PROTEOLYTIC PROCESSING OF THE ARTERIVIRUS REPLICASE

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INTRODUCTION

Arteriviruses are enveloped positive-stranded RNA viruses which belong to the so-called 'coronaviruslike superfamily'^{1,2,3,4}. At present, the arterivirus group is comprised of equine arteritis virus (EAV, the prototype of the group), lactate dehydrogenase-elevating virus (LDV), porcine reproductive and respiratory syndrome virus (PRRSV, also known as 'Lelystad virus'), and simian haemorrhagic fever virus (SHFV). Their isometric nucleocapsid core contains a nonsegmented genome of 12.7-15.1 kb^{1,5,6}. The morphological characteristics and genome size of EAV are most comparable to those of togaviruses and flaviviruses. However, the arterivirus replication strategy is similar to that of coronaviruses, which possess 25-31 kb positive-stranded RNA genomes. Among their common features are the polycistronic genome organization, the same basic gene order (5'-replicase gene-envelope protein genes-nucleocapsid protein gene-3'), and the production of a 3'-coterminal nested set of 4 to 7 subgenomic mRNAs. The 5' part of the genomes of these viruses is occupied by two large open reading frames (ORF1a and ORF1b) which encode the viral replicase^{1,5-12}. Both ORF1a and ORF1b are expressed from the genomic RNA, the latter by means of ribosomal frameshifting^{1,13}. The ORF1b products of various members of the coronaviruslike group contain a number of homologous domains^{1,6,9} which indicate that the replicase genes of these viruses are evolutionarily related (Fig. 1).

The large coronaviruslike replicase gene product (345K-420K in arteriviruses, 740K-810K in coronaviruses) is a multidomain precursor which is posttranslationally cleaved into smaller functional units. Sequence comparison revealed the presence of multiple putative protease domains in the ORF1a polyproteins of both arteriviruses and coronaviruses^{1,5,6,10,14}. One of the coronavirus proteases, responsible for the liberation of the 28K N-terminal replicase product of mouse hepatitis virus (MHV), has

Figure 1. Location of conserved and possibly conserved domains in the replicase proteins of various members of the coronaviruslike superfamily. Abbreviations: PCP, papainlike cysteine protease; 3CL-CP, 3C-like cysteine protease; POL, polymerase motif; HEL, helicase motif; CTD, conserved C-terminal ORF1b domain; CP, cysteine protease; SP, serine protease; IBV, infectious bronchitis virus; HCV, human coronavirus 229E; BEV, Berne torovirus; other virus abbreviations: see text.

recently been characterized¹⁵. Furthermore, the identification of a number of (putative) coronavirus replicase cleavage products has been reported¹⁶⁻¹⁹. In the case of the arteriviruses, detailed information on the posttranslational processing of the ORF1a protein has now been obtained. Six proteolytic cleavages and four different viral proteases, all residing within the ORF1a protein, have been documented. The various arterivirus protease domains have been characterized in considerable detail. In this brief review, we will summarize our current knowledge of the complex proteolytic processing of the arterivirus replicase ORF1a protein.

PROCESSING SCHEME OF THE EAV ORF1A PROTEIN

The production of specific antisera, directed against various parts of the EAV ORF1a protein, has enabled us to study its proteolytic processing in infected cells and transient expression systems²⁰. Western blotting, immunoprecipitation, and pulse-chase experiments revealed that the EAV ORF1a protein is subject to (at least) five proteolytic cleavages. These generate processing products of 29K, 61K, 22K, 31K, 41K, and 3K, which were named nonstructural protein (nsp) 1 through 6, respectively²⁰ (Fig. 2). Furthermore, a number of intermediate processing products was detected, of which only the smallest three have been identified so far: nsp3456, nsp34, and nsp56 (Fig. 2). After the relatively rapid cleavage of the nsp1/2, nsp2/3, and nsp4/5 junctions, the final processing steps (at the nsp3/4 and nsp5/6 sites) were found to be extremely slow²⁰ (Fig. 2). This suggests that, as in other viral systems, processing intermediates fulfil specific functions in the arterivirus replication cycle.

THE ARTERIVIRUS PAPAINLIKE CYSTEINE PROTEASES

Due to its activity in an *in vitro* translation assay, the papainlike cysteine protease in EAV nsp1, which had been predicted by comparative sequence analysis¹ (Fig. 3), could be identified and characterized before ORF1a protein-specific antisera had been obtained²¹. Residues Cys-164 and His-230 were confirmed as members of the putative catalytic dyad of the EAV nsp1 protease, which was shown to cleave the nsp1/2 site extremely rapidly and exclusively in *cis*²¹. A Gly-Gly dipeptide at position 260-261 was identified as the nsp1/2 cleavage site²¹. The involvement of the nsp1 protease in the nsp1/2 cleavage was recently

Figure 2. (A) SDS-PAGE of samples from a pulse-chase analysis of EAV ORF1a protein processing in virus-infected cells. A 15 minutes pulse-labeling was followed by chase periods of 0-180 minutes. The lane indicated with TOT shows the results of a continuous 3 hour labeling. Cells were lysed and viral nsps were immunoprecipitated using a mixture of ORF1a-protein specific antisera²⁰. (B) Schematic representation of EAV ORF1a protein processing. The location of the six EAV nsps and the order of proteolytic cleavages is shown. The three EAV proteases and their (putative) relation to cleavage sites are indicated and will be discussed below.

confirmed in an *in vivo* expression system²⁰. The nsp1 protease appears to have no other function in replicase processing than the liberation of the N-terminal ORF1a product. Therefore, it can be added to a rapidly growing family of so-called 'leader' proteases^{22,23}, which is comprised of papainlike autoproteases encountered in various positive-stranded RNA virus groups.

In contrast to the C-terminal half of the ORF1a protein and the ORF1b protein, the N-terminal part of the arterivirus ORF1a product is relatively variable, both in size and in sequence^{6,23}. Comparative sequence analysis (Fig. 3) and the data obtained with the EAV nsp1 protease suggested that both PRRSV and LDV might contain an additional PCP domain in the N-terminus of the ORF1a protein. Recent experiments have revealed that this is indeed the case²³. PRRSV and LDV each contain two PCP domains, provisionally named PCP α and PCP β , each of which mediates an autoproteolytic cleavage at its own C-terminus, just downstream of the active site His residue. This means that, compared with EAV, these viruses both produce an additional ORF1a protein cleavage product. To avoid differences in nomenclature of highly conserved downstream ORF1a-encoded nsps, we have named the two N-terminal products of PRRSV and LDV nsp1 α and nsp1 β (Fig. 5). The sizes of these proteins differ slightly between PRRSV (20K and 27K) and LDV (22K and 26K). Both pairs of predicted active site Cys residues (Cys-76 and Cys-276 in PRRSV, Cys-76 and Cys-269 in LDV) were subjected to site-directed mutagenesis and were shown to be essential for proteolytic activity of a PCP domain²³. Furthermore, the probable catalytic His residues of the PRRSV PCP α and PCP β proteases were identified (His-146 and His-345, respectively). Sequence alignments indicated that the PCP β domain corresponds to the EAV nsp1 PCP domain. An equivalent of the PCP α domain may have been present in EAV²³ (Fig. 3).

THE NSP2 CYSTEINE PROTEASE

Only the EAV nsp1 protease was found to be active in an *in vitro* translation system²¹ We therefore continued our processing analysis using polyclonal rabbit antisera, EAV-infected cells, and an *in vivo* expression system The latter was based on vaccinia virus recombinant vTF7-3, which produces the T7 RNA polymerase²⁴, and transfection of T7 expression plasmids containing (parts of) EAV ORF1a²⁰

The analysis of both deletions and site-directed mutations in the EAV ORF1a protein indicated that, in addition to the nsp1 and nsp4 proteases, a third protease was involved in its processing When the nsp1 and nsp4 sequences were deleted from the polyprotein²⁵, or when the active site residues Cys-164 and Ser-1184 of the nsp1 and nsp4 proteases, respectively, were mutated²⁵, the processing of the nsp2/3 cleavage site was not affected Our deletion mutagenesis indicated that, if viral, the protease responsible for cleavage of the nsp2/3 junction should be located within nsp2 or nsp3 Although we have not yet been able to completely exclude the involvement of a host protease in the nsp2/3 cleavage, the data available at this moment are most consistent with a proteolytic function for the conserved N-terminus of nsp2 This 100 amino acid domain contains the only nsp2/nsp3 conserved histidine (His-332 in EAV), a residue which is involved in catalysis in most types of proteases A number of additional observations, including the results of site-directed mutagenesis, suggest that Cys-270 and His-332 (Fig 3) may be active site residues of a proteolytic enzyme which represents a novel group of cysteine proteases However, our studies of the nsp2 protease are hampered by the fact that processing of the nsp2/3 junction appears to be very sensitive to changes in nsp2 Large or small deletions in the region which separates the putative protease and its cleavage site all abolished cleavage of the nsp2/3 site

the arterivirus nsp1 proteases

			*			*
papain		qgscgsCwafsa	127	kvdHavaavgyn		
		*		*		
LDV	PCPa	crPgGmCWLss1	62	navHvSdesFPG		
PRRSV	PCPa	ctPsGcCWLsav	61	nsmHvSdqpFPG		
EAV	ex-PCP?	pVpVgHkfl1gw	40	stdHaSakrFPG		
		*		*		
LDV	PCPB	dtkfkskCWek1f	62	y1rHvsragepv		
PRRSV	PCPB	dvfdgkCW1scf	60	w1rH1t-ldddv		
EAV	PCP	qeodgfCW1k11	67	rawH1ttrscK1		
		*		*		

the arterivirus nsp2 proteases

			*			*
LDV	CP	gYsPPgDgaCG1hc1sAm1N	42	CPsA1Yk1dcvnqHWtV	11	LapdC1rGvC
PRRSV	CP	tYsPPtDGsCGwhv1aA1mN	44	CPnAkY11k1ngvHWvV	10	LsreCvvGvC
EAV	CP	gYnPPgDgaCGYrc1-AfmN	39	CPnAkYamicdkqHWrV	9	LdesCfrG1C
		*		*		

the arterivirus nsp4 proteases

			*			*
trypsin		wVvsAaHcy	39	1t1nnD1	86	scmGDSGgp-----vvcsgklqG1vswgsg
		*		*		*
LDV	SP	vVvTAsH11	17	FKcaGDy	45	TkcGDSSGSp 9 G1HTGSNkrGsgmVTTh
PRRSV	SP	tVvTAaHv1	17	FKtnGDy	46	TncGDSSGSp 9 G1HTGSNK1Gsg1VTTp
EAV	SP	vV1TAsHvv	18	FKknGDf	48	TtsGDSSGsa 8 GvHTGSNTsGvayVTTp
		*		*		*

Figure 3. Comparative sequence analysis of the four arterivirus protease domains Absolutely conserved amino acid residues are shown in capitals Putative active site residues are indicated with asterisks For comparison, the cellular proteases papain and trypsin have been included in the alignment of the nsp1 and nsp4 proteases, respectively

This was also the case when three additional conserved Cys residues in the N-terminal domain of nsp2 were substituted. On the other hand, a 10K heterologous insertion in the central part of nsp2 was tolerated²⁰ and a separately expressed putative protease domain was shown to be able to induce cleavage of the nsp2/3 site in trans²⁵. It appears that a specific folding or posttranslational organization of the nsp2-nsp3 complex is required for proteolysis of the nsp2/3 site. Deleterious effects on the organization of the protease domain and/or the cleavage site region may explain the results obtained with many of our nsp2 mutants carrying amino acid substitutions or deletions.

Both the presence of nsp2 and the cleavage of the nsp2/3 junction appear to be extremely important for processing of the arterivirus ORF1a protein. Preliminary observations from ORF1a *in vivo* expression experiments indicate that the nsp4 protease is inactive when nsp2 is absent or when nsp2 contains mutations which abolish cleavage at the nsp2/3 junction. Under these conditions, only the nsp1 protease is able to function normally²⁶. To increase our understanding of the role of nsp2 in ORF1a protein processing, we are currently studying two properties of the nsp2-nsp3 region which may be related to its structure. First, the conserved nsp2 N-terminus, the conserved nsp2 C-terminus, and nsp3 each contain a cluster of conserved cysteine residues²⁰, suggesting that disulfide bridges may be involved in the organization of this part of the ORF1a protein. Second, immunofluorescence experiments have revealed that, in infected cells, nsp2 is associated with intracellular membranes²⁶.

THE NSP4 SERINE PROTEASE

Comparative sequence analysis produced a convincing alignment of the arterivirus nsp4 region and the proteases that belong to the superfamily of chymotrypsinlike serine proteases and 3C-like cysteine proteases^{1,6,27,28}. A putative catalytic triad of His, Asp and Ser was identified. For EAV, this prediction is now supported by the results from site-directed mutagenesis of two of these putative active site residues, His-1103 and Ser-1184. Their replacement abolishes the processing of the nsp3456 region, but does not affect the production of nsp1 and nsp2²⁶. Together with data obtained from the mutagenesis of putative SP cleavage sites, these results indicate that the nsp3/4, nsp4/5, and nsp5/6 cleavages are all mediated by the nsp4 protease. Since the SP is able to cleave multiple cleavage sites, albeit with different kinetics (Fig. 2), it is the most likely candidate protease to be involved in the processing of the ORF1b product. The coronavirus 3C-like cysteine protease, which is located at a comparable relative position in the replicase, has recently been shown to be involved in processing of the coronavirus ORF1b protein¹⁹.

CLEAVAGE SITES

Six different proteolytic cleavages have now been documented in the various arterivirus ORF1a proteins (Fig. 5). However, only one cleavage site has been determined by protein sequence analysis, the nsp1/2 site between Gly-260 and Gly-261 in EAV²¹. For the corresponding PRRSV and LDV nsp1 β /2 sites, processing is predicted to occur at a Tyr-Gly dipeptide, which is present in both viruses and which can be aligned with the EAV nsp1/2 cleavage site²³ (Fig. 4). This prediction is supported by the sizes which have been observed for the PRRSV and LDV cleavage products after *in vitro* translation of cDNA constructs²³. A suitable cleavage site candidate for the nsp1 α /1 β site in PRRSV and LDV has not been identified, although a quite accurate estimate of its location was obtained from the same *in vitro* translation experiments²³.

	<i>nsp1</i> ▼ <i>nsp2</i>		<i>nsp2</i> ▼ <i>nsp3</i>		
LDV	fqtrkyY GYsPPgD	LDV	glrfvsG GqIadv		
PRRSV	fgahkwY Gaagkra	PRRSV	istkttG Gasytla		
EAV	lpagnyG GYnPPgD	EAV	pgfrliG Gwiygic		
	<i>nsp3</i> ▼ <i>nsp4</i>		<i>nsp4</i> ▼ <i>nsp5</i>	<i>nsp5</i> ▼ <i>nsp6</i>	
LDV	nfgsvlE Gslrtrg	LDV	eslpalE Galssmq	LDV	igesdlE Aerltvd
PRRSV	avgsllE Gaftrhk	PRRSV	asvpvE Gglstvtq	PRRSV	kpdnc1E AaklslE
EAV	eggmvfE Glfrspk	EAV	dglsnrE Sslsgpq	EAV	lgkgsyE Glldqkv

Figure 4. Comparison of possible cleavage sites in arterivirus ORF1a proteins.

The expression of an *nsp2* C-terminal deletion mutant²⁵ revealed that the EAV *nsp2/3* site should be situated near amino acid 825 of the ORF1a protein. A Gly-Gly dipeptide (residues 831-832) appears to be the most attractive candidate (Fig. 4): it is conserved in PRRSV and LDV and it is followed directly by a very hydrophobic domain, which would in this case be the N-terminus of *nsp3*.

Cleavage site predictions for the arterivirus serine protease were already published before the proteolytic activity of *nsp4* and the processing map of the ORF1a protein had been addressed experimentally⁶. On basis of the putative relationship of the *nsp4* protease with the so-called 3C-like cysteine protease group, a set of conserved cleavage sites was proposed which is characterized by a Glu (or Asp) at the P1 position, a Gly (or Ser) at the P1' position, and a bulky hydrophobic residue (e.g. Val, Phe, or Leu) at the P3' position. For the EAV ORF1a protein, SP-directed cleavages at the C-terminal side of aa 1064 (Glu/Gly), 1268 (Glu/Ser), and 1430 (Glu/Gly) were predicted (Fig. 4). We have recently substituted the Glu residues at each of these sites with Pro. Subsequently, these mutations were tested in an ORF1a cDNA construct which was expressed *in vivo*. Replacement of Glu-1064 abolished cleavage of the *nsp3/4* junction²⁶. Since this position in the ORF1a protein sequence also corresponds well with the estimated sizes of *nsp3* and *nsp4*, this residue may indeed be located at (or near) the *nsp3/4* cleavage site. This preliminary identification of the *nsp3/4* junction is supported by mutagenesis of another conserved Glu residue, located at position 1677 in EAV. When this Glu, which is followed by Gly in EAV and Ala in PRRSV/LDV, was replaced by Pro, the EAV *nsp5/6* site was no longer processed²⁶. The two putative candidates for the *nsp4/5* cleavage site are more problematic: the estimated N-terminus of *nsp5* (44K from the C-terminus of the ORF1a protein) is in the middle between the two predicted cleavage sites at residues 1268 and 1430, which are 53K and 32K from the ORF1a C-terminus, respectively. A Glu1430→Pro substitution did not have an effect on the generation of any of the currently known ORF1a protein cleavage products²⁶. On the other hand, the Glu1268→Pro replacement completely abolished processing of the *nsp4/5* junction²⁶. If the *nsp4/5* site would indeed be located at residue 1268, the sizes of both *nsp4* and *nsp56* would differ considerably from the size values estimated from SDS-PAGE: *nsp4* would have to be 31K instead of 22K, *nsp56* would not be 44K but 53K. Since the Glu1268→Pro substitution also abolished cleavage at the *nsp34* junction, this mutation, which is relatively close to the SP active site, may affect the structure of the protease rather than the *nsp4/5* cleavage site. Additional experiments, which will also include possible cleavage sites in the ORF1b protein, are now in progress.

ARTERIVIRUS ORF1A PROCESSING SCHEMES AND PRODUCTS

Our current knowledge of the proteolytic processing of the arterivirus ORF1a protein, and the viral proteases involved in it, is summarized in Fig. 5. The three available arterivirus

Figure 5. Comparison of the (partially putative) proteolytic processing schemes of the EAV, LDV and PRRSV replicase ORF1a proteins. The position of the four arterivirus proteases and the approximate location of cleavage sites are indicated.

replicase sequences are highly similar in the region which covers the C-terminus of nsp2, nsp3 through 6, and the ORF1b product^{6,23}. We therefore expect that in this part of the replicase the proteolytic processing will be identical or largely similar for EAV, LDV, and PRRSV. The processing of one of the two relatively variable regions, the N-terminal domain of the ORF1a protein containing PCP α , has now been analyzed²³. This leaves the variable central region of nsp2 to be studied. The high sequence similarity between the three arteriviruses abruptly ends downstream of the putative nsp2 cysteine protease domain. It is restored about 250 amino acids downstream in EAV, with a domain that contains a cluster of six invariant cysteines. However, the size (600 and 730 amino acids in LDV and PRRSV, respectively) and the sequence of the interlying central region of nsp2 are variable^{6,20}. The presence of a hydrophobic domain appears to be the only common property. Obviously, we cannot exclude that the proteolytic processing of this region of the PRRSV/LDV ORF1a proteins differs from that of EAV.

The presence of hydrophobic domains at both sides of the putative main protease, either the SP domain or the 3C-like protease, is a property shared by arteriviruses and coronaviruses. Almost the entire arterivirus nsp3 sequence is hydrophobic. The only hydrophobic domain contains a cluster of conserved cysteine residues. Depending on the exact location of the nsp4/5 cleavage site (see above), a hydrophobic region downstream of the SP motif could form a C-terminal hydrophobic domain of nsp4 or an N-terminal hydrophobic region of nsp5. A more downstream hydrophobic part between amino acids 1420 and 1550 in EAV, which is again conserved in PRRSV and LDV, should definitely be within nsp5. Little can be said about the small nsp6. The extreme C terminus of the ORF1a protein is not very conserved. Although nsp6 itself may be a functional protein, the slow nsp5/6 cleavage could also serve to liberate the ORF1b-encoded proteins from the ORF1ab protein which is produced by ribosomal frameshifting¹.

CONCLUDING REMARKS

Our analysis of the processing of the arterivirus replicase has revealed the involvement of (at least) four viral proteases, an unprecedented number among positive-stranded

RNA viruses Three of these appear to be autoproteases, mediating only the liberation of nsp1 α , nsp1 β (or nsp1 in EAV), and nsp2 from the rest of the replicase polyprotein Although a detailed processing map of the coronavirus ORF1a protein is not yet available, a general pattern for coronaviruses and arteriviruses seems to emerge The functions encoded from the central region of ORF1a to the 3'-end of ORF1b appear to be the 'core' of the coronaviruslike replicase polyprotein the well-conserved domains (protease - polymerase - helicase - C-terminal 'unique' ORF1b domain) are within this area, and only small insertions and deletions in this part of the replicase can be detected within the coronavirus or arterivirus groups In comparable positions, the ORF1a proteins of both virus groups contain a domain belonging to the protease superfamily that comprises the chymotrypsin-like and picornavirus 3C-like proteolytic enzymes Although the predicted catalytic nucleophile of the coronavirus protease (Cys) differs from that in the arterivirus proteases (Ser), this domain may still be a remnant from a common ancestor of both virus groups The exchange of Cys for Ser at the active site of the enzyme is considered to be feasible^{27 28} Furthermore, the predicted cleavage site sequences are remarkably similar for both virus groups

The N-terminal half of the ORF1a protein, on the other hand, is quite variable, suggesting that species-specific, rather than group-specific, functions have been added to the set of basic functions provided by the 'core' replicase It seems that in both corona- and arteriviruses multiple cysteine autoproteases mediate the processing of this least conserved part of the coronaviruslike replicase

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REFERENCES

- 1 Den Boon, J A , Snijder E J , Chirnside, E D , de Vries, A A F , Horzinek, M C , Spaan, W J M Equine arteritis virus is not a togavirus but belongs to the coronaviruslike superfamily *J Virol* 1991,65 2910-2920
- 2 Snijder, E J , Horzinek, M C , Spaan, W J M The coronaviruslike superfamily *Adv Exp Med Biol* 1993,342 235-244
- 3 Snijder, E J , Horzinek, M C Toroviruses replication, evolution and comparison with other members of the coronaviruslike superfamily *J Gen Virol* 1993,74 2305-2316
- 4 Plagemann, P G W , Moennig, V Lactate dehydrogenase-elevating virus, equine arteritis virus, and simian haemorrhagic fever virus a new group of positive-stranded RNA viruses *Adv Virus Res* 1991,91 99-192
- 5 Meulenberg, J J M , Hulst, M M , de Meijer, E J , Moonen, P L J M , den Besten, A , de Kluyver, E P , Wensvoort, G , Moormann, R J M Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV *Virology* 1993,192 62-72
- 6 Godeny, E K , Chen, L , Kumar, S N , Methven, S L , Koonin, E V , Brinton, M A Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus *Virology* 1993,194 585-596

- 7 Bournsnel, M E G , Brown, T D K , Foulds, I J , Green, P F , Tomley, F M , Binns, M M Completion of the sequence of the genome of the coronavirus avian infectious bronchitis virus J Gen Virol 1987,68 57-77
- 8 Bredenbeek, P J , Pachuk, C J , Noten, J F H , Charite, J , Luytjes, W , Weiss, S R , Spaan, W J M The primary structure and expression of the second open reading frame of the polymerase gene of the coronavirus MHV-A59 Nucleic Acids Res 1990,18 1825-1832
- 9 Snijder, E J , den Boon, J A , Bredenbeek, P J , Horzinek, M C , Rijnbrand, R , Spaan, W J M The carboxyl-terminal part of the putative Berne virus polymerase is expressed by ribosomal frameshifting and contains sequence motifs which indicate that toro- and coronaviruses are evolutionary related Nucleic Acids Res 1990,18 4535-4542
- 10 Lee, H J , Shieh, C K , Gorbalenya, A E , Koonin, E V , la Monica, N , Tuler, J , Bagdzhadzhyan, A , Lai, M M C The complete sequence (22 kilobases) of murine coronavirus gene 1 encoding the putative protease and RNA polymerase Virology 1991,180 567-582
- 11 Herold, J , Raabe, T , Schelle-Prinz, B , Siddell, S G Nucleotide sequence of the human coronavirus 229E RNA polymerase locus Virology 1993,195 680-691
- 12 Bonilla, P J , Gorbalenya, A E , Weiss, S R Mouse hepatitis virus strain A59 RNA polymerase gene ORF1a heterogeneity among MHV strains Virology 1994,198 736-740
- 13 Brierley, I , Diggard, P , Inglis, S C Characterization of an efficient coronavirus ribosomal frameshifting signal requirement for an RNA pseudoknot Cell 1989,57 537-547
- 14 Gorbalenya, A E , Koonin, E V , Donchenko, A P , Blinov, V M Coronavirus genome prediction of putative functional domains in the non-structural polyprotein by comparative amino acid sequence analysis Nucleic Acids Res 1989,17 4847-4861
- 15 Baker, S C , Shieh, C K , Soe, L H , Chang, M F , Vannier, D M , Lai, M M C Identification of a domain required for autoproteolytic cleavage of murine coronavirus gene A polyprotein J Virol 1989,63 3693-3699
- 16 Brierley, I , Bournsnel, M E G , Binns, M M , Bilmoria, B , Rolley, N J , Brown, T D K , Inglis, S C Products of the polymerase-encoding region of the coronavirus IBV Adv Exp Med Biol 1990,276 275-278
- 17 Denison, M R , Zoltick, P W , Leibowitz, J L , Pachuk, C J , Weiss, S R Identification of polypeptides encoded in open reading frame 1b of the putative polymerase gene of the murine coronavirus mouse hepatitis virus A59 J Virol 1991,65 3067-3082
- 18 Denison, M R , Zoltick, P W , Hughes, S A , Giangreco, B , Olson, A L , Perlman, S , Leibowitz, J L , and Weiss, S R Intracellular processing of the N-terminal ORF1a proteins of the coronavirus MHV-A59 requires multiple proteolytic events Virology 1992,189 274-284
- 19 Liu, D X , Brierley, I , Tibbles, K W , Brown, T D K A 100-kilodalton polypeptide encoded by open reading frame (ORF) 1b of the coronavirus infectious bronchitis virus is processed by ORF 1a products J Virol 1994,68 5772-5780
- 20 Snijder, E J , Wassenaar, A L M , Spaan, W J M Proteolytic processing of the replicase ORF1a protein of equine arteritis virus J Virol 1994,68 5755-5764
- 21 Snijder, E J , Wassenaar, A L M , Spaan, W J M The 5' end of the equine arteritis virus replicase gene encodes a papainlike cysteine protease J Virol 1992,66 7040-7048
- 22 Gorbalenya, A E , Koonin, E V , Lai, M M C Putative papain-related thiol proteases of positive-strand RNA viruses FEBS Lett 1991,288 201-205
- 23 den Boon, J A , Faaberg, K S , Meulenberg, J J M , Wassenaar, A L M , Plegmann, P G W , Gorbalenya, A E , Snijder, E J Processing of the N-terminal region of the arterivirus replicase ORF1a protein identification of a second papainlike cysteine protease Submitted
- 24 Fuerst, T R , Niles, E G , Studier, F W , Moss, B Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase Proc Natl Acad Sci USA 1986,83 8122-8126
- 25 Snijder, E J , Wassenaar, A L M , Spaan, W J M , Gorbalenya, A E The arterivirus nsp2 protease In preparation
- 26 Snijder, E J , Wassenaar, A L M Unpublished observations
- 27 Gorbalenya, A E , Donchenko, A P , Blinov, V M , Koonin, E V Cysteine proteases of positive strand RNA viruses and chymotrypsin-like serine proteases a distinct protein superfamily with a common structural fold FEBS Lett 1989,243 103-114
- 28 Bazan, J F , Fletterick, R J Viral cysteine proteases are homologous to the trypsin-like family of serine proteases structural and functional implications Proc Natl Acad Sci USA 1988,85 7872-7876