The Nucleocapsid Protein of Murine Hepatitis Virus Type 3 Induces Transcription of the Novel fgl2 Prothrombinase Gene*  

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Using a set of parental and recombinant murine hepatitis virus strains, we demonstrate that the nucleocapsid protein induces transcription of the novel fgl2 prothrombinase gene and elevated procoagulant activity in those strains that produce fulminant hepatitis. Chinese hamster ovary cells cotransfected with a construct expressing nucleocapsid protein from susceptible strains and with a luciferase reporter construct containing the fgl2 promoter showed a 6-fold increase in luciferase activity compared with nontransfected cells or cells cotransfected with a construct expressing nucleocapsid protein from resistant strains. Two deletions found at coding sites 111–123 and 1143–1145 of structural domains I and III, respectively, of the nucleocapsid gene may account for the differences between pathogenic and nonpathogenic strains. Preliminary mapping of the fgl2 promoter has defined a region from −372 to −306 upstream from the ATG translation initiation site to be responsive to nucleocapsid protein. Hence, mapping of genetic determinants in parental and recombinant strains demonstrates that the nucleocapsid protein of strains that induce fulminant hepatitis is responsible for transcription of the fgl2 prothrombinase gene. These studies provide new insights into the role of the nucleocapsid gene in the pathogenesis of viral hepatitis.

Murine coronavirus infection is recognized as one of the best models for studying acute and chronic hepatitis of humans. MHV1-3 infection in BALB/cJ mice causes fulminant hepatic failure, which is characterized by macrophage activation and marked production of proinflammatory mediators. Especially intriguing is the ability of MHV-3 to induce de novo synthesis of a unique procoagulant, the fgl2 prothrombinase, encoded by the fgl2 gene located on mouse chromosome 5 (1, 2). Several lines of evidence implicate expression of this gene product in the pathogenesis of fulminant murine hepatitis. First, levels of this prothrombinase activity correlate with the severity of the disease (3, 4). Second, treatment of mice with a neutralizing monoclonal antibody to the MHV-3-induced prothrombinase prevents the lethality associated with MHV-3 infection (5). Concordant with these observations, expression of fgl2 prothrombinase in liver accounts for widespread fibrin deposition in hepatic blood vessels and hepatocellular necrosis (6).

The MHV genome is a single-stranded nonsegmented RNA of approximately 32 kb (7). The RNA genome contains seven or eight genes encoding three or four structural proteins and four nonstructural proteins (8). An important aspect of MHV biology is the high frequency of RNA-RNA recombination between strains of MHV (9–11). RNA recombination may contribute to viral pathogenesis and also provides a useful tool for the study of genetic control of the biologic properties of viruses. Studies using recombinant viruses derived from MHV-JHM and MHV-A59 have demonstrated that the 3’-portion (about 25%) of the viral genome, representing RNA genomic regions encoding for all of the structural proteins, controls biologic properties such as organotropism of the virus, the pattern of the virus-induced central nervous system pathology in mice, plaque morphology, and virus yield in tissue culture (12, 13). In this paper, we have defined the genetic basis for induction of fgl2 gene transcription by MHV-3.

EXPERIMENTAL PROCEDURES

Mice

Female BALB/cJ mice, 6–8 weeks of age, from Charles River Laboratories (St. Constant, Quebec) were kept in microisolated cages, housed in the animal facilities at the Toronto Hospital, and fed a standard laboratory chow diet and water ad libitum.

Virus

MHV-3 was obtained from American Type Culture Collection (ATCC), Rockville, MD and plaque purified on monolayers of DBT cells. Parental viruses A59, JHM, MHV-2, and two sets of recombinant viruses have been described previously (14). The schematic representations of oligonucleotide maps of the recombinant viruses are presented in Fig. 1. To ensure that the recombinant virus strains are clonal, ML3 and ML11 were plaque purified three times in 17Cl-1 cells. The purified strains were used for creating nucleocapsid (N) gene expression constructs and transfection experiments.

Cells

Peritoneal macrophages were harvested from BALB/cJ mice 4 days after intraperitoneal administration of 1.5 ml of 3% thioglycolate (Difeo Laboratories) as described previously (6). Macrophages were resuspended in RPMI 1640 (ICN Biomedicals Inc., Costa Mesa, CA) supplemented with 2% glutamine (Sigma) and 2% heat-inactivated fetal calf serum (Flow Laboratories, Mississauga, Ontario, Canada). Macrophages were greater than 95% in purity as determined by morphology...
and nonspecific esterase stain. Viability exceeded 95% by trypan blue exclusion. Chinese hamster ovary cell line (CHO cells) were from ATCC.

**Procoagulant Activity (PCA)**

MHV and recombinant virus-infected macrophages, at a multiplicity of infection of 2.5, were incubated for 8 h in RPMI 1640 supplemented with 2% fetal bovine serum and 200 μM glutamine. Mock-infected macrophages and MHV-3-infected macrophages represented negative and positive controls, respectively. Macrophages were evaluated for functional PCA in a one-stage clotting assay, as described previously (15). After incubation, samples to be assayed for PCA were washed three times with unsupplemented RPMI 1640 and resuspended at a concentration of 10^6/ml. Samples were assayed for the ability to shorten the spontaneous clotting time of normal citrated human platelet-poor plasma. Milliunits of PCA were assigned by reference to a standard curve generated with serial log dilutions of a standard rabbit brain thromboplastin (Dade Division, American Hospital Supply Co., Miami, FL).

**RT-PCR**

Expression of fgl2 was detected by RT-PCR. Freshly isolated macrophages, at a multiplicity of infection of 2.5, were infected with different strains of viruses for 6 h. 1 x 10^7 macrophages were pelleted in 1.5-ml Eppendorf tubes, and total cellular RNA was isolated by 8 M acid-guanidium hydrochloride extraction in a modified procedure as described previously (16). The quantity and quality of RNA were examined by spectrophotometry and on a 1% analytical agarose DNA gel. RNA (5 μg) was reverse transcribed using Moloney murine leukemia virus reverse transcriptase in 20-μl reactions using Moloney murine leukemia virus reverse transcriptase in 20-μl reactions. As recommended by the manufacturer, PCR was then performed in 50-μl reactions using 1-μl portions of cDNA and the primers fgl2-318 (TGC CCA GGC CCA TCA TCA A) corresponding to nucleotides 318–336 of BALB/cJ (M15761) and fgl2-1224 (GAG ACA ACG ATC GGT ACC CCT) corresponding to nucleotides 1224–1244 of BALB/cJ fgl2 cDNA (M16235), which yield a 906-base pair band in 1% agarose DNA gel. Amplification products were not obtained when reverse transcriptase was omitted (data not shown). RT-PCR for glyceraldehyde-3-phosphate dehydrogenase was also set up as an internal control to assess the quality of first strand synthesis.

**Creation of N Gene and fgl2 Promoter Constructs**

Restriction enzymes used to create constructs were obtained from Life Technologies, Inc. All plasmids were purified using Qiagen Maxiprep Kits and grown in DH5α Escherichia coli bacteria (Life Technologies, Inc.).

**N Gene Expression Constructs**—The entire N gene coding regions and 3'-untranslated regions of MHV-A59, MHV-2, ML3, and ML11 were amplified by RT-PCR. RNA was originally extracted from infected macrophages. The sense primer AGC ATG TCT TTT GTT CCT GGG was phosphorylated chemically at position 1 to achieve directed insertion and ligation of PCR products to its vector; the antisense primer at position 1654 TTT TTT TGT ATT CTT CCA had a poly(T) group to match the poly(A) tail at the 3'-end of nucleocapsid genomic RNA. The N gene fragments were subcloned into the 5.0-kb expression vector pCR3.1 (Invitrogen), under the control of the cytomegalovirus promoter and bovine growth hormone 3'-processing signals. External restriction endonuclease EcoRI, EcoRV were used to analysis the size and orientation of N gene insert in recombinant plasmid constructs.

**Luciferase Reporter Constructs**—A 1.3-kb DNA fragment flanking the 5'-end of mouse fgl2 was released by restriction digestion with EcoRV and SphI from a subclone pBluescript-m166 (pml166) of mouse genomic P1 plasmid (Genome System Inc.) which contains the entire mouse fgl2 gene. This fragment was sequenced by cycle sequencing on an automated DNA sequencer (model 377, Applied Biosystems) using dideoxy dye terminator chemistry. This sequence has been deposited into GenBank with the Accession number AF025817 (Fig. 2) (17). This 1.3-kb fragment was inserted into Smal and Xhol sites of the pGL2-basic luciferase reporter vector (Promega) to form pfgl2-1328LUC. 5'-Deletion constructs of fgl2 promoter were made by first amplifying the specific fragment using pm166 as template and then cloned into pCR2.1 cloning vector (Strategene) and resubcloned into pGL2-basic plasmid at XhoI and HindIII sites. The reverse primer (GCC ACA ACC AAC CAG GAA G) was used to make all deletion constructs by PCR amplification. The upstream primers used were: GAG CTG AGT GAT GGG GAA GGA for pfgl2-693LUC, GCA CTG AGT ATT ACA TGG CC for pfgl2-625LUC, GGA CCT TTG TTC TGA TTA GGG GC for pfgl2-511LUC, GCA AGA CAT TTA GAC GTT CC for pfgl2-372LUC, and GCG CAC TGG TAT AAC CTT GG for pfgl2-306LUC. All promoter-luciferase report constructs were sequenced to confirm the orientation and to verify the sequence. Positive control, pGL2 control plasmid with SV40 promoter, and Rous sarcoma virus β-galactosidase vector were from Promega. A 2-kb tissue factor promoter construct pTF(-2kb)LUC was a kind gift of Dr. Nigel Mackman (18).

**N Gene Sequence of Different Virus Strains**

The N gene sequence of multiple clones for each strain of virus was determined using primer-directed strategies by cycle sequencing on an automated DNA sequencer, using the Applied Biosystems PRISM™ dRhodamine Terminator Cycle Sequencing Reading Reaction kit (model 377, PR Applied Biosystems). The T7 primer and pCR3.1 reverse primer were used for 5' to 3' and 3' to 5' sequence, respectively. A new T7 primer AGG GCT TTT ATG TTT GTA GAG (MHV-ND557) at position 557 was also designed based on the outcome of sequencing and the published cDNA sequence of MHV-A59 to complete the sequencing. Extension products were purified by ethanol/sodium acetate precipitation. Samples were subjected to electrophoresis on the PRISM 310. The sequence was analyzed using the DNAsis for windows, sequence anal-
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**Fig. 2. Schematic diagram of the organization of the fgl2 gene is shown in the upper panel. The lower panel shows the sequence of the 1.3-kb DNA-flanking '5-end of fgl2.** The sequence shows the overlapping 400 base pairs at the '3-end of this fragment and the '5-end of the published sequence by Koyama et al. (17) with the accession number M15761 are consistent. The putative cis-elements responsive to N protein and initiating ATG for translation are indicated in bold and underlined.

**RESULTS**

Expression of BALB/cJ Macrophage Functional PCA Induced by Parental Viruses and Their Recombinants—Studies were undertaken using parental A59, JHM, and MHV-2 strains and two sets of recombinant viruses between them (A59 × JHM, A59 × MHV-2) (Fig. 1). MHV-A59 infection of macrophages resulted in a marked elevation of functional PCA, similar to what was reported previously for MHV-3 (1, 6), whereas JHM and MHV-2 failed to induce PCA (Fig. 3A). A59 × JHM-derived recombinant B1, RL1, and IL27, in which the '3'-portion of the genome is derived from MHV-A59, induced high level of functional PCA. In contrast, CA13 and CA43, two recombinants in which the '3'-portion of the genome is derived from MHV-JHM, did not induce PCA, suggesting that the '3'-portion of the MHV-A59 genome may contain a viral genetic determinant needed for induction of functional PCA, which is lacking in the corresponding region of MHV-JHM. To delineate better the candidate genes required for induction of fgl2, we next studied a set of recombinants derived from MHV-A59 × MHV-2, which have multiple crossovers in a single genome, particularly within the '3'-portion of the genome. Recombinants ML3 and ML10, which contain a viral genetic determinant needed for induction of functional PCA, similar to what was reported previously for MHV-3 (1, 6), whereas JHM and MHV-2 failed to induce PCA (Fig. 3A).

**Western Blot**

At 48 h post-infection with N gene and pfgl2(−1328)LUC, cultures of 1 × 10⁶ CHO cells were collected and lysed in 100 μl of Western blot lysis buffer with protease inhibitor. 20 μl of lysate was resolved by SDS-polyacrylamide gel electrophoresis and then transferred to a nitrocellulose membrane. After blocking in 4% milk and phosphate-buffered saline for 1 h, membranes were incubated with goat anti-mouse IgG labeled with horseradish peroxidase for 1 h and washed five times in 2% milk, phosphate-buffered saline, and Tween. Substrates luminol and Enhancer were added and incubated for 1 min. The membrane was then exposed to Kodak XAR-5 film with intensifying screens for 10 min.

**Statistical Analysis**

Data are expressed as mean ± S.D. where applicable. Student’s t test for unpaired samples (two-tailed) was used to analyze the data.

**Expression of the BALB/cJ Macrophage Functional PCA**

| Gene        | ATG | Exon I | Exon II |
|-------------|-----|--------|---------|
| EcoRV       |     |        |         |
| TAGATCATGTA | GATGTCATTAG | TAGAGCTCGGT | AGAGCAAGGC |
| ATAGGACGCGT | TGGCAAAATAT | TGGCTATGGA | TATGCTCGCT |
| TGGTATATTT | CTATGCGACA | AAAATAATTA | TCAAACTTCT |
| GAGCGGCTAG | CAAACCGCAAT | ACAGTGGTATG | TGCAGCTTGA |
| TGCAGAGCTC | TGCAGTGCGGA | AGGTGGCGCTG | CCGCCTGCGC |
| AGTCCGCTTA | AGCATTTATGA | AGATGCGCTG | CCGCCTGCGC |
| AGAAGTACGA | AGAGAAGGCA | AGGCGCCTGC | GCGGCAACAT |
| CAAAGCAGCAG | AGACACACAC | AGAGACACCA | TTAATGAGGC |
| AGCAAGAATG | AGAGACACCA | AGAGACACCA | TTAATGAGGC |
| CAAAACAGGA | GAGGACACAC | AGGCGCCTGC | GCGGCAACAT |
| AGCAAGAATG | AGAGAAGGCA | AGGCGCCTGC | GCGGCAACAT |
| AGAAGTACGA | AGAGAAGGCA | AGGCGCCTGC | GCGGCAACAT |
| CAAAGCAGCAG | AGACACACAC | AGAGACACCA | TTAATGAGGC |
| AGCAAGAATG | AGAGAAGGCA | AGGCGCCTGC | GCGGCAACAT |
| CAAAACAGGA | GAGGACACAC | AGGCGCCTGC | GCGGCAACAT |

**Transfection**

CHO cells were cultured in six-well plates until 50–80% confluence. 1 μg of N gene construct DNA or 1 μg of empty pCR3.1 vector, 0.5 μg of fgl2 promoter-luciferase reporter construct DNA, and 0.25 μg of β-galactosidase DNA (as a marker for transfection efficiency by β-galactosidase assay) in 100 μl of Opti-DMEM were mixed by vortexing with 3.5 μl of LipofectAMINE™ (2 μg/μl) in 100 μl of Opti-DMEM medium. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells. After incubation of the mixture at room temperature for 30 min, 1.8 ml of incubated mixture was distributed into one of the duplicated wells with CHO cells.
with the published sequence of A59 in GenBank (M35156), the sequence of MHV-A59 used in this study had two point mutations at nucleotides 441 (T \rightarrow A) and 1613 (T \rightarrow C); ML3 had three point mutations at nucleotides 405 (C \rightarrow A), 441 (T \rightarrow A), and 1613 (T \rightarrow C). Sequence differences between the N genes of MHV-A59 and MHV-2 (AF061835) were mainly within two regions corresponding to nucleotides 400–500 and 1100–1200 of the MHV-A59 sequence. In addition, MHV-2 had a 12-nucleotide deletion at 111–123 and a 3-nucleotide insertion at 1143–1145 compared with MHV-A59. In contrast, MHV-2 N gene expression did not enhance fgl2 expression. To confirm the specificity of the effect of N protein on fgl2 promoter activity, pTF (−2kb)LUC and a pGL2-control vector under SV40 promoter were each cotransfected with the MHV-A59 N gene construct. There was no significant increase in luciferase expression when these two constructs were cotransfected with or without the MHV-A59 N gene construct (Fig. 6).

Mapping of the fgl2 Promoter—To characterize the region in the fgl2 promoter which is responsive to N protein of MHV-A59, constructs containing progressive deletions of the −1328 base pair fragment were cotransfected with either N gene construct from A59 and a murine pfgl2(1–1328)LUC were performed. Multiple clones from each construct were sequenced. T7 primer and pCR3.1 reverse primer were used for 5′ to 3′ and 3′ to 5′ sequence, respectively. A new primer at position 557 was also designed to accomplish the cloned entire N gene sequence as described under "Experimental Procedures." The sequence was analyzed using the DNAsis for Windows sequence analysis software.

### Table 1

| Identity comparison of N gene sequence | %     | %     |
|--------------------------------------|-------|-------|
| MHV-A59 (1.666 Kb)                   | 99    | 93    |
| MHV-ML3 (1.666 Kb)                   | 99    | 92    |
| MHV-ML11 (1.657 Kb)                  | 93    | 99    |

**Fig. 3. Expression of BALB/cJ macrophage functional PCA induced by parental viruses and their recombinants derived from MHV-A59 × JHM (panel A) or MHV-A59 × MHV-2 (panel B).** Macrophages from BALB/cJ were infected with viruses at a multiplicity of infection of 2.5 for 8–10 h and harvested for measurement of PCA activity. Values represent the mean ± S.D. of three separate experiments done in triplicate. *represents p < 0.01 compared with unstimulated macrophages.

**Fig. 4. Expression of BALB/cJ macrophage fgl2 transcripts induced by parental viruses and their recombinants by RT-PCR.** 5 µg of total cellular RNA extracted from infected macrophages was reverse transcribed, and then PCR was performed using specific fgl2 primers as described under "Experimental Procedures." Lane 1, parental viruses + MHV-3; lane 2, parental viruses + PCA activity. Values represent the mean ± S.D. of three separate experiments done in triplicate. *represents p < 0.01 compared with unstimulated macrophages.

**Fig. 5. Expression of N protein in transfected cells by Western blot analysis.** 2 × 10^6 of cell lysis post-transfection were loaded in a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were then probed with antibodies as described under "Experimental Procedures." Lane 1, CHO + pfgl2(−1328)LUC + A59 N gene construct; lane 2, CHO + pfgl2(−1328)LUC + MHV-2 N gene construct; lane 3, CHO + pfgl2(−1328)LUC + empty pCR3.1 vector; lane 4, 17Cl-1 cells + MHV-3.

**Fig. 6.** Expression of BALB/cJ macrophage fgl2 transcripts induced by parental viruses and their recombinants by RT-PCR. 5 µg of total cellular RNA extracted from infected macrophages was reverse transcribed, and then PCR was performed using specific fgl2 primers as described under "Experimental Procedures." Lane 1, parental viruses + MHV-3; lane 2, parental viruses + PCA activity. Values represent the mean ± S.D. of three separate experiments done in triplicate. *represents p < 0.01 compared with unstimulated macrophages.

**Fig. 5.** Expression of N protein in transfected cells by Western blot analysis. 2 × 10^6 of cell lysis post-transfection were loaded in a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were then probed with antibodies as described under "Experimental Procedures." Lane 1, CHO + pfgl2(−1328)LUC + A59 N gene construct; lane 2, CHO + pfgl2(−1328)LUC + MHV-2 N gene construct; lane 3, CHO + pfgl2(−1328)LUC + empty pCR3.1 vector; lane 4, 17Cl-1 cells + MHV-3.
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Fig. 6. Effect of N protein on fgl2 promoter. 0.5 μg of N gene construct from MHV-A59, MHV-2 was cotransfected with 0.5 μg of pGL2−1328LUC or pTFI−326LUC in CHO cells for 40–44 h, and cells were harvested and freeze-thawed three times for measurement of luciferase activity. Values represent the mean ± S.D. of five separate experiments done in triplicate. * represents p < 0.01 compared with cells cotransfected with empty pCR3.1 vector.

Studies using a model of viral hepatitis induced by infection with MHV-3 have provided significant insight into the mechanisms underlying the pathogenesis of this disease and have suggested novel approaches to therapy (2, 19, 20). Furthermore, recent studies have demonstrated the role of the selective expression of the fgl2 prothrombinase in the pathogenesis of MHV-3-induced fulminant liver failure (1, 2, 5, 6). The studies presented here demonstrate that infection of macrophages with MHV-A59 resulted in transcription of fgl2 and elevated functional prothrombinase activity, a result similar to that observed during infection of macrophages with MHV-3. This contrasts with the results obtained with MHV-JHM and MHV-2, which do not induce elevations of prothrombinase in infected macrophages. Recombinant viruses (n = 5) which derive a portion of their genomic RNA from the 3′-region of MHV-A59 induce functional prothrombinase, whereas those containing a 3′-region derived from JHM or MHV-2 (n = 5) do not, suggesting that the 3′-portion of the genome may play an important role in the induction of fgl2 transcription. The sequence differences in the genome in the 3′-area of interest lie mainly within the 5′-end of the N gene. The sequence differences in the N gene between inducers and noninducers are a 12-nucleotide deletion at nucleotides 111–123 of the coding region for the structural domain I and a 3-nucleotide insertion at 1143–1145 of the coding region for structural domain III, which may account for the inability of JHM or MHV-2 to induce fgl2.

CHO cells cotransfected with the N gene construct from A59 and with the fgl2 promoter construct showed a 6-fold increase in luciferase activity in contrast to base-line or MHV-2-cotransfected cells.

These findings strongly suggest that the N protein is responsible for fgl2 induction. It should be noted that induction of fgl2 could not be explained by differences in virus replication, as MHV-2, a noninducer, replicates to higher titers than A59, whereas A59 replicates to higher titers than JHM. It is also of great interest that all of the recombinants of MHV-2 × A59 have the MHV-2-derived leader sequence, whereas the majority of recombinants between A59 × JHM contain the A59 leader (21). It is not clear whether the leader sequence differences among viruses are responsible for the altered growth property of the virus.

MHV N protein has been proposed to consists of three conserved structural domains (I basic, II basic, and III acidic) which are tethered to each other by two regions of variable amino acid composition (designated A and B) (22). The sequence differences in the N gene between A59 and MHV-2 lie within two regions corresponding to domain I (nucleotides 400–500) and domain III (nucleotides 1100–1200), respectively. These differences may account for the inability of MHV-2 to induce the fgl2 gene.

Cotransfection of an N gene expression construct with the fgl2 promoter/reporter luciferase construct in the CHO cell line confirms that the N gene of A59 accounts for induction of fgl2. To confirm the specificity of the induction of N protein for the fgl2 promoter, the same experiment was performed with two irrelevant constructs. Neither showed enhancement of luciferase activity when cotransfected with fgl2 promoter construct. However, this does not suggest that the N protein will not induce the expression of other proinflammatory genes, which are important in the pathogenesis of MHV-3-induced hepatitis (23). Possible mechanisms by which N protein induces fgl2 prothrombinase expression include the transport of the N protein into the nucleus of infected cells, acting as a transcription activator for fgl2, or modulation of signal transduction pathways that regulate host transcription machinery, or production of additional transcription factors thereby increasing the steady-state levels for fgl2 transcripts. Recent work by both nuclear runoff assays and transient transfection experiments have demonstrated that the induction of fgl2 mRNA by MHV-3 infection is at least in part attributable to new transcription (18). An alternative mechanism may be N protein binding directly to fgl2 mRNA, thus altering the rate of fgl2 transcript degradation. Further experiments are now in progress to clarify the mechanisms involved.

Preliminary mapping of the fgl2 promoter has defined a region from −372 to −306 to be responsive to the N protein. At present, we have not determined the precise cis-element(s) that are necessary for transcription. However, we have identified three putative cis-elements. Of particular interest, previous reports have suggested that LF-A1 is typical of a liver-specific gene and liver-enriched transcription factor. Furthermore, LF-A1 is known to regulate gene expression of coagulation factors IX, X, and XII (24–26). Additionally, granulocyte macrophage colony-stimulating factor is released by activated T lymphocytes, monocytes, endothelial cells, and fibroblasts treated with proinflammatory cytokines (27, 28). IE1.2 has been implicated as human cytomegalovirus immediate-early gene 1 and 2 regulatory elements, providing a link between virus infection and inflammation (29). These data do not exclude the presence of other cis-elements that might contribute to transcription of fgl2, and studies now ongoing will firmly define relevant elements necessary for fgl2 transcription.

Recent studies have shown that the severity of hepatic injury in patients with hepatitis B is related to the synthesis and
expression of nucleocapsid protein of the hepatitis B virus, suggesting that the accumulation of hepatitis B core antigen may damage hepatocytes directly or may serve to stimulate cell-mediated immune responses (30, 31). We have recently cloned and sequenced the human prothrombinase gene (hfgl2) gene and have shown its expression in the liver of three patients with fulminant hepatic failure.2 Studies are now in progress to determine if hepatitis B core antigen induces transcription of hfgl2.

In conclusion, mapping of genetic determinants in parental and recombinant MHV strains demonstrates that the N protein of strains of MHV which induce fulminant hepatic favor is responsible for enhanced transcription of the fgl2 prothrombinase gene. These studies may provide significant insights into the viral pathogenesis of human diseases such as hepatitis B and C in which core antigen (nucleocapsid protein) influences disease activity.

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Fig. 7. Panel A, transient expression of luciferase activity by deletion constructs of the fgl2 promoter in response to MHV-A59 N protein in CHO cells. A series of the fgl2 promoter constructs containing varying lengths of the fgl2 promoter sequence was cotransfected with a MHV-A59 N gene construct into CHO cells. Relative luciferase activity is expressed in fold increase compared with CHO cells cotransfected with the fgl2 promoter constructs with empty pCR3.1 vector. PGL2-basic vector was used as a negative control. All luciferase assays represent the mean ± S.D. of six or more independent experiments. * represents p < 0.01 compared with cells cotransfected with empty pCR 3.1 vector. Panel B, schematic representation of the putative regulatory elements in the putative (−372 to −306) fgl2 promoter responsive to N protein. Also shown are the ATG translation initiation site and the location of the TATA box.
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