other than morphological techniques.

The evidence is therefore growing that there is indeed a plug-insertion mechanism analogous to complement lysis involved in lymphocyte killing. This is presumably distinct from the oxygen-radical killing mechanisms largely used by myeloid cells and it remains to be seen whether individual cell types always use only one or other of these methods or whether they may use both. Oxygen-radical killing is characteristically accompanied by chemiluminescence and can be inhibited by superoxide dismutase or catalase or strict anaerobiosis. There is fairly general agreement that these procedures do not inhibit T-cell killing. It has been claimed, however, that NK cells do kill by an oxygen metabolite mechanism[27], that they are of myeloid origin[28] and that their killing mechanism is distinct from that of cytotoxic T cells[29]. This is not immediately compatible with the view that they are 'plug inserters' and give identical lesions to those produced by cytotoxic T cells. This conflict remains to be resolved. □

P.J. Lachmann is Honorary Director of the MRC Mechanisms in Tumour Immunity Unit, University Medical School, Hills Road, Cambridge CB2 2QH.

1. Frank, M.M., Rapp, H.J. & Borsos, T. in *Complement* (Ciba Fdn Symp.; eds Wolstenholme, G.E.W. & Knight, J.) 120 (Churchill, London, 1964).
2. Lachmann, P.J., Munn, E.A. & Weissman, G. *Immunology* **19**, 983 (1970).
3. Kinsky S.C., Bonsen, P.P.M., Kinsky, C.B., van Deenen, L.M. & Rosenthal, A.F. *Biochim. biophys. Acta* **233**, 815 (1971).
4. Lachmann, P.J., Bowyer, D.E., Nicol, P.A.E., Dawson, R.M.C. & Munn, E.A. *Immunology* **24**, 135 (1973).
5. Borsos, T., Dourmashkin, R.R. & Humphrey, J.H. *Nature* **202**, 251 (1964).
6. Tranum-Jensen, J. & Bhakdi, S. *Molec. Immun.* **19**, 1406 (1982).
7. Mayer, M.M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2954 (1972).
8. Esser, A.F., Kolb, W.P., Podack, E.R. & Muller-Eberhard, H.J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1410 (1979).
9. Esser A.F. in *Biological Membranes* Vol.4 (ed. Chapman, D.) 277 (Academic, New York, 1982).
10. Tschopp, J., Muller-Eberhard, H.J. & Podack, E.R. *Nature* **298**, 534 (1982).
11. Podack, E.R., Tschopp, J. & Muller-Eberhard, H.J. *J. exp. Med.* **156**, 268 (1982).
12. Biesecker, G., Podack, E.R., Halveson, C.A. & Muller-Eberhard, H.J. *J. exp. Med.* **149**, 448 (1979).
13. Tschopp, J., Engel, A. & Podack, E.R. *J. biol. Chem.* (in the press).
14. Ramm, L.E., Whitlow, M.B. & Mayer, M.M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4751 (1982).
15. Lint, T.F., Zeitz, H.J. & Gewurz, H. *J. Immun.* **125**, 2252 (1980).
16. Mayer, M.M., Hammer, C.H., Michaels, D.W. & Shin, M.L. *Immunochemistry* **15**, 813 (1979).
17. Simone, C.B. & Henkart, P. *J. Immun.* **124**, 954 (1980).
18. Martz, E. & Benacerraf, B. *Ad. Biosci.* **12**, 37 (1973).
19. Perlmann, P., Perlmann, H. & Lachmann, P.J. *Scand. J. Immun.* **3**, 77 (1974).
20. Sundsmo, J.S. & Muller-Eberhard, H.J. *J. Immun.* **122**, 2371 (1979).
21. Lachmann, P.J., Hobart, M.J. & Woo, P. *Clin. exp. Immun.* **33**, 193 (1978).
22. Lachmann, P.J. *Behring Inst. Mitt.* **63**, 25 (1979).
23. Dourmashkin, R.R., Deteix, P., Simone, C.B. & Henkart, P. *Clin. exp. Immun.* **42**, 554 (1980).
24. Henkart, M.P. & Henkart, P.A. *Mechanisms of Cell-Mediated Cytotoxicity* (eds Clark, W.R. & Golstein, P.G.) 227 (Plenum, New York, 1982).
25. Podack, E.R. & Dennert, G. *Nature* **302**, 442 (1983).
26. Dnnert, G. & Podack, E. *J. exp. Med.* **157**, 1483 (1983).
27. Helfand, S.L., Workmeister, J. & Roder, J.C. *J. exp. Med.* **156**, 492 (1982).
28. Ortaldo, J.R., Sharrow, S.O., Timonen, T. & Herberman, R.B. *J. Immun.* **127**, 2401 (1981).
29. Patek, P.Q., Collins, J.L. & Cohn, M. *Eur. J. Immun.* **13**, 433 (1983).
30. Inai, S. & Akagaki, Y. reported at the *International Symposium on Frontiers of Complement*, Kashigofima, Japan (1983).

Virology

Molecular biology of the coronaviruses

from Brian W.J. Mahy

THE rapid advances being made in understanding the molecular biology of the coronaviruses took centre stage at a recent international workshop* on these viruses, overshadowing studies of the pathogenesis of the diseases they cause: respiratory infections and colds in man, and numerous acute and chronic diseases in animals. Of particular interest were studies revealing novel mechanisms employed by coronaviruses for the synthesis of glycoproteins and mRNA. These studies have concentrated mainly on mouse hepatitis virus (MHV) and avian infectious bronchitis virus (IBV).

Glycoproteins

Coronaviruses contain two envelope glycoproteins, termed E_1 and E_2 in MHV. E_1, the matrix protein, is a transmembrane glycoprotein with its N-terminus exterior to the envelope and its C-terminus associated internally with the nucleocapsid. E_2 or spike protein which forms the peplomers on the virion surface attaches to host cells and elicits neutralizing antibodies during infection. Virions are formed by budding into intracytoplasmic vesicles from membranes of the rough endoplasmic reticulum and the Golgi apparatus, and not from the plasma membrane where most enveloped viruses bud. Coronaviruses thus provide a unique opportunity to study protein structural features which influence the site of budding.

Budding is determined by restricted intracellular migration of the E_1 membrane glycoprotein; incorporation of E_2 is a late event, not necessary for budding to occur (K. Holmes, NIH; H. Neimann, Justus-Liebig Universität, Giessen). The carbohydrate moiety of E_1 is O-glycosydically linked in murine and bovine coronaviruses; in the presence of tunicamycin (which blocks the N-linked glycosylation of E_2), E_1 continues to be synthesized and spikeless particles bud intracellularly. O-glycosylation of E_1 occurs late, in the Golgi apparatus, and is not essential for virus maturation. Niemann reported that addition of the glycoprotein transport inhibitor monensin to murine coronavirus-infected cells blocked glycosylation of E_1 but allowed accumulation of enveloped virions in the endoplasmic reticulum. Similar results were reported for human coronavirus 229E (M.C. Kemp, University of Colorado). Glycosylation of E_1 is therefore not co-translational, in contrast with E_2 which is formed by synchronous membrane insertion and glycosylation of the nascent polypeptide chain, involving recognition and cleavage of an N-terminal signal sequence.

E_1 has been synthesized *in vitro* (P. Rottier, University of Utrecht) by translation of MHV mRNA in the presence of dog pancreatic microsomes, and the disposition of the protein in the membrane determined by accessibility to proteases and selective N-terminal labelling. The bulk of the E_1 protein is buried in the membrane, with only small portions from the N- and C-termini expressed in the lumenal and cytoplasmic domains respectively. Addition of microsomes at different times after synthesis shows that the protein can enter the membrane at any stage during synthesis of the first 150 amino-acid residues. O-glycosylation does not occur *in vitro*, and there is no evidence for a cleavable signal sequence. The nucleotide sequence of the E_1 gene (J. Armstrong, EMBL, Heidelberg) confirms the strongly hydrophobic nature of the protein, and the N-terminal sequence (Met-Ser-Ser-Thr-Thr-Glu . . . . .) reveals four potential O-glycosylation sites. From a preliminary sequence of the matrix protein gene of IBV (M. Boursnell and T. Brown, Poultry Research Station, Houghton), the avian virus protein appears similarly hydrophobic, but the sequence predicts no serine or threonine residues near the N-terminus. In agreement with this, IBV matrix protein has been found to have N- rather than O-linked carbohydrate side chains (D. Stern, MIT and D. Cavanagh, Poultry Research Station, Houghton). Further comparative studies of coronaviruses would be of interest in this respect, but clearly the existence of this unusual O-linked glycoprotein in murine and bovine Coronaviridae does not provide a hallmark for all members of the family.

The availability of cloned DNA copies of the E_1 protein gene has opened the way for site-directed mutagenesis which may soon reveal, at least *in vitro*, which features of the protein govern its interaction with membranes of various cellular compartments.

Generation of coronavirus mRNAs

The coronavirus genome is a linear single-stranded RNA molecule about 20 kilobases (kb) long which is polyadenylated at the 3′ end, capped at the 5′end and infectious. During replication, mRNA of genome length is synthesized together with six subgenomic mRNA molecules which range in size from 2 to 10 kb. Previous T_1-oligonucleotide mapping studies showed that these mRNAs form a 3′-co-terminal nested

*The second international workshop on coronaviruses, organized by P. Rottier, B. van der Zeijst, W. Spaan and M. Horzinek, was sponsored by EMBO and held in Zeist, The Netherlands, 8-10 June 1983.

0028-0836/83/400474-02$01.00

© 1983 Macmillan Journals Ltd

set extending towards the 5′ end of the genome. Each appears to be translated independently to produce a single protein which corresponds in size to the unique 5′-terminal sequences not present in the next smallest RNA (reviewed in ref.1).

M. Lai (University of Southern California, Los Angeles) reported T_1-oligonucleotide mapping data showing that, in addition to the common 3′ sequences, a common 5′-terminal 'leader' sequence is present on each of the mRNAs, derived from a 5′-terminal sequence present only once in the genome RNA which must become translocated onto the mRNAs during their synthesis or generated by a splicing mechanism. Two alternative approaches have confirmed the existence of leader sequences on coronavirus mRNAs. Hybridizing cDNA molecules (W. Spaan, University of Utrecht; H. Delius, EMBL, Heidelberg) transcribed from the smallest subgenomic mRNA (7) to genome RNA and examining the hybrids by electron microscopy after cytochrome *c* spreading shows that the bulk of the cDNA hybridizes to the 3′-terminal region of the genome. However, about 50 nucleotides from the 5′ terminus of genome RNA also hybridizes to the cDNA, resulting in large looped structures about 19 kb long. Direct sequence analysis of mRNA 7 and the corresponding region of the genome has also been carried out (J. Armstrong, EMBL; M. Skinner, University of Würzburg; Spaan).

This work confirms the existence of a 'fusion sequence' since the nucleotide sequence of the 5′ region of mRNA 7 diverged from the corresponding genome region upstream from the *N*-gene initiation codon. Fusion of leader and body sequences occurs within the sequence 5′ AAUCUAAUCUAAACU 3′, which does not include the consensus established[2] for splice junctions in viral and cellular mRNAs. Conventional splicing also seems to be ruled out since murine coronaviruses replicate in enucleated cells, and transcriptional mapping data show that the UV target size of each mRNA corresponds to its physical size[2]. The template for mRNA synthesis is a single genome-length negative-stranded RNA molecule (Lai), so each mRNA must be individually transcribed at its own initiation point on the negative-strand template.

Two possible mechanisms by which leader sequences could become fused to mRNA body sequences during transcription were considered at the meeting. The first involves bringing together non-contiguous sequences on the negative-strand RNA template by secondary structure alterations which lead to looping or folding of the molecule. The polymerase could read the leader sequence then jump across a postulated gap in the template, joining the leader to mRNA body sequences in the process. Such a jumping mechanism, which requires ribonucleoprotein structure to bring together non-contiguous sequences, may be involved in the generation of defective interfering RNAs during influenza virus transcription[3,4]. The second mechanism involves reinitiation of transcription near the start of each gene on the template; the leader RNA sequence remains attached to the polymerase after its own synthesis and primes transcription at six different regions along the template. This mechanism has recently been invoked to explain mRNA synthesis from overlapping genes in a negative-stranded bunyavirus[5]. Although data are not yet available for murine coronavirus, Boursnell and Brown have found two regions of homology on the IBV genome which might be the primer-attachment points.

Lai presented evidence in favour of the second mechanism. Only full-length double-stranded replicative-form molecules were found in infected cells after ribonuclease treatment. If the first mechanism were correct, multiple replicative forms would be expected to be generated. Also, only one species of replicative intermediate, migrating faster than genome RNA, was found by gel electrophoresis. This species was 40–60 per cent resistant to ribonuclease, and its structure suggested six single-stranded tails on each full-length template RNA.

The nature of the RNA-dependent RNA polymerase responsible for these events is still unclear. The current hypothesis would favour separate polymerase activities for the synthesis of negative and positive strands. Considerable further work on these enzymes is needed to establish the events involved in the unique RNA synthetic mechanism induced by coronavirus infection. □

Brian W. J. Mahy is Huddersfield Lecturer in Special Pathology at the Department of Pathology, University of Cambridge, Cambridge CB2 2QQ.

1. Siddell, S., Wege, H. & ter Meulen, V. *J. gen. Virol.* **64**, 761 (1983).
2. Mount, S.M. *Nucleic Acids Res.* **10**, 459 (1982).
3. Fields, S. & Winter, G. *Cell* **28**, 303 (1982).
4. Jennings, P.A., Finch, J.T., Winter, G. & Robertson, J.S. *Cell* (in the press).
5. Patterson, J.L. *et al. Cell* **33**, 791 (1983).

Earth science

Suspect terranes

from Peter J. Smith

AN unexpected revelation of the past decade or so in the field of global tectonics, but one rather slow to penetrate the consciousness of the wider geological community, is the degree to which continental drift can be a messy process. The simple view of the 'Wegenerian' drift of the past 200 million years is one of large more-or-less recognizable landmasses moving slowly across the Earth's surface in the context of spreading ocean floors and a lithosphere divided into a number of discrete plates. Such landmasses may be in the process of splitting apart, may have their outlines modified slightly by the offshore deposition of sediment, may collide and thus become severely distorted along their leading edges, may stretch and contract a little as they find themselves moving around an Earth of variable curvature, and may — although this is more dubious — grow slowly and steadily by accretion around their edges; but since the breakup of Pangaea they have, by and large, retained their original shapes and sizes. This turns out to be an oversimplification, and nowhere more so than in relation to western North America.

The North American continent that split away from Eurasia all those millions of years ago was 20–30 per cent smaller than the North America of today, but the subsequent growth took place not slowly by accretion around the edges but rapidly by the addition of lumps chiefly to one, the western, edge. A 500-km or so strip of the Pacific coast from Alaska to San Francisco, a rather narrower strip running south into Mexico and almost the whole of Alaska itself comprise a mosaic of 'suspect terranes' that were grafted onto the original stable craton of North America in ones and twos at various times between 200 and 50 Myr ago.

These suspect terranes, of which about a hundred have been identified, range in size from hundreds to tens of thousands of square kilometres. Most are apparently of oceanic origin — islands, plateaus, ridges and arcs — although a few are continental slivers. Some were identified, or at least regarded as 'suspect', even before continental drift became widely accepted, largely because they were found to have palaeontologies quite uncharacteristic of the region and quite different from those of adjacent terranes. Subsequent geological studies have confirmed the extent to which adjacent terranes are stratigraphically unrelated. More recently, palaeomagnetic studies have revealed that many of the terranes have not only come from up to thousands of kilometres to the south and west but have been rotated through up to 70°, before, during or since accretion. Some apparently joined North America individually; others amalgamated before reaching their current resting place; and still others have, since joining, split and separated from each other. Their status in plate tectonic terms is uncertain. Some of the larger terranes appear to be genuine 'microplates', comprising segments of the complete lithosphere down to the asthenosphere; some of the smaller ones may be comparatively thin blocks that have become decoupled from the lower lithosphere.

As the laborious task of defining,

0028-0836/83/400475-02$01.00

© 1983 Macmillan Journals Ltd