date   Chairperson, Graduate Committee

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ABSTRACT

Mengovirus is a small neurotropic picornavirus that may also cause myocarditis and diabetes. The ability of the virus to bind and enter particular cell types is the major determinant of tissue tropism and depends' on surface features of the virus capsid and the presence of suitable cell receptors. Hemagglutination, the ability of the virus capsid to agglutinate erythrocytes, is a model system for cell-virus interactions. The goal of this thesis is to determine the molecular basis of mengovirus hemagglutination.

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INTRODUCTION

Picornaviruses, with their small sizes and comparably small genomes, have proved to be valuable models for a variety of biological studies. The application of classical genetic methods to the study of mutated viruses has helped elucidate how genes function. Now, with advances in molecular biology, picornavirus models can be even more informative. The actual nucleotide differences between variants can be determined by sequencing complementary DNA (cDNA) or RNA and infectious cDNAs can be constructed. Using recombinant DNA techniques or *in vitro* mutagenesis, changes can be introduced almost at will into infectious clones making no part of the viral genome inaccessible to study. Major advances have also been made in understanding the physical structure of the picornavirus virion. X-ray crystallographic studies have provided detailed information about the atomic structure of the virion providing insight into how capsids are assembled and held together, how they come apart, how they bind to cells, potential immunogenic sites for vaccine development, mechanisms and design of antiviral agents, and the evolution of biological structures.

Picornaviruses

The Picornaviridae are a large and diverse family of small, nonenveloped RNA viruses which include poliovirus, foot-and-mouth disease virus (FMDV), and human rhinoviruses (HRV), all important

pathogens of man and animals. In the last decade, over a dozen members of the family have been cloned and sequenced (21,22,25,26,91,98, 101,112,120). In the last five years, the atomic structures of several picornaviruses including poliovirus (59), human rhinovirus 14 (HRV14) (103), FMDV (1), and mengovirus (75) have been determined by X-ray crystallography providing a three-dimensional comparison of the capsid structures and surfaces.

Classification

The Picornaviridae were divided into four genera based on physical and serological properties of the virions. Now, as the genomic RNA sequences of more members become available, genomic organization has become an important determinant in their classification. Table 1 below lists the current picornavirus genera and some properties which differentiate them (105).

Table 1.   Classification of the Picornaviridae.

Group	pH Stability	Buoyant Density of Virions in CsCl	Poly(C) Tract in Genome	Leader Sequences
Enterovirus	stable	1.34	–	–
Cardiovirus	stable	1.34	+	+
Rhinovirus	labile	1.39-1.42	–	–
Aphthovirus	labile	1.43-1.45	+	+

The enterovirus group contains the coxsackieviruses and echoviruses as well as poliovirus, probably the most studied and best

understood virus of man. Rhinoviruses, the most frequent cause of the
common cold, are closely related to the enteroviruses but can be
distinguished from them by their acid lability and disease pathology.
Like the enteroviruses, cardioviruses are relatively insensitive to acid
conditions. They can be differentiated from enteroviruses by the
presence of a polycytidylate-rich region, the poly(C) tract, and a
leader sequence in their genome. Aphthoviruses also have poly(C) tracts
and leader sequences, but, unlike cardioviruses, are easily inactivated
by acids. A fifth group, containing the hepatitis A virus, currently
classified as an enterovirus, is likely to be added soon since its
genomic organization differs considerably from the enteroviruses (128).
Theiler's murine encephalomyelitis virus is also currently included in
the enteroviruses although it has more sequence homology with
encephalomyocarditis virus, a cardiovirus, than with any of the
enteroviruses (88,92,98). Unlike the cardioviruses, it lacks a poly(C)
tract in the genome. Table 2 lists some other examples from each
picornavirus group.

Nomenclature

The nomenclature for the genes and gene products of picornaviruses
used in this thesis are those adopted by the European Study Group on the
Molecular Biology of Picornaviruses in 1983 (106). The traditional
designation of the cardiovirus capsid proteins as α, β, γ, δ, and ε have
been replaced by 1D, 1B, 1C, 1A, and 1AB respectively. The equivalent
protein designations still often used for poliovirus are VP1 (1D), VP2
(1B), VP3 (1C), VP4 (1A), and VP0 (1AB).

Using this system the proper name of the small viral protein linked to the 5'-end of the genomic RNA is 3B, however, since it is almost universally referred to as VPg (for Viral Protein, genome), I will use that term.

Table 2.   Members of the Picornaviridae. (adapted from reference 105)

Group	Examples
Enterovirus	poliovirus, 3 serotypes coxsackievirus, type A, 23 serotypes type B, 6 serotypes echovirus, 32 serotypes hepatitis A virus (HAV) Theiler's murine encephalomyelitis virus
Cardiovirus	mengovirus encephalomyocarditis virus (EMCV) Maus Elberfeld virus Columbia SK virus
Rhinovirus	human rhinoviruses (HRV), 100 serotypes
Aphthovirus	foot-and-mouth disease virus (FMDV), 7 serotypes

The Picornavirus Genome

The picornavirus genome is a single-stranded RNA molecule which ranges in size from 7102 bases for HRV2 (93) to 8450 bases for FMDV (22). The RNA is plus strand, messenger-sense RNA with one long open reading frame which can be translated directly into a single, large polyprotein product. The polyprotein coding region has been divided into three regions corresponding to the first protein products seen during poliovirus replication. The P1 region contains the coding region for the 1A, 1B, 1C, and 1D capsid proteins. In cardioviruses and

aphthoviruses, a short leader sequence precedes the P1 region. The P2 and P3 regions code for the nonstructural proteins including viral proteinases and the viral polymerase.

Like cellular messages, the viral RNA is polyadenylated at the 3'-end. The length of the poly(A) region is quite variable both within and between viral variants. Its length ranges from an average length of 35 bases in EMCV (3) to 100 bases in FMDV (105). Infectivity of the viral RNA increases with increased length of the poly(A) region (63,109).

A small 2 kd protein, VPg, is covalently attached to the 5'-end of the genomic RNA via a phosphodiester bond to a phenolic hydroxyl group of a tyrosine residue of VPg (64,123). VPg is not required for infectivity of viral RNA but appears to be important in initiation of viral RNA synthesis (89) and may function in RNA packaging (90).

The 5'-noncoding or nontranslated region of picornaviruses ranges in length from 624 bases in HRV 14 to 1,199 bases in FMDV (105) and is quite long compared to 5'-noncoding regions in cellular messages. This region is highly conserved among the picornavirus groups (93) and stable stem and loop secondary structures are predicted for the region (102). This region has been shown to play a role in determining host range of picornaviruses since cellular initiation factors important in protein synthesis bind to the region (66,97,118,119). A single base change in this region increases the neurovirulence of the Sabin strain of poliovirus type 3 (45). Other point mutations can alter translation efficiency of the RNA (115).

The genomes of cardioviruses and aphthoviruses contain a poly(C) tract (15,50) located about 150 bases from the 5'-end of the RNA between

VPg and the site of initiation of translation (96). The size of the
poly(C) tract varies in length from 80 to 250 bases in EMCV, from 60 to
90 bases in mengovirus, and is even longer in aphthoviruses (13). The
poly(C) tract is thought to function in pathogenicity since reducing the
length of the tract decreases the *in vivo* virulence of infectious clones
(41,86).

The 3'-noncoding region is shorter than the 5'-noncoding region
and ranges in size from 47 bases in HRV14 to 126 bases in EMCV (105).
The function of this region is unknown but alteration of the region can
change the phenotype of the virus (110).

Figure 1 shows the basic structure of the picornavirus genome.

Figure 1.   The general structure of picornavirus genomic RNA. The
             poly(C) tract and leader (L) sequence are found only in
             cardioviruses and aphthoviruses.
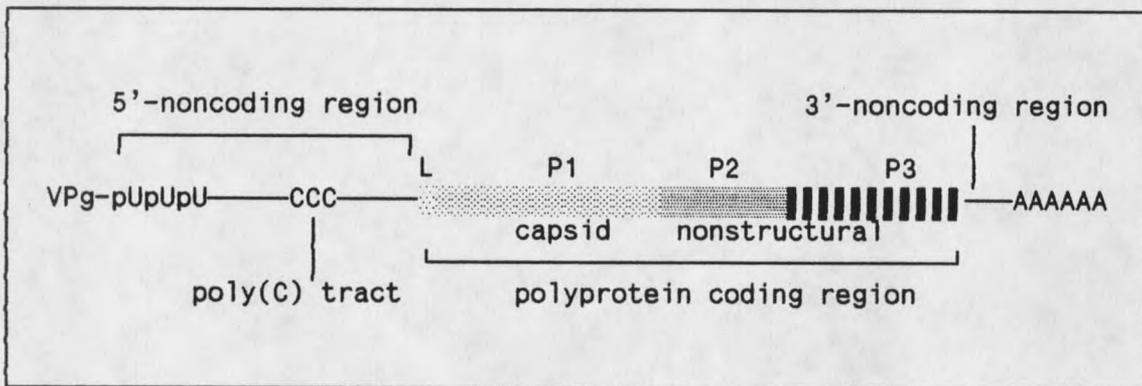
Translation and Processing of Viral Proteins

The translation product of the single long open reading frame of
the genomic RNA is a single, large polyprotein that is post-
translationally cleaved to produce the P1, P2, and P3 proteins. These
initial products undergo additional processing to produce the structural

and nonstructural proteins required for capsid formation, polyprotein processing, and viral RNA replication and packaging (87,121).

The capsid proteins 1A, 1B, 1C, and 1D are produced by cleavage of the P1 product. Figure 2 shows the posttranslation processing of the cardiovirus capsid proteins in more detail. The amino- and carboxyl-termini of the capsid proteins, except for the amino terminus of 1A which is blocked by the myristic acid residue, have been determined by partial protein sequencing of purified capsid proteins (127). Except for the carboxyl terminus of the 1D protein, which has been found to vary depending on the virion isolation procedure (14) the protein termini are not trimmed or modified after cleavage.

The leader-1A and 1C-1D cleavages occur at glutamine-glycine amino acid pairs and the 1B-1C cleavage occurs at a glutamine-serine amino acid pair. Both types of cleavages result from the activity of a viral proteinase, 3C^{pro}. The 1D-2A cleavage occurs at a glutamic acid-cysteine amino acid pair and may involve a separate proteinase activity.

The 1A-1B cleavage at an alanine-aspartic acid amino acid pair does not occur until capsid maturation when genomic RNA is packaged (65). Analysis of the three-dimensional structure of the picornavirus capsid has led to a suggestion that a serine residue in 1B may act in conjunction with virion RNA in producing this cleavage (103).

In aphthoviruses, the cleavage of the leader appears to be an autocatalytic event (65). In the cardioviruses, this cleavage is thought to result from 3C^{pro} activity (95). The function of the leader sequence is still unclear.

Figure 2.   Posttranslation processing of mengovirus capsid proteins.
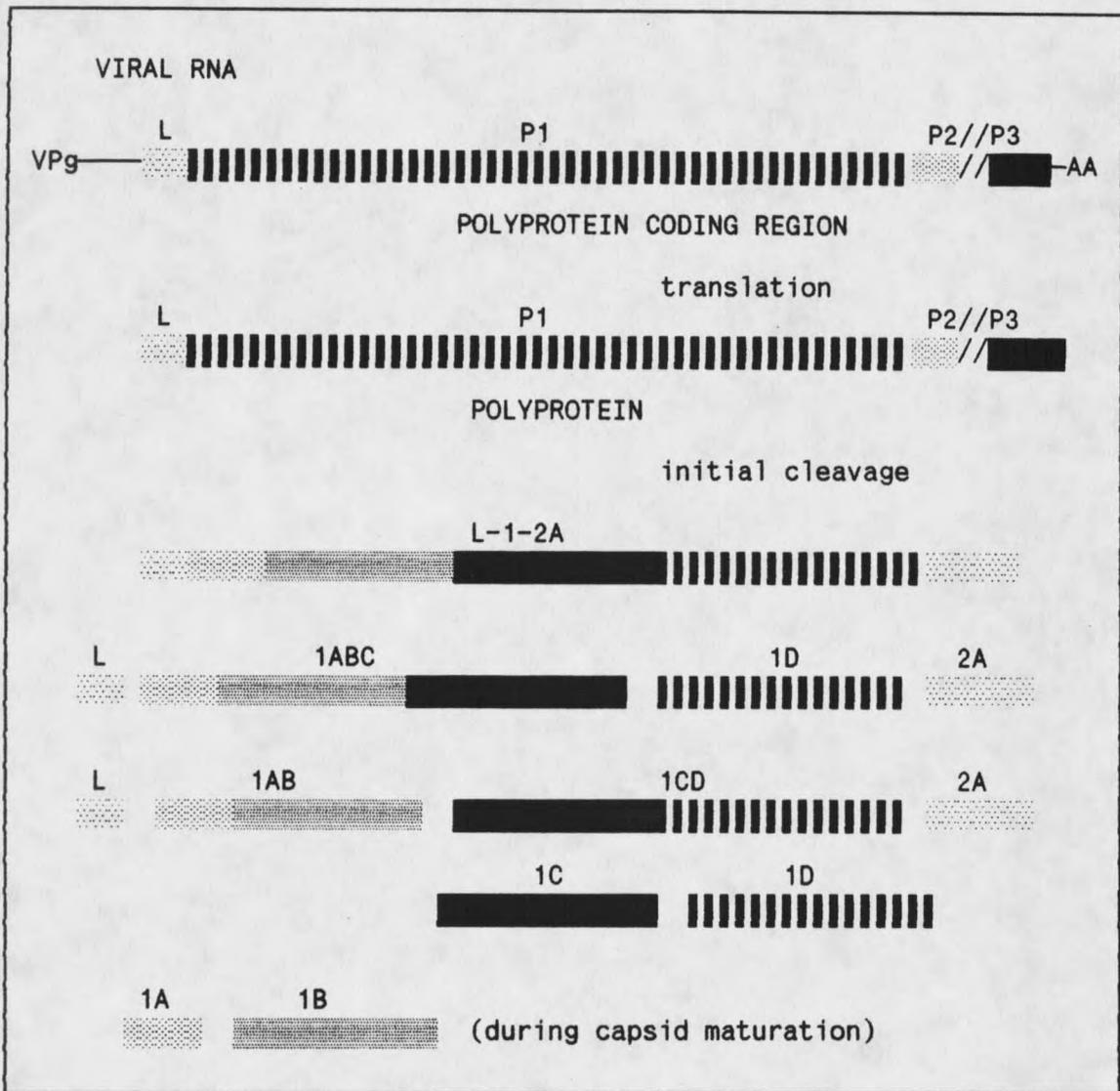
The P2 region codes for the 2A, 2B, and 2C nonstructural proteins. In poliovirus, the 2A protein appears to have two functions. It has been identified as the proteinase responsible for inactivation of the host cell translation initiation factor eIF-4F and shutoff of host protein synthesis.   The 2A protein of poliovirus is also responsible for the P1-P2 cleavage during posttranslation processing of the

polyprotein. Neither of these 2A activities have been found in the cardioviruses (74). In the aphthoviruses and cardioviruses, the initial cleavage occurs between 2A and 2B (87). The roles of the other products of the P2 region are not well understood. They are thought to function in RNA replication (74).

The P3 region codes for the 3A, 3B, 3C ,and 3D nonstructural proteins. The role of 3A is unclear. The 3B protein is VPg, the small protein covalently attached to genomic RNA. Aphthoviruses are unique in this region having 3 copies of 3B in tandem (49). The 3C protein is a cysteine proteinase, $3C^{pro}$, which is responsible for the majority of the cleavages in the processing of the polyprotein (121). Protein 3D is the RNA dependant RNA polymerase, $3D^{pol}$, which is the RNA-dependent RNA polymerase (48). In poliovirus-infected cells, the alternative cleavage products 3C' and 3D' are found (73). The role of these alternative products is unclear and they are not found in the cardiovirus-infected cells (19,20).

The Picornavirus Capsid

The picornavirus virion has a diameter of about 30 nm and is composed of a protein capsid which surrounds and protects an RNA core. Early X-ray diffraction patterns of virus crystals determined that the picornavirus capsid had icosahedral symmetry indicating that the virion was composed of identical protein subunits with fivefold, threefold, and, twofold axes of symmetry (47).

The capsid is assembled from protomers composed of one copy of 1AB, 1C, and 1D. Five protomers associate to form a pentamer and 12 pentamers associate to form the procapsid. When RNA is packaged, the

final cleavage of 1AB to 1A and 1B occurs producing a mature and infectious virion (65). Sixty copies of each of the 1A, 1B, 1C, and 1D capsid proteins are found in the mature virions. The cleavage of 1AB, the precursor of 1A and 1B, is not complete and traces of 1AB, averaging two molecules per capsid, are also found (104).

The sizes of the capsid proteins of poliovirus are 33.5 kilodaltons (kd) (1D), 30.0 kd (1B), 26.4 kd (1C), and 7.39 kd (1A) but the sizes vary between the picornavirus genera (105). In aphthoviruses 1D, 1B, and 1C are nearly equal in size (114). The 1A protein of HAV is much smaller at 1.73 kd than the 1A proteins of other viruses in the family (25). The 1A and 1AB proteins have hydrophobic myristic acid residues covalently attached to their 5'-ends (23).

X-ray crystallography of virions has shown that the basic structure of the 1B, 1C, and 1D proteins are similar to each other and to the capsid protein of icosahedral plant viruses (59,103). The basic structure is a wedge-shaped β-barrel formed by the interaction of eight regions of β-sheet structure in the capsid proteins. Two conserved α-helical regions are also seen along with less conserved regions forming loops which connect the β-sheet regions. The 1D protein is the most surface exposed capsid protein while the 1A protein is buried within the virion.

The Picornavirus Multiplication Cycle

The multiplication cycle of mengovirus and other picornaviruses begins with attachment of the capsid to cell surface receptors. Initially the attachment appears to be rather loose and reversible. The attachment becomes progressively tighter causing irreversible

conformational changes in the capsid structure. Virions which are released at this stage have lost the 1A protein from the capsid and are no longer infectious (61). Receptor-mediated endocytosis appears to play a role in virus entry into cells since infection is inhibited by agents which interfere with that process (77). How the RNA leaves the capsid is still speculative but it has been suggested that the RNA is released through an opening at the vertex of a capsid pentamer with the extruded 1A molecules forming a pore through the endocytotic membrane via hydrophobic interaction of the myristate tails with the membrane lipids (59,103).

The viral RNA functions both as a template for viral RNA synthesis and as a messenger RNA for translation by the cellular protein synthesis machinery. Before replication can begin, the virus-specific proteins needed for replication of the RNA and processing of the polyprotein must be produced by the translation of the virion RNA. One product is the viral RNA dependent RNA polymerase, $3D^{pol}$, which forms a replication complex with other viral and cellular factors. The replication complex is associated with the smooth endoplasmic reticulum and initially functions to produce minus-strand copies of the RNA. From the minus-strand copies, additional plus-strand copies for translation and packaging as virion RNA are made.

As the infection continues a pool of structural proteins accumulates in the cytoplasm and self assemble into procapsids. When viral RNA covalently linked to VPg is packed into the procapsid, the final cleavage occurs in the 1AB procapsid protein producing mature virions. The virions are released by lysis of the cell.

Host Range and Tissue Tropism

The ability of a virus to infect and multiply in a particular organism determines its host range. Cell tropism refers to the ability of the virus to preferentially infect and multiply in certain cell types within the host. A major determinant in the host range and tissue tropism of a virus is the ability of the virus to adsorb to and enter particular types of cells (60,79). Binding characteristics are dependent on properties of the capsid surface of the virion and the presence of suitable cell receptors on the target cell.

The determinants of host range and cell tropism are best understood for poliovirus (30). The normal host range of poliovirus is restricted to primates but variants have been adapted to mice, rats, hamsters, and chick embryos. Poliovirus normally infects and multiplies in cells of the nasopharynx and gut of man but can readily infect and damage cells of the central nervous system. Attenuated poliovirus strains are less able to infect neural cells but retain the ability to infect and multiply in the nasopharynx and gut (100). The host range of poliovirus is determined by the presence of suitable cellular receptors on primate cells (60). A wide range of other cell types which lack the primate receptor can support a single round of poliovirus replication if the requirement for cell attachment is bypassed by transfection of viral RNA directly into the cell (62).

Alteration of the poliovirus capsid proteins can alter their ability to bind cellular receptors. The Mahoney type 1 poliovirus is virulent for man but does not cause disease in mice even when inoculated directly into the brain. Replacement of 6 amino acids in the 1D protein

with the corresponding residues found in the Lansing type 2 poliovirus variant, a neurovirulent mouse variant which is no longer virulent for man, gave the virus the ability to bind mouse cells and altered the host range and neurovirulence to include mice (85).

Alteration of capsid proteins has been found to affect the host range of rhinoviruses. When host range mutants human rhinovirus 14 (HRV14) were examined, alterations were found in the nucleotide sequence of the capsid coding region (125).

The factors that determine neurovirulence are less well understood. The study of the molecular changes responsible for the attenuation of the poliovirus vaccines have shown that neurovirulence maps to several regions of the poliovirus genome (24). Both coding and noncoding mutations are involved. Comparison of the genomic RNA sequences of the virulent Mahoney poliovirus type 1 variant and the avirulent Sabin poliovirus type 1 variant indicate that they differ at 55 nucleotide positions (91). Analysis of the effect of these changes in recombinant poliovirus clones show that while changes affecting attenuation occurred throughout the genome, the strongest attenuation was obtained in recombinants with a single base change at position 480 in the 5'-noncoding region of the genome (69).

Only ten sequence differences were found between the avirulent Sabin poliovirus type 3 and the Leon poliovirus type 3, its virulent parent. Each mutation was incorporated into an infectious cDNA and analyzed separately for its effect on virulence (122). Two strongly attenuating mutations were found. One occurred in the 5'-noncoding region at position 472. *In vivo* confirmation of the role of this

mutation in attenuation has been found in isolates isolated from cases of vaccine-associated poliomyelitis. In several cases the only back mutation found in these neurovirulent isolates is at position 472 (45). The second strongly attenuating mutation is a coding change in the 1C capsid protein which results in the replacement of a serine residue in the virulent variant with phenylalanine.

Cellular Receptors and the Canyon Hypothesis

The three-dimensional structural analysis of the surface of the HVR14 (103) and poliovirus (59) capsids reveals the presence of a canyon or cleft surrounding each of the fivefold vertices. The placement of the cellular receptor binding site in a cleft inaccessible to antibodies helped explain the conservation of the cellular receptor binding site under the pressure of the immune system. The canyon hypothesis proposed that the cellular receptor binding site was within the canyon (103). Evidence for this hypothesis has been obtained by several means. When the residues of the canyon of HRV14 are changed by site directed mutagenesis, the binding affinity to cellular receptors is changed (27). The binding of antiviral agents in a hydrophobic pocket of the canyon of HRV14 alters the canyon conformation and inhibited binding to cellular receptors (99).

The major cellular receptor for HVR14 has been identified as the intercellular adhesion molecule 1 (ICAM-1), a member of the immunoglobulin superfamily (52,113,116). ICAM-1 is able to bind to an integrin molecule on lymphocytes and is known to facilitate antigen presentation to T-cells. The receptor for poliovirus has recently been

cloned and sequenced (82) and is also a member of the immunoglobulin superfamily.

Several members of the picornavirus family including group B coxsackieviruses, echoviruses, rhinovirus, FMDV, EMCV, and mengovirus agglutinate erythrocytes (105). The significance of hemagglutination is unknown since attachment to erythrocytes is not part of the disease process.

The Mengovirus Model

Mengovirus, a small neurotropic picornavirus, continues to be a good model system for the examination of virus structure and function. Variants of mengovirus have been isolated which cause myocarditis (117) or diabetes (126). The determination of the three-dimensional capsid structure makes examination of mutations affecting capsid structure particularly rewarding. The mengovirus cellular receptor molecule has not been identified but its binding probably involves sialic acid residues of the receptor since free sialic acid binds in the putative cellular receptor binding site of the capsid (70). Mengovirus is able to agglutinate erythrocytes from several different animal species, including type O cells of man. Hemagglutination, while not required for mengovirus infection, is the result of the interaction of the viral capsid with cellular surface molecules and provides a model for virus-cell receptor interaction.

Discovery and Isolation

Mengovirus was first isolated in 1946 by Dick, Smithburn, and Haddow (36,38) from a paralyzed rhesus monkey at the Yellow Fever

Research Institute in Entebbe, Uganda. Named mengo encephalomyelitis virus after the Mengo district in which it was isolated and the disease produced in infected animals, it was recovered over the next two years from *Taeniorhynchus* mosquitos, a mongoose, another rhesus monkey and man.

Only one human case of mengovirus encephalomyelitis has been reported (37). G. W. A. Dick, one of the scientists who first isolated the virus, also contracted it either naturally or as the result of laboratory exposure. The incubation period was estimated to be between 5 and 9 days. The initial symptoms included fever, headache, and irritability that progressed to delirium which lasted several days. Late in the infection, he developed some weakness in muscles on the right side and deafness of the right ear. Aside from slight residual nerve deafness in one ear, the eventual recovery was complete.

A small survey on human sera, done at the Yellow Fever Research Institute at the time mengovirus was first isolated, showed only a small percentage (0.8%) of the population of the surrounding area to have antibodies specific for mengovirus.

Mengovirus is pathogenic for mice and was first isolated from spinal tissue from the paralyzed monkey by passage in mice. Initial isolates killed most animals in an average of 4 days when inoculated intracerebrally. The first symptom of infection was paralysis of one or more hind limbs. Later isolates, adapted by continuous passage in mice, produced more rapid paralysis and death, killing most animals within two days.

Serologically, the members of the cardiovirus group are indistinct. Despite difference in biological activity and receptor attachment, mengovirus has been considered to be closely related to EMCV (84). Infectious RNAs have been prepared from hybrid clones linking the first 299 bases from the 5'-end of mengovirus with a 7424 base EMCV sequence (41). Strains of both viruses have now been sequenced (Palmenburg and Duke, personal communication, (94) and have a 93% peptide sequence identity (93) but share less than 80% overall nucleotide identity (41).

The Atomic Structure of Mengovirus

The atomic structure of the M variant of mengovirus has been determined to a 3 angstrom (Å) resolution by X-ray crystallographic studies by Luo et al. (75). This study confirms the basic structural similarity of the capsid proteins of mengovirus to other picornaviruses such as poliovirus and human rhinovirus 14 (HRV14). The three capsid proteins 1D, 1C, and 1B share the eight-stranded antiparallel β-barrel structure. Major differences between HRV14 and mengovirus in regions of 1D not involved in the β-barrel structure increase the exposure of the 1D protein on the surface of the mengovirus virion. The addition of two regions in 1D, loop I and II, and modification of the puff region in 1B change the putative cellular receptor region from the canyon-like structure in HRV14 to a series of five pits 22 Å deep by 30 A wide on the virion surface. Capsid protein 1A of mengovirus, which is entirely internal, has an altered relationship with the other capsid virions compared to HVR14. However, position of the carboxyl-terminus of 1A,

which is generated by the autocatalytic cleavage of 1AB to 1A and 1B during maturation of the virion, is conserved.

Relationship of the M and 37A Variants of Mengovirus

The M variant of mengovirus used in the X-ray crystallography determination of capsid structure and the 37A variant used in this work were derived from the same stock in the early 1960s but have been separated since that time. They share an ability to agglutinate human O cells but differ markedly in their virulence for mice.

The M (medium plaque size) variant of mengovirus was isolated by Ellem and Colter in the early 1960s (43) from stock obtained from Smithburn (38). The M variant was differentiated from the S (small plaque size) and L (large plaque size) variants by its intermediate plaque size, plaque morphology in L cells, and ability to agglutinate human O erythrocytes. The M variant was relative nonvirulent (28) when injected intraperitoneally into mice with a LD_{50} of 10,000 to 50,000 plaque forming units (PFU) compared to a LD_{50} of 1 PFU for the L variant by the same route. When inoculated intracerebrally, all three variants had LD_{50}s of less than 10 PFU.

The genome of the M variant of mengovirus has been sequenced (Dr. Ann Palmenberg, personal communication) and is about 7800 bases in length with a polyprotein region of 6879 bases coding for a polyprotein of 2283 amino acids. The 5'-noncoding region is 758 bases long. The poly(C) tract contains 60 C residues (41) and has the sequence $C_{50}UC_{10}$. The 3'-noncoding region is 124 bases long.

The 37A strain of mengovirus is a heat stable variant derived by Brownstein and Graham (16) also in the early 1960s from wild-type mengovirus stock obtained from Colter. Like the M variant, 37A also possesses the ability to agglutinate human O erythrocytes. Unlike the M variant it is virulent in mice with a LD_{50} of 1500 PFU when injected intraperitoneally and a LD_{50} of 7 PFU when injected intracranially (7).

Mutants and Revertants of the 37A Mengovirus Variant

Mengovirus mutants 205 and 280 were isolated from cells infected with the 37A variant of mengovirus and treated with acriflavine by Dr. M. A. Gill (personal communication). Selected for small plaque size in temperature shift analysis of infected L cell monolayers, they were originally thought to be temperature-sensitive mutants. Further studies were unable to demonstrate significant, temperature-dependent differences in virus yields or adsorption to cell monolayers between the mutants and the parental 37A variant indicating the mutation or mutations are not dependent on temperature (7).

Extensive biological characterization of 37A and mutants 280 and 205 was undertaken by Anderson and Bond (7). There are significant differences in phenotypes between the mutants and 37A. These differences are summarized in Table 3 below. The mutants have a significantly smaller plaque size than 37A and have lost the ability to agglutinate human O erythrocytes. The mutants were avirulent in mice by either the intraperitoneal or intracranial route as indicated by the difference in the LD_{50} data. Mice injected intracranially with 10^7 PFU of either 205 or 280 showed no signs of infection.

When the extent of multiplication of mutants 280 and 205 in brain tissue was determined, it was observed that mice infected with mutant 205 had detectable titers more often than mice infected with 280. Plaque isolates from the brain suspensions were tested to determine their hemagglutination phenotype. All the isolates from mice infected with 37A were hemagglutination-positive as expected. None of the isolates from 280 were hemagglutination-positive. When the isolates from mice infected with 205 were tested, 20 of the 36 isolates were hemagglutination-positive.

Table 3.   Biological differences between 37A and mengovirus mutants 205 and 280 (adapted from reference 7).

Property	37A	205	280
Plaque size[a]	3.99 ± 1.08	1.56 ± 0.37	1.49 ± 0.44
Hemagglutination Titer[b]	2048	0	0
Ability to bind glycophorin[c]	+	−	−
LD_{50}[d]			
ip	1500	$> 10^7$	$> 10^7$
ic	7	$> 10^6$	$> 10^6$

[a]   Mean plaque diameter in mm measured at 48 hours.
[b]   Hemagglutination titers are expresses as the reciprocal of the end point dilution.
[c]   As measured by a change in migration of purified virions mixed with glycophorin in sucrose density gradients.
[d] LD_{50} titers are expressed as PFU injected intraperitoneally (ip) or intracranially (ic).

The plaque sizes of the isolates from mice infected with 205, whether hemagglutination-positive or negative were significantly smaller