RNA SEQUENCE ANALYSIS OF THE E2 GENES OF WILDTYPE AND NEUROATTENUATED MUTANTS OF MHV-4 REVEALS A HYPERVARIABLE DOMAIN

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INTRODUCTION

Murine hepatitis virus 4 (MHV-4) is a neurotropic coronavirus (1-4). Infection of the CNS in susceptible mice strains results in a fatal encephalitis with destruction of neurons accompanied by demyelination. The few mice that survive the acute infection develop a chronic demyelinating disease characterized by episodes of demyelination followed by remyelination (2,5). The extensive white matter disease is believed to arise as the consequence of viral infection and destruction of oligodendrocytes (2,3,6).

There is substantial evidence indicating that the MHV-4 E2 glycoprotein plays a crucial role in determining the neurovirulence of an MHV-4 infection. E2, the major constituent of the viral spike or peplomer (7,8), is initially synthesized as a 180 kDa peripheral membrane glycoprotein and is subsequently cleaved into two non-identical 90 kDa subunits compromising the amino terminal (S_1 or 90B) and the carboxy terminal (S_2 or 90A) regions of the protein (9). The E2 glycoprotein mediates the attachment of the virion to susceptible cells, is responsible for cell to cell fusion after infection and is the major target on the virus for neutralizing antibodies (9-12). Passive transfer of neutralizing MAbs to E2 alters the course of MHV-4 induced disease from a fatal encephalitis to a chronic demyelinating disease (13). Furthermore, variants of MHV-4 selected for their ability to escape neutralization by anti-E2 MAbs are neuroattenuated and induce chronic demyelination (14-16).

The selection of neuroattenuated variants by antibodies specific for E2 provides some of the strongest evidence that E2 plays a pivotal role in the outcome of a CNS infection. To localize the genetic alteration(s) in E2 responsible for neuroattenuation we have sequenced the viral RNA encoding the E2 gene of MHV-4 and of the neuroattenuated variants. The carboxy terminal half of E2 was found to be highly conserved whereas in contrast, the amino terminal half is

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very polymorphic and contains deletions of up to 159 amino acids in the antibody resistant variants.

METHODS

The parental strain of MHV-4 was originally obtained from L.P. Weiner and propagated on Sac- cells as previously described (7). Neuroattenuated variants V5A13.5(86) and V4B11.3(86) were isolated from a 1986 plaque purified stock of MHV-4 by their ability to resist neutralization by MAb 5A13.5 (epitope E2B) and 4B11.6 (epitope E2C) respectively, as previously described (14). V5A13(88) was recently isolated from a 1988 plaque purified stock of MHV-4 (17). JHM-X, a variant of MHV-JHM which has been shown to have a smaller E2 gene as well as a truncated E2 glycoprotein compared to wildtype (18) was kindly provided for sequence analysis by Dr. M.M.C. Lai.

The sequence of the viral RNA was determined by the dideoxy primer extension method using radiolabeled synthetic oligonucleotides 20 bases in length as primers (19,20). Viral RNA was isolated from infected cells by guanidine isothiocyanate extraction (21). Sequence data was compiled and analyzed using the University of Wisconsin Genetics Computer Group sequence analysis software package (22).

RESULTS AND DISCUSSION

Direct RNA sequence analysis of the E2 gene of the neuroattenuated variants of MHV-4 and the JHM-X revealed that the variants had large deletions ranging in size from 426 to 477 nucleotides in the 5' coding region of the E2 gene. As a consequence of the nucleotide deletions, the E2 glycoproteins of the variants have large deletions ranging from 142 amino acids in the case of V5A13.1(86) to 159 amino acids in the case of V4B11.3(86). Table 1 lists the size and location of the deletions. As a consequence of the deletion in the E2 glycoprotein of V5A13.1(86), there is a lysine to asparagine substitution at amino acid 433 at the 5' boundary of the deletion (Fig. 1). The deletions in E2 all map to a localized region in the amino terminal half of the protein (Fig. 1) thus defining the location of major epitopes determining both neutralization and neurovirulence.

Table 1. Location of Deletions in E2

Virus	Nucleotide Deletion	Amino Acid Deletion
MHV-JHM	423 (1,359-1,781)	141 (454-594)
JHM-X	458 (1,336-1,794)	153 (446-598)
V5A13.1(86)	426 (1,298-1,723)	142 (434-575)
V5A13(88)	447 (1,307-1,753)	149 (436-585)
V4B11.3(86)	477 (1,285-1,761)	159 (429-586)

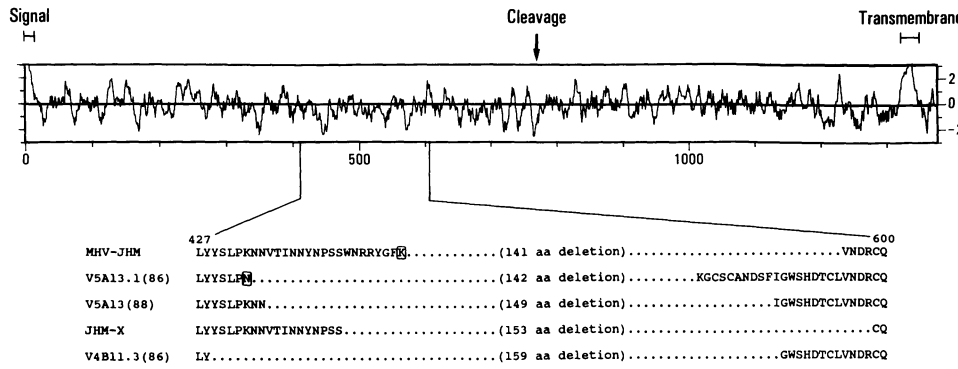
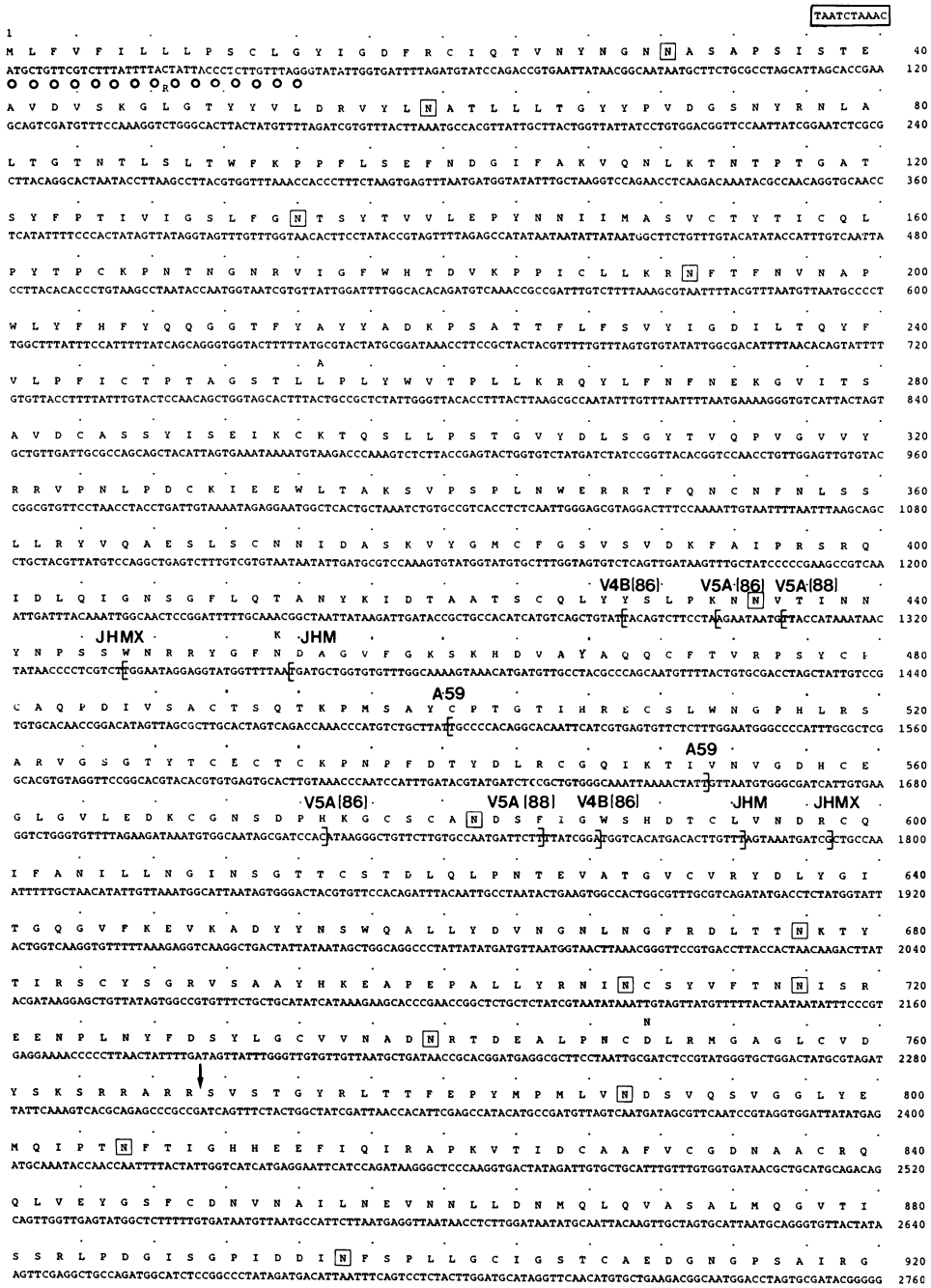


Fig. 1. Hydropathicity plot of the MHV-4 E2 glycoprotein according to the analysis of Kyte and Doolittle (40) and localization of the deletions in the amino terminal domain of MHV-4 E2. The vertical scale is the average hydropathicity (+2 to -2) index for each residue over a window of 9 amino acids. Hydrophobic sequences appear above the midline and hydrophilic sequences appear below the midline. Below is an alignment of comparable amino acid sequences from MHV-JHM (29), JHM-X, and MHV-4 variants V8A13.1(86), V4B11.3(86) and V5A13(88). The numbering is relative to the MHV-4 E2 amino acid sequence. Dots indicate deletions and boxed amino acids indicate sequence changes from MHV-4 E2. The signal sequence, putative cleavage site and transmembrane domains of E2 are indicated.

Selection by MAbs of viral escape mutants with large deletions is uncommon. Most often it has been shown that MAb selected variants have point mutations which affect antibody binding to a given epitope (23-28). The E2 glycoprotein may be unique in that it can accommodate large deletions while retaining functions necessary for virus growth both in vitro and in vivo.

A comparison of the amino acid sequence of E2 for our parental strain of MHV-4 (see Fig. 2 for the complete sequence of E2 for MHV-4) with that of MHV-JHM (29), and of MHV-A59 (30) reveals that the carboxy terminal region is highly conserved whereas the amino terminal region of E2 is very heterogeneous with respect to size. Of importance is the finding that the E2 glycoprotein of MHV-4 contains an additional 141 amino acids (aa 454-594) as compared to MHV-JHM (Fig. 2). This clearly demonstrates that MHV-4 and MHV-JHM which were previously considered to be synonymous, are in fact distinct viruses. Given the near sequence identity of E2 for the two viruses, MHV-JHM may actually be a deletion variant of MHV-4. The E2 glycoprotein of MHV-4 also contains an additional 52 amino acids (501-552) compared to MHV-A59. This heterogeneity in E2 maps to the same region of the protein which is deleted in the variants of MHV-4 (Fig. 2). Heterogeneity in terms of size of E2 has also been reported to occur as a result of the in vitro and in vivo passage of MHV where variants with both a smaller E2

(31) as well as variants with a larger E2 than the parental strain have been described (32,33). As the sequence data becomes available for these variants it will be of interest to determine if these differences represent deletions or insertions of the polymorphic sequences that we have described.



Recombination between heterologous strains of coronaviruses has been reported to occur at a relatively high frequency (34-36) and is believed to occur as a consequence of the discontinuous mechanism of "leader primed transcription" that has been described for MHV replication. Presumably, during MHV replication RNA transcription frequently pauses at sites of secondary structure on the template RNA releasing the transcriptional complex and the nascent RNA strand which subsequently rejoins the template and reinitiates transcription (37). Deletions of genomic RNA could occur by the same mechanism whereby the transcriptional complex reinitiates at a distant site on the template RNA from where the initial disassociation event occurred. Because E2 can accommodate large deletions, the progeny virions resulting from a recombination in the E2 may require site specific recombination without the accompanying loss of genetic information.

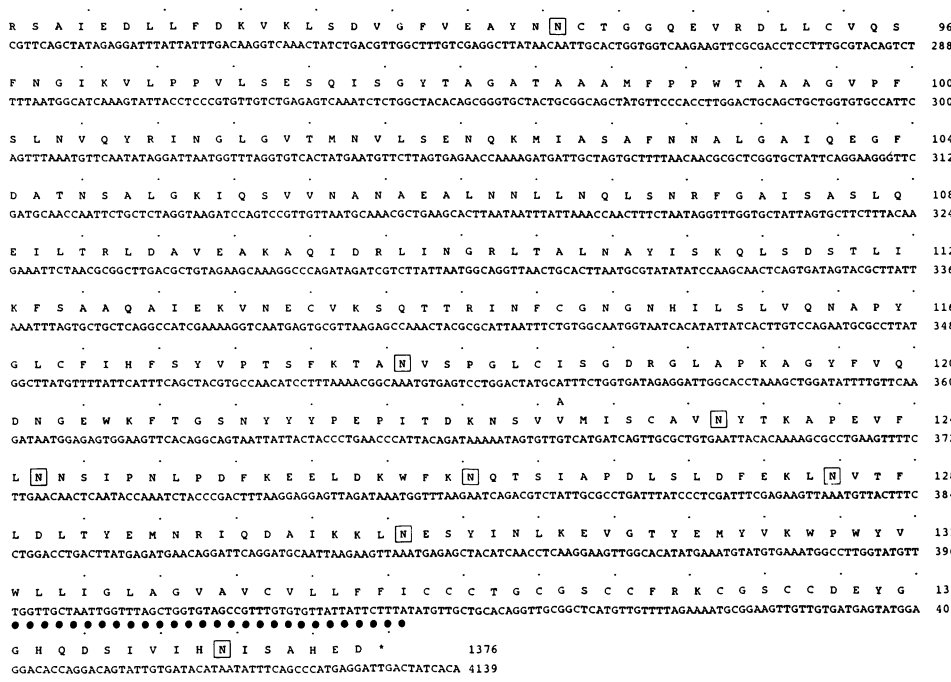


Fig. 2. Nucleotide and predicted amino acid sequence of the MHV-4 E2 gene. The numbering starts at the ATG codon. Open circles indicate the N-terminal signal sequence (calculated according to the algorithm of Von Heijne [38], as described by Fazakerley and Ross [39]) and closed circles indicate the hydrophobic carboxy-terminal transmembrane domain. Potential glycosylation sites are indicated by boxed asparagine residues. The intergenic homology sequence TAATCTAAAC is boxed. The putative proteolytic cleavage site between the amino terminal and carboxy terminal domain is indicated by an arrowhead. The MHV-JHM amino acid sequence (29) is indicated where differences occur. Brackets denote the boundaries of the deletion for the indicated viruses.

By direct RNA sequence analysis of the E2 gene of wild-type MHV-4 and of neuroattenuated variants we have demonstrated that the E2 glycoprotein of MHV is very heterogeneous with respect to deletions in a localized region of the amino terminal half of the protein. Sequences localized within this polymorphic region of the protein are important in determining the neurovirulence of an MHV-4 infection of the CNS. Studies are currently underway to further assess the role of this domain in an *in vivo* infection.

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