J. gen. Virol. (1983), **64**, 761–776. *Printed in Great Britain*

Key words: *Coronaviruses/structure/replication/pathogenesis*

REVIEW ARTICLE

The Biology of Coronaviruses

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Introduction

The Coronaviridae is a monogeneric family comprising 11 viruses which infect vertebrates. Members of the group are responsible for diseases of clinical and economic importance, in particular respiratory and gastrointestinal disorders (Table 1). The group was originally recognized on the basis of a characteristic virion morphology (Tyrrell *et al.*, 1968), but can now be defined by biological and molecular criteria. Various aspects of coronavirus biology have been dealt with in recent reviews (Robb & Bond, 1979; Siddell *et al.*, 1982; Wege *et al.*, 1982).

Structure

Morphology

Coronavirions are pleomorphic, although generally spherical, 60 to 220 nm in diameter and bear widely spaced, club-shaped surface projections about 20 nm in length. Complete virions have a density in sucrose of about 1·18 g/ml. In thin sections the virion envelope may be visualized as inner and outer shells separated by a translucent space. In negatively stained preparations of avian infectious bronchitis virus (IBV) an inner tongue-shaped membrane is visible (Bingham & Almeida, 1977; Fig. 1). The internal ribonucleoprotein (RNP) component of coronavirions has been visualized as a long strand of 1 to 2 nm diameter (Davies *et al.*, 1981) or as a helical RNP condensed into coiled structures of varying diameter, normally 10 to 20 nm (Caul *et al.*, 1979; Macnaughton *et al.*, 1978; Massalski *et al.*, 1982). Presumably, the different forms seen represent different states of RNP relaxation. After disruption of virions with detergent, nucleocapsid structures with a density of 1·27 to 1·28 g/ml in sucrose can be isolated.

Genome RNA

The coronavirus genome is a linear molecule of single-stranded RNA which is polyadenylated and infectious. The murine hepatitis virus (MHV) genome has also been shown to be capped. The genome RNA has a mol. wt. of 5×10^6 to 7×10^6, corresponding to about 15000 to 20000 nucleotides. T_1-resistant oligonucleotide fingerprinting of genome RNA and intracellular viral mRNA (see *Coronavirus-directed RNA synthesis*) confirms the positive polarity of the genome and indicates that it does not have extensive sequence reiteration (Brian *et al.*, 1980; Clewley *et al.*, 1981; King & Brian, 1982; Lai & Stohlman, 1978, 1981*a*; Lai *et al.*, 1981; Leibowitz *et al.*, 1981; Macnaughton, 1978; Macnaughton & Madge, 1978; Spaan *et al.*, 1981, 1982; Stern & Kennedy, 1980*a*, *b*; Wege *et al.*, 1978, 1981*a*, *b*; Weiss & Leibowitz, 1981).

Virion proteins

In addition to RNA the coronavirion nucleocapsid contains a non-glycosylated protein of 50000 to 60000 mol. wt. This protein is phosphorylated and purified MHV virions have been shown to contain a protein kinase activity. Coronavirions contain two major envelope proteins. The matrix protein is a transmembrane glycoprotein of 20000 to 35000 mol. wt., the carbohydrate moiety of which is known for MHV and bovine coronavirus (BCV) to be O-glycosydically linked. The glycosylated region of the protein is exterior to the virion envelope and in many cases matrix proteins with different degrees of glycosylation are incorporated into virions. Glycosylation is most likely to be at the N-terminus of the polypeptide. The second

0022-1317/83/0000-5501 $02.00

Table 1. *Coronaviruses*

Virus member	Natural host	Disease
Infectious bronchitis virus (IBV)	Chicken	Respiratory disease, nephritis, gonaditis
Murine hepatitis virus (MHV)	Mouse	Hepatitis, encephalomyelitis, enteritis, vasculitis
Bovine coronavirus (BCV)	Cattle	Enteritis
Human coronavirus* (HCV)	Man	Respiratory disease
Transmissible gastroenteritis virus (TGEV)	Pig	Enteritis
Haemagglutinating encephalomyelitis virus (HEV)	Pig	Vomiting and wasting disease, encephalomyelitis
Probable virus member		
Canine coronavirus (CCV)	Dog	Enteritis
Feline infectious peritonitis virus (FIPV)	Cat	Peritonitis, granulomatous inflammations in many organs
Possible virus member		
Rat coronavirus, sialodacryoadenitis virus† (RCV, SDAV)	Rat	Respiratory disease, adenitis
Turkey coronavirus (TCV)	Turkey	Enteritis
Porcine epidemic diarrhoea virus‡ (PEDV)	Pig	Enteritis

* Human enteric coronaviruses (HECV) have also been reported (Robb & Bond, 1979; see also Vaucher *et al.*, 1982) but there is insufficient evidence to justify their inclusion in the group (Macnaughton & Davies, 1981). Coronavirus-like agents reported from foals (Bass & Sharpee, 1975), non-human primates (Smith *et al.*, 1982), rabbits (Small *et al.*, 1979; Lapierre *et al.*, 1980) and other animals (see Macnaughton & Davies, 1981) require further identification, as do isolates recovered from mouse tissues after inoculation with material from human brain (Burks *et al.*, 1980) or shearwaters (Nuttall & Harrap, 1982). Earlier reports of coronavirus isolates from parrot and ticks are probably incorrect (Hirai *et al.*, 1982; Wege *et al.*, 1982).

† Maru & Sato (1982).

‡ Pensaert (1981).

coronavirion envelope protein, which constitutes the surface peplomer, is responsible for eliciting neutralizing antibodies during infection (Collins *et al.*, 1982; Hasony & Macnaughton, 1981, 1982; Schmidt & Kenny, 1981, 1982). In many cases different molecular weight forms (80000 to 200000 mol. wt.) of the protein are incorporated into virions. The protein is acylated and complex and mannose-rich carbohydrate side-chains are N-glycosydically linked to the polypeptide. Virions grown in cells treated with tunicamycin lack the peplomer protein and are unable to attach to cells or initiate infection. There are indications that for some coronaviruses proteolytic processing of the peplomer protein during morphogenesis may be involved in activating functions such as virus-induced cell fusion (Dea *et al.*, 1980; Otsuki & Tsubokura, 1981; Storz *et al.*, 1981*b*).

In addition to these characteristic proteins, others which do not appear to fit into any consistent pattern have been described, notably a 14000 mol. wt. protein described for IBV and MHV, and glycoproteins of about 60000 to 70000 mol. wt. described for MHV, BCV and porcine haemagglutinating encephalitis virus (HEV). IBV, human coronavirus (HCV) and porcine transmissible gastroenteritis virus (TGEV) virions have been shown to lack RNA polymerase activity. Fig. 2 shows a schematic model of the MHV coronavirion and the relationship of its RNA and protein components (Callebaut & Pensaert, 1980; Cavanagh, 1981; Dennis & Brian, 1982; Garwes & Reynolds, 1981; Holmes *et al.*, 1981; King & Brian, 1982; Lai & Stohlman, 1981*b*; Laporte & Bobulesco, 1981; Lomniczi & Morser, 1981; Niemann & Klenk, 1981*a*; Obert *et al.*, 1981; Rottier *et al.*, 1981*a*, *b*; Schmidt, 1982; Schmidt & Kenny, 1982; Schochetman *et al.*, 1977; Siddell, 1982; Siddell *et al.*, 1981*a*, *b*; Stern *et al.*, 1982; Stohlman & Lai, 1979; Storz *et al.*, 1981*a*; Sturman *et al.*, 1980; Tannock & Hierholzer, 1978).

Lipids

The lipid envelope of TGEV has been studied in detail. The virion envelope contains phospholipids, glycolipids, cholesterol, di- and triglycerides and free fatty acids in proportions

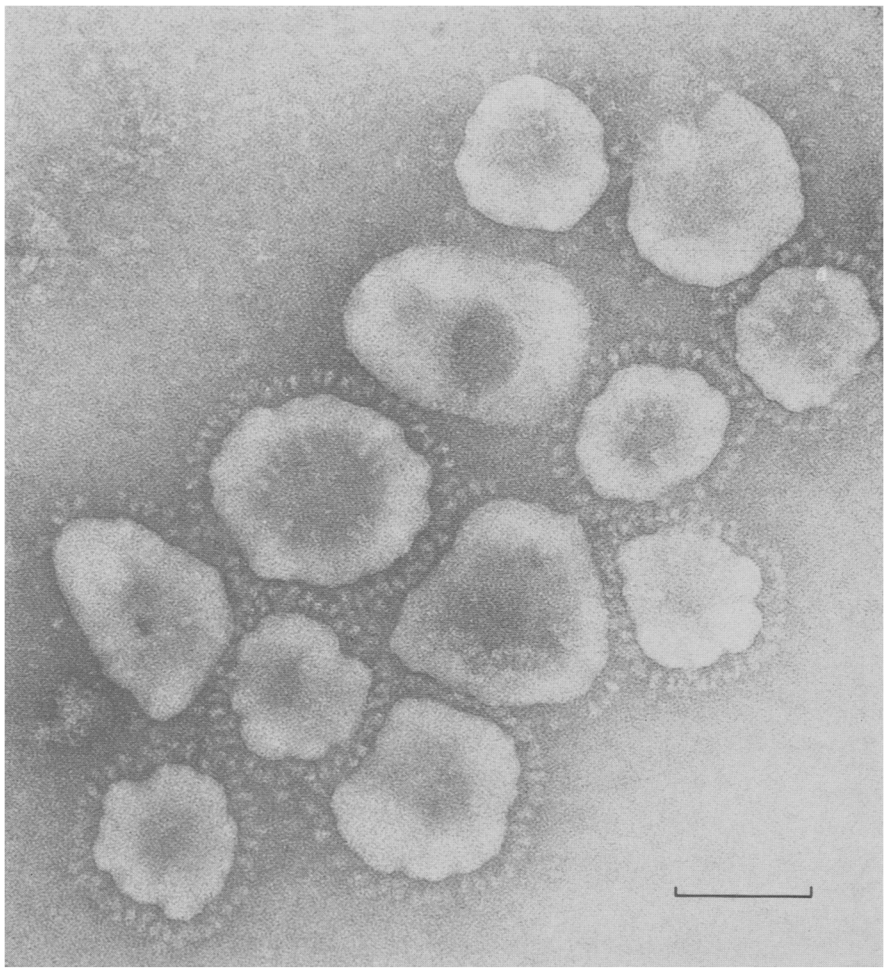

Fig. 1. Coronavirion morphology. A negatively stained preparation of infectious bronchitis virus (courtesy of J. Almeida). Bar marker represents 200 nm.

approximately corresponding to those in the cell membrane. Cholesteryl and fatty acid esters present in cell membranes are selectively depleted in the virion membrane. When grown in different cell types the virion envelope reflects the lipid content of the host cell membrane (Pike & Garwes, 1977).

Relationships between coronaviruses

(i) *Molecular.* The relationships between coronaviruses have been studied by molecular and immunological methods but the data is fragmentary. Molecular hybridization using cDNA representative of the majority of the MHV genome or the 3′ end of the genome (which represents the nucleocapsid protein gene; see *Coronavirus-directed protein synthesis*) indicates extensive sequence homology amongst MHV strains (Cheley *et al.*, 1981*b*; Weiss & Leibowitz, 1982). This homology is reflected in T_1-resistant oligonucleotide fingerprints of MHV genomes (Lai & Stohlman, 1981*b*) or chymotryptic peptide fingerprinting of MHV nucleocapsid proteins (Cheley *et al.*, 1981*b*). In contrast, T_1-resistant oligonucleotide fingerprinting of the genome RNA of 13 isolates of IBV (Clewley *et al.*, 1981) produced 11 quite distinct fingerprints.

(ii) *Antigenic.* Coronavirions contain three major antigens each corresponding to one of the three types of virion protein. The antigens may be distinguished by antibodies against virion

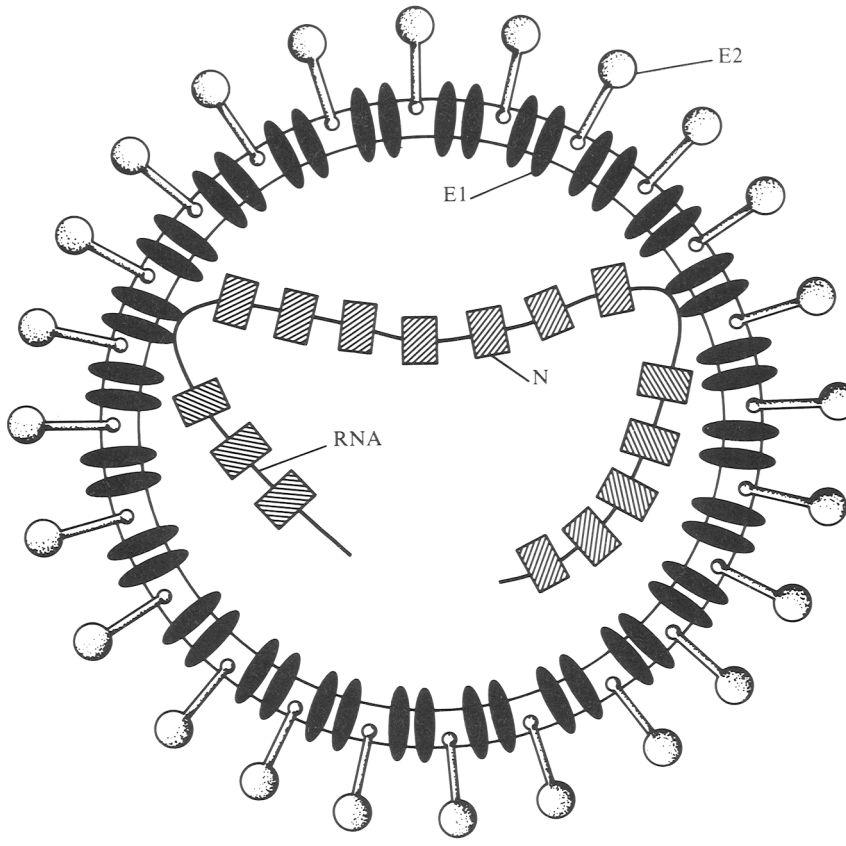

Fig. 2. A schematic model of coronavirion structure. Genome (RNA), nucleocapsid protein (N), matrix protein (E1) and peplomer protein (E2) are shown (courtesy of L. Sturman).

Table 2. *Antigenic relationships of coronaviruses**

	Group 1	Group 2
Mammalian	HCV 229E and other isolates TGEV (1 serotype) CCV (1 serotype) FIPV (1 serotype)	HCV OC43 and other isolates MHV (many serotypes) RCV (SDAV) (1 serotype) BCV (1 serotype) HEV (1 serotype)
Avian	IBV (at least 8 serotypes)	TCV (1 serotype)

* PEDV remains unclassified.

subcomponents (Collins *et al.*, 1982; Hasony & Macnaughton, 1981, 1982; Schmidt & Kenny, 1981, 1982; Yaseen & Johnson-Lussenburg, 1981). Studies on the antigenic relationships of coronaviruses present a complex pattern, but the data suggest that the family can be divided into four distinct groups, and these are shown in Table 2 (Gerna *et al.*, 1981; Horzinek *et al.*, 1982; Macnaughton, 1981; Macnaughton *et al.*, 1981; Maru & Sato, 1982; Pedersen *et al.*, 1978; Pensaert *et al.*, 1981; Reynolds *et al.*, 1980; Schmidt & Kenny, 1981).

Replication

Early events

Infection of tissue culture cells with coronaviruses is initiated with one-hit kinetics and the one-step growth curve at 37 °C is about 10 to 12 h. Infection is often accompanied by cellular

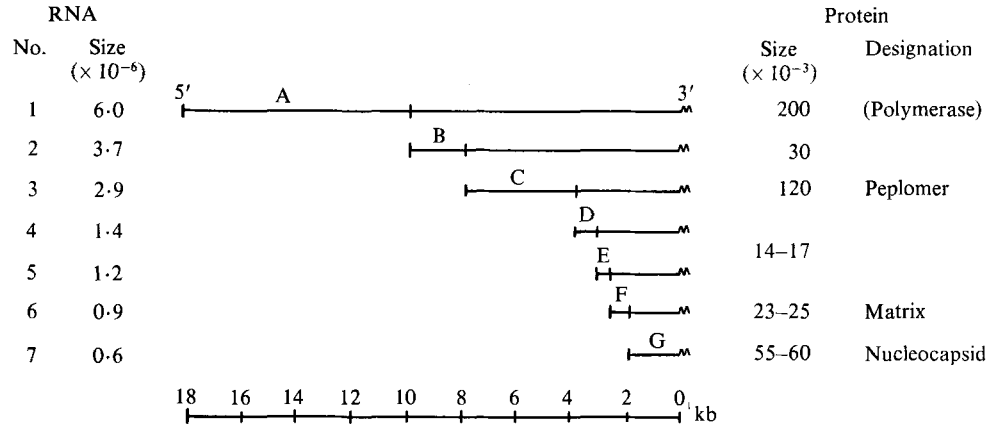

Fig. 3. The structure and expression of the murine hepatitis virus genome.

cytopathic changes, in some cases syncytium formation but most frequently vacuolation followed by disintegration. There are very few data on the early events associated with coronavirus infection. Patterson & Macnaughton (1981) have shown that on HCV 229E-infected cell monolayers, virions initially attach over the whole cell surface but are then rapidly redistributed away from the cell periphery by an energy-requiring process. The reason for this redistribution is unknown. Krzystyniak & Dupuy (1981) have shown that MHV3 uptake into cells is rapid and temperature-dependent. Uptake is not related to the phagocytic capacity of the cells and may therefore involve a mechanism such as receptor-mediated endocytosis, as has been reported for other systems (Helenius *et al.*, 1980).

Coronavirus-directed RNA synthesis

The synthesis of coronavirus RNA within the first few hours of infection is not easily detected. Brayton *et al.* (1982) have, however, recently shown that in cells treated with actinomycin D prior to infection with MHV A59, a small but reproducible synthesis of RNA can be demonstrated (see also Cheley *et al.*, 1981*a*). This synthesis presumably reflects the translation of the incoming genome RNA to produce proteins which then produce negative-stranded template. This assumption seems to be correct since pulse labelling of coronavirus-infected cells with [^{3}H]uridine in the presence of actinomycin D after the initial phase of infection reveals the synthesis of positive-stranded viral RNA. It is characteristic of coronaviruses that genomic-sized and multiple (4 to 6) subgenomic positive-stranded RNAs are synthesized in infected cells. These RNAs are synthesized in non-equimolar amounts but in relatively constant proportions and their synthesis is dependent on continued protein synthesis throughout infection. They range in size from 0·6 × 10^6 to genomic size (the largest RNA is termed RNA 1 and smaller RNAs are numbered accordingly, see Fig. 3) and form a 3′ co-terminal nested set extending towards the 5′ end of the genome. The RNAs are polyadenylated and all subgenomic and a proportion of the genomic-sized RNA molecules are associated with polysomes throughout infection. For MHV, the messenger function of these positive-stranded RNA species has been demonstrated *in vitro* (see *Coronavirus-directed protein synthesis*). No structural difference between the genome RNA and genome-sized intracellular mRNA has been described (Brayton *et al.*, 1982; Cheley *et al.*, 1981*a*; Dennis & Brian, 1982; Jacobs *et al.*, 1981; Lai *et al.*, 1981, 1982*a*; Leibowitz *et al.*, 1981; Mahy *et al.*, 1983; Spaan *et al.*, 1981, 1982; Stern & Kennedy, 1980*a*, *b*; Wege *et al.*, 1981*b*, *c*; Weiss & Leibowitz, 1982).

The 5′ ends of all MHV A59 mRNAs share a common sequence (5′-cap-N-UAAG) and a more extensive 5′ sequence homology is probable (Lai *et al.*, 1982*a*; Spaan *et al.*, 1982). It is unlikely, however, that this 'leader' sequence is produced by a conventional splicing mechanism. The replication of MHV is not impaired in cells treated with actinomycin D or α-amanitin (Brayton *et al.*, 1981; Lai *et al.*, 1981; Mahy *et al.*, 1983; Spaan *et al.*, 1981) and

replication has been reported in enucleated cells (Brayton *et al.*, 1981; Wilhelmsen *et al.*, 1981). Also, the synthesis of each mRNA is inactivated by u.v. irradiation in proportion to its own length (Jacobs *et al.*, 1981; this is also true for IBV mRNAs, Stern & Sefton, 1981). Thus, the subgenomic mRNAs are not apparently produced by the processing of a larger RNA. Lai *et al.* (1982*b*) have recently demonstrated that the negative-stranded template from which MHV mRNAs are copied is of genome size.

In contrast to MHV, oligonucleotide fingerprinting of the genome and mRNAs of IBV gives no evidence of leader sequences, although short 5′ homologies cannot be excluded (Stern & Kennedy, 1980*a*, *b*). Also, it has been reported that the replication of IBV is impaired in enucleated, u.v.-irradiated or α-amanitin-treated BHK-21 cells (Evans & Simpson, 1980). Whether these results indicate that there are significant differences in the replication of avian and murine coronaviruses remains to be seen.

Coronavirus-directed protein synthesis

Shortly after the onset of subgenomic mRNA production in the infected cell the synthesis of intracellular precursors to the virion peplomer, nucleocapsid and matrix proteins can be detected. The major intracellular viral polypeptide synthesized is the phosphorylated nucleocapsid precursor of 50000 to 60000 mol. wt. Kinetic experiments indicate that a large intracellular pool of this polypeptide is built up during infection, probably corresponding to the nucleocapsid structures which accumulate in some cells. For MHV, the matrix precursor polypeptide is synthesized in a non-glycosylated form (20000 to 25000 mol. wt.) and then undergoes post-translational glycosylation (in some cases to differing degrees) at the Golgi complex. In contrast, the peplomer precursor (150000 mol. wt.) is co-translationally glycosylated at the rough endoplasmic reticulum and subsequently modified to yield virion proteins by oligosaccharide processing, elongation and proteolytic cleavage during transfer through the Golgi apparatus. The coronavirus envelope proteins will provide useful models for the investigation of synthesis, glycosylation and intracellular transport of both N- and O-linked glycoproteins (Anderson *et al.*, 1979; Bond *et al.*, 1981; Cheley & Anderson, 1981; Collins *et al.*, 1982; Gerdes *et al.*, 1981; Holmes *et al.*, 1981; Niemann & Klenk, 1981*b*; Niemann *et al.*, 1982; Rottier *et al.*, 1981*a*, *b*; Siddell, 1982; Siddell *et al.*, 1980, 1981*a*, *b*, 1982; Stern *et al.*, 1981).

A number of other polypeptides synthesized in coronavirus-infected cells have been identified as virus-specific. In MHV-infected cells treated with tunicamycin the non-glycosylated polypeptide core (120000 mol. wt.) of the peplomer protein has been identified (Niemann & Klenk, 1981*b*; Rottier *et al.*, 1981*b*; Siddell, 1983). MHV-specific polypeptides of 14000 to 17000 mol. wt. and 30000 mol. wt. which are virus-coded (see below) have also been detected (Siddell *et al.*, 1981*b*).

In addition to the proteins described above, virus-specific RNA polymerase activities have been isolated from coronavirus-infected cells, although their polypeptide components have not been identified either *in vivo* or *in vitro* (Brayton *et al.*, 1982; Dennis & Brian, 1982; Mahy *et al.*, 1983). Usually, these activities have been isolated late in infection and their membrane location and association with replicative intermediate RNA has been demonstrated. In one case, a virus-specific polymerase activity has been isolated from MHV-infected cells before the onset of mRNA production and it was found to have different ion and pH requirements compared to late polymerase activity (Brayton *et al.*, 1982). Lai *et al.* (1982*b*) have suggested that these two different activities are responsible for negative- and positive-strand RNA synthesis respectively but it is not known how the polarity of RNA synthesis is dictated or if different or modified enzymes are involved. Interestingly, Leibowitz *et al.* (1982*a*) and Koolen *et al.* (1983) have found that the majority of MHV temperature-sensitive (*ts*) mutants have a mRNA$^-$ phenotype and these mutants can be divided into five or six non-overlapping complementation groups. Whether these groups represent different polymerase components, presumably encoded within the unique sequences at the 5′ end of genome RNA (and RNA 1, see below), or other viral gene products in addition, is not known.

The finding of multiple subgenomic positive-stranded RNAs and a corresponding number of virus-specific polypeptides in infected cells, immediately suggests that each RNA has a mRNA

function and encodes one viral protein. In the case of MHV this supposition has now been confirmed. In MHV-infected cells the synthesis of each viral polypeptide is initiated independently (Cheley & Anderson, 1981; Siddell, 1983) and polyadenylated RNA isolated from infected cells directs the synthesis *in vitro*, or in oocytes, of viral polypeptides. The translational activities encoding these polypeptides can be fractionated by sedimentation or electrophoresis and each corresponds to one of the subgenomic viral RNAs. The smallest RNA, RNA 7, encodes the intracellular nucleocapsid polypeptide (60000 mol. wt.). The next smallest, RNA 6, encodes the matrix protein polypeptide (23000 mol. wt.) *in vitro*, or its glycosylated counterpart (25000 mol. wt.) in oocytes and the third major intracellular RNA, RNA 3, encodes the peplomer protein core (120000 mol. wt.) *in vitro* or the co-translationally glycosylated peplomer precursor (150000 mol. wt.) in oocytes (Cheley *et al.*, 1981*a*; Leibowitz *et al.*, 1982*b*; Rottier *et al.*, 1981*a*; Siddell, 1983; Siddell *et al.*, 1980). The translation products of two further MHV RNAs, RNA 2 and RNA 4/5, have been identified as corresponding to 30000 and 14000 to 17000 mol. wt. intracellular viral polypeptides respectively (Leibowitz *et al.*, 1982*b*; Siddell, 1983).

The *in vitro* translation of the coronavirus intracellular genome-sized mRNA 1 has not yet been reported. However, in the case of MHV, its virion counterpart, the genome RNA, has been translated *in vitro* to produce a group of polypeptides of greater than 200000 mol. wt. (Leibowitz *et al.*, 1982*b*). The identity of these products is not yet known but it seems reasonable to assume that they are related to the components of the virus-specific RNA polymerase found in infected cells. *In vitro* translation studies with other coronaviruses are preliminary. It has been reported that the smallest IBV-specific intracellular RNA encodes the IBV nucleocapsid protein (Stern *et al.*, 1982) but many more data will be needed before the conclusions reached for MHV can be extended to other coronaviruses.

Replication strategy

The results described in the previous two sections lead to a model for the replication strategy of coronaviruses. This model is depicted for MHV in Fig. 3. The essential features are: (i) the expression of coronavirus information in the cell is mediated through multiple subgenomic mRNAs which form a 3′ co-terminal nested set; (ii) each mRNA directs the translation of only one protein; (iii) the size of the translation product for each RNA corresponds approximately to the coding potential of the 5′ sequences which are absent from the next smallest RNA. Although it has not been proven, these features and the inability of ribosomes to initiate translation at internal sites on eukaryotic mRNA (Kozak, 1981) suggest that only the 5′ sequences of each mRNA (depicted as genes A, B, C, etc. in Fig. 3) are translated into protein. This strategy has many obvious parallels with the strategies of other positive-stranded RNA viruses. For example, it appears that in general subgenomic mRNAs extend inwards from the 3′ end of genomes (see also Kääriäinen & Söderlund, 1978; Davies & Hull, 1982). Also, the coronavirus strategy appears to be a flexible one, allowing for the control of viral protein synthesis at the levels of both transcription and translation.

Virion assembly

Morphogenetic studies on the maturation of coronaviruses have revealed that assembly is restricted to the cytoplasm where progeny virions are formed by a budding process from membranes of the rough endoplasmic reticulum. The virions acquire their lipid envelope from the cells, excluding host cell proteins in the process, and are subsequently transported through and accumulate in the Golgi complex and smooth walled vesicles. There is an absence of budding from the plasmalemma (Beesley & Hitchcock, 1982; Ducatelle *et al.*, 1981; Holmes *et al.*, 1981; Massalski *et al.*, 1981, 1982). The mechanism of virus release has not been elucidated.

Pathogenesis

Transmission

Coronaviruses are probably distributed worldwide. In many cases they replicate in the respiratory tract and transmission of the virus is usually from this site, although virus is also shed

with the faeces. The diseases associated with coronaviruses are shown in Table 1. Most coronaviruses cause clinical disease in the species from which they were isolated but natural and experimental transmission to other species is possible. Human and avian viruses are transmissible to mice by the intracerebral route and TGEV can replicate in dogs, foxes and cats. Feline and canine coronaviruses are also infectious for pigs. Transmission to other hosts often leads to inapparent infections or diseases which do not occur under natural conditions (Wege *et al.*, 1982).

Acute infections

Coronaviruses primarily infect the respiratory system [HCV, IBV, MHV and rat coronavirus (RCV)] or the gastrointestinal tract [BCV, canine coronavirus (CCV), TGEV, turkey coronavirus (TCV) and some MHV strains]. Also, many strains of MHV cause hepatitis or encephalomyelitis. These infections are generally acute and it is likely that a lytic infection which destroys the host cell is the basic pathogenetic mechanism involved. Respiratory infection is usually confined to the ciliary epithelium of the trachea, nasal mucosa and alveolar cells of the lungs. A local immune response resulting in secretion of IgA is normally sufficient to overcome the acute phase of disease and the development of a systemic humoral immunity hinders a severe involvement of other organs. The enteropathogenic coronaviruses selectively infect absorptive cells and crypt cells of the intestinal mucosa resulting in atrophy of the villi. Virus strains differ in their predilection for a particular site (small or large intestines, colon) and cell type (absorptive epithelium and/or crypt cells) and the severity of disease varies from mild, transient enteritis to a rapidly progressing fatal diarrhoea. As with respiratory infection, the local immune response provides the most important line of defence against enteric infection. Maternal antibodies and lymph cells provide some protection to immunologically immature animals (Bhatt & Jacoby, 1977; Carthew & Sparrow, 1981; Chhabra & Goel, 1980; Doughri & Storz, 1977; Frederick & Bohl, 1976; Gonder *et al.*, 1976; Hierholzer *et al.*, 1979; Klobasa & Werhahn, 1981; Pensaert *et al.*, 1970; Purcell & McFerran, 1972; Shepherd *et al.*, 1979; Shimizu & Shimizu, 1979; Stone *et al.*, 1976; Taguchi *et al.*, 1976).

In one case, the pathogenic mechanism of an acute coronavirus infection appears to be quite different. The infection of pigs by haemagglutinating encephalomyelitis virus results in a disease which is characterized by vomiting, sometimes accompanied by encephalomyelitis. The disease is initiated by an inapparent infection of the respiratory tract, tonsils and intestines, which spreads along nerve tracts to peripheral ganglia and the central nervous system. Subsequently, the infection of neurons which regulate peristaltic functions of the intestinal tract results in disease and young animals in particular may die of starvation (Andries & Pensaert, 1980*a*, *b*; Andries *et al.*, 1978).

Chronic infections

Coronaviruses readily establish persistent infection in animals, often leading to diseases of a subacute or chronic nature. They also readily establish persistent infections in tissue culture (Chaloner-Larsson & Johnson-Lussenburg, 1981; Lucas *et al.*, 1977, 1978; Stohlman *et al.*, 1979; Yoshikura & Tejima, 1981). Very little is known of the mechanisms governing persistent infections *in vivo* or *in vitro*, but some factors which influence the outcome of the infection in animals have been identified.

(i) *Murine hepatitis virus.* Inbred mouse strains interact differently with different MHV strains and factors which are important in determining the outcome of both acute and persistent infections have been widely investigated in this system. Resistance of mice to acute infection is inherited by recessive genetic traits involving one gene for MHV2 and MHV3 and possibly two genes for MHV JHM. These genes are not H-2-linked. In contrast, chronic infection with MHV3 is additionally regulated by a gene which is associated with the H-2 region responsible for T cell functions. The expression of genetic resistance is manifested at the level of the mature macrophage, and since these cells are found at the sites of primary virus replication their interaction with the virus strongly determines the outcome of infection. Basically, virus replication in macrophages from resistant mice is restricted whereas virus replicates well in macrophages from susceptible mice. Additionally, genetic resistance can be expressed at the

level of the target cell type, for example, neuronal cells during MHV JHM infection or hepatocytes during MHV3 infection. Infections can also be modulated by lymphokines and interferons secreted from T lymphocytes and by corticosteroid hormones. Also macrophages secrete interferon, complement, chemotactic substances and prostaglandins, all of which influence viral replication, and these functions can be impaired or enhanced during chronic infections (Arnheiter *et al.*, 1982; Bang & Warwick, 1960; Dupuy *et al.*, 1975; Knobler *et al.*, 1981*b*; Krzystyniak & Dupuy, 1981; Lahmy & Virelizier, 1981; Levy-Leblond & Dupuy, 1977; Levy-Leblond *et al.*, 1979; Sheets *et al.*, 1978; Shif & Bang, 1970; Stohlman & Frelinger, 1978; Stohlman *et al.*, 1980; Taguchi *et al.*, 1976; Tardieu *et al.*, 1980; Taylor *et al.*, 1981; Virelizier & Allison, 1976; Virelizier & Gresser, 1978; Virelizier *et al.*, 1976; Weiser & Bang, 1976, 1977; Weiser *et al.*, 1976).

The disease processes which result from persistent infection by murine coronaviruses are also of interest pathologically. Infection of C3H/He or A2G mice with MHV3 leads to a persistent infection of the central nervous system accompanied by a chronic neurological disease. After the acute stage of infection the majority of animals survive but later develop clinical signs of incoordination and paralysis. Neuropathologically, a chronic chorioependymitis and hydrocephalus (A2G mice) or a diffuse vasculitis in kidney, liver, spleen, brain and spinal cord (C3H/He mice) is found. Perivascular infiltrations by polymorphonuclear lymphocytes and fibrinoid necrosis develop around blood vessels and these changes are accompanied by destruction of myelin and neuronal axons. Viral antigens have never been demonstrated in neuronal cells, but only in the endothelial cells of affected veins or arteries (Le Prevost *et al.*, 1975*a*, *b*; Virelizier *et al.*, 1975).

The neurotropic MHV JHM strain also induces persistent infections of the central nervous system in rodents, especially if attenuated (*ts*) virus is used. In mice, there is an absence of clinical disease after the acute phase of infection but mild lesions of demyelination in the brain are detectable by electron microscopy. Viral antigens are detectable only in oligodendroglia cells, in contrast to an acute encephalomyelitis where there is a destruction of neurons and glia cells. If rats are infected with MHV JHM, a subacute to chronic demyelinating encephalomyelitis can develop after an incubation period of several weeks to months. Typically, plaques of primary demyelination develop after the acute phase and in chronically diseased rats both fresh and old lesions are detectable. Infectious virus can be reisolated from the brain tissue of overtly diseased animals. These infections are of interest as experimental models for virus-induced demyelinating diseases (Haspel *et al.*, 1978; Knobler *et al.*, 1981*a*, *b*, 1982; Nagashima *et al.*, 1978, 1979; Sorensen *et al.*, 1980; Stohlman & Weiner, 1981; Wege *et al.*, 1983).

(ii) *Feline infectious peritonitis virus.* FIP virus infects a high percentage of cats causing mild respiratory and intestinal disorders. However, a small percentage of animals develop a fatal disease after an incubation period of several weeks to months. The disease starts with symptoms such as loss of appetite and elevated temperature and may be accompanied by swelling of the abdomen and dehydration. The infection involves many organs, especially the peritoneum, lungs, lymphoid tissue, liver and kidneys. Histologically, a severe peritonitis and pleuritis is seen and affected organs may reveal severe focal necrosis and granulomatous inflammation. FIP virus can be recovered throughout the clinical course from affected organs and a persistent infection is established in macrophages and cells of the reticuloendothelial system.

Experimental results strongly suggest that the pathological changes during this chronic coronavirus infection are due to an immune-complex disease. The disease develops faster in animals with high antiviral antibody titres than in seronegative kittens. In seropositive kittens organ lesions contain viral antigen bound to IgG, free viral antigen and complement. Deposits of immunoglobulins and complement, especially C3, are detectable in renal glomeruli and the kinetics of antiviral antibody formation, circulating immune complexes and complement concentration correlate with the stage of disease. Also, the passive transfer of antiviral immunoglobulin or vaccination with inactivated FIP virus results in a marked aggravation of the disease upon subsequent challenge with live virus (Horzinek & Osterhaus, 1979; Hoshino & Scott, 1978; Jacobse-Geels *et al.*, 1980, 1982; Loeffler *et al.*, 1978; Pedersen, 1976; Pedersen & Boyle, 1980; Weiss *et al.*, 1980; Weiss & Scott, 1981).

The persistence of FIP virus in the presence of high antibody levels has parallels in other

coronavirus infections. For example, chickens inoculated with certain vaccine strains of IBV can recover from a transient mild respiratory distress but several months later develop a rapidly progressing fatal disease. In these animals the virus persists, especially in the caecal lymph nodes, and a high titre of antiviral antibodies and severe kidney lesions are found (Alexander & Gough, 1977; Alexander *et al.*, 1978).

To many virologists coronaviruses will be of interest because they provide models for the study of chronic disease processes. Some of the major factors which determine the outcome of these processes are related to virus replication, for example virulence and tropism, whilst others are determined by the host, for example the immune response. This review testifies to the progress made in understanding the basic molecular features of coronavirus replication, although there remain obvious gaps in our knowledge. In particular, information on the molecular aspects of persistent coronavirus infections is rudimentary. Hopefully, the use of virus mutants, monoclonal antibodies and the techniques of genetic engineering will allow us to identify and study in detail the viral genes and gene products associated with pathogenesis. Some preliminary experiments in this direction have already been reported (Stohlman *et al.*, 1982). With regard to factors determined by the animal clearly the immune response plays a crucial role in determining both the nature of the infection, and thereby the ensuing disease process, or as is seen in the FIP virus model the response itself can be the pathogenetic mechanism from which disease develops. The molecular details and genetic basis of this response in these different situations are clearly of great interest.

The authors acknowledge financial support from the Deutsche Forschungsgemeinschaft and thank Helga Kriesinger for typing the manuscript.

Note added in proof. Stern & Sefton (*J. Virol.* **44**, 794–803; **44**, 804–812) have recently shown that O-linked oligosaccharides are not a universal feature of the small coronavirus membrane proteins. The IBV, p23 family of proteins contains both simple and complex type oligosaccharrides which are N-glycosidically linked and most likely attached to the nascent polypeptide. The same authors have also shown that the IBV peplomer protein is comprised of two polypeptide chains derived from a common intracellular precursor. Interestingly, one polypeptide chain is selectively lost from the IBV virion; whilst the second has a marked tendency to form aggregates. The number of molecules comprising the morphological peplomar structure remains unknown.

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