GENOME STRUCTURE OF MOUSE HEPATITIS VIRUS: COMPARATIVE ANALYSIS BY OLIGONUCLEOTIDE MAPPING

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ABSTRACT

Several natural variants of mouse hepatitis viruses have been compared by the T_1-oligonucleotide fingerprinting technique. In general, they have diverged quite extensively. However, MHV-3, a hepatotropic strain, and A59, a nonpathogenic strain, were found to be extremely related. Yet, each of them contains 2-4 specific oligonucleotides. One of the MHV-3-specific oligonucleotides was mapped in 30S poly(A)-containing RNA or 6-7 Kb from the 3-end and the other near the 5'-end of the genome. These two genetic regions might be associated with viral pathogenicity. In addition, two JHM plaque variants, DL producing large plaques and DS producing small plaques, were also compared. They share almost all T_1-oligonucleotides, but each contains one unique spot. The DL-specific oligonucleotide was mapped in 21S poly(A)-containing RNA or at about 4 kb from the 3'-end. Finally, the MHV genome was found to contain the "cap" structure, confirming that it is a positive-stranded RNA.

INTRODUCTION

Mouse hepatitis viruses (MHV) are members of the coronavirus group which includes viruses infecting a variety of animals (McIntosh, 1973). MHV infections occur primarily as natural, latent infections of mice (Gledhill and Niven, 1955) and have been isolated from many laboratory strains of mice (Calisher and Row, 1966). Experimentally, they induce a variety of pathological findings in animals: while most MHV strains cause fulminating hepatitis, a few strains are able to induce acute and chronic neurological diseases (Herndon et al, 1975; Virelizier et al, 1975). For

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instance, the JHM strain induces demyelinating encephalomyelitis (Weiner, 1973) and MHV-3 strain induces chronic choriodoependymitis and meningitis in some strains of mice (Virelizier et al, 1975). Still another strain, A59, has very low pathogenicity (Robb and Bond, 1978). The diversity in the disease potential of MHV offers a system for the study of mechanism of viral pathogenesis.

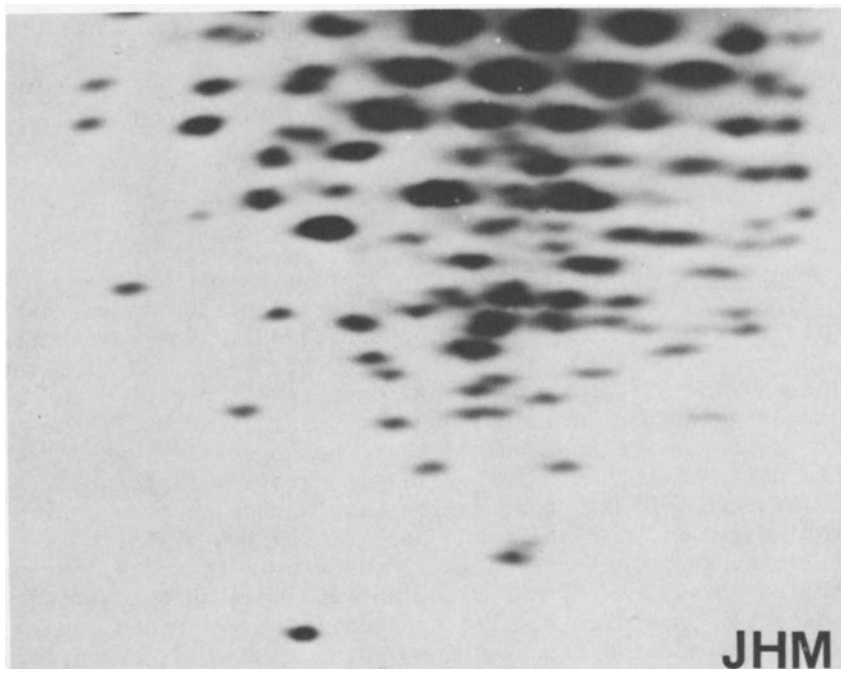
The genome of MHV has been shown to consist of a single piece of single-stranded 60S RNA (Lai and Stohlman, 1978; Wage et al, 1978). At least one third of the genomic RNA contains polyadenylated sequences and is infectious. Therefore, the MHV genome appears to be positive-stranded, although the presence of negative-stranded RNA in the MHV virion has not been ruled out. To study the genetic basis of viral pathogenicity, we have first characterized the RNA genome of various MHV strains by oligonucleotide mapping. We have identified some gene sequences which might be associated with pathogenicity of the different diseases caused by MHV. This approach thus proves to be useful for the study of molecular basis of viral pathogenicity.

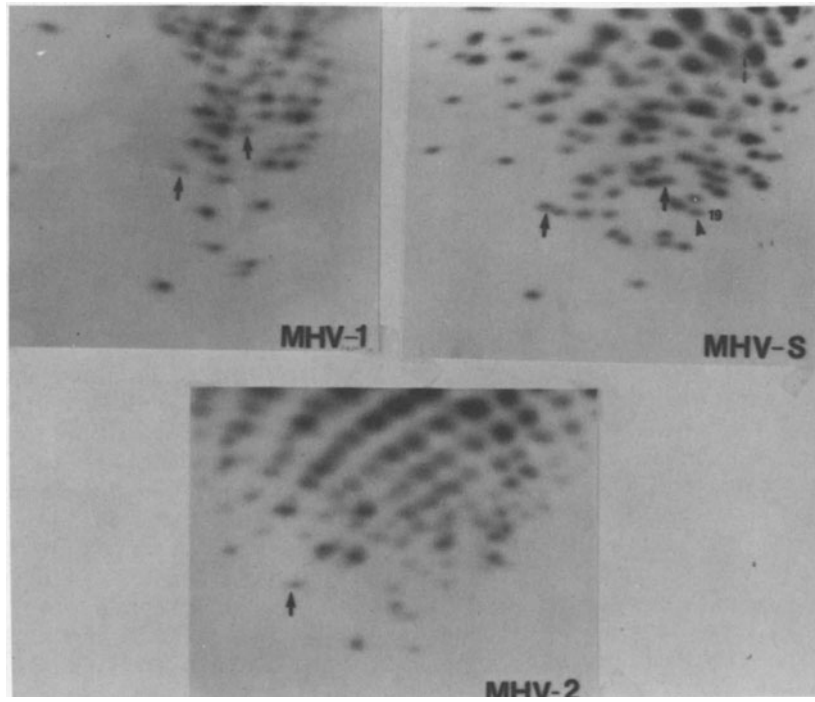
MATERIALS AND METHODS

Viruses: MHV-1, MHV-3, and MHV-S were originally isolated from mice dying of acute hepatitis (Gledhill and Andrews, 1951; Dick, Niven, and Gledhill, 1956; and Rowe et al, 1963) and were obtained from Dr. Michael Collins, Microbiological Associates, Bethesda, Maryland. MHV-2 and MHV-A_{59} were also isolated from animals with acute hepatitis (Nelson, 1952; Manaker et al, 1961) and were obtained from K. Fujiwara, Tokyo, Japan and L. Sturman, Albany, N.Y. respectively. MHV-JHM was originally isolated from a mouse with hindleg paralysis and demyelination (Cheever et al, 1949; Bailey et al, 1949). The origin of plaque variant, designated DL, has been previously described (Lai and Stohlman, 1978). The DS variant was obtained from the 8th suckling mouse brain passage (Weiner, 1972) and cloned to homogeneity by 8 passages of single plaques using DBT cells and was used at the 6th subsequent in vitro passage in DBT cells for this study.

Oligonucleotide Mapping: Growth and purification of virus and extraction of RNA were carried out as previously described (Lai and Stohlman, 1978). The ^{32}P-labeled RNA was exhaustively digested with RNase T_1 and analyzed by two-dimensional polyacrylamide gel electrophoresis according to a modification of the pro-

Fig. 1 Oligonucleotide fingerprints of the genomic RNA of different MHV strains: The spots indicated by triangles are the oligonucleotides shared with the MHV-3 specific spots. The spots indicated by arrows are the oligonucleotides shared with the A59 specific oligonucleotides (see Fig. 3 for comparison).


JHM


MHV-1
MHV-S
MHV-2

cedure by deWachter and Fiers (1972). The first dimension was performed on 8% polyacrylamide gel slabs (30 x 10 x 0.15 cm.) at pH 3.3, 700 $\bar{v}$ for 4 hours. The second dimension was performed on 22% polyacrylamide gel slabs (40 x 35 x 0.075 cm.) at pH 8.0, 650 $\bar{v}$ for 16 hours.

RESULTS

Comparative Oligonucleotide Fingerprinting of Different MHV Strains: Several MHV strains have been isolated independently from different mouse colonies (McIntosch, 1973). They are serologically separable (Hierholzer et al, 1979; Childs and Stohlman, unpublished) and differ in their pathogenicity for mice. To study their genetic relationship and to possibly identify the genetic basis for the spectrum of their pathogenicity, we first compared different MHV strains by oligonucleotide fingerprinting. The ^{32}P-labeled 60S RNAs from various virus strains were exhaustively digested with RNase T_1 and analyzed by two-dimensional polycrylamide gel electrophoresis. As shown in Figure 1, pathogenic MHV strains MHV-1, MHV-2, MHV-S and MHV-JHM have quite distinct and apparently unrelated T_1-oligonucleotide fingerprints, suggesting that all these strains isolated independently have diverged very extensively. In an attempt to understand the genetic basis for pathogenicity, we have compared these strains with a nonpathogenic strain MHV-A59. The fingerprints of the RNA mixtures of MHV-A59 and the other viruses (pictures not shown) were compared with the fingerprints of each individual viral RNA. As shown in Figure 2, these viruses share various degrees of homology with MHV-A59. If we assume that the large T_1-oligonucleotides are representative of the viral genome, the degree of sequence relatedness of different virus strains to MHV-A59 varies from 29% to 59%, except for MHV-3 (Figure 3), which has 97% sequence homology to A59 (Table 1). We therefore further studied MHV-3 and MHV-A59 extensively.

MHV-3 and MHV-A59 share about 95 out of 98 T_1-oligonucleotides which can be identified (Figure 3). But MHV-3 contains two spots, a and b, which are not present in MHV-A59, and MHV-A59 contains 4 spots, #13, #28, #19 and #9 which are MHV-A59 specific (the numbers of each spot are shown in Figure 5). Since these two strains are closely related antigenically (Childs and Stohlman, unpublished) and have similar protein profiles (Stohlman and Lai, 1979) but differ in their pathogenicity, some or all of these unique oligonucleotides might be derived from the genetic sequences which are associated with the pathogenic properties of the virus. If this is indeed the case, then all of the MHV strains having similar pathogenicity might contain the identical or related oligonucleotides. To test this possibility, we examined all MHV strains for the presence of these MHV-A59- or MHV-3-specific

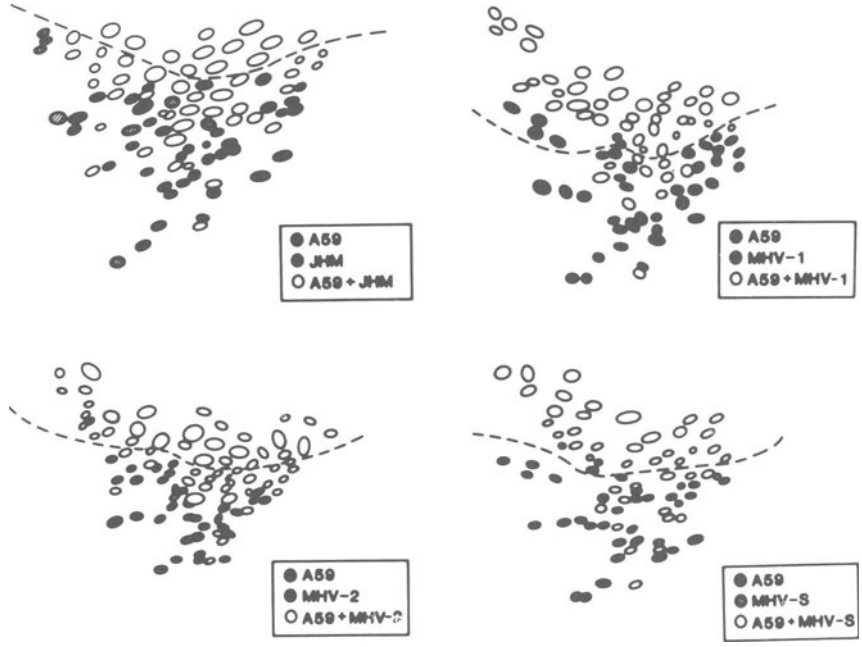


Fig. 2 Schematic drawing of the fingerprints of the RNA mixtures of A59 and other MHV strains: Equal amounts of ^{32}P-labeled RNAs from A59 and other MHV strains were mixed, digested with Rnase T_1 and analyzed by two-dimensional polyacrylamide gel electrophoresis. The origin of each spot was determined by comparing these fingerprints with the fingerprints of individual RNAs.

Table 1

Sequence relationship between MHV-A59 and other MHV strains

	The number of oligonucleotide spots shared with A59[a]	
MHV-1	8/28	(28%)
MHV-2	24/43	(55%)
MHV-3	95/98	(97%)
MHV-S	14/28	(50%)
JHM	32/54	(59%)

a) only the large T_1-oligonucleotides below the dashed lines in each fingerprints in Figure 2 were considered

oligonucleotides. Examination of Figure 1-3 reveals that one of the MHV-3 specific spots, a, is present in all 4 hepatotropic strains, but not in MHV-A59 or MHV-JHM, both of which cause minimal hepatitis (Table 2). The other spot, b, is present in MHV-1, MHV-3, and MHV-S, but not in MHV-2, A59 or JHM (Table 2). Therefore, these spots, particularly, spot a, appear to be widely preserved in and unique to most of the hepatotropic strains. On the other hand, among the 4 MHV-A59-specific oligonucleotides, #19 is present also in MHV-S, a hepatotropic strain of reduced virulence. #9 was occasionally seen in MHV-3; therefore, it is probably not a real MHV-A59 specific oligonucleotide. Thus, only #13 and #28 are unique to the nonpathogenic MHV-A59.

Mapping of MHV-3- or A59-specific oligonucleotides on the RNA genome: Since MHV-3 causes hepatitis in most strains of

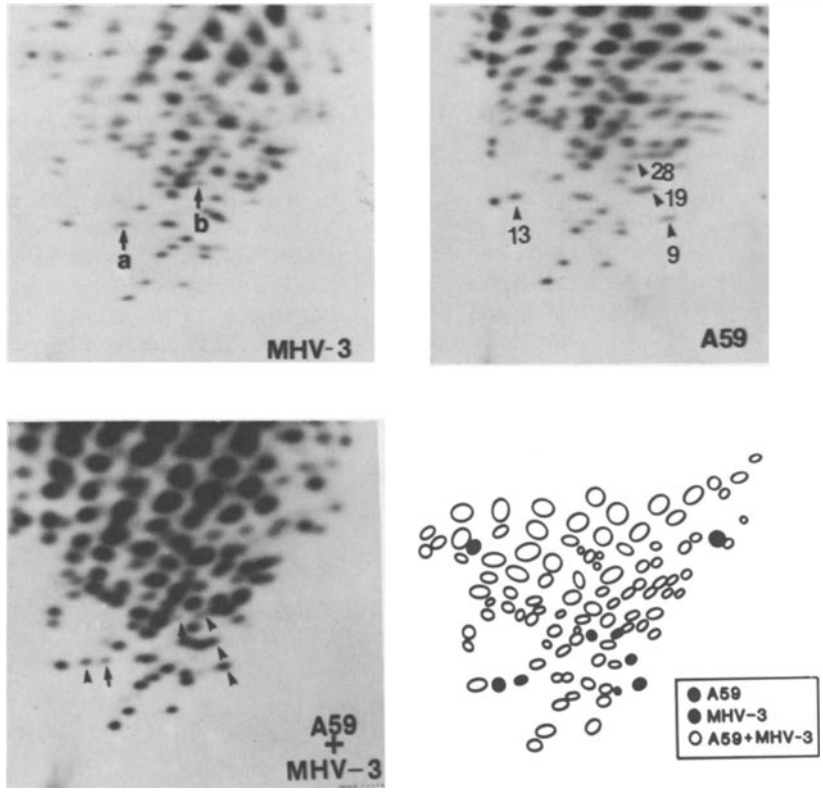


Fig. 3 Oligonucleotide fingerprints of MHV-3, A59 and their schematic drawing: The spots indicated by triangles and arrows are MHV-3 and A59 specific oligonucleotides, respectively.

Table 2

Distribution of MHV-3 and A59-specific oligonucleotides in different MHV strains*

	a	b	13	28	19	9
MHV-3	+	+	-	-	-	±
MHV-1	+	+	-	-	-	-
MHV-2	+	-	-	-	-	-
MHV-S	+	+	-	-	+	-
A59	-	-	+	+	+	+
JHM	-	-	-	-	-	-

* The data were obtained from Figure 1-3

mice and MHV-A59 has very low pathogenicity (Robb and Bond, 1978), the oligonucleotide spots unique to either virus could come from a region of the RNA genome which is associated with viral pathogenicity. It is, therefore, of interest to understand the localization of these specific spots on the RNA genome. To map these oligonucleotides, the ^{32}P-labeled 60S RNA was briefly degraded and passed through oligo(dT) cellulose column (Aviv and Leder, 1973). The poly(A)-containing RNA fractions were selected and separated by sucrose gradient sedimentation. The RNAs of different size fractions were then analyzed individually by oligonucleotide fingerprinting. Such an analysis of MHV-A59 is shown in Figure 4. From the oligonucleotides present in different size fractions of poly(A)-containing RNA, a map of oligonucleotide spots on the genome was obtained (Figure 5). It is seen that among the four probably MHV-A59-specific oligonucleotides, #28 is located near the extreme 5'-end of the genome; #13 is located in 30S poly(A)-containing RNA, or at about 6-7 kb from the 3'-end, and the other two spots, #19 and #9 are located in the 5'-half of the genome. Therefore, these 4 spots appear not to be contiguous on the genome.

Similar studies have been performed with MHV-3 RNA, and the oligonucleotide map is also presented in Figure 5 for comparison with MHV-A59. Since MHV-3 and MHV-A59 share most of the oligonucleotide spots, the oligonucleotides of MHV-3 were given numbers as corresponding to the spots identified in MHV-A59, except for the two MHV-3 specific spots. All of the shared oligonucleotides in MHV-3 were localized at exactly the same map positions as the corresponding spots in MHV-A59, confirming the genetic homology between the two viruses (The MHV-3 oligonucleotides which are identical to those of MHV-A59 are not represented in Figure 5).

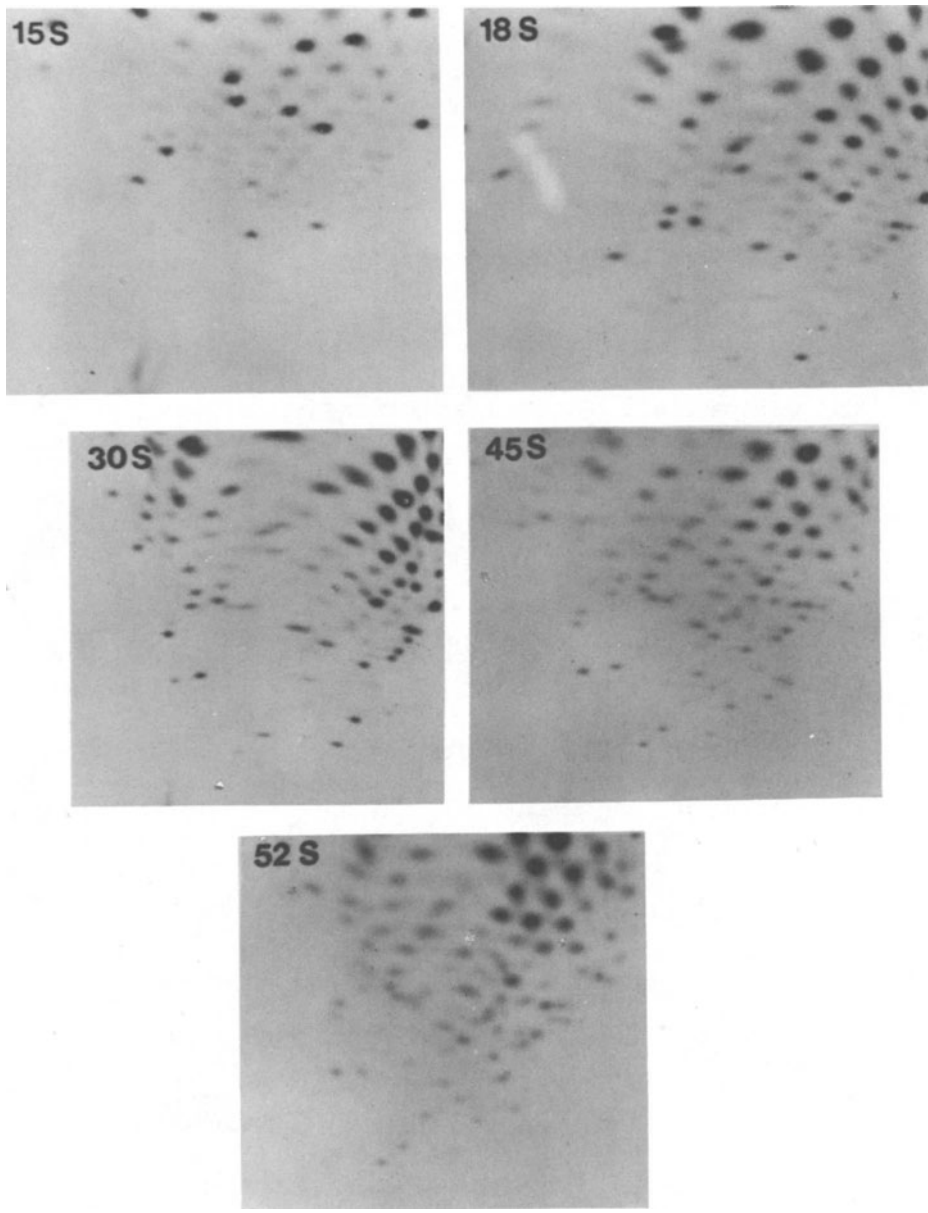


Fig. 4 Oligonucleotide fingerprints of the different size fractions of poly(A)-containing RNA of A59: The ^{32}P-poly(A)-containing A59 RNA was selected by oligo dT column, separated by sucrose gradient sedimentation and each size fraction was fingerprinted individually.

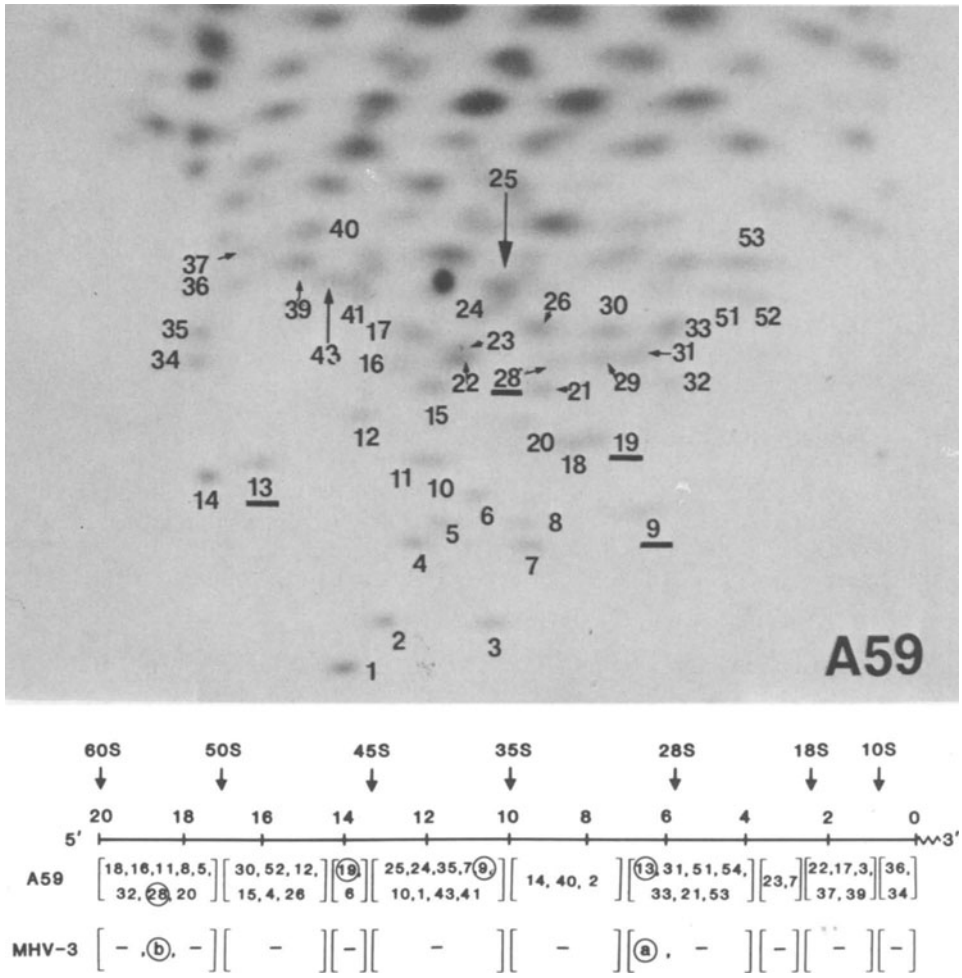


Fig. 5 Maps of the oligonucleotides on the RNA genome of A59 and MHV-3: The numbers of oligonucleotides were arbitrarily assigned. The numbers circled are A59-specific spots. The position of each oligonucleotide on the genome was estimated from the sedimentation values of different RNA size fractions. The map positions of all of the MHV-3 spots are identical to those of A59. Only the MHV-3-specific spots, a and b, are shown on the map.

As shown in Figure 5,one of the MHV-3 specific spots, a, was mapped at exactly the same location as the MHV-A59-specific spot #13, while the other MHV-3 specific spot, b, was mapped at the 5'-end, the same location as the MHV-A59-specific spot, #28. This suggests that a and #13, and b and #28 are probably related

in their sequences, and represent the corresponding genetic regions in A59. It also suggests that these two regions, the 5'-end and the region at 6-7 kb from the 3'-end are associated with viral pathogenicity, possibly virus-induced hepatitis.

Oligonucleotide fingerprinting of the two JHM plaque variants: MHV-JHM infection of mice results in acute encephalomyelitis with both acute and chronic demyelination following intracerebral inoculation (Herndon et al, 1975; Weiner, 1973). It provides a useful model for studying virus-induced demyelination. To gain insight into the genetic basis for MHV-JHM's neurotropism, we have studied by oligonucleotide fingerprinting the genome structure of two JHM variants which were recently isolated in our laboratories. One variant, DL, produces large plaques while the other, DS, produces small plaques. JHM-DL is much more virulent than JHM-DS, having about 200-fold lower LD_{50} for mice. JHM-DS also induces both acute and chronic demyelination at higher efficiency than either JHM-DL or parental MHV-JHM (unpublished observation). As shown in Figure 6, the fingerprints of these two variants show that JHM-DS contains an oligonucleotide, x, which is missing in JHM-DL. On the other hand, JHM-DL also contains a specific spot, Z, which is missing in JHM-DS. These minor differences between these two plaque variants could possibly account for the difference in their biological properties *in vitro* and *in vivo*. The genetic localization of these two oligonucleotides is being investigated. Preliminary data showed that the JHM-DL specific spot, Z, was mapped in 21S poly(A)-containing RNA, or at 4 kb from the 3'-end (data not shown). The JHM-DS specific spot, x, was probably localized in 35S poly(A)-containing RNA or 9-10 kb from the 3'-end. Which of these two genetic regions is responsible for plaque size or demyelination is being studied.

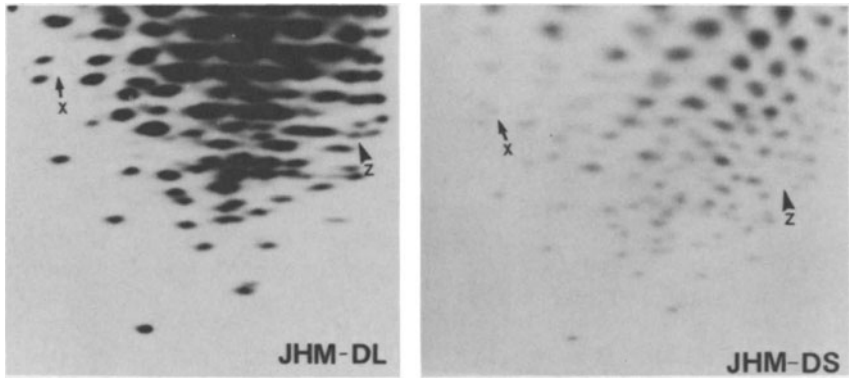


Fig. 6 Oligonucleotide fingerprints of JHM-DS and JHM-DL: The spots indicated by arrows are JHM-DS and JHM-DL specific spots.

The MHV RNA contains 5'-"cap" structure: Since the 60S RNA of MHV is a positive-stranded RNA, we wanted to know whether it contained a "cap" structure at its 5'-end (Shatkin, 1974; Furuich and Miura, 1975). The ^{32}P-labeled 60S MHV RNA was digested with a mixture of Rnase A, T1 and T2 and then electrophoresed on DEAE-cellulose paper at pH 3.5. Under this condition, the entire sequences of the RNA are digested into mono- or di- nucleotides except the "cap" structure, which has a characteristic electrophoretic mobility (Shatkin, 1974; Wang et al, 1977). As shown in Figure 7, the MHV RNA contains a "cap" structure which has similar electrophoretic mobility to that of the cap structure in Rous sarcoma virus (Wang et al, 1977; Keith and Fraenkel-Conrat, 1975), providing further evidence that MHV genome is a positive-stranded RNA.

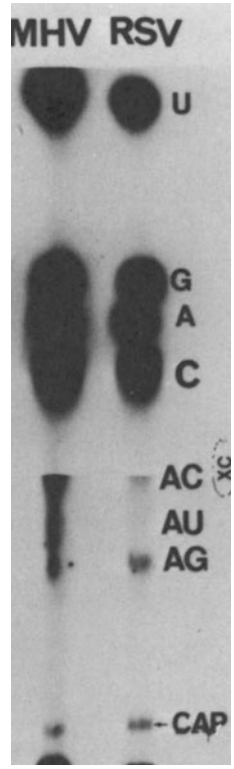


Fig. 7 Electrophoretic separation of RNA digests of A59: The ^{32}P-A59 RNA was digested with RNase A, T_1, and T_2 and then separated by electrophoresis on DEAE cellulose paper at pH 3.5 at 1500 $\bar{v}$ for 3 hours. The position of the "cap" structure was indicated on the electrophoregram. The lower part of the electrophoregram was exposed with an intensifying screen.

DISCUSSION

The studies presented in this communication represent our first attempts to understand the molecular basis of the pathogenesis of the diseases produced by MHV. Two major groups of diseases are caused by these viruses: hepatitis and demyelinating neurological disorders (McIntosch, 1973; Weiner, 1973; Herndon et al, 1974). Our oligonucleotide fingerprinting studies on a hepatotropic strain, MHV-3, and a nonpathogenic strain, MHV-A59, suggest that the genetic sequences associated with hepatitis might be localized at the region around 6-7 kb from the 3'-end, and possibly also at the 5'-end region. The latter location was inferred from the location of one of the MHV-3 specific oligonucleotides, b. However, this spot is not present in all of the hepatotropic strains and may be correlated with other diseases caused by MHV-3 (Virelizier et al, 1975). Therefore, the biological significance of the 5'-end sequences is questionable at the moment. In contrast, the region at 6-7 kb from the 3'-end contains the other MHV-3-specific oligonucleotide, a, which is present in all of the hepatotropic strains. If mouse hepatitis virus has the same mRNA structure as avian coronavirus, Infectious Bronchitis Virus, i.e. various virus-specific mRNAs have nested structure (Stern and Kennedy, 1980), it will suggest that the genetic sequences associated with viral pathogenicity will be present in the mRNA species with molecular weight of about $2.0\text{-}2.5 \times 10^6$, or 30S, and probably also the mRNA of the genome size. The translation products of such mRNA will be candidate proteins responsible for the difference in the biological and pathogenic properties of MHV-3 and MHV-A59.

The interpretation of our data is complicated by the fact that MHV-3 and A59 are natural variants of MHV. Therefore, it could not be ruled out that these two strains might differ in more respects than pathogenicity alone. Thus, the difference detected in their genome sequences does not necessarily reflect the pathogenicity. However, since pathogenicity is the only major difference between these two strains, it is likely that at least one of these two genetic locations might be associated with either hepatitis or other diseases caused by MHV-3 (Virelizier et al, 1975). More definitive studies will have to be performed with laboratory-derived virus mutants.

Studies on the neurotropic properties of MHV-JHM plaque variants had just begun. The small difference in their oligonucleotide fingerprints suggests that the difference between them is very minor, possibly involving only minor base substitutions. Since these two variants vary both in plaque size *in vitro* and in pathogenicity *in vivo* it is possible that they differ in more than one genetic location. Preliminary data mapped the two unique oligonucleotides at two different locations. The biological

functions of these genetic regions is being investigated.

ACKNOWLEDGEMENTS

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