THE POLYMERASE GENE OF CORONA- AND TOROVIRUSES: EVIDENCE FOR AN
EVOLUTIONARY RELATIONSHIP

Peter J. Bredenbeek, Eric J. Snijder, Ans F.H. Noten, Johan
A. den Boon, Wim M.M. Schaaper¹, Marian C. Horzinek and
Willy J.M. Spaan

Institute of Virology, Department of Infectious Diseases and
Immunology, State University of Utrecht, P.O. Box 80.165
3508 TD, Utrecht, The Netherlands, ¹Central Veterinary
Institute, P.O. Box 65, 8200 AB Lelystad, The Netherlands

SUMMARY

In this paper we demonstrate that the organization of the polymerase gene of toroviruses and coronaviruses is similar. The polymerase gene of both virus families consists of at least two large ORFs (1a and 1b). Four domains of conserved amino acid sequences have been identified in nearly identical positions in the 3' ORF of the pol gene of toroviruses and coronaviruses. The most 3' conserved domain which is still unique for these viruses encodes a 33-kDA protein in MHV-A59, which is cleaved from a precursor protein. Expression of ORF1b of the pol gene of both virus families occurs by ribosomal frameshifting. A predicted stem-loop structure and pseudoknot are conserved in the ORF1a/ORF1b overlap of toro- and coronaviruses. On the basis of these results we postulate that toro- and coronaviruses are ancestrally more related to each other than to other families of positive stranded RNA viruses.

INTRODUCTION

Ancestral relationships between RNA viruses were first indicated by similarities in genome organization and expression (1). The increasing amount of sequence data on positive stranded RNA viruses has enabled scientists to compare these viruses on the level of genetic information. The presence of extensive sequence homologies and the similarities in genome organization and expression have led to "supergroups" containing more than one virus family which are thought to reflect ancestral relationships among seemingly divergent viruses (see 2 and 3 for a review). Among the positive stranded RNA viruses two superfamilies have been recognized; the alphavirus like and picornavirus like families. Detailed analysis of the nucleotide sequences has also revealed short stretches of amino acid sequence similarity in most of the replicase proteins of positive stranded plant and animal RNA viruses. This indicates that viruses related in this way may have descended from a common ancestor (3). The two most prominent amino acid motifs conserved in the replicase proteins are the "GDD" or polymerase motif (4) and the "GKS/T" or nucleotide triphosphate (NTP) binding motif (5, 6). Both motifs consist of several

stretches of conserved amino acids interspersed by variable numbers of non-conserved amino acids.

Coronaviruses have not been classified in either of the two superfamilies due to their different genome organization and expression. Infectious bronchitis virus (IBV) is still the only coronavirus of which the genome has been completely sequenced (7). The polymerase (pol) gene of IBV is about 20 kb in length and contains two large open reading frames (ORF) ORF1a and ORF1b (previously named F1 and F2) which potentially encode polypeptides of 441-kDa and 300-kDa, respectively. The second ORF is not expressed from a subgenomic mRNA but by ribosomal frameshifting (8, 9). Analysis of the predicted amino acid sequence of the IBV polymerase has revealed the presence of both amino acid motifs described above in ORF1b of the IBV pol gene (5, 10).

Two other groups of enveloped, positive stranded RNA viruses express their genetic information from a nested set of multiple subgenomic mRNAs; the arteriviruses (11; unpublished results) and the proposed family of Toroviridae (12). Equine arteritis virus, the prototype arterivirus, possesses an icosahedral nucleocapsid which contains a genomic RNA of approximately 13 kb. Initially, toroviruses were considered to be coronavirus-like, but analysis of the structure and replication of the family prototype Berne virus (BEV) has clearly distinguished them from coronaviruses. BEV contains an elongated tubular nucleocapsid which contains an RNA of approximately the same size as the coronavirus genome (13). In infected cells the genetic information of BEV is expressed by synthesis of four subgenomic mRNAs. These mRNAs are 3' co-terminal, but in contrast to the coronaviral mRNAs, no common leader sequence has been detected at their 5' end. From U.V. transcription mapping data it could be concluded that the synthesis of BEV mRNAs does not involve conventional cis-splicing (14)

In this paper we describe the analysis of the nucleotide sequence of a substantial part of the polymerase genes of both mouse hepatitis virus (MHV strain A59) and BEV. We present evidence demonstrating that in both toro- and coronaviruses expression of the polymerase gene involves ribosomal frameshifting and that both viruses are ancestrally related.

MATERIALS AND METHODS

cDNA synthesis, cloning and sequencing

cDNA was prepared from MHV-A59 genomic RNA and intracellular BEV poly(A)-selected RNA using oligo-(dT) and pentanucleotides as a primer. cDNA synthesis, cloning and sequencing was performed using standard procedures. Full details will be published elsewhere.

Computer analysis

Sequence data were analyzed using the computer programs of Staden (15) and Wisconsin (version 5, 1987). Comparison to the National Biomedical Research Foundation (NBRF) protein identification resource was made using the program FASTP (16).

Production of antisera

A peptide (CTRKEVFGDSLNVK) covering the 14 carboxyl terminal amino acids encoded by the MHV-A59 pol gene, linked to an amino-terminal cysteine residue to couple the peptide to keyhole limpet hemacyanin (KLH) was synthesized. Rabbits were injected intramuscularly and subcutaneously with 1 mg KLH coupled peptide suspended in 1 ml Freund's complete adjuvant. Sera were collected starting 6 weeks after immunization.

Figure 1. Construction of the plasmids pWAP0 and pWAP1. The position of the T7 promoter is indicated as a black box. The nucleocapsid gene of BEV, the MHV-A59 polymerase fragment and 30-kDa ns protein encoding fragment are shown as a hatched, an open and a cross-hatched box, respectively. The amino acids encoded by a part of the polylinker in pWAP1 are indicated by a dashed box. S = SalI; P = PstI; H = HindIII and Sa = SacI. The position of the stopcodon for the translation of the fusion protein in pWAP1 is shown.

Construction of pWAP1

To test the obtained sera for the presence of antibodies against the MHV pol gene encoded product(s) the plasmid pWAP1 was constructed. An HindIII fragment containing 1.85 kb of the 3' end of the MHV pol gene and the complete first ORF of the unique region of mRNA2 was isolated from cDNA clone 96 (17) and cloned downstream of the nucleocapsid gene of BEV in pBSN1 (18). Plasmid DNA isolated from transformants was digested with PstI to confirm the depicted orientation of the HindIII fragment (Fig. 1). The internal Sal fragment was deleted by digestion of pWAP0 with SalI and subsequent religation resulted in pWAP1, in which the MHV pol gene DNA fragment was fused in frame to the N-terminal part of the BEV nucleocapsid gene by making use of a number amino acids encoded by the polylinker of the pBSN1 plasmid. Transcription of the BEV N/MHV pol hybrid gene is under the control of the T7 promoter.

In vitro transcription and translation

Plasmid DNA of pWAP1 was linearized with SacI. Full details of the in vitro transcription and translation will be described elsewhere (18).

Radioactive labeling of viral intracellular proteins

Monolayer cultures of Sac(-) cells were infected at a m.o.i. of 30 with MHV-A59 or mock infected using standard conditions. At 4 h after infection (p.i.) the medium was replaced by methionine deficient MEM, containing 50 μ Ci/ml [35 S]-methionine. At 8 h p.i. the medium was removed and the cells were lysed (19).

Immunoprecipitation of proteins

Immunoprecipitations of the [35 S]-methionine labeled proteins were essentially performed as described (19); 4 μ l of in vitro translation mixtures or 100 μ l of the cell lysates were used. Immunoprecipitated proteins were analyzed by electrophoresis on SDS- polyacrylamide gels.

RESULTS AND DISCUSSION

Nucleotide sequence analysis

The nucleotide sequence of 8.6 kb located in the 3' half of the polymerase gene of MHV-A59 was determined. Analysis of the sequence data revealed the 3' end of an ORF (ORF1a) overlapping with a large ORF (ORF1b) covering the 3' half of the pol gene. ORF1b has a length of 8199 nucleotides and potentially encodes a protein of 309-kDa (2733 amino acids). The first potential translation initiating codon is located at position 405 in ORF1b and the ORF terminates just upstream of the junction sequence AAAUCUAUAC. This region separates the gene encoding the 30-kDa non-structural protein from the polymerase gene in MHV-A59 (17). ORF1a overlaps ORF1b for 75 nucleotides.

Sequence analysis of the 3' half of the polymerase gene of BEV revealed that the organization of this gene in toroviruses is very similar to coronaviruses. An ORF of 6873 nucleotides (2291 amino acids) covering the 3' half of the BEV pol gene was identified. This large ORF (ORF1b) has a 12 nucleotide overlap with the 3' end of a preceding ORF.

Computer analysis of coronavirus and BEV polymerase genes

Dot matrix comparison of the predicted amino acid sequence of ORF1b of MHV-A59 and IBV showed that almost the entire open reading frame is extremely well conserved (Fig 2A). The positional identity in an alignment of the amino acid sequence of ORF1b of MHV and IBV is 57%. Based on the sequence analysis of a small part of the 3' end of the polymerase gene of the feline coronavirus FIPV, which was found to be very similar to MHV-A59 as well as IBV (De Groot, unpublished results), we predict that the ORF1b encoded product of coronaviruses is well conserved. No similarity could be detected in the predicted amino acid sequence of the 5' end of ORF1a of the IBV and MHV-A59 pol genes (20). However the putative carboxyl terminal region of the ORF1a translation product of MHV-A59 and IBV is well

Figure 2. Proportional dot matrix comparisons. Numbers of the amino acid residues are indicated at the axis. A) ORF1b of IBV-M42 and MHV-A59. B) ORF1b of MHV-A59 and BEV.

conserved (data not shown). No similarity has been observed in the other MHV and IBV non-structural proteins while the structural proteins only showed significant similarity in relative small regions (reviewed by 21). These data suggest a selective pressure against mutations in the second ORF of the polymerase gene.

Remarkably, a proportional dot matrix comparison of the deduced amino acid sequences of BEV and MHV-A59 ORF1b showed a clustered but significant similarity (Fig. 2B). Identical results were obtained when the predicted amino acid sequences of IBV and BEV were compared. The overall identity between BEV and either MHV or IBV is approximately 19%. No similarity between the structural proteins of BEV and coronaviruses has been identified (18, unpublished results).

Detailed analysis of the regions in the pol products of toro- and coronaviruses which exhibit significant similarity revealed that they could be separated into four domains (Fig. 3). Three of these domains (domain 1-3 of Fig. 3) have also been described recently for ORF1b of IBV (10). Domain 1 contains the "GDD" motif. However, in contrast to the amino acid sequence GDD, which is thought to be the core of this motif, both coronaviruses and BEV contain the amino acid sequence SDD at this position. Although occasionally a M, C, V or L residue has been reported in the position of the G residue (4), no serine residue has been reported. immediately upstream of the two conserved aspartic acid residues. Domain 2 is characterized by a stretch of amino acids which contain a large number of conserved cysteine residues and a few conserved histidines. It is therefore tempting to speculate that this domain forms a metal binding "finger" structure of the cys-his type which could interact with nucleic acid (22) as pointed out by Gorbalenya et al. (10). However, the model proposed by Gorbalenya et al. (10) requires modification since some of the cysteine and histidine residues involved in their proposed "finger" structure are not conserved between MHV and IBV. Domain 3 represent the "GKS/T" motif or NTP binding motif (5, 10). The fourth conserved region is located at the carboxyl terminus of the product encoded by ORF1b and is still unique for toro- and coronaviruses since no significant similarity with any other amino acid sequence has been discovered.

Domains 1 and 3 have also been reported as present in the replicase proteins of viruses belonging to the alphavirus- and picornavirus-like superfamilies (3), but as indicated in fig. 3, the positional identity between toro- and coronaviruses is much higher as compared to viruses belonging to one of the two supergroups. In addition, the GDD motif (domain 1) is located downstream of the GKS/T motif (domain 3) in viruses belonging to the alphavirus- or picornavirus-like superfamily (3).

Analysis of the ORF1a/ORF1b overlapping sequence

It has been shown that the nucleotide sequence of the ORF1a/ORF1b overlapping region of the pol gene of IBV is capable of inducing ribosomal frameshifting in vitro and in vivo (8, 9). A predicted, stable stem-loop structure and RNA pseudoknot (Fig. 4A) were shown to be essential for accurate translational frameshifting in IBV (9). Comparison of the nucleotide sequence of the ORF1a/ORF1b overlap of MHV-A59 and IBV-M42 revealed a well conserved stretch of nucleotides. A nearly identical stem-loop structure and pseudoknot can be predicted for the MHV ORF1a/ORF1b overlapping region. The significance of these proposed secondary and tertiary RNA structures is emphasized by the presence of co-variation as indicated in figure 4A. Mutations in one part of the stem or in the nucleotide sequence of the predicted loop are compensated by complementary mutations in either the stem or in the downstream sequence involved in the potential pseudoknot (Fig. 4A). In both IBV and MHV the stop codon for the translation of ORF1a is located in this hairpin structure.

1) BEV 514 CLIGVSK-Y-GLK--FSKFLKD-9-VF-GSDYTKCDRTFP-LSFR-37-LLNKPGGTSSGDA
 IBV 585 VVIGTTKFYGGWDMRLRLIQG-4-ILMGWDYPKCDRAMPNL-LR-41-IYVKPGGTSSGDA
 MHV 594 VVIGTTKFYGGWDDMLRLIKD-4-VLMGWDYPKCDRAMPNI-LR-41-YYVKPGGTSSGDA
 * * * *

TTAHSNTFYN-51-FLNFLSDDSFIF-34-KGHIEEFCSAH-II-KTDGEYHFLP--SRGRLL 744 BEV
 TTAYANSVFN-58-SLMILSDDGVVC-41-KGP-HEFCSQHTMLVEVDGDPKYLPPDPSPRIL 836 IBV
 TTAFANSVFN-58-SMMILSDDGVVC-41-KGP-HEFCSQHTMLVKMDGDEVLYLPPDPSPRIL 845 MHV
 * ** * ** * ** *

2) BEV 845 ANFDKVCFCPPNPAVSVCEECYVPLPLCAYCYVHVVISNHSKVEDKFKCFGGQDNIRELYI
 IBV 929 LQSCGVCVVCNSQTILRCGNCIRKPFLCCKCCYDHVMHTDHKNVLSINPYICSQLGCGE-AD
 MHV 938 LQSVGACVVCSSQTSRLRCGSCIRKPLLCCKCAYDHVMSTDHKYVLSVSPYVNCSPGC-DVND
 * * * * * *

V--LNNSICMYQCKNCVESDRLRI 928 BEV
 VIKLYLGGMYSYFCGNHKKPLSIPL 1013 IBV
 VTKLYLGGMSSYCEDHKKPQYSFKL 1022 MHV
 * *

3) BEV 1099 VMGPPGTGKTTTFVYD--TYLSKA
 IBV 1210 VQPPPGSGKSHFAIGLAVYFSSA
 MHV 1218 VQPPPGTGKSHLAIGLAVYYCTA
 * * * * *

-53-THNTLPFIKSAVLIADDEVSLI-15-VVLLGDPFQLSP-24-YLTACYRCPPQILSAFS-64-LGDVT-TI
 -56-TINALPEVSCDILLVDEVSM-16-VVYVGDPALPA-28-FLAKCYRCPEIVDTVS-75-LGLNVQTV
 -56-TINALPELVTDIIIVDEVSM-16-VVYIGDPAQLPA-29-FLGTCYRCPEIVDTVS-72-LGLQTV
 ** ** ** * *** *

DSSQG-19-VNRVIVGCSRS 1367 BEV
 DSSQG-19-INRFNVALTRA 1500 IBV
 DSAQG-19-VNRFNVAITRA 1506 MHV
 ** * **

4) BEV 1921 HVFSGDFTVEGTDIGGVHHVVAL-29-
 IBV 2274 HILYGEVDK--PQLGGLHTVIGM-38-
 MHV 2322 HVVYGSFNQ--KIIGGLHLLIGL-38-

TLVDVCANQLYEKVK- 8-SKVIFVNIDFQDVQFMVFANGEDDIQTFYP-23-LKNYGQNPFTMP-3-NFAKYT
 TVVDLLDDFLELLR-10-SKVVTVSIDYHSINFMTWFE-DGSIKTCYP-27-IPNYG-VGITLP-5-NVAKYT
 TVIDLDDDFVDIVK- 7-SKVNVNVNDFKDFQFMLWCN-EEKVMTFYP-29-LWNYG-KPITLP-5-NVAKYT

QICTFI QDHVKVARNALVWHLGAAGVDGCSPGDIVL-32-NLIVSDIY-16-LALGGTIVFKTTESS-17-FF
 QLCQYLSKTTICVPHNMRVMHFGAGSDKGVPAGSTVL-39-DLVIDSMY-39-LALGGSFAVKVTETS-17-FC
 QLCQYLSSTTTLAVPANMRVLHLGAGSDKGVPAGSAVL-39-DLIISDMY-28-LALGGSVAIKITEFS-17-FC

TAGVNTSSSEVFVV 2209 BEV
 TA-VNASSSEAFI 2598 IBV
 TN-VNASSSEGFLI 2641 MHV

Fig. 3. Localization (A) and amino acid sequences (B) of the conserved domains in ORF1b of the pol gene of BEV and the coronaviruses. The amino acids which are identical or conserved in these viruses are underlined. Domain 1; the "GDD" or polymerase motif, amino acids conserved according to (10) are indicated by asterisks. Domain 2; the cys-his motif, conserved cysteine and histidine residues are marked with asterisks. Domain 3; the "GKS/T" or helicase motif, asterisks indicate the amino acids conserved according to (5). Domain 4; the carboxyl terminal motif.

Figure 5. Identification of a 33-kDa protein encoded by the pol gene of MHV-A59. A) Lane 1, pWAP1 RNA *in vitro* translation products; pWAP1 translation products immunoprecipitated with antiserum (lane 2) and pre-serum (lane 3). B) Immunoprecipitation with the anti-peptide serum on lysates of MHV-A59 infected (lane 1) or mock infected (lane 2) Sac(-) cells. Lane 3 and 4 immunoprecipitations using pre-serum and lysates of infected and mock infected cells, respectively (exposure 7 days. C) Enlargement of the section containing proteins exceeding 200-kDa the autoradiograph shown in section B (exposure 3 days).

sequence has also been shown to function as a site for ribosomal frameshifting in Rous sarcoma virus (23). Recently we have demonstrated that the ORF1a/ORF1b overlapping regions of both MHV and BEV are able to direct ribosomal frameshifting *in vitro* as well as *in vivo* (data not shown). The efficiency of this translational frameshifting was 40% and 25% for MHV and BEV, respectively.

As in retroviruses the BEV and coronaviral polymerase is probably produced as a fusion protein. Based on amino acid sequence analysis it is postulated that domain 1 of ORF1b (fig. 3), which is located downstream of the ribosomal frameshifting sequence, is the core domain of the replicase

Tabel 1. Predicted 3C protease-like cleavage sites in the carboxyl terminal region of ORF1b of the pol genes of IBV (10), MHV-A59 and BEV.

Virus	predicted cleavage site	position Q residue in the sequence
IBV	TCYPQLQ/SAWLGGY	2389
MHV	MTFYPLQ/AAADWKP	2434
BEV	NGEDDIQ/TFYPQKD	2021

of both corona- and toroviruses. It is tempting to speculate that the fusion protein is the functional polymerase, cleavage of this functional polyprotein will result in an inactive polymerase protein. This would explain the observed requirements for continuous de novo protein synthesis (24, 25).

A 33-kDa cleavage product from the putative MHV polymerase contains conserved domain 4.

To test the specificity of the antiserum raised against a peptide representing the carboxyl terminus encoded by ORF1b of MHV-A59, in vitro translation products of pWAP1 RNA were used in immunoprecipitations. Direct analysis of the in vitro translation products showed a large number of different sized proteins (Fig. 5A, lane 1). Most of these products probably result from premature termination of translation since only two proteins of the expected size (60-kDa and 62-kDa) were precipitated by the antiserum (Fig. 5A, lane 2). None of the translation products were recognized by the preimmune serum (Fig. 5A, lane 3). This antiserum was used to identify virus encoded protein(s) in infected cells and immunoprecipitated a 33-kDa protein from lysates of MHV-A59 infected cells (Fig. 5B, lane 1). This 33-kDa protein was not detected in mock infected cells (Fig. 5B, lane 2), or in similar immunoprecipitations using the preimmune serum (Fig. 5B, lane 3 and 4). Based on its size the 33-kDa protein should contain the complete fourth conserved domain. Enlargement and careful inspection of the upper region of the gel revealed several proteins with a high molecular weight which were only precipitated by the antisera from lysates of the infected cells (Fig. 5C). These observed products are likely to be precursors of the 33-kDa protein.

Recently Gorbalenya et al. (10) have described a putative protease domain in ORF1a of IBV which exhibits similarity to the well conserved 3C protease of picornaviruses. Based on the cleavage site of the picornavirus 3C proteases (26) potential cleavage sites in the pol products encoded by ORF1a and ORF1b of IBV were postulated. Inspection of the region in MHV ORF1b encoding the 33-kDa protein revealed that a potential 3C protease-like cleavage site present in a nearly identical position in MHV (tabel 1). If this cleavage site is used in MHV a 33-kDa carboxyl terminal protein is predicted from the sequence. In addition an other potential 3C protease-like cleavage site can be found in the amino acid sequence of this region in ORF1b of BEV (tabel 1). Usage of this cleavage site in BEV will result in 31-kDa protein containing the conserved domain 4.

CONCLUDING REMARKS

Recent studies on the molecular biology of corona- and toroviruses have revealed several unique similarities. Members of both virus families express their genetic information from a nested set of multiple subgenomic mRNAs. Sequence analysis and in vitro translation studies clearly demonstrate that they have an identical gene order: 5'POLYMERASE-PEPLOMER-MEMBRANE PROTEIN-NUCLEOCAPSID PROTEIN-3'. These similarities already indicate a possible evolutionary relationship between toro- and corona

viruses. The data presented in this paper on the organization and expression of the pol gene and the significant level in the similarity in the products encoded by ORF1b suggest that these viruses are ancestrally more related to each other than to other families of positive stranded RNA viruses. If in the near future the toroviruses are classified as a new family of positive stranded RNA viruses the toro- and coronaviruses would then form a third superfamily of positive stranded RNA viruses.

ACKNOWLEDGMENTS

The help of Willem Luytjes during the initial stages of this work is greatly acknowledged. P. B. was supported by the Dutch Foundation for Chemical Research with financial aid from the Dutch Organization for the Advancement of Pure Research (project number 331-025). E. S. was supported by the Division for Health Research TNO in cooperation with the Foundation for Medical Research MEDIGON (project number 900-502-081).

REFERENCES

1. Strauss, J.H., and Strauss, E.G. (1983). *Curr. Top. Microbiol. Immunol.* 105, 1-98.
2. Goldbach, R., and Wellink, J. (1988). *Intervirology* 29, 260-267.
3. Strauss, J.H., and Strauss, E.G. (1988). *Ann. Rev. Microbiol.* 42, 657-683.
4. Argos, P. (1988). *Nucl. Acids Res.* 16, 9909-9916.
5. Hodgman, T.C. (1988). *Nature* 335, 22-23.
6. Gorbalenya, A.E., and Koonin, E.V., Donchenko, A.P., and Blinov, V.M. (1988). *FEBS LETT.* 235, 16-24.
7. Boursnell, M.E., Brown, T.D., Foulds, I.J., Green, P.F., Tomley, F.M., and Binns, M.M. (1987). *J. Gen. Virol.* 68, 57-77.
8. Brierley, I., Boursnell, M.E., Binns, M.M., Bilimoria, B., Blok, V.C., Brown, T.D. and Inglis, S.C. (1987). *EMBO. J.* 6, 3779-3785.
9. Brierley, I., Diggard, P. and Inglis, S.C. (1989). *Cell* 57, 537-547.
10. Gorbalenya, A.E., and Koonin, E.V., Donchenko, A.P., and Blinov, V.M. (1989). *Nucl. Acids Res.* 12, 4847-4861.
11. Van Berlo, M.F., Horzinek, M.C., Van der Zeijst, B.A.M. (1982). *Virology* 118, 345-352.
12. Horzinek, M.C. and Weiss, M. (1984). *Zbl. Vet. Med. B.* 31, 649-659.
13. Snijder, E.J., Ederveen, J., Spaan, W.J.M., Weiss, M. and Horzinek, M.C. (1988). *J. Gen. Virol.* 69, 2135-2144.
14. Snijder, E.J., Horzinek, M.C. and Spaan, W.J.M. *J. Virol.* in press.
15. Staden, R. (1986). *Nucl. Acids Res.* 14, 217-233.
16. Lipman, D.J. and Pearson, W.R. (1985). *Science* 227, 1435-1441.
17. Luytjes, W., Bredenbeek, P.J., Noten, A.F.H., Horzinek, M.C., and Spaan, W.J.M. (1988). *Virology* 166, 415-422.
18. Snijder, E.J., Den Boon, J.A., Spaan, W.J.M., Verjans, G.M.G.M. and Horzinek, M.C. *J. Gen. Virol.* in press.
19. De Groot, R.J., Ter Haar, R.J., Horzinek, M.C. and Van der Zeijst, B.A.M. (1987). *J. Gen. Virol.* 68, 995-1002.
20. Pachuk, C.J., Bredenbeek, P.J., Zoltick, P.W., Spaan, W.J.M., and Weiss, S.R. (1989). *Virology* 71, 141-148.
21. Spaan, W.J.M., Cavanagh, D., and Horzinek, M.C. (1988). *J. Gen. Virol.* 69, 2939-2952.
22. Klug, A. and Rhodes, D. (1987). *TIBS* 12, 464-468.
23. Jacks, T., Madhani, D.H., Masiarz, F.R., and Varmus H.E. (1988). *Cell* 55, 449-458.
24. Sawicki, S.G., and Sawicki, D.L. (1986). *J. Virol.* 57, 328-334.
25. Compton, S.R., Rogers, D.B., Holmes, K.V., Fertsch, D., Remenick, J., and McGowan, J.J. (1987). *J. Virol.* 61, 1814-1820.
26. Kräusslich, H.-G. and Wimmer, E. (1988). *Ann. Rev. Biochem.* 57, 701-754.