POSITIVE STRAND RNA VIRUSES

Margo A. Brinton and Roland Rueckert, Organizers

April 20 — April 26, 1986

Plenary Sessions

Poster Sessions

Late Additions

Genome Structure and Viral Evolution

Q1 EXPRESSION OF NATURAL AND MODIFIED RNA VIRUS SEQUENCES FROM CLONED cDNA, Paul Ahlquist, Biophysics Laboratory and Plant Pathology Dept., University of Wisconsin, Madison, Wisconsin 53706.

Brome mosaic virus (BMV) is an isometric, positive strand RNA virus of cereal plants, related by substantial nonstructural protein homologies and features of RNA replication to a variety of other plant virus groups and to the animal alphaviruses. In vitro transcripts of complete cDNA clones of the three BMV genomic RNAs can be used to infect either whole plants (1) or protoplasts (2). 50% or more of inoculated protoplasts can be routinely infected with appropriately tailored BMV transcripts, allowing direct examination of virus replication in primarily infected cells. Such expressible BMV cDNA clones have been used to alter the virus for a variety of studies on gene expression and replication.

Transcript inoculations show that the two largest BMV genomic RNAs (BMV 1 and 2) are both necessary and sufficient for viral RNA replication in protoplasts. Transcripts of the third genomic RNA, BMV3, are not required for replication in protoplasts, but are readily replicated when co-inoculated with BMV 1 and 2. Numerous changes, such as linker insertions and extensive deletions, have been made in the sequences of BMV3 while preserving its ability to be replicated. The coat protein gene of BMV3 has also been replaced with foreign genes, yielding virus derivatives which serve as efficient expression vectors in transfected protoplasts (2).

Systematic deletion analysis has been used to identify cis-acting sequence elements which direct efficient replication and accumulation of BMV RNA3 in protoplasts. These studies show that in addition to expected requirements for terminal or near-terminal portions of the 5' and 3' noncoding sequences, there is a strong requirement for a defined internal sequence for efficient accumulation of RNA3 progeny. Some of the required terminal sequences are involved in initiation of (-) strand RNA synthesis, and others may function in (+) strand initiation and/or RNA stability. The role of the required internal sequence in RNA3 replication and accumulation is under investigation.

1. Ahlquist, P., French, R., Janda, M. and Loesch-Fries, L. S. (1984) Proc. Natl. Acad. Sci. USA 81: 7066-7070.

2. French, R., Janda, M. and Ahlquist, P. (1986) Science (in press).

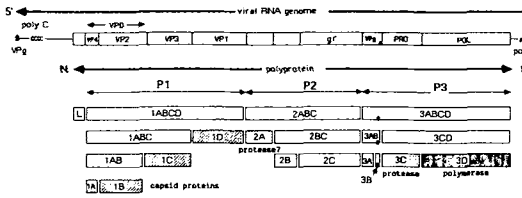
Q2 REPLICATION AND SELECTION KINETIC OF SHORT-CHAINED RNA SPECIES, Christof K. Biebricher, Max-Planck-Institut fur Biophysical Chemie, D-3400 Gottingen, Fed. Rep. of Germany

In vitro replication of short-chained self-replicating RNA species is a suitable model system for the study of evolution and selection at the molecular level. Phonotypic expression of a genotype is reduced to its ability to be effectively replicated by $Q\beta$ replicase added as highly purified preparation and constituting an environmental factor. The high template specificity of $Q\beta$ replicase is due to kinetic control of formation of initiation complexes. A minimal replication mechanism can be written by compiling the elementary steps of chain initiation, elongation and termination. The effects of double strand formation between plus and minus strands, of asymmetric synthesis in the plus and the minus cycles and the complicated competition behavior between several RNA species have been determined by mathematical analysis, leading to compact equations and by computer simulations. The computed concentration profiles agreed with the experimental profiles.

A virtually unlimited number of different self-replicating RNA species can be obtained by template-free RNA synthesis with $Q\beta$ replicase: after long lag times non-reproducible self-replicating RNA species are produced de novo. When amplification of RNA is suppressed by ommission of one triphosphate a slow condensation of triphosphates to oligonucleotides is observed to be catalysed by $Q\beta$ replicase.

Q3 COMPARATIVE ORGANIZATION AND GENOME STRUCTURE IN PICORNAVIRUSES, Ann C. Palmenberg, Biophysics Laboratory, University of Wisconsin, Madison, WI 53706

Although physically among the smallest of positive-strand viruses, the picornaviruses are of major historic, economic and medical importance. The family contains a diverse variety of highly virulent human and animal pathogens, including: polio-, rhino- (common cold), hepatitis A, coxsackie-, murine encephalomyocarditis (mengo), swine vesicular disease and foot-and-mouth disease (FMD) virus. In spite of the disparate afflictions caused by these agents, recent advances in molecular virology, and X-ray crystallography have shown that all picornaviruses share a great degree of physical similarity in their particle structure and genome organization (1).



Four non-identical polypeptides (VP1, VP2, VP3 and VP4) comprise each of the 60 symmetrical subunits found in the icosohedryl particle. Translation of genomic RNA produces a single, large polyprotein molecule, which is cleaved proteolytically into the capsid and other non-structural proteins (see map). At least two viral peptides have been identified as the proteases responsible for polyprotein cleavages. Peptide 2A (in poliovirus) can cleave autocatalytically between 2A and 2B. Most of the remaining cleavages are then subsequently carried out by peptide 3C. Capsid precursor 1AB (VP0) may also be involved in autocatalytic processing during virion morphogenesis.

Picornaviruses are usually divided into 4 subgroups (entero-, rhino-, cardio-and aphthoviruses) on the basis of properties such as pH lability, buoyant density and thermostability. However, new comparisons of genome organization, nucleotide sequence, base composition, codon preference and computer-generated protein alignments, strongly suggest the viruses should be re-divided into somewhat different catagories. For example, the rhino and polioviruses are very closely related to each other, but share little homology with hepatitis A (traditionally an enterovirus). Theiler's virus looks remarkably like the cardioviruses EMC and mengo, and probably represents a new serotype of this genus rather than its previous classification as an enterovirus. Possible suggestions for revised picornaviral divisions include: Group 1 (all FMDVs), Group 2 (all previous cardioviruses and Theiler's virus), Group 3 (hepatitis A), Group 4 (rhinoviruses and all other previous enteroviruses).

(1) Palmenberg, A.C. (1986) Genome Organization, Translation and Processing in Picornaviruses. In: "The Molecular Biology of Positive Strand RNA Viruses." D.J. Rowlands, B.W.J. Mahy and M. Mayo, eds., Academic Press, London, in press.

Antigenic and Functional Analyses of Virion Proteins and Cellular Receptors

Q4 HUMAN RHINOVIRUS ATTACHMENT REQUIRES A SPECIFIC CELLULAR RECEPTOR PROTEIN, Richard J. Colonno, Joanne E. Tomassini, and Pia L. Callahan, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486

Human rhinoviruses (HRVs) are members of the picornaviridae family and are the major causative agent of the common cold in man. Previous studies have indicated that HRVs can be divided into 2 groups based on receptor specificity. One group, designated the major HRV group because it represents 90% of the known HRV serotypes, utilizes a single cellular receptor on human cells. The remaining 10% of HRV serotypes form a second group, designated the minor HRV group, which compete with each other for a cellular receptor distinct from that used by the major HRV group. Using HeLa cells as an immunogen, a mouse monoclonal (IgG-1) antibody was isolated which specifically blocked attachment of the major group of HRVs. The receptor antibody showed a strong avidity for the cellular receptor as evidenced by its rapid binding kinetics and ability to displace previously-bound HRV from receptors. Attempts to bypass the receptor antibody blockage by infecting HeLa cells at a high multiplicity of infection (>3000) were unsuccessful. In addition, no natural viral variants have been isolated which can utilize an alternate route of entry into cells.

Detergent treatment of HeLa cell membranes solubilized a receptor complex which eluted from gel filtration columns with an apparent molecular weight of 440,000. Neuraminidase treatment of the receptor protein suggested the existence of sialic acid as the terminal sugar moiety. Using immune affinity chromatography, we were able to isolate a 90,000 MW glycoprotein which we believe to be the cellular receptor protein for the major group of HRVs. Polyclonal antiserum prepared against this isolated receptor protein specifically inhibited the attachment of the major group of HRVs. The normal cellular function of this receptor remains unknown. Prolonged exposure of cells to receptor antibody showed no inhibitory effect on cell growth or division.

The efficacy of the receptor antibody in preventing HRV infections was successfully tested in a chimpanzee animal model.

Q5 EPITOPE MAPPING OF A FLAVIVIRUS GLYCOPROTEIN,
Franz X.Heinz, G.Winkler, W.Tuma, C.Mandl, F.Guirakhoo, C.Kunz,
Institute of Virology, University of Vienna, Vienna, Austria.

Tick-borne encephalitis (TBE) virus represents a member of the family flaviviridae. By the use of monoclonal antibodies the serological specificities, functional activities and spatial relationships of antigenic determinants on the TBE virus surface glycoprotein have been defined. Simultaneous binding studies of antibody pairs revealed that antibodies may not only block each other but in contrast may even enhance the binding of other antibodies. This leads to a complex network of interactions between antibodies to distinct nonoverlapping epitopes which can either be unidirectional or bidirectional. The quantitytive evaluation of binding data revealed that the observed phenomenon is due to an up to six-fold increase of antibody avidity and is not dependent on antibody bivalency. It is assumed that binding of antibodies to certain epitopes induces conformational changes in distant parts of the molecule which can result in increased avidity of antibodies directed to conformationally changed epitopes.

Strong cooperative effects were also observed in functional assays, neutralization and passive protection. Most interestingly, nonneutralizing and nonprotective antibodies could strongly enhance the functional activities of certain antibodies, thus emphasizing the importance and potential of antigenic determinants which are regarded as functionally irrelevant when analyzed alone.

The role of carbohydrate in the antigenic structure of the glycoprotein was investigated by the use of specific endoglycosidases. As shown by Endo F digestion TBE virus as well as Murrey Valley encephalitis virus contain a single carbohydrate side chain. Removal of carbohydrate does not change any of the epitopes defined by monoclonal antibodies. The corresponding envelope protein of another flavivirus, West Nile virus, completely lacks carbohydrate.

Several monoclonal antibodies cluster to form antigenic domains which reveal great differences with respect to their denaturation sensitivity. One of these domains is highly resistant to denaturation due to stabilization by disulfide bridges which have a strong tendency for renaturation after reduction. Experiments are under way to assess the relevance of these disulfide bridges as a general structural feature of flavivirus envelope proteins.

Q6 CELL BIOLOGY OF VIRUS ENTRY, Ari Helenius, Margaret Kielian, Sandra Schmid, Con Beckers, Mark Marsh and Ira Mellman, Department of Cell Biology, Yale University School of Medicine, New Haven, CT, 06510.

Many enveloped and nonenveloped animal viruses enter cells by receptor-mediated endocytosis. In most cases uptake is followed by delivery to acidic prelysosomal vacuoles (endosomes) and secondary lysosomes. In the two cases studied in detail in our laboratory, Influenza virus and Semliki Forest virus, penetration takes place in endosomes (1,2). It depends on acid-triggered, irreversible conformational changes in the spike glycoproteins (3). These changes can be monitored in vivo and in vitro by changes in proteolytic sensitivity in various spike glycopolypeptides and by appearance or disappearance of specific antibody binding sites. Kinetic and morphological studies implicate both peripheral and perinuclear endosomes as sites of viral penetration. Viruses or virus mutants which fuse at relatively low pH (~pH 5.3) fuse in BHK cells 30-40 minutes later than viruses which are triggered around pH 6, confirming the notion that incoming ligands encounter gradually decreasing pH within the endosome compartment.

We have isolated endosomes from CHO cells at about 80% purity using a combination of free-flow-electrophoresis and density gradient centrifugation. The isolated endosomes remained active as judged by their capacity to acidify in the presence of ATP. The overall protein composition of endosomal membranes was clearly different from that of plasma membranes and lysosomes. More than 90% of cell associated virus particles are endocytozed into these organelles and about half of them deliver their nucleocapsids to the cytoplasmic compartment. The nucleocapsids delivered to the cytoplasm do not share the hydrophobic properties of acid-treated, isolated nucleocapsids, suggesting that they do not get exposed to acidity during entry. Nevertheless, they are likely to undergo subtle changes during entry; we found that capsids isolated from virus (unlike the naked viral RNA) were not infective when artifically injected into cells. This result suggests that the nucleocapsids have to enter by a normal cellular pathway to be infective. We are presently trying to define the changes in nucleocapsids occurring during entry.

(1) Marsh, M., Bolzau, E. and Helenius, A. (1983) Cell
(2) Yoshimura, A. and Ohnishi, S.-I. (1984) J. Virol. 51:497-504
(3) White, J. Kielian, M. and Helenius, A. (1983) Quart. Revs. Biophys. 77.

Supported by grants from NIH and the Swebelius foundation.

Q7 The three dimensional structure of poliovirus: implications for assembly and immune recognition. James M. Hogle *, Marie Chow **, and David J. Filman *, * Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037, and ** Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

The three dimensional structure of the Mahoney strain of type 1 poliovirus has been determined at 2.9 Å resolution by x-ray crystallographic methods (1). The three large capsid proteins, VP1, VP2, and VP3, are structurally similar. Each consists of a conserved core (an eight stranded antiparallel beta barrel with two flanking helices), with variable connecting loops and amino and carboxy terminal extensions. The smaller protein, VP4, has an extended structure, except for a short two-stranded antiparallel beta sheet at its amino terminus. The cores of the large subunits form the continuous shell of the virion. The amino terminal extensions and VP4 form an extensive network on the inner surface of the capsid that may direct assembly. The connecting loops and carboxy terminal extensions form prominent features on the outer surface of the virion. In the virus, five copies of VP1 cluster around the particle fivefold axes, while VP2 and VP3 alternate around the particle threefold axes, forming a T=1 icosahedral shell. At the fivefold axis, the pronounced tilt of VP1 outward along the axis produces a prominent radial protrusion surrounded by a broad deep valley. The less prominent protrusion formed by VP2 and VP3 at the threefold axis is ringed by two sets of radial projections. The larger of the two is formed by large loops of VP1 and VP2 and by the carboxy terminus of VP2. The smaller is formed by a loop in VP3. The neutralizing antigenic sites of the virus (as defined by monoclonal release mutations) map to the prominent surface features of the virus. These sites have been grouped by proximity and by function into three major clusters. The capsid proteins of poliovirus are topologically identical and structurally similar to those of several T=3 plant viruses whose structures are known. Moreover, the packing of the subunits and the functional roles of the cores, loops, and terminal extensions are similar. Although these similarities strongly indicate an evolutionary relationship, the exact nature of the relationship is unclear, since the gene order, mechanisms for the control of gene expression, details of the assembly process, and other aspects of the life cycle are significantly different.

(1) Hogle, J. M., Chow, M., and Filman, D. J. (1985) Science 229:1358-1365.

Q8 STRUCTURE, FUNCTION AND EVOLUTION OF HUMAN RHINOVIRUS 14. Michael G. Rossmann[1], Edward Arnold[1], James P. Griffith[1], Greg Kamer[1], Ming Luo[1], Anne G. Mosser[2], Ann C. Palmenberg[2], Roland R. Rueckert[2], Barbara Sherry[2] and Gerrit Vriend[1]. [1]Department of Biological Sciences, Purdue University, W. Lafayette, Indiana 47907 and [2]Biophysics Lab, University of Wisconsin, 1525 Linden Drive, Madison, Wisconsin 53706.
The structure of human rhinovirus 14 (HRV14) has been determined in three dimensions to atomic detail. The course of all four capsid polypeptides has been traced and correlated with the known amino acid sequences. The tertiary structures of the three larger proteins (VP1, VP2 and VP3) are each strikingly similar to those of the known icosahedral plant RNA viruses, as is also their quaternary organization in the virus coat. Four neutralizing immunogenic regions have been identified by sequencing mutants selected for their ability to survive in the presence of neutralizing antibodies. The altered amino acids, as well as corresponding antigenic sequence in the homologous polio and foot-and-mouth disease viruses, reside on protrusions. A large cleft, spanning the center of each icosahedral face, is most probably the host cell receptor binding site. The carboxy end of VP4 (the small internal coat protein) is very close to Ser 10 of VP2, providing the basis for the autocatalytic cleavage of VP0 during maturation of the virus. The intertwining of VP0, VP1 and VP3 shows the nature of the 6S and 12S protomeric assembly units and perhaps a basis for the steps in their post-translational cleavage. The evolution of picornaviruses and RNA viruses can be assessed on the basis of sequence and structural alignments. Conserved amino acids important in protein folding and viral function can be recognized.

Gene Expression

Q9 GENE EXPRESSION IN TURNIP YELLOW MOSAIC VIRUS, Anne-Lise Haenni, Marie-Dominique Morch, Gabrièle Drugeon, Rosaura Valle, Rajiv L. Joshi and Térèse Marie Denial, Institut Jacques Monod, C.N.R.S. and Université Paris VII, 2 Place Jussieu, 75251 Paris Cedex 05, France

Turnip yellow mosaic virus (TYMV), the type-member of the tymovirus group contains a monopartite RNA genome of 2 X 10^6 daltons. A subgenomic RNA of 0.24 X 10^6 daltons deriving from the 3' region of the genomic RNA is also encapsidated; it codes for the viral coat protein. Various aspects of the viral RNA are being examined. 1) Expression. As seen by *in vitro* studies, several strategies are utilized by this virus to synthesize its non-structural proteins. These include read-through of a UAG codon, arrest of translation at the level of a stop signal distinct from a termination triplet, utilization of two overlapping reading frames, and post-translational cleavage (1). To determine the nature of the signals that enable the virus to synthesize its different proteins from a unique RNA species, and in particular the unconventional stop signal, the following approaches are being used: sequencing of the genomic RNA via cDNA synthesis, cloning and sequencing by the dideoxynucleotide method, identification of a tRNA capable of overcoming the stop signal, and determination of the C-terminal amino acids of the protein whose synthesis is interrupted by the stop signal so as to locate more precisely the position of this signal on the genome. A suppressor tRNA capable of recognizing the UAG codon has been isolated from calf liver and is being sequenced. 2) tRNA-like Region. The 3' region of TYMV RNA can be aminoacylated *in vitro* and *in vivo* with valine. The size of the shortest 3'-terminal RNA fragment capable of interacting with various tRNA-specific enzymes (tRNA nucleotidyltransferase, valyl-tRNA synthetase, elongation factor) has been determined, and has contributed to the establishment of a possible folding of the viral tRNA-like region (2). The effect of various tRNA-specific enzymes on viral RNA replication *in vitro* has been examined. The tRNA-like region synthesized by *in vitro* transcription of the corresponding cloned cDNA is being used to examine its interaction with tRNA-specific enzymes and with the viral RNA replicase.

(1) M.D. Morch, W. Zagorski & A.L. Haenni (1982). Proteolytic maturation of the turnip-yellow-mosaic-virus polyprotein coded *in vitro* occurs by internal catalysis. *Eur. J. Biochem.* 127, 259-265.

(2) S. Joshi, R.L. Joshi, A.L. Haenni & F. Chapeville (1983). tRNA-like structures in genomic RNAs of plant viruses. *Trends Biochem. Sci.* 8, 402-404.

Q10 TOROVIRIDAE: A PROPOSED NEW FAMILY OF ENVELOPED RNA VIRUSES, Marian C. Horzinek, Institute of Virology, Veterinary Faculty, State University Utrecht, Yalelaan 1, 3508 TD Utrecht, The Netherlands

Toroviruses are enveloped RNA viruses containing an elongated tubular nucleocapsid of presumably helical symmetry. The capsid may be bent into an open torus conferring a disk- or kidney- shaped morphology to the virion (largest diameter 120 to 140 nm) or straight, resulting in a rod-shaped particle (dimension 35x170 nm). Virus maturation occurs predominantly at Golgi vesicles by budding of preformed tubular nucleocapsids. The major structural protein of 20 kD is phosphorylated and participates in nucleocapsid architecture. Glycoproteins of about 120 kD and 80 kD (probably forming the peplomers) have been identified; their synthesis in inhibited by tunicamycin. This is also true for a 200kD glycoprotein which is present only in the infected cell. The viral genome has a molecular weight of 6x10*6, is polyadenylated and infectious. In infected cells 6 unique polyadenylated RNAs have been demonstrated with molecular weights of 2.6, 1.2, 1.0, 0.35, 0.27, and 0.22 x 10*6. The three smallest RNAs also appear in an extracellular particle of 50 S. The results described have been obtained with Berne virus, isolated from a horse; it is the only torovirus that can be propagated in cell culture until now. An antigenically related virus (Breda virus) has been recognized in calves with diarrhea. Seroepidemiologic evidence indicates that toroviruses infect ungulates, lagomorphs, rodents and man.

Q11 MURINE CORONAVIRUS GENE EXPRESSION, Stuart G. Siddell, Institute of Virology, University of Würzburg, Versbacher Str. 7, 8700 Würzburg, F.R.G.

The MHV virion is comprised of a positive-stranded RNA genome associated with a basic phosphoprotein, N, to form a helical nucleocapsid. This structure lies within a lipoprotein envelope which contains a largely integral transmembrane glycoprotein, M, and a peripheral glycoprotein, S, which assembles to form the surface structures of the virion. The organization and expression of the MHV genome can be summarized as follows 1) The virus structural genes encoding the N, M and S proteins are ordered within the 3' half of the genome. These genes are interspersed by putative non-structural genes. 2) The viral RNA polymerase activity is encoded in the 5' distal region of the genome. The number and arrangement of ORFS within this region are unknown. 3) The MHV genes which have been identified are non-overlapping. 4) The expression of MHV genes is mediated by multiple subgenomic mRNAs which form a 3' coterminal nested set. 5) Only the "unique" 5' regions of the subgenomic mRNAs are known to be translationally active and only a single polypeptide, representing one gene, is translated from each mRNA. The genes encoding the MHV-JHM N, M and S proteins have been cloned and sequenced. The predicted primary sequences of each protein reveal characteristic features. The N protein displays clustering of basic and acid residues as well as specific regions of high serine content. The M protein characteristically displays three N terminal regions of high hydrophobicity. The predicted amino acid sequence of the S protein displays regions characteristic of an N terminal signal sequence, an internal protopolypeptide cleavage site, a C-terminal membrane anchoring domain as well as the specific clustering of potential glycosylation sites and cysteine residues. The significance of these regions in the structural and functional organization of these proteins will be discussed.

Q12 MULTIPLE SUBGENOMIC mRNA's ARE INVOLVED IN THE GENE EXPRESSION OF EQUINE ARTERITIS VIRUS, A NON-ARTHROPOD BORNE TOGAVIRUS. Bernard A.M. Van der Zeijst*, M.F.van Berlo, W.C.Vooys, W.J.M.Spaan, P.Bredenbeek and M.C. Horzinek, Institute of Virology, Veterinary Faculty, State University Utrecht, Yalelaan 1, 3508 TD Utrecht, The Netherlands.

Equine arteritis virus (EAV), although structurally similar , differs completely from other togaviruses in its mode of replication. In addition to the genome (RNA1; 4.3 x 10^6 mol.wt) 5 subgenomic RNA's are present in infected cells with the following molecular weights: 1.3 x 10^6 (RNA2), 0.9 x 10^6 (RNA3), 0.7 x 10^6 (RNA4), 0.3 x 10^6 (RNA5) and 0.2 x 10^6(RNA6) [1]. The RNA's have a messenger activity but the viral proteins have not been mapped yet to the RNA's. There are a number of parallels with coronavirus replication [2]. In both cases the genomic and subgenomic RNA's form a nested set sharing 3' sequences. However, in the case of coronaviruses the sequence extensions in the larger RNA's are unique; in EAV this is only true up to RNA2. RNAse-T1 fingerprinting reveals 19 "unique" oligonucleotides in the genome, 6 more than in RNA2. But still considerably less than the expected number of 44, suggesting large repeats in RNA1 of regions also present in RNA2. The mechanism by which the RNA's are generated is also different in both cases. UV-transcription mapping experiments excluded that coronavirus RNA's are processed or spliced from a common precursor of genome length [3], but the same approach demonstrated that the EAV RNA's do arise from such a precursor. Splicing therefore seems to be a possible mechanisme. Since EAV is an RNA virus, replicating in the cytoplasma, such a splicing process should be an unorthodox one. Sequence studies are in progress to establish more precisely the homologies between the various RNA's and the points of sequence divergence.

1). Van Berlo, M.F., Horzinek, M.C., Van der Zeijst, B.A.M. (1982). Equine arteritis virus-infected cells contain six polyadenylated virus-specific RNA's. Virology 118, 345-352.
2). Siddell, S., Wege, H. and Ter Meulen, V. (1983). The biology of coronaviruses. J.gen.Virol. 64, 761-776.
3). Jacobs, L., Spaan, W.J.M., Horzinek, M.C. and Van der Zeijst, B.A.M. (1981) Synthesis of subgenomic mRNA's of mouse hepatitis virus is initiated indepently: Evidence from UV transcription mopping. J.Virol. 39, 401-406.

* Present address: University of Utrecht, Dept of Infectious Diseases, Section Bacteriology, P.O. Box 80.171, 3508 TD Utrecht, The Netherlands

Q13 COTRANSLATIONAL DISASSEMBLY OF PLANT VIRUS NUCLEOCAPSIDS, T. Michael A. Wilson, Department of Virus Research, John Innes Institute, Colney Lane, Norwich, NR4 7UH, U.K. and John G. Shaw, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, U.S.A.

Release of positive-sense RNA genomes from rod-shaped plant viral nucleocapsids requires a mechanism for the complete disassembly of the virus particles while maintaining some protection for the emerging RNA. Recent observations that otherwise-stable, tobacco mosaic virus (TMV) particles appear to uncoat cotranslationally in cell-free protein synthesizing systems (1,2) may provide some clues about the virus disassembly process in vivo (3). The discovery of active in vitro translation complexes "striposomes", in which the 5'-portion of the viral RNA is associated with several 80S ribosomes while the 3'-end of the RNA remains encapsidated, suggested to us methods for detecting such species in TMV-inoculated tobacco cells. Thus the progressive, stepwise removal of coat protein subunits during ribosome translocation would achieve the necessary release of viral RNA while affording some degree of continued protection against RNases and ensuring complete expression of the early (non-structural) viral genes. Using either $^{32}P/^{3}H$ double-labeled TMV particles, or ^{35}S-methionine and unlabeled virions, we can detect "striposome-like" intermediate complexes in tobacco epidermal cells within 30 min. of inoculation (4). Several control experiments which support this conclusion will be described. Comparable results from other host-virus inoculum combinations will also be described.

Other in vitro experiments indicate that regions of strong RNA-protein interaction may inhibit ribosome translocation (5) and that free coat protein subunits may impede, to a significant extent, the cotranslational disassembly process. The implications of these observations for the expression of packaged, subgenomic RNAs in vivo will be discussed.

References

(1) Wilson, T.M.A. (1984a) Virology 137, 255-265.
(2) Wilson, T.M.A. (1984b) Virology 138, 353-356.
(3) Wilson, T.M.A. and Shaw, J.G. (1985) Trends in Biochem. Sci. 10, 57-60.
(4) Shaw, J.G., Plaskitt, K.A. and Wilson, T.M.A. (1986) Virology (in press).
(5) Wilson, T.M.A. and Watkins, P.A.C. (1985) Virology 145, 346-349.

Proteolytic Processing of Viral Proteins

Q14 BIOSYNTHESIS AND PROTEOLYTIC PROCESSING OF RETROVIRAL POLYPROTEINS, S. Oroszlan, LBI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD.

The major internal structural proteins and replicative enzymes as well as the protease of all replication competent retroviruses are synthesized via the translation of the genome-size 35S mRNA. A smaller 28S spliced mRNA codes for the viral envelope proteins. In murine and feline viruses the primary translational products of the gag and gag-pol genes of the 35S RNA are $Pr65^{gag}$ (p15-p12-p30-p10') and $Pr180^{gag\text{-}pol}$ (p15-p12-p30-p10-protease-reverse transcriptase-endonuclease) respectively. Both are initiated with the same AUG of the 5' gag, and occasional in-frame readthrough of the gag amber terminator translated as glutamine is responsible for the synthesis of the pol gene derived portion of $Pr180^{gag\text{-}pol}$. After assembly and budding, proteolytic processing starts with a probably autocatalytic cleavage of $PR180^{gag\text{-}pol}$ between the protease and the reverse transcriptase. Additional cleavage at structurally conserved sites of both gag and pol products is accomplished by the viral protease. The product of the env mRNA, $gPR85^{env}$, is cleaved after the last arginine of the consensus sequence Arg-X-Arg/Lys-Arg by a cellular enzyme to generate the surface glycoprotein $gp70^{env}$ and the membrane anchored $Prel5^{env}$ which is further processed by the viral protease into $p15^{env}$ and the internal $p2^{env}$. All proteolytic cleavages are necessary for virus maturation and important for replication. Translational suppression, and the biosynthetic and proteolytic pathways in avian, bovine and human retroviruses will also be discussed.

Q15 REPLICATION OF ALPHAVIRUSES AND FLAVIVIRUSES: PROTEOLYTIC PROCESSING OF POLY PROTEINS, James H. Strauss, Ellen G. Strauss, Chang. S. Hahn, Young S. Hahn, Ricardo Galler, Winters R. Hardy and Charles M. Rice[1], Division of Biology, California Institute of Technology, Pasadena, California 91125

The genomes of the type alphavirus, Sindbis virus[2] (family Togaviridae) and of the type flavivirus, yellow fever virus[3] (family Flaviviridae) have been sequenced in their entirety and details of the genome organizations of these viruses determined. It appears clear that in both virus families proteolytic processing of polyprotein precursors is extensively used to produce the final protein products required for virus replication. We have proposed that these cleavage events fall into two categories[4]. The first category includes cleavages that occur in the cytosolic phase of the infected cell, that may involve production of nonstructural proteins required for RNA replication or in some cases structural proteins required for virion assembly. These we hypothesize are performed by virus encoded proteases. The second category includes cleavages that occur in subcellular organelles, the lumen of the endoplasmic reticulum or the Golgi apparatus, that are catalyzed by cellular proteases. These cleavages are restricted to viral proteins that have access to these compartments, which for the most part are structural glycoproteins. The nature of the cleavage sites used and the enzymes responsible for the cleavages are being probed by a combination of techniques including comparative sequencing of viruses, sequencing of temperature sensitive mutants defective in the protease at a nonpermissive temperature[5], and site-specific mutagenesis. For the alphaviruses, two mRNAs are translated into polyproteins. We propose that at least two virus encoded proteases are required to cleave these polyproteins, one a serine autoprotease to release the capsid protein from the structural polyprotein, the second a nonstructural protease to separate the four nonstructural proteins. The glycoproteins are hypothesized to be cleaved by signalase and a Golgi-associated protease. In flaviviruses only one mRNA is utilized and the resulting polyprotein is cleaved in a manner analogous to the alphavirus polyproteins, using a virus-encoded nonstructural protease and cell organelle bound proteases for the structural region.

[1]Present address: Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110.

[2]E. G. Strauss, C. M. Rice, and J. H. Strauss (1984) Virology 133, 92-110.
[3]C. M. Rice, E. M. Lenches, S. R. Eddy, S. J. Shin, R. L. Sheets and J. H. Strauss (1985) Science 229, 726-733.
[4]C. M. Rice and J. H. Strauss (1981) Proc. Natl. Acad. Sci. USA 78, 2062-2066.
[5]C.S. Hahn, E.G. Strauss and J.H. Strauss (1985) Proc. Natl. Acad. Sci. USA 82, 4648-4652.

Q16 PROTEOLYTIC PROCESSING IN PICORNAVIRUS REPLICATION, Eckard Wimmer, Martin J. H. Nicklin, Haruka Toyoda and Michael G. Murray, Department of Microbiology, SUNY at Stony Brook, Stony Brook, NY 11794

Poliovirus polyprotein, the only polypeptide encoded by the viral genome, undergoes numerous proteolytic cleavages to yield structural and non-structural proteins. Proteolytic processing occurs at three types of amino acid pairs: Q-G, Y-G and N-S. Cleavages at the Y-G and Q-G sites are catalyzed by the two virus-encoded proteinases called 3C and 2A, respectively (see Fig. 1). The activity cleaving at the N-S pair, an event occurring only during virion maturation, is not known. Cleavage specificity is not governed by the nature of the amino acid pair alone; other determinants such as the amino acid at the -4 position and polypeptide folding may also play a crucial role. Polypeptides 3C and 2A appear to be capable of intramolecular catalysis. For example, experimental evidence supports a model by which 2A cleaves the Y-G pair between the P1 and P2 region as soon as the ribosomes passed the coding region for 2A and the polyprotein is still in statu nascendi. The same is likely to be true for polypeptide 3C. Both activities must also be able to cleave in trans. Interestingly, the cleavage pathway of picornavirus polyproteins is different for different genera of Picornaviridae: entero- and rhinoviruses appear to have very similar cleavage patterns, whereas cardoviruses (EMC) and aphthoviruses (foot-and-mouth disease virus, FMDV) each exhibit some variation of the scheme identified by us for poliovirus. In EMC the polypeptide 2A does exist but its enzymic activity is uncertain. FMDV does not code at all for 2A. It appears that in polypeptide processing of FMDV a leader polypeptide (preceding the P1 region) has proteolytic activity cleaving the FMDV polyprotein between the L and P1 region. This leader polypeptide may also assume the role of polio's 2A.

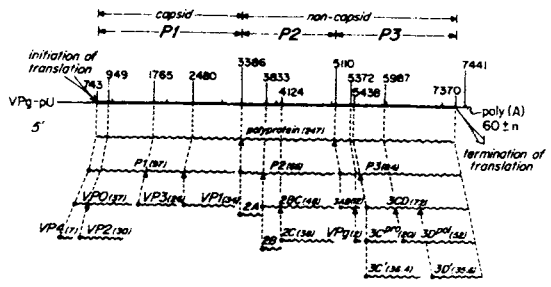


Fig. 1 Gene Organization and Polyprotein Processing of Poliovirus. Closed triangles: Q-G; open triangles: Y-G; open diamond: N-S cleavage sites.

Genome Replication

Q17 IN VITRO STUDY OF FLAVIVIRUS REPLICATION COMPLEXES
Margo A. Brinton and Janet B. Grun
The Wistar Institute, Philadelphia, PA 19104

The components of flavivirus replication complexes are being characterized in West Nile Virus (WNV) infected BHK21/WI2 cells. Cell fractionation studies have indicated that the perinuclear membrane fragments are enriched in viral polymerase activity and also contain cellular adenylyl and uridylyl-specific terminal transferase activities. However, no specific role for either of these cellular enzymes in the initiation of flavivirus RNA transcription can be postulated, since flavivirus template RNAs do not contain poly A or poly U sequences at their 3'-termini. Instead, a stable stem and loop structure is present at the 3'-termini of the genome RNA. Although the size and shape of this terminal secondary structure is conserved among flaviviruses, sequence conservation is restricted to the loop regions. A similar secondary structure is not present at the 3'-terminus of the complementary minus strand template, indicating that initiation of plus and minus strand templates may differ.

It has been demonstrated for at least two other types of plus-strand RNA viruses, picornaviruses and RNA bacteriophages, that certain cellular proteins function as part of the viral replication complexes. In both cases, a viral-encoded enzyme provides the elongation function, but cellular proteins initiate the viral RNA templates. The existence of a murine flavivirus-specific host resistance gene which acts at the level of viral RNA synthesis, implies that one or more cellular proteins may also be involved in flavivirus RNA replication. Cellular proteins which can bind viral RNA are currently being studied to assess their involvement in flavivirus RNA synthesis.

Q18 REPLICATION AND TRANSCRIPTION OF MURINE CORONAVIRUS RNA, Michael M.C. Lai, Shinji Makino, Ralph S. Baric, James G. Keck, Lisa Soe, Chien-Kou Shieh and Stephen A. Stohlman Department of Microbiology, University of Southern California, School of Medicine. Los Angeles, California 90033

The genomic RNA of murine coronaviruses is a single piece of nonsegmented RNA, which is transcribed into seven mRNAs of nested-set structure in infected cells. All of these mRNAs contain a stretch of 72-nucleotide leader sequences, which is encoded from the 5'-end of the genomic RNA. Various biochemical and biological evidence suggests that this leader RNA is not joined to the mRNAs by conventional RNA splicing. We have proposed a leader-primed transcription model in which the leader RNA is tanscribed independently, falls off and rejoins the RNA template and then serves as the primer for transcription of the various coronavirus mRNAs. We will present evidence in support of this model: (1) Free leader RNA species of various size have been detected in MHV-infected cells. Some of these RNAs were separated from the RNA template. (2) We have isolated a temperature-sensitive mutant which synthesizes only the leader RNA but not mRNAs. (3) During mixed infection, the leader RNA sequences of a virus can be freely reassorted to the mRNAs of a co-infecting MHV. These data prove that free leader RNA participates in the transcription of MHV mRNAs. (4) Expression of an anti-sense leader RNA in L cells inhibited the transcription of subgenomic mRNAs of a superinfecting virus. These data suggest that the leader RNA is required for the initiation of MHV mRNA transcription.

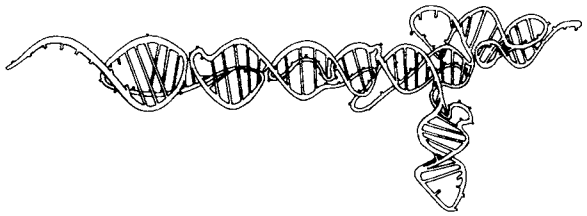
In addition to the leader-sized RNA species, large leader-containing RNA species have also been found in MHV-infectd cells. The presence of these discrete-sized RNA intermediates suggests that MHV RNA replication might proceed in a discontinuous and nonprocessive manner, generating RNA intermediates as a result of transcriptional pausing. These discrete RNA intermediates could lead to generation of RNA recombinant viruses by a copy-choice mechanism. Indeed, we have detected several such recombinants between A59 and JHM strains. By using a mixed infection involving a temperature-sensitive mutant of A59 and wild-type JHM, we have also shown that recombination frequency between coronaviruses could be higher than 10%. Such a high frequency recombination is reminiscent of RNA reassortment in segmented RNA viruses. These data are in support of discontinuous, nonprocessive mode of MHV RNA replication.

The viral RNA polymerase is being studied by cDNA cloning and sequencing of the 5'-end of the RNA genome, which encodes RNA polymerase.

Q19 THE SPATIAL FOLDING OF THE 3' NONCODING REGION OF AMINOACYLATABLE PLANT VIRAL RNAs, Cornelis, W.A. Pleij, Jan Pieter Abrahams, Alex van Belkum, Krijn Rietveld and Leendert Bosch, Department of Biochemistry, University of Leiden, The Netherlands

Three-dimensional models of the tRNA-like structure at the 3' terminus of turnip yellow mosaic virus (TYMV), brome mosaic virus (BMV) and tobacco mosaic virus (TMV) RNA were proposed earlier on the basis of chemical modification studies, enzymatic digestions and sequence comparisons[1]. An essential feature in the construction of the aminoacyl acceptor arm of all tRNA-like structures studied so far is the presence of a so-called pseudoknot. This novel RNA folding principle is based on a Watson-Crick base pairing of a region from a hairpin or bulge loop with a single stranded complementary sequence elsewhere in the RNA chain[2].

Experimental analysis of the region located upstream of the tRNA-like structure of TMV RNA revealed 4 more pseudoknots arranged in tandem. All these 5 pseudoknots together give rise to a compact folding of the 3' terminal 200 nucleotides of TMV RNA. A model for the spatial folding was proposed (see fig.)[3]. The presence of these pseudoknots was strongly supported by sequence comparisons among tobamoviral RNAs.



A systematic survey of the 3' noncoding regions of other (aminoacylatable) plant viral RNAs showed that similar "stalk-like" structural elements, consisting of several consecutive pseudoknots, appear to be present in other RNAs as well (e.g. RNAs of the bromovirus and hordeivirus groups). The strong conservation of these tandemly arranged pseudoknots suggests an important biological role. The presence of similar pseudoknotted structures in the 3' noncoding regions of poly(A) containing RNA viruses could not be demonstrated so far.

References

1. K. Rietveld, K. Linschooten, C.W.A. Pley and L. Bosch (1984) EMBO J. 3, 2613-2619.
2. C.W.A. Pley, K. Rietveld and L. Bosch (1985) Nucl. Acids Res. 13, 1717-1731.
3. A. van Belkum, J.P. Abrahams, C.W.A. Pley and L. Bosch (1985) Nucl. Acids Res. 13, 7673-7686.

Q20 REPLICATION AND PACKAGING SEQUENCES IN DEFECTIVE INTERFERING RNAs OF SINDBIS VIRUS, Sondra Schlesinger, Robin Levis, Barbara G. Weiss, Manuel Tsiang and Henry Huang, Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, MO 63110

Defective interfering (DI) genomes provide a valuable tool for identifying those sequences in a viral genome essential for replication and packaging. DI genomes are less complex than the genome of an infectious virion; although they must contain recognition sequences for replication and packaging, they need not have any coding information. We have been analyzing a DI genome of Sindbis virus to identify essential sequences. To achieve this goal, we cloned a cDNA copy of a complete DI genome directly downstream from the promoter for the SP6 bacteriophage DNA dependent RNA polymerase. The cDNA was transcribed into RNA which was transfected into chicken embryo fibroblasts in the presence of helper Sindbis virus. After one to two passages the DI RNA became the major viral RNA species in infected cells. Using this transfection and amplification assay we are examining a series of deletions covering the entire DI genome. The results of this study will be presented.

Q21 STRUCTURE-FUNCTION RELATIONSHIPS IN THE GLYCOPROTEINS OF ALPHAVIRUSES, Ellen G. Strauss, Division of Biology, California Institute of Technology, Pasadena, CA 91125; Alan Schmaljohn, Department of Microbiology, University of Maryland School of Medicine, Baltimore, MD 21201; and Diane Griffin, Department of Medicine and Neurology, Johns Hopkins University, Baltimore, MD 21205

The alphaviruses consist of an icosahedral core containing the genomic RNA complexed with about 200 molecules of the capsid protein (C) surrounded by a lipid bilayer containing two integral membrane proteins, E1 and E2. The core particle self-assembles in the cytoplasm of infected cells and diffuses to the periphery of the host cell, where it interacts with cytoplasmic domains of the transmembranous E1 and E2. The capsid then buds through modified plasmalemma containing exclusively virus-specified polypeptides, releasing the mature enveloped virion into the extracellular space. We have been studying the mechanisms by which these components are synthesized, processed, modified in various ways, directed to their proper cellular destination, and interact to form infectious virions, using a number of virus variants whose interactions have been altered by changes in the primary amino acid sequence. We have been identifying and mapping these changes by sequencing the appropriate regions of the 26S RNA which encodes them. These have been sequenced both from cDNA copies by chemical sequencing or directly from the viral RNA chain termination methods by using reverse transcriptase and specific oligonucleotide primers. Three types of variants are being examined. First, we have examined a number of temperature sensitive mutants belonging to the three RNA^+ complementation groups of Sindbis virus. Mutants of group C affect capsid protein and its autoproteolytic cleavage from the nascent polyprotein precursor[1], mutants of group D affect processing and transport of E1[2] and mutants of group E affect E2[3]. Secondly, we have examined a number of antigenic variants of Sindbis virus which were selected by their resistance to neutralization by monoclonal antibodies to purified glycoproteins E1 and E2. Thirdly, we are examining Sindbis variants selected for altered neurovirulence for mice. Determination of the amino acid changes responsible for antigenic variation and altered pathogenicity can help to identify functional domains of the viral proteins and regions in which these proteins interact with one another and with their hosts.

[1]Hahn, C. S., E. G. Strauss, and J. H. Strauss (1985). Proc Natl. Acad. Sci. USA 82, 4648-4652.
[2]Arias, C., J. R. Bell, E. M. Lenches, E. G. Strauss, and J. H. Strauss (1983). J. Mol. Biol. 168, 87-102.
[3]Lindqvist, B. H., J. DiSalvo, C. M. Rice, J. H. Strauss, and E. G. Strauss (1986). Virology, in press.

Translation, Protein Modification, and Assembly

Q22 SYNTHESIS OF THE STRUCTURAL PROTEINS OF SEMLIKI FOREST VIRUS, Henrik Garoff, Paul Melançon and Daniel Cutler, European Molecular Biology Laboratory, Box 102209, 6900 Heidelberg, FRG.

All structural proteins of Semliki Forest virus (SFV) are translated from a 26S mRNA molecule using a single initiation site. The capsid protein (C) is made first and this is followed by two membrane glycoproteins, p62 and E1. In addition, the 26S mRNA encodes a 6kD nonstructural peptide. Its coding region is interspaced between those of the two glycoproteins. We have used directed mutagenesis of the cloned polyprotein cDNA to study the various protease cleavages and peptide signals which are required for the correct synthesis of the viral proteins. The most important results and conclusions are summarized below.

I. The earlier suggestion that the C protein is an autoprotease (1,2) has been confirmed in an experiment in which we inserted a DNA linker close to the region encoding the proposed active center of this protein. Expression of the mutant polyprotein in vitro yields a 130kD uncleaved product.

II. A hybrid protein was created by replacing the coding region for capsid and most of the p62 with that for the secretory protein lysozyme. Expression of this hybrid in vitro in the presence of RER microsomes yields a transmembrane lysozyme-p62 fusion molecule, the 6kD peptide and glycosylated E1. This demonstrates that the C protease does not catalyse the cleavages of the 6kD peptide.

III. The membrane binding region of the p62 protein was mutated as follows: (1) A charged residue was introduced in the middle of the hydrophobic transmembrane peptide of p62. (2) Neutral or acidic residues were substituted for the basic ones in the flanking cluster of basic aminoacid residues. When expressed in vivo the mutant proteins displayed normal topology and were transported to the cell surface. This suggests that the peptide signals which are involved in the generation of a transmembrane topology of the p62 protein are degenerate.

IV. A series of internal deletions were introduced in the SFV polyprotein cDNA. The effect on E1 synthesis and translocation was analyzed in vitro. The results suggested that the signal for E1 translocation was localized to the COOH-terminal third of the 6kD peptide. This was confirmed by fusing the putative signal region to α-globin and showing translocation in vitro. Further characterization demonstrated that the E1 translocation signal required SRP similar to a cleavable signal peptide.

1. Aliperti et al. (1978) Virology 90:366.
2. Boege et al. (1981) Virology 113:293.

Q23 MECHANISM OF IN VITRO ASSEMBLY OF A T=3 PLANT VIRUS

Harrison, S.C., Sorger, P.K., Stockley, P.G. Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

The structure of turnip crinkle virus (TCV) has been determined at molecular resolution, using the structure of the closely related tomato bushy stunt virus as a starting point. TCV can be dissociated and reassembled in vitro. The products of dissociation at elevated pH and ionic strength are free coat-protein dimers and an RNP complex that contains the viral RNA, six coat-protein subunits, and one p80 (a covalently linked coat-protein dimer found in one copy per virion). This "rp-complex" is stable at high salt and pH 8.5. Reassembly of TCV, selective for viral RNA, can be accomplished under physiological conditions, using isolated coat protein and either rp-complex or protein-free RNA. Electron microscopy shows that assembly proceeds by continuous growth of a shell from an initiating structure. These and other data lead to a model for the mechanism of TCV assembly, with rp-complex as the specific initiating structure.

Q24 THE MYRISTYLATION SITE IN $Pr65^{gag}$ IS REQUIRED FOR ASSEMBLY OF MOLONEY MURINE LEUKEMIA VIRUS, A. Rein, M. McClure, N. Rice, A. Schultz, LBI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

The polyprotein precursor ($Pr65^{gag}$) of the core proteins of mammalian type C retroviruses is modified by the addition of myristic acid to the N-terminal glycine (1,2). We have used oligonucleotide-directed mutagenesis to change the glycine codon at the 5' end of the gag gene of Moloney MuLV to an alanine codon; we have also specifically deleted the glycine codon.

Infectivity assays on cells transfected with these mutant MuLV genomes show that the mutants are replication-defective. The transfected cells contain $Pr65^{gag}$ molecules which are not myristylated; thus the N-terminal glycine is essential both for myristylation and for production of infectious progeny. Significantly, we find no evidence for virus particle production by these cells, even with sensitive immunological assays. These results show that the myristylation site is required for release of Moloney MuLV.

Preliminary cell-fractionation experiments indicate that, in contrast to wild-type $Pr65^{gag}$ molecules, little or no mutant (unmyristylated) $Pr65^{gag}$ is membrane-bound.

Type C viruses normally assemble at the plasma membrane. Our results suggest that myristic acid plays a role in the association of $Pr65^{gag}$ with the membrane, and that this association is an essential early step in type C virus assembly.

1. Henderson, L., H. Krutzsch, and S. Oroszlan; PNAS 80:339, 1983.
2. Schultz, A., and S. Oroszlan; J. Virol. 46: 355, 1983.

(Research sponsored by National Cancer Institute under contract NO1-CO-23909 with Litton Bionetics, Inc.)

Q25 STRUCTURE AND BIOGENESIS OF CORONAVIRUS MEMBRANE GLYCOPROTEIN E1, Peter J.M. Rottier, The Salk Institute, San Diego, La Jolla, CA 92138

Coronaviruses are enveloped animal viruses with a single positive-stranded RNA. One interesting property of these viruses is that they bud from intracellular membranes rather than from the plasma membrane. The lipid envelope of mouse hepatitis virus strain A59, the most intensively studied member of the Coronaviridae, contains two membrane-associated glycoproteins, E1 and E2. E1 plays a key role in the intracellular budding process. This protein lacks covalently bound fatty acids and contains O-linked oligosaccharides. After its synthesis on membrane-bound ribosomes it stays in internal membranes. We have undertaken a study on the synthesis and structure of the E1 glycoprotein. Translation of viral mRNA in the presence of dog pancreatic microsomes revealed

i) that the insertion of E1 into membranes requires signal recognition particle (SRP),
ii) that no signal sequence is cleaved off, and
iii) that a large part of the protein becomes buried within the lipid bilayer.

On the basis of these data and of the amino acid sequence of the E1 protein, a structure model was developed. In addition, the availability of a cDNA clone for E1 allowed us to start experiments on the expression of the gene the results of which will be discussed.

Special Lecture

Q26 GENETIC ANALYSIS OF POLIOVIRUS, David Baltimore, Harris Bernstein, Karla Kirkegaard, Barbara Nelsen and Peter Sarnow, Whitehead Institute, Nine Cambridge Center, Cambridge, MA 02142 and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Although much has been learned about the biochemistry of the poliovirus life cycle and physiology over the last 30 years, many events are poorly understood, many polypeptides have not been associated with a function and there are certainly hidden surprises. To crack these previously recalcitrant nuts we have used infectious poliovirus cDNA as a substrate for making defined mutational alterations in the virus. These have been used both to develop a formal genetics of poliovirus and to provide a genetic wedge into various physiological phenomena. Mutants have been made by either insertion of 3-nucleotides segments, insertion of linker DNA or deletion of random DNA. The most surprising mutant is one with a highly temperature-sensitive phenotype resulting from an alteration in the 3'-noncoding region. With this mutant and a complex guanidine resistance mutation, high resolution intertypic recombination studies have proved possible. These have shown no obvious site preference, many occurring at sites of limited local RNA homology. Complementation between various pairs of mutations has been demonstrated. Mutants have revealed a protein involved in inhibition of host cell protein synthesis and a new locus involved in RNA synthesis. A number of morphogenesis mutants have been made by alterations in the capsid proteins. Clearly, genetic analysis of RNA viruses is now possible and can offer many new insights.

Pathogenesis and Virulence

Q27 MOLECULAR DETERMINANTS OF CNS VIRULENCE OF THE CORONAVIRUS MOUSE HEPATITIS VIRUS-4, Michael J. Buchmeier, Robert G. Dalziel, M.J.M. Koolen, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Infection of mice with the coronavirus mouse hepatitis virus-4 (JHM strain) has provided one of the best experimental models of virus-induced demyelinating disease. A high percentage of mice infected intracerebrally with wild type MHV-4 succumb within 7 days due to an acute encephalomyelitis. In the small fraction of animals surviving this initial acute infection demyelination is evident in the brain and spinal cord white matter. Over the past decade a number of laboratories have reported the isolation of temperature sensitive mutants and spontaneously arising variants of the virus which are attenuated with respect to encephalomyelitis but retain the ability to induce demyelinating disease. These models have consistently shown that in the attenuated (with respect to neurovirulence) infection, neurons in the CNS are spared but infection of oligodendroglial cells occurs. The oligodendrocyte is the cell responsible for elaboration and maintenance of CNS myelin sheaths, and infection results in loss of these cells and consequent demyelination. Genetic lesions responsible for loss of virulence in these models have not been determined. Our laboratory has attempted to define the factor(s) which determine neurotropism and limit the spread of infection <u>in vivo</u> in the CNS. We have used monoclonal antibodies (MAb) of defined specificity in a passive transfer model to define viral proteins which are important determinants of neurovirulence. Using this approach we have shown that MAb against two topographically distinct sites on the E2 peplomer glycoprotein of MHV-4 are able to block development of fatal encephalitis following ic infection with wild type virus. Although neurons were spared, oligodendrocytes were infected and demyelination resulted. Having shown that sites on E2 were involved, we selected variant strains of MHV-4 which contained mutations in E2 and were resistant to E2 directed neutralizing MAb. These mutant strains of virus were attenuated by a factor of 2-3 logs in LD_{50}, but continued to induce chronic demyelinating disease characterized by mild inflammation, primary demyelination, remyelination and recurrent demyelination over a prolonged observation period. Thus we have directly demonstrated that the E2 glycoprotein is an important determinant of neurovirulence in MHV-4 infection. Ongoing studies are directed toward identifying the nature of the lesions in the variant viruses and their effect on tropism and virulence. Supported by USPHS grants NS12428 and AI16102. M.J.M. Koolen is the recipient of a long-term fellowship from EMBO.

Q28 CELLULAR RECEPTORS IN COXSACKIEVIRUS INFECTIONS, Richard L. Crowell, K-H Lee Hsu, Maggie Schultz and Burton J. Landau, Department of Microbiology and Immunology, Hahnemann University School of Medicine, Philadelphia, PA 19102.

Cellular receptors are important determinants of virus tropism in the pathogenesis of human and animal diseases. This concept is derived from the observation that the presence or absence of specific receptors is the predominant determinant of the host range of a virus. In addition, the receptor specificity for the different species of picornaviruses places these viruses within sub-groups according to their original classification which was based on the type of disease and histopathology produced in humans or animals. This receptor specificity was determined by competition between viruses for a given receptor (1). More recently, monoclonal antibodies prepared against cellular receptors for picornaviruses have confirmed this receptor specificity (2-5). A receptor protein, Rp-a, of 49.5 kd which binds to group B cosackieviruses (CB) has been isolated from HeLa cells (6). A rabbit antiserum prepared against the receptor preparation was found to block binding and protect cells against CB1, CB4, CB5, CB1-RD, and echo 6, but not against poliovirus T1 or CB6. Surprisingly, this rabbit antiserum resembled the activity of a monoclonal antibody (2) which was active against receptors for CB1, CB3, CB5 and their respective RD variants, echovirus 6 and CA21, but not against CB2,CB4,CB6, polioviruses T1-3 or 12 other picornaviruses. These and other antibodies should prove useful in determining the relationship between the diverse receptors and their structures on different cell types. Because the CB-RD variant viruses may have acquired a second site for binding to an additional receptor (7) and because CB3 was unique among the parental CB viruses for binding to receptors of the rat L_8 myogenic cell line, more than one type of receptor for binding virus may exist on different cell types which can influence the cellular host range and virus tropism (8). The distribution and function of these receptors on different cell types during stages of differentiation remains to be determined.

1. Crowell, R.L. and Landau, B.J. 1983. Comp. Virology 18: 1-42.
2. Crowell, R.L., et al. 1986, J. Virol. 57 (Feb).
3. Colonno, R.J., et al. 1986, J. Virol. 57: 7-12
4. Minor, P.D., et al. 1984, Virus Res. 1: 203-212.
5. Campbell, B.A. and Cords, C.E. 1983, J. Virol. 48: 561-564.
6. Mapoles, J.E., et al, 1985, J. Virol. 55: 560-566.
7. Reagan, K.J., et al, 1984. J. Virol. 49: 635-640.
8. Crowell, R.L., et al, 1985, Banbury Rep. 22, Cold Spring Harbor Lab. 147-161.

Q29 RESPONSES OF PLANT CELLS TO INFECTION BY POSITIVE-STRAND RNA VIRUSES, R. I. B. Francki, Department of Plant Pathology, Waite Agricultural Research Institute, University of Adelaide, South Australia 5064

Our understanding of the molecular events leading to the synthesis of plant viruses has advanced spectacularly in recent years. However, the cellular localization of these events and the responses of the cell to infection remain largely obscure. Much of the data concerning infected cells, whether structural or physiological, are largely descriptive. For example, the molecular events leading to the synthesis of tobacco mosaic virus (TMV) are at least in part understood, yet the mode of entry of the virus into cells and the sites of RNA replication are largely a matter of conjecture. The function of some TMV-induced structures such as the X-bodies are no clearer now than they were at the turn of the century when they were first observed.

Perusal of the literature concerning the cytology and physiology of virus-infected plant cells indicates that many of the observed changes following infection are manifestations of virus-induced senescence whereas others appear to be concerned directly with virus multiplications. One of the most consistent structural changes in plant cells supporting the multiplication of positive-sense RNA viruses is the development of vesicles containing what apepars to be ds-RNA. The cellular location of these structures vary with different viruses. They include nuclei (e.g. pea enatian mosaic virus), chloroplasts (e.g. turnip yellow mosaic virus), mitochondria (e.g. cucumber green mottle mosaic virus), peroxisomes (e.g. tomato bushy stunt virus), tonoplasts (e.g. cucumber mosaic virus) and the endoplasmic reticulum (e.g. cowpea mosaic virus). The possible significance of these structures to virus replication will be discussed in detail.

Q30 STUDY ON VIRULENCE OF POLIOVIRUS TYPE 1 USING IN VITRO MODIFIED VIRUSES.

Akio Nomoto[1], Michinori Kohara[1,2], Shusuke Kuge[1], Mineo Arita[3], Toshihiko Komatsu[3], Shinobu Abe[2], Bert L. Semler[4], Noriyuki Kawamura[1], Eckard Wimmer[5], and Heihachi Itoh[2]. [1]Department of Microbiology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113; [2]Japan Poliomyelitis Research Institute, Tokyo 189; [3]NIH of Japan, Tokyo 190-12; [4]Univ. of California, Irvine, CA 92717; [5]State Univ. of New york at Stony Brook, New York 11794.

Poliovirus, the causative agent of poliomyelitis, is a human enterovirus of the Picornaviridae, and contains a single-stranded RNA genome of messenger-sense. The attenuated Sabin 1 strain, oral live vaccine strain, was derived from the virulent Mahoney strain of type 1 poliovirus by spontaneous mutations occurred during attenuation process. In addition to their differences in the potential for causing disease, these two strains differ in many biological characteristics. These include the sensitivity of viral multiplication at elevated temperature (rct marker), the size of plaques produced in infected monolayers of primate cells, and the sensitivity to low concentration of bicarbonate under agar overlay (d marker).

Different biological characteristics of these two strains must be due to the differences in genome structures. The elucidation of the total nucleotide sequences of the RNA genomes of both the strains has revealed 55 nucleotide substitutions within the total of 7441 heteropolymeric bases. To identify the nucleotide changes contributing to the different characteristics, a number of recombinant viruses between the virulent Mahoney and the attenuated Sabin 1 strains were constructed in vitro using infectious cDNA clones of both the strains, and tested for their biological characteristics.

Determinants of neurovirulence and rct marker were spread over several areas of the viral genome, including the 5' noncoding region. The temperature-sensitivity had some correlation with the attenuation, suggesting that viral multiplication rate in the central nervous system of monkey might be one of the important factors contributing to the neurovirulence. Determinants of plaque size and d marker appeared to exist in the genome region encoding viral capsid proteins. Viruses carrying Sabin-derived capsid proteins had tendency to show less neurovirulence. This suggested that some characters of Sabin capsid proteins might affect to the viral attenuation. However, virion surface function such as immunogenicity had little correlation with neurovirulence. These results indicated that expression of the attenuation phenotype of the Sabin 1 strain required a number of biological characteristics.

Strategies for Control of Positive-Strand Virus Diseases

Q31 CONTROL OF FOOT-AND-MOUTH DISEASE: THE PRESENT POSITION AND FUTURE PROSPECTS.

F. Brown, Wellcome Biotechnology Ltd, Beckenham, Kent, UK.

Foot-and-mouth disease is the most important virus disease of farm animals. Not only does it cause loss of productivity of up to 25% but the consequential losses following an outbreak can be even more devastating. Control is by slaughter (the stamping-out policy) where the disease does not normally occur or by vaccination in those countries where the disease is endemic.

Vaccination is complicated by the occurrence of the virus as seven serotypes. Moreover, the antigenic diversity within serotypes often means that, although a vaccine will give good protection against the homologous virus it may afford only limited protection against other viruses of the same serotype. Consequently, vaccines must be chosen which are closely related antigenically to the current isolates.

Recent work on the molecular biology of the virus has provided detailed information on the immunogenic sites of the virus and enabled us to test the possibility of using synthetic peptides as vaccines. As little as 100μg of a peptide containing the amino acid sequence 141-160 of capsid protein VP1 will protect guinea pigs against challenge infection and preliminary results with cattle using the same peptide are encouraging. In addition we have some evidence that a composite peptide comprising the 141-160 sequence and a sequence at the C terminus of VP1 is more efficacious in reacting with neutralizing antibody and eliciting an immune response.

The advantages of a totally synthetic vaccine which avoids the handling of infectious virus and the downstream processing of antigens produced in cells are considerable, both practically and intellectually. Clearly there are many problems still to be solved but the recent 3-D structural work on the picornaviruses should provide the means to do this.

Q32 RESISTANCE OF PLANTS TO POSITIVE-STRAND RNA VIRUSES, B. D. Harrison, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, U.K.

Recent work has enabled several types of virus resistance in plants to be distinguished:
1. Inoculated plants show no evidence of infection, and few if any protoplasts of these plant genotypes become infected when inoculated in suspension. Example, potato virus Y in potato carrying gene Ry.
2. Inoculated plants develop no symptoms but isolated protoplasts are infected normally. The virus seems unable to move from cell to cell in intact tissue. Example, tobacco mosaic virus in tomato carrying gene Tm-1.
3. Inoculated plants develop necrotic local lesions but are not invaded systemically. Isolated protoplasts are infected normally. Example, potato virus Y in potato carrying gene Ny.
4. Inoculated plants become systemically infected but virus replication is limited and many cells remain uninfected. Symptoms may be mild, and infected plants are poor inoculum sources. Isolated protoplasts can be infected. Example, potato leafroll virus in potato cv. Pentland Crown.
5. Plants are difficult to infect except by graft-inoculation. Example, raspberry leaf spot virus in raspberry cv. Glen Clova.

Plant breeders utilize all these types of resistance, and aim to increase the durability of resistance by combining the different types in the same plant cultivar. The improved characterization and categorization of resistance mechanisms should enable this objective to be attained more readily than before.

Another kind of virus-specific resistance, known as cross-protection, is shown by plants that are already infected with another strain of the virus in question. Although recent evidence indicates that, with nepoviruses, more than one virus gene is involved in cross-protection, it is hypothesized that one gene may be mainly responsible. In a novel approach to resistance breeding, plants are being transformed with individual virus genes in an attempt to simulate cross-protection. In a related approach, other plants are being transformed so as to enable them to produce antisense RNA that could interfere with the replication or translation of virus RNA. Reactions of these transformed plants to virus inoculation will be described and the implications for resistance breeding discussed.

Q33 PROSPECTS FOR DEVELOPMENT OF HEPATITIS A VACCINES, Robert H. Purcell, Hepatitis Viruses Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Hepatitis A virus is a picornavirus that shares characteristics with other picornaviruses, especially the entero- and cardioviruses, but is distinct from all recognized genera by hybridization and sequence analysis. Hepatitis A virus accounts for approximately 25% of clinical hepatitis in the United States and, although its importance is diminishing in developed countries, it is still highly endemic in much of the world and the most important cause of viral hepatitis in some developing countries. At present, only one serotype of HAV is recognized. There is a need for immunoprophylaxis against HAV and several approaches are being pursued. Candidate live HAV vaccines have been developed by serial passage of the virus in tissue culture, with resultant attenuation for susceptible primate species. Such vaccines are in preclinical trials. Killed whole-virus vaccines have also been developed. Although expensive because of the relatively poor growth of this virus in tissue culture, killed vaccines would have an added margin of safety. Killed vaccines are in early clinical trials. Recombinant DNA technology offers a third approach to hepatitis A vaccine development. As with other picornaviruses, HAV cDNA should be infectious and attempts to construct viable full length cDNA clones are in progress. These could then be modified by site-specific mutagenesis or generation of deletions to yield attenuated live virus vaccines. Attempts to express HAV capsid proteins in prokaryotic or eukaryotic expression systems are also being explored, although efficient expression of antigenic proteins of other picornaviruses has been difficult to achieve. Finally, synthetic peptides representing different regions of the capsid proteins, especially VP1, have been synthesized as part of a search for neutralizing epitopes. Although some neutralizing epitopes have been identified, it is not clear yet which are the most important for the neutralization of HAV or whether a vaccine consisting of one or more synthetic peptides is feasible. It is premature to determine which, if any, of these approaches to HAV vaccine development will be fruitful, but the basic virology of HAV deriving from these studies is yielding much new information about the nature of the hepatitis A virus.

Variation of Nucleic Acid and Protein

Q34 THE DETECTION OF RHINOVIRUSES BY DNA-RNA HYBRIDIZATION, W. Al-Nakib[1], G. Stanway[2], M. Forsyth[1], P. Hughes[2], J. Almond[3] and D.A.J. Tyrrell[1]; MRC Common Cold Unit, Salisbury[1] and the Departments of Microbiology at the Universities of Leicester[2] and Reading[3], U.K.

The entire genome of human rhinovirus (HRV) 14 has recently been cloned and sequenced. A SS M13 probe comprising 800 nucleotide sequences from the 5' end non-coding region of HRV14 has been constructed and labelled with 32-Phosphorus. This probe was used in DNA-RNA hybridization procedures to detect various rhinovirus serotypes. Of the 35 human rhinoviruses so far studied, 32 or 91% were detected using this probe indicating close genetic relationship with HRV14 in this region of the genome. The probe did not react with RNA from other respiratory viruses such as Influenza A, B, Parainfluenza 3 or coronavirus 229E or DNA from herpes simplex virus type 1. Preliminary data suggests that rhinoviruses could be detected in nasal washings using DNA-RNA hybridization procedures. Details of these findings and their implications are discussed.

Q35 Sequence Variation Among cDNA Clones of TMV, David L. Beck, David A. Knorr, and William O. Dawson, Univ. of Calif., Riverside, Riverside, CA 92521

The sequence heterogeneity of the 5' terminal nucleotides of tobacco mosaic virus (TMV) strain U1 was studied. There have been conflicting reports on the polymorphism of this region of TMV U1. Four independent cDNA clones of TMV were sequenced through the 5' terminal 250 nucleotides and all were identical. The lack of variation in the 5' untranslated region was confirmed by direct RNA sequencing using the dideoxy chain terminating method. The sequence determined for the 5' terminus of TMV U1 was 100% homologous to the shorter of two reported variants [Proc. Natl. Acad. Sci. USA (1982) 79, 5818].

We also determined the proportion of infectious, wild type virus in a native virus population. Transcripts obtained from three of four full-genomic TMV cDNA constructions were infectious. The progeny virus from plants infected with cDNA transcripts appeared identical to the wild type virus both biologically and physically. All of the cDNA clones that were sequenced and/or extensively analyzed with restriction endonucleases fit a single map. These results suggest that the majority of the viral RNAs in the virus population are similar, infectious, and express the wild type phenotype.

Q36 THE JAPANESE ENCEPHALITIS VIRUS GENOME: STRUCTURE AND RELATEDNESS TO OTHER FLAVIVIRUS RNAs. P.C. McAda[1], P.W. Mason[1], L.J. Niedzwiec[1], C.S. Schmaljohn[2], J.M. Dalrymple[2], T.L. Mason[1] and M.J. Fournier[1]. Dept. of Biochemistry, Univ. of MA, Amherst, MA 01003[1] and Dept. of Viral Biol., USAMRIID, Frederick, MD 21701[2].

Approximately 95% of the estimated 11 kb genome of the Japanese encephalitis virus (JEV) has been cloned as overlapping cDNA fragments of 2.5-3.5 kb. Physical and functional maps are being developed by DNA sequencing (~60% complete) and in vivo expression and immunological screening analyses, the latter in E. coli with the λgtll vector (see P. Mason et al., this meeting). The results have identified the genomic segments encoding the capsid (C), membrane (M) and envelope (E) structural proteins (S) and the non-structural (NS) proteins NS1, NS3 and NS5. The JEV genome is strikingly similar in size and organization to that of the yellow fever (YF) virus and the sequenced C-M portion of the West Nile virus. The six major JEV cistrons occur in the same order in YFV i.e., C-M-E-NS1---NS3---NS5 and an open reading frame extends at least through the C-M-E-NS1 coding region, consistent with possible co-expression of a S-NS polyprotein precursor. The encoded proteins also exhibit a high degree of relatedness . A segment of the E protein region determined to be rich in epitopes for both virus-neutralizing and flavivirus cross-reactive monoclonal antibodies shows 45% sequence homology with the corresponding YF protein and an essentially identical hydropathy profile. Together, these results suggest the possibility that other, perhaps even all flavivirus genomes will be organized in similar fashion and that the genetic relatedness will also extend to the proteins encoded.

Q37 OUTBREAK OF PARALYTIC POLIOMYELITIS IN FINLAND IN 1984 ASSOCIATED WITH ANTIGENIC VARIATION OF POLIOVIRUS TYPE 3, Tapani Hovi, Anita Huovilainen, Esko Kinnunen, Tuija Pöyry, Merja Roivainen, Nina Salama and Mirja Stenvik, Natl Public Health Inst, Helsinki

Nine cases of paralytic poliomyelitis and widespread circulation of wild-type poliovirus 3 were found in Finland between August 1984 and February 1985. These were the first reported cases of poliomyelitis since 1964 and this was the first occation of recognized circulation of poliovirus in the population since the early 1960s. After 1.5 million extra doses of inactivated poliovirus vaccine (IPV) to children and an oral poliovirus vaccine campaign covering about 93% of the entire population (4.8 millions) the outbreak and the circulation of the epidemic strain now appear to be over. Three different precipitating factors can be seen for the outbreak.(1) A considerable part of children under 15 years did not have neutralizing antibodies to poliovirus type 3. (2) The fraction of population with deficient mucosal immunity had been gradually increasing because of the use of IPV and lack of virus circulation. (3) The most important factor as regards the timing of the outbreak was probably the altered antigenic character of the virus. Antigenic and molecular analysis of several isolates at the National Institute for Biological Standards and Control, London, has shown that the strain associated with the outbreak differed markedly form the vaccine strains and from most previous wild-type strains of poliovirus 3 (Magrath et al., manuscr.). Further variation of the strain during the outbreak was documented by demonstrating antigenic shifts in the neutralization determinants in consecutive isolates from several persons (Huovilainen et al., mauscr.).

Q38 CHARACTERIZATION OF SINDBIS VIRUS STRAINS WITH MONOCLONAL ANTIBODIES, Alan C. Jackson, Jeff Stanley and Diane E. Griffin, Johns Hopkins University, Baltimore, MD

Sindbis virus (SV) causes fatal acute encephalitis in neonatal mice and nonfatal encephalitis in weanling mice. A neuroadapted strain (NSV), produced by serial intracerebral inoculation of mice, causes fatal encephalitis in weanling mice. A panel of anti-SV monoclonal antibodies (mabs) has been developed. Biological domain maps have been constructed for the neutralization and hemagglutination inhibition sites of the two envelope glycoproteins, E1 and E2 (J. Virol. 56: 110, 1985). A variety of attenuated and neuroadapted strains of SV have been characterized with anti-SV mabs. The pathogenicity of these strains varies from one that is avirulent in neonatal mice to a neuroadapted virus with a 50% lethal dose of 2-20 PFU. In addition, five neutralization-resistant variants were selected in vitro from a 5-fluorouracil-mutagenized NSV stock. The anti-E2 selecting mab neutralized NSV much better than SV. All variants grew efficiently in cell culture. Purified variant viruses were characterized by binding of serially diluted mabs on ELISA. Some variants showed alterations only in the biological domain of the selecting antibody, while others showed extensive changes which involved the capsid and envelope glycoproteins. All of the domains examined showed alterations in some variants. Decreased binding to variants was most marked for mabs recognizing the E2 neutralization epitopes. All of the neutralization-resistant variants retained virulence. This evidence suggests that the neutralizing epitopes defined by this panel of mabs are probably not essential for virulence.

Q39 FUNCTIONAL MAPPING OF THE JAPANESE ENCEPHALITIS VIRUS GENOME, P.W. Mason[1], P.C. McAda[1], D.S. Burke[2], J.M. Dalrymple[3], C.S. Schmaljohn[3], M.J. Fournier[1], and T.L. Mason[1]. Univ. of Mass., Amherst, MA 01003[1], Walter Reed Army Inst. Res., Wash., DC 20307[2], and U.S. Army Med. Res. Inst. Infect. Dis., Frederick, MD 21701[3]

JEV protein coding sequences were identified by expression of cloned cDNA (see McAda et al., this meeting) in *E. coli* using the λgtll expression vector. Recombinants expressing viral protein sequences as β-galactosidase fusion proteins were selected immunologically with monoclonal antibodies (mcAbs) and polyclonal antiviral hyperimmune mouse ascites fluid (HMAF). cDNA sequences expressed by 15 recombinants detected with E and M protein mcAbs were mapped to the 5' end of the genome. cDNA sequences expressed by ≈100 λ-clones reactive with HMAF but not with the E/M mcAbs were located to other portions of the genome. Identification of these immunoreactive protein sequences was achieved by using the fusion proteins to affinity purify antibodies from HMAF. These antibodies were then used to identify homologous, viral proteins in western blots of JEV-infected mosquito cells.

These methods have been used to: (1) map the proteins M, E, NS1 (NV3), and NS3 (NV4) to the viral genome, (2) map the epitopes recognized by 9 anti-E mcAbs; these epitopes, mapped thus far to a 140 amino acid sequence, are also clustered on the surface of the intact virion, (3) obtain functional evidence for a translational open reading frame between M, E, and NS1, (4) identify in infected mosquito cells higher MW forms of NS1 that contain additional COOH terminal hydrophobic sequences.

Q40 SEQUENCE STUDIES OF THEILER'S MURINE ENCEPHALOMYELITIS VIRUS (TMEV), Sorachai Nitayaphan, Daniel Omilianowski*, Steven B. Stein, Yoshiro Ohara, Marcia M. Toth, and Raymond P. Roos, University of Chicago, Chicago, IL; *University of Wisconsin, Madison, WI.

TMEV are a group of mouse picornaviruses which cause enteric infection and varied neurological disease in mice. The TO subgroup TMEV strains (which includes DA) cause a chronic persistent white matter demyelinating infection in mice, while the GDVII subgroup strains cause gray matter neuronal disease and neither persist nor demyelinate. We have analyzed the structure of TMEV in order to delineate molecular determinants of the virus' biological activities.

Following HPLC purification of DA strain capsid proteins, we analyzed the first 17 amino acids (aa's) of the amino (N)-terminus of DA VP1. The aa sequence was more similar to encephalomyocarditis virus (EMC) a member of the cardiovirus genus than to other picornaviruses. Nucleic acid sequencing of the polymerase region of cDNA clones of DA demonstrated greater homology at the amino acid level to EMC than to other picornaviruses. The results suggest that TMEV is a member of the cardiovirus genus not the enterovirus genus as previously suspected. These studies will be important in construction of a full-length clone for recombinant infectious cDNA manipulations.

Q41 **GENETIC RELATIONSHIPS AMONG COXSACKIE B VIRUS ISOLATES DETERMINED BY GENOMIC SEQUENCING.** **Mark A. Pallansch, Charlotte Freeman, and Olen M. Kew, Center for Disease Control, Atlanta, GA 30333**

The Coxsackie B viruses comprise 6 serotypes within the enterovirus group. Surveillance data within the United States has shown different prevalence of the serotypes in different years. In addition, certain serotypes, particularly Coxsackie B5, appear in an epidemic pattern with few isolates in non-epidemic years. Other serotypes, such as Coxsackie B4, appear in an endemic pattern with nearly the same number of isolates in every year. Preliminary studies using oligonucleotide fingerprinting of Coxsackie B5 showed great similarities among isolates within an epidemic year but differences between isolates of different epidemics. In order to increase the sensitivity for observing changes over long periods within a serotype and to study similarities between serotypes, isolates of all six serotypes were examined by genomic sequencing. These data show geographic and temporal changes within a serotype and demonstrate significant similarities between serotypes.

Q42 IS ECHOVIRUS 6 PERSISTENCE ASSOCIATED WITH ALTERATIONS IN VIRAL CAPSID PROTEINS? V. Fay Righthand and Robert V. Blackburn, Wayne State University, Detroit, MI 48201

The structural polypeptides of purified echovirus 6 (m+), recovered from acutely infected WISH cells, were immunoprecipitated by polyclonal anti-echovirus 6 serum (D'Amori) and separated on the basis of molecular weights and isoelectric points by polyacrylamide gel electrophoresis. The apparent molecular weights of the viral polypeptides were 31.5, 27, 21 and 9.5 kilodaltons. At least 7 different species of these polypeptides were detected by isoelectric focusing, and 2 of these species migrated at the molecular weight of VP1 (27 kd). Peptide analyses by partial proteolysis of isolated viral proteins indicate that the two smaller polypeptides are processed from the largest polypeptide (VP0) and are thus designated as VP2 (21 kd) and VP4 (9.5 kd). Viral RNA is detected within 4 hours after infection by hybridization with cDNA prepared from purified virions. By 7 hours after infection, most of VP0 has been processed, and the other 4 structural viral proteins (VP1-VP4) are detectable in equal concentrations. Although genome size echovirus 6 RNA is present in cloned, persistently infected cells, only VP0, VP1 and VP3 are detected in either cellular extracts or in viral particles recovered from these cells. The absence of VP2 and VP4 may be due to either incomplete processing of VP0 or to instability and degradation of the processed VP0 products during persistent echovirus 6 infection.

Q43 MECHANISMS OF IN VITRO NEUTRALIZATION OF ALPHAVIRUSES BY MONOCLONAL ANTIBODIES, John T. Roehrig and James H. Mathews, Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins, CO 80526

We have previously identified at least eight epitopes on the E2 glycoprotein of Venezuelan equine encephalomyelitis (VEE) virus using monoclonal antibodies (MAbs). Some of these antibodies identified a critical neutralization (N)-site. Passive transfer of MAbs reactive with this site were efficient at protecting animals from a lethal viral encephalitis. Antibody fragmentation studies determined that an intact Fc was required for in vivo protection, and bivalent antibody was required for in vitro virus N. We are currently characterizing virus-cell interactions, and the mechanisms by which neutralizing MAbs inhibit virus replication in vitro. Adsorption studies were performed with radioactively labelled VEE virus on Vero and human diploid cells. Dilutions of homologous and closely related heterologous viruses could compete for binding with radioactive marker virus for adsorption in a typical dose-response relationship. Incubation of virus with various preparations of MAbs prior to adsorption, in conjunction with monitoring viral RNA synthesis, suggested three related mechanisms of virus N. Ten µg of MAbs which define the critical N-site inhibits 90% of a 5 µg (1 x 10^7 pfu) dose of virus from binding to cells. MAbs which define epitopes spatially proximal to the critical N-site (with low but demonstrable N titers), are less efficient at blocking (50-60% inhibition), but equally efficient at suppressing a productive infection. A third mechanism involves a MAb which enhances binding of virus to cells, however the infection is significantly inhibited.

Q44 NUCLEOTIDE SEQUENCE OF SAINT LOUIS ENCEPHALITIS VIRUS GENOME ENCODING THE STRUCTURAL PROTEINS AND NONSTRUCTURAL PROTEIN NS-1, Dennis W. Trent,[1] Richard M. Kinney,[1] Barbara J. B. Johnson,[1] Joyce A. Grant,[1] A. Vance Vorndam,[1] and Charles M. Rice,[2] [1]Centers for Disease Control, Fort Collins, CO 80522 and [2]California Institute of Technology, Pasadena, CA 91125

The nucleotide sequence of 3.7 kilobases at the 5'-terminus of Saint Louis encephalitis (SLE) virus RNA has been determined from cloned cDNA encoding the capsid, membrane, and envelope structural genes and nonstructural protein NS-1 (NV3). Analysis of this sequence reveals a single long open reading frame of 3636 nucleotides coding for the viral proteins in the gene order 5'-C-prM (M)-E-NS1-3' in a polypeptide of 1212 amino acids. Comparison of the genome organization of SLE virus with Murray Valley encephalitis (MVE), West Nile, and yellow fever (YF) flaviviruses reveals conservation in: (1) the size of the structural proteins; (2) cystene residues in the structural region; (3) N-terminal cleavage sites; and (4) glycosylation sites in the pM and NS1 proteins. The envelope glycoprotein contains two Asn-X-Thr/Ser glycosylation sites at amino acids 153 and 313 which are not conserved with YF or MVE viruses. Amino acid sequence homology between SLE and YF polyproteins in the region sequenced is 58% whereas the homology with serologically unrelated yellow fever is 43% in the structural genes. Hydrophobicity plots of the flavivirus structural proteins are similar and suggest that most of the E glycoprotein is external to the membrane and that about half of M is embeded in the envelope.

Q45 MOLECULAR BASIS OF VARIATION IN FOOT-AND-MOUTH DISEASE VIRUS, Vikram N. Vakharia and Douglas M. Moore, USDA, ARS, Plum Island Animal Disease Center, Greenport, NY 11944.

Foot-and-mouth disease virus (FMDV) is an antigenically variable picornavirus grouped into 7 major serotypes and many subtypes. Even among subtypes there exist naturally occuring antigenic and plaque variants. There is considerable evidence that most of the major immunogenic sites are situated on the capsid protein VP1 and that VP1 itself retains at least several antigenic sites in isolated, purified form. Therefore, it is important to know the nucleotide sequence to compare the variability of the amino acid sequence between the variants and correlate these with observed differences in biological properites. Here we report the nucleotide sequences from cloned cDNA segments coding for the FMDV immunogenic protein VP1 for three different variants of subtype A12 displaying plaque or antigenic variation: large plaque 'b' (LP b), large plaque 'c' (LP c), and small plaque. The derived amino acid sequences was compared with the known sequence of the reference A12 strain, large plaque 'ab' (LP ab). A very limited number of nucleotide changes were observed among the variants indicating the close relationship of these variants. Of 18 base changes among three variants, 11 resulted in change of codon. These changes occurred in three areas along the VP1 sequence: a single substitution at position 26; significant substitutions at three positions in the highly variable region of VP1 (147, 152 and 154); and near the C-terminus (198 and 201). Each variant displayed specific changes mainly in the highly variable region, but there were common sequences permuted among the variants, as well. A potent neutralizing monoclonal antibody specific for the variable region of VP1 distinguished all of the variants from LP ab. All of the variants contained substitution(s) in the known binding site of the antibody. The small plaque variant possessed three unique substitutions in the variable region, but was identical to LP ab elsewhere on VP1. The results indicate there are several areas on VP1 subject to variation, but that a significant contribution to antigenicity and plaque size resides in the highly variable region of VP1, positions 140 through 160.

Q46 MOLECULAR CLONING AND SEQUENCE ANALYSIS OF THE GENES CODING FOR THE STRUCTURAL PROTEINS OF RUBELLA VIRUS, Gabrielle Vidgren, Leevi Kääriäinen and Ralf F. Pettersson Recombinant DNA Laboratory, University of Helsinki, Helsinki, Finland

We have previously shown that rubella virus (RV), the sole member of the *Rubivirus* genus within the *Togaviridae* family, contains three structural proteins, E1 and E2 (a and b), (membrane glycoproteins) and C (nucleocapsid protein) (1). These proteins are coded for by a 24S subgenomic mRNA (about 3,500 bases long) corresponding to the 3' one third of the genomic 40S RNA (about 11,000 bases) (2). The gene order is: NH_2-C-E2-E1-COOH (3). Using the 40S RNA as a template, we have synthesized and cloned in *E. coli* ds cDNA covering the 24S mRNA region. Three partially overlapping clones, 1500, 1950 and 1000 bp in length, have been characterized by RE-mapping and sequencing. All clones were colinear with the 40S RNA. The 1500 bp-clone corresponds to the 3' end of the genome including part of the poly(A)-tract. Since the NH_2-terminal amino acid sequences of E2 and E1 are known (4), we were able to localize the E2 and E1 genes within these clones.

The general strategy of the gene expression of RV is strikingly similar to that of the alphaviruses. Once the sequence of the RV 24S RNA has been completed, it will be of particular interest to determine the evolutionary relatioship between the alphaviruses and RV.

(1) Oker-Blom, C., N. Kalkkinen, L. Kääriäinen and R.F. Pettersson. J.Virol. 46:964-973(1983)
(2) Oker-Blom, C., I. Ulmanen, L. Kääriäinen and R.F. Pettersson. J.Virol. 49:403-408 (1984)
(3) Oker-Blom, C. J.Virol. 51:806-812 (1984).
(4) Kalkkinen, N., C. Oker-Blom and R.F. Pettersson. J. Gen.Virol. 65: 1549-1557 (1984).

Gene Expression

Q47 ACTIVATION OF PROTO-ONCOGENE DURING NON A NON B HEPATITIS (NANBH) VIRUS-MEDIATED CONVERSION OF HEPATOCYTES TO HEPATOCELLULAR CARCINOMA. **A.A.Hakim**, M.G.Durkin, C.E. Joseph*and C.M. Siraki. Loyola University Medical Center, Maywood. Illinois 60153 & *University Southern California, Los Angeles, California 90007.

Some viral hepatitis (VH) infection, develop into hepatocellular carcinoma. Both the infected hepatocytes and the hapatocellular carcinomas synthesize and secrete the viral surface antigens. The present studies examined if the DNA complementary to the positive strand RNA viruses possess the capacity to directly confer neoplastic phenotype on hepatocytes. Serum specimen from 45 patients each with clinically, histologically and (by exclusion) serologically confirme NANBH were examined. Control sera were obtained from 50 heathy adults. Each in quadruplicates, serum samples, negative control (fetal bovine serum) and positive control material (Rous Sarcoma virus) were centrifuged in 35% glycerol in 0.5 mM Tris.HCl, pH 7.0 at 77,000g for 1 hr at 4°C to pellet the viral particles. Then, a solution containing 60 m mol tris.HCl, $MgCl_2$, KCl, dithiothreitol, actinomycin D and 80 umol/l each of unlabelled deoxyribonucleoside triphosphate, and ^{3}H-thymidine triphosphate. The tubes were vortexed, then incubated at 37°C for 1 hr. After incubation 10^6/ml human embryonic liver (HEL) cells were added to each tube and incubation was continued for 6hrs at 37°C in 5% CO_2 humidified atmosphere. HEL cells in the absence of viral pellets and the viral pellets alone were incubated and used as controls. Hepatocellular carcinoma PLC/PRF/5 cells were run in parallel. All Nu/Nu mice which received HEL cells preincubated with the viral pellets, 25% of the animals which received the PLC/PRF/5 cells, and 15% of the animals which received the pellts developed tumors. These reults indicate the activation of Proto-Oncogene to Oncogen.

Q48 Analysis of avian coronavirus messenger RNA function.
S.C. Inglis*, M.E.G. Boursnell+, M.M. Binns+ and T.D.K. Brown+.
*Division of Virology, Department of Pathology, University of Cambridge, U.K.
+Houghton Poultry Research Station, Houghton, Huntingdon, Cambridge, U.K.

The avian coronavirus infectious bronchitis virus (IBV) specifies at least 5 subgenomic messenger RNAs in infected cells, in addition to full length genome RNA. Of these subgenomic mRNAs, which are known to form a 3' co-terminal 'nested set', three are known to encode structural proteins, but the coding function of the other two has not been established. Nucleotide sequencing studies on the IBV genome indicate that each of these mRNAs contains more than one open reading frame which is not present on the next smallest subgenomic mRNA, raising the possibility that these mRNAs are functionally polycistronic unlike the mRNAs for the structural proteins which appear to translate into single polypeptides. This possibility has been investigated through in vitro translation studies on naturally and artificially synthesised IBV messenger RNAs.

Q49 EXPRESSION OF SEMLIKI FOREST VIRUS CAPSID PROTEIN WITH SV 40 AND BOVINE PAPILLOMA VIRUS VECTORS, Anu Jalanko, Ismo Ulmanen and Hans Söderlund, Recombinant DNA Laboratory, University of Helsinki, Finland.

The N-terminal capsid protein of Semliki Forest virus (SFV) is proteolytically cleaved from the nascent structural polyprotein (C-E3-E2-E1) in infected cells. To evaluate if this processing takes place in the absence of other viral polypeptides, we cloned cDNA fragments coding for SFV capsid protein including the proteolytic processing site into SV 40 late region. The SV 40 recombinants produced virus stocks by complementation with an early region mutant in CV-1 cells, but with some indications that the inserted gene had toxic properties. As analysed by immunoprecipitation and gel electrophoresis, all recombinants yielded capsid protein indistinquishable from the virion derived capsid protein indicating that the nascent cleavage occurs also with the truncated proteins. Immunofluorescence staining with anti capsid antiserum indicated that the produced recombinant SFV capsid proteins accumulated mainly in the nucleus. We have also cloned a capsid cDNA fragment into a BPV vector under the control of an inducible promoter. By transfection we produce cell lines expressing SFV capsid protein. The capsid protein appears to be involved in the switch off of host-specific protein synthesis in virus infected cells, possibly by binding to initiation factor eIF-4B and to the cap-binding protein (van Steeg H., et al. (1984) Eur.J.Biochem. 138:473). The cell lines which can be induced to synthesize c-protein will be used to test the hypothesis that the capsid protein has a major role in the control of host cell protein synthesis.

Q50 STRUCTURAL ANALYSIS OF DENGUE VIRUS TYPE-2 GENOME, Radha Padmanabhan, Tazuko Yaegashi, Vikram Vakharia, Robert Feighny+, University of Kansas Medical Center, Kansas City, KS 66103, +WRAIR, Washington D.C. 20317

Dengue viruses are members of the flavivirus family which contain positive stranded non-segmented RNA genome approximately 11 kilobases in length. The viruses are classified into four different serotypes which are distributed throughout the world. The knowledge of entire sequence of the viral genome and the deduced amino acid sequences of the polypeptides is required to understand the replication and translation strategies used by the virus. Using recombinant DNA techniques, cDNA clones of Dengue virus type 2 (DEN-2) RNA were constructed. The complete nucleotide sequence of three cloned cDNAs pVV1, 9, 17, totalling 4.6 kb were determined using the chemical method. Translation of the DNA sequences of the two overlapping clones pVV1 and 9 revealed a long open reading frame (ORF1) which could encode a polypeptide which is 868 amino acids long. Translation of DNA sequence of pVV17 gave rise to ORF2 with a coding potential for a polypeptide containing 643 amino acids. Alignment of these two polypeptides with YF polyprotein using dot matrix homology program showed that ORF1 spanned from amino acid #1050 - #1918 and ORF2 mapped in a region between #2101-2744 of YF polyprotein. The comparison of protein sequences between YF and DEN-2 in the two regions, #1050-1500 and #1505-1918 showed that the two viruses shared a high degree of homology in the latter region encoding NS3 but much less homology in the region encoding NS2a and NS2b. These data support the notion that NS3 protein is probably involved in an essential function for the life cycle of the virus. This project was supported by U.S. Army Medical Research and Development Command Contract DAMD 17-82-C2051.

Q51 REGULATED EXPRESSION OF SINDBIS AND VESICULAR STOMATITIS VIRUS GLYCOPROTEINS IN S. CEREVISIAE, Milton J. Schlesinger, Duanzhi Wen and Mingxaio Ding, Washington University School of Medicine, St. Louis, MO 63110

cDNAs encoding either the structural proteins (capsid and glycoproteins E1 and E2) of Sindbis virus or the glycoprotein (G) of VSV were fused to the S. cerevisiae galactose kinase gene (GAL-1) promoter and inserted into a yeast shuttle vector. After addition of galactose to yeast transformed with this vector, 2.5 to 3% of total yeast protein synthesis was detected as virus proteins by specific antivirus protein antibodies. In cells containing the Sindbis virus structural genes, - the virus capsid protein was effectively released from the nascent polypeptide and two endoglycosidase F sensitive glycoproteins were produced. One of these was identical in its gel mobility to E1 and the other appeared to be p62, a precursor to E2. A low level of E1 protein was detected on the cell's surface membranes. A single molecular weight species of glycosylated VSV G was produced and half of the total protein could be detected at the surface membranes of yeast. Addition of long mannose chains and acylation of the virus proteins with fatty acids were not observed. The virus glycoproteins formed high mol. wt. aggregates of disulfide-linked monomers and these could be separated from most of the yeast proteins after extraction of cells with 1% SDS. Formation of virus proteins was also examined in yeast secretory mutants; one of these (sec 53) failed to glycosylate the virus proteins.

Q52 MOLECULAR CLONING AND SEQUENCING OF HEPATITIS A VIRUS RNA, Klaus von der Helm, Richard Ostermayr, Axel Lezius*, Eckard Wimmer**, Minqhan Zhou, F. Deinhardt, University of Munich, D-8000 Munich 2, *University of Münster, FRG, **Dept. of Microbiology, SUNY at Stony Brook, USA

Hepatitis A virus (HAV) is a picornavirus which replicates slowly in cell cultures and induces no cythopathic effects. To study the molecular biology of this virus and to elucidate why HAV has a replication pattern different from that of most other picornaviruses we cloned the RNA of the euro-african isolate (MBB) of HAV. cDNA clones of smaller than full genome length obtained were mapped along the genomic RNA. The pattern of cleavage by restriction enzymes of the clones was very similar to that of published cloned HAV isolates (Ticehurst et al, PNAS 80 (1983) 5885; Linemeyer et al, J Med Virol 54 (1985) 247); it differed partly in that region of the genome which codes for the capsid proteins. The nucleotide sequences of part of the MBB genome was done; more than 85% of it was homologous, the amino acid sequence deduced from this nucleotid sequence was more than 95% homologous to the sequence of the mentioned HAV isolates. cDNA clones representing the VP_1 region were expressed in E. coli as ß-galactoside-fusion proteins; they exhibited HAV-antigenicity as demonstrated by reaction to anti-HAV serum. -- When viral RNA from newly HAV infected cell cultures was hybridised with single (+)strand cDNA probe we detected (-)strand HAV RNA 1-2 days p.i. This implies that the replication of viral RNA occurs relatively early compared to the appearance of viral proteins so that a possible lesion in the replication cycle of HAV in cultured cells affects the synthesis of proteins rather than the RNA transcription.

Q53 VPg OF HEPATITIS A VIRUS (HAV): DETECTION AND CHARACTERIZATION, M. Weitz, B. Baroudy, L. Maloy, J. Ticehurst, and R. Purcell, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

The presence of a VPg in HAV was deduced from the predicted HAV amino acid (aa) sequence corresponding to the P3 polyprotein region. Its sequence includes a tyrosine residue in the third position from its putative N-terminus. A tyrosine in this location is not only the site of covalent binding to RNA in poliovirus (PV) but is common to all known picornaviral VPg aa sequences. Antibodies, raised in rabbits to a synthetic peptide that contained 10 aa from the predicted C-terminus of VPg, precipitated VPg-bound RNA from purified and disrupted HAV and from HAV-infected cells. This synthetic aa sequence is therefore colinear with the VPg gene and HAV has the genomic organization of at least part of the picornaviral P3 region. Comparison of VPg aa sequences of HAV and members of the four picornavirus genera revealed three conserved regions. The sequences of foot and mouth disease virus VPgs are most closely related to that of HAV. Computer analysis of VPg aa sequences predicted both similarities and differences in the structural and physical features of the proteins. Among the unique properties of HAV VPg is a predicted isoelectric point (pI) at pH 7; VPgs of other picornaviruses have pIs 10-12.

Viral RNA Transcription and Replication and Virus Families Workshop

Q54 SINGLE-CYCLE GROWTH KINETICS OF HEPATITIS A VIRUS IN BSC-1 CELLS.

David A. Anderson, Stephen A. Locarnini, Ian D. Gust,
Virus Laboratory, Fairfield Hospital, Fairfield 3078, Vic., Australia.

Although officially classified as an Enterovirus, hepatitis A virus (HAV) exhibits some growth properties atypical of this genus. Many isolates of HAV cause persistent infections of susceptible primary and continuous cell lines of primate origin, and such cultures have been maintained for years with continuous production of infectious virions. In contrast, cell culture-adapted HAV strain HM175 causes a degenerative CPE in acutely infected BSC-1 cells maintained at 34° in serum-free medium, with a peak yield of infectious HAV after 2-3 days. Addition of 2% fetal calf serum or 1 mM Guanidine.HCl to the medium prevents the development of a CPE and results in a persistent infection. Guanidine prolongs the eclipse phase from 12 to 24 hours in treated cultures, but does not affect the yield of infectious HAV in acutely infected cultures. Hydroxy-benzyl benzimidazole has no effect on HAV replication, but 0.5 mM $ZnCl_2$ reduces the yield of infectious HAV 1000-fold. The kinetics of HAV RNA and antigen synthesis in these cultures has been examined by indirect immunofluorescence, in situ radioimmunoassay and dot-blot hybridization with HAV-specific cDNA probes, in parallel with a radioimmunofocus assay for infectious HAV. The results suggest that viral RNA synthesis may be a rate-limiting step in the replication of HAV in BSC-1 cells.

Q55 PARTIAL MOLECULAR CLONING AND NUCLEOTIDE SEQUENCE ANALYSIS OF cDNA ENCODING THE STRUCTURAL PROTEINS OF DENGUE 2 VIRUS, GENETIC VARIETY OF JAMAICA 1981, Vincent Deubel, Richard M. Kinney, and Dennis W. Trent, Centers for Disease Control, Fort Collins, CO 80522

The nucleotide sequence of 2.4 kilobases to the 5'-terminal half of dengue 2 virus has been determined and the encoded proteins compared with those of other flaviviruses, Saint Louis encephalitis, yellow fever, and Murray Valley encephalitis. The amino acid sequence homology between the polyprotein of the viruses coding for the structural proteins is 36%. The structural proteins are organized in the gene order 5'-C-prM(M)-E-3' and are in a single open reading frame following the first AUG codon ending the 5'-noncoding region. The dengue virus structural proteins are similar in size and composition to those of the other flaviviruses and on the base of amino acid homologies and proteolytic cleavage sites reveal a basic capsid protein and envelope protein with the N-terminus outside and a C-terminal membrane spanning region.

Q56 DIFFERENCES IN VIRUS-SPECIFIC POLYPEPTIDES IN CELLS INFECTED BY CYTOPATHIC AND NONCYTOPATHIC BIOTYPES OF BOVINE VIRUS DIARRHEA-MUCOSAL DISEASE VIRUS, Ruben O. Donis and E. J. Dubovi, Diagnostic Laboratory, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Two biotypes of bovine virus diarrhea-mucosal disease virus are present in nature: one that induces cytopathology in bovine cell cultures and other that infects cells without overt cytopathology. Both are productive infections that yield similar amounts of infectious progeny virus. Field and laboratory isolates of both biotypes of bovine virus diarrhea-mucosal disease virus were analyzed by radioimmunoprecipitation and polyacrylamide gel electrophoresis of infected cell extracts. Noncytopathic biotype isolates can be consistently differentiated from cytopathic biotype isolates on the basis of peculiar polypeptide profiles induced in the infected cells: noncytopathic biotype isolates lack an 80 kD polypeptide which is the most prominent polypeptide present in cytopathic biotype-infected cells. We put forward the hypothesis that the major difference between cytopathic and noncytopathic biotype isolates is a difference in the proteolytic processing of the 118 kD polypeptide precursor. In addition the sizes and ratios of the glycoproteins induced by all virus isolates showed marked variation. We present evidence indicating that there is remarkable heterogeneity among field viral isolates.

Q57 SYNTHESIS OF PLUS AND MINUS STRAND RNA FROM POLIOVIRION RNA TEMPLATE IN VITRO, Timothy D. Hey, O.C. Richards and Ellie Ehrenfeld, University of Utah School of Medicine, Salt Lake City, Utah 84132

The poliovirus RNA polymerase, 3D ^{pol}, has been used to synthesize RNA in vitro in the presence of a host factor preparation from uninfected HeLa cells and poliovirion RNA as template. The transcription products include molecules approximately twice the length of the template, apparently resulting from hairpin formation and template directed elongation, as previously reported (J. Virol. 54:256-264). Other polyadenylated template RNAs also yield products that are twice the template in length. The polarity of the products synthesized from plus strand polio RNA template was analyzed by Southern blotting using labeled product RNA to probe single-stranded polio DNAs cloned into M13 vectors. The results demonstrate that host factor-mediated polymerase products contain nely-synthesized plus strand sequences as well as the expected minus strand sequences. Polymerase products primed with oligo(U) are all of minus strand polarity.

Q58 RNA RECOMBINATION IN POLIOVIRUS, Karla Kirkegaard and David Baltimore, Whitehead Institute for Biomedical Research, Cambridge MA

We have selected and characterized mutant virus bearing determinants conferring resistance to guanidine (gua^R) and PTH7387, a very tight temperature sensitive mutation in the 3'-noncoding region of poliovirus RNA constructed by P. Sarnow. Upon coinfection of the serotype I PTH7387gua^R virus and wild type virus of either serotype I or serotype II, recombinant progeny that can grow in the presence of guanidine but are no longer temperature sensitive can be selected. The junctions between the type I and type II sequences have been sequenced in 14 intertypic recombinants. The conclusions from these studies are that intertypic RNA recombination is not site-specific, nor does it require extensive homology between the recombining parents at the crossover site. In order to discriminate between breaking-joining and copy choice mechanisms of RNA recombination, we have inhibited the replication of the recombining parents independently. Recombination occurs at a normal frequency when wild type virus replicates in the presence of high levels of nonreplicating PTH7387gua^R virus. However, no recombination occurs when PTH7387gua^R virus replicates in the presence of high levels of nonreplicating wild type virus. The asymmetry of this effect is difficult to reconcile with any simple breaking and rejoining recombination model and is perfectly consistant with a copy choice mechanism, in which template switching occurs during negative strand synthesis.

Q59 INITIATION OF POLIOVIRUS PLUS-STRAND RNA SYNTHESIS IN VITRO, Richard J. Kuhn, Naokazu Takeda, Chen-fu Yang, Haruka Toyoda, and Eckard Wimmer. Department of Microbiology, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

A crude membrane fraction from poliovirus-infected HeLa cells has been shown, in vitro, to be capable of synthesizing the nucleotidyl-proteins, VPgpU and VPgpUpU, which represent the 5'-terminal structure of nascent RNA molecules. Following the formation of VPgpU, longer VPg containing nucleotidyl-proteins can be detected by assaying for the formation of the ultimate 5' ribonuclease T1-resistant oligonucleotide VPgpUUAAAACAGp. In the presence of the four NTPs, the nucleotidyl-protein, VPgpUpU, could be chased to VPgpUUAAAACAGp. Treatment of this fraction with DEAE-cellulose resulted in an increase in VPgpUpU synthesis and a decrease in the 5' T1-oligonucleotide formed. The elongation activity could be restored by the addition of a soluble fraction (S10) isolated from uninfected HeLa cells. Our data suggest that VPgpU can function as a primer in the membraneous replication complex and that the elongation reaction is stimulated by a host cellular factor. In addition, site-directed mutagenesis of the poliovirus replication proteins using an infectious molecular clone is currently underway to investigate the role of virus-specific proteins in the process of RNA initiation and subsequent chain elongation.

Q60 ANALYSIS OF THE INTERNAL PROMOTOR FOR TRANSCRIPTION OF THE SUBGENOMIC RNA4 OF BROME MOSAIC VIRUS, Loren E. Marsh, Theo W. Dreher and Timothy C. Hall, Texas A&M University, College Station, TX 77843.

Brome mosaic virus has three genomic RNAs and a subgenomic RNA, (RNA4). RNA4 serves as the mRNA for the coat protein and is generated in vivo from the 3' end of the dicistronic genomic RNA3. Plus strand subgenomic RNA can be synthesized in vitro by a replicase (RNA dependent RNA polymerase) from infected barley leaves using minus strand RNA3 as a template (Nature (1985) 313, 68). The minus strand template is generated by in vitro transcription with SP6 and T7 polymerase of cloned RNA3 cDNA. The internal promotor used for initiation of the subgenomic RNA synthesis from the minus strand template has been delineated by mutational analysis. Initially, deletion mutations were constructed in cDNA transcriptional plasmids. Analysis of these mutations has limited the promotor to about twenty bases upstream of the initiation site. The internal promotor has been subsequently further analyzed by site directed mutagenesis within the region.

Q61 STRUCTURAL SIMILARITIES IN THE 3' NONCODING REGIONS OF POLY(A) CONTAINING RNA VIRUSES, C.W.A. Pley, Department of Biochemistry, University of Leiden, The Netherlands

We recently have proposed models for the three-dimensional folding of the tRNA-like structures which are present at the 3' end of a number of plant viral RNAs[1].
Since the presence of tRNA-like structures in the genomic RNAs of Mengo virus and encephalomyocarditis virus (EMC) was suggested by others, I have looked for such structures in the 3' noncoding regions of picornaviral RNAs. So far no tRNA-like structures were found. However, some common features in the secondary structure at the 3' terminus of a number of poly(A)-containing viral RNAs may exist.
The secondary structure which will be presented for the 3' terminus of poliovirus RNA is strongly supported by the comparison with the sequences of the related enteroviruses coxsackie B_3 and swine vesicular disease virus (SVDV). Interestingly, the human rhinovirus RNAs and the two genomic RNAs of the plant virus cowpea mosaic virus (CPMV) can be folded into secondary structures which have features in common with the enteroviruses. In all secondary structures proposed, the poly A tract takes part in the formation of these 3' terminal secondary structures. Other picornaviruses show less (EMC) or no (FMDV) structural relationship at their 3' end, which is in agreement with findings of others on the relationships among these viruses.

Reference
1. K. Rietveld, K. Linschooten, C.W.A. Pley and L. Bosch (1984) EMBO J. 3, 2613-2619.

Q62 What Determines the Rate of Minus and Plus Strand Synthesis During Alphavirus Replication? D. Sawicki and S. Sawicki, Medical College of Ohio, Toledo, OH 43699.

Normally, during the replication of the Alphaviruses, minus strand synthesis stops at the same time that plus strand synthesis reaches a maximal rate. We have studied many of the RNA negative ts mutants of Sindbis HR virus to characterize viral RNA synthesis, and in addition to identifying ts mutants with specific defects in minus strand synthesis and its regulation, we have isolated and characterized revertants of Sindbis HR ts24 that replicate efficiently at nonpermissive temperature but do not regulate minus strand synthesis at 40°C. Although failure to regulate minus strand synthesis resulted in continued synthesis and accumulation of minus strands, the rate of plus strand synthesis was not increased concertedly. Minus strands synthesized late in infection after plus strand synthesis became constant were demonstrated to be utilized as templates for 26S mRNA synthesis. Thus, the change from an increasing to a constant rate of plus strand synthesis during the alphavirus replication cycle was not governed solely by the regulation of synthesis of minus strand templates. Moreover, these results suggest the ts defect in ts24 affecting minus strand synthesis may reside in a function normally responsible for the stable association of alphavirus minus strand RNA in a transcription complex. In ts24 and ts24 revertant infected cells, minus strand templates would become displaced or detached from complexes at 40°C but not at 30°C, allowing a plus strand to engage as a template for minus strand synthesis. The temporal cessation of alphavirus minus strand synthesis thus may involve the preferential binding of minus strands by the components of the transcription complex.

Q63 TOWARDS AN *IN VITRO* SYSTEM FOR PICORNAVIRUS ASSEMBLY: PURIFICATION OF MENGO VIRUS 14S CAPSID PRECURSOR PARTICLES, Douglas G. Scraba, Doreen S.-W. Ko and Ulrike Boege, University of Alberta, Edmonton, Canada T6G 2H7

Mengo virus 14S capsid precursor particles generated in infected L-cells and in a cell-free translation system primed with Mengo virus RNA were purified by sucrose gradient centrifugation and immunoaffinity chromatography. The preparations from both sources contained essentially pure proteins ε, γ and α (1AB, 1C and 1D) as was demonstrated in terms of virus-specific proteins (by autoradiography) and total protein content (by silver staining of SDS-polyacrylamide electrophoresis gels). These purified proteins sedimented as discrete particles at the 14S position when re-centrifuged in sucrose gradients.

When [^{35}S]labeled 14S particles purified from infected cells were incubated with Mengo virus genome RNA, part of the label was transfered to a structure cosedimenting with mature Mengo virus. When labeled 13.4S particles ($[\alpha\beta\gamma]_5$) obtained from dissociated virus were incubated under the same conditions, no such structure could be detected.

Q64 COMPLETE NUCLEOTIDE SEQUENCE OF THE NONSTRUCTURAL PROTEIN GENES OF SEMLIKI FOREST VIRUS

Kristiina Takkinen, Nisse Kalkkinen and Hans Söderlund, Recombinant DNA Laboratory, University of Helsinki, Finland

The whole nucleotide sequence coding for the nonstructural protein genes of Semliki Forest virus (SFV) has been determined from cDNA clones. The total length of the nonstructural region is 7381 nucleotides, it contains an open reading frame starting at position 86 and ending at an UAA stop codon at position 7379-7381. This open reading frame codes for a 2431 amino acids long polyprotein, from which the individual ns proteins are formed by proteolytic processing steps, so that nsP1 is 537, nsP2 798, nsP3 482 and nsP4 614 amino acids. The positions of the genes for the individual nonstructural proteins has been determined by partial aminoterminal sequencing of the radiolabeled ns proteins. In the closely related Sindbis and Middelburg viruses there is an opal stop codon (UGA) between the genes for nsP3 and nsP4 (Strauss et al. (1983) PNAS 80, 5271-5275). Interestingly no such stop codon is found in frame in this region of the SFV 42S RNA. In other aspects the amino acid sequence homology between Sindbis, Middelburg and SFV nonstructural polyproteins is highly significant.

Q65 RUBELLA VIRUS ASSOCIATED WITH CYTOSKELETON (RUBELLA VACS) PARTICLES - RELEVANT TO SCRAPIE?

D. Van Alstyne, M. DeCamillis, P. Sunga and R.F. Marsh. Quadra Logic Technologies Inc., Vancouver, B.C., Canada, and the University of Wisconsin, Madison, Wisconsin, U.S.A.

Persistent rubella virus (RV) infection in the central nervous system (CNS) has been associated with some chronic, degenerative neurologic disorders where there is a long initial period of latency. However, the mechanism underlying RV persistence as it relates to CNS disease remains unclear. To further our ongoing investigations of persistent rubella infection in the CNS we have produced hybridomas which secrete monospecific antirubella antibodies. In the course of their characterisation, it was observed that an immunosorbent column of monoclonal antirubella IgG linked to Sepharose beads could be employed to affinity-purify all RV proteins simultaneously from RV-infected L cell lysates. The viral proteins were eluted as rod-like structures averaging 10-20 x 50-100 nm and were shown to react positively with antiactin actibody. They were then shown to be associated with 10^5-10^6 Kd single-stranded (ss) RNA, rendering the RNA nuclease resistent. Therefore, the rods were likely composed of RV associated with actin-containing structures, such as microfilaments, and have been designated rubella VACS (Virus Associated with Cytoskeleton) particles. These structures were further shown to be infectious and to demonstrate some unique biological properties unlike those of the virus stocks from which they were derived. Particles could also be affinity-purified from lysates of VACS-infected cells. These particles were distinct from VACS-1 and are termed VACS-2; they were composed predominantly of a 24-26 Kd protein and they reacted positively with antitubulin antibody. These in vitro data suggest that RV possesses an alternate mode of infection, mediated by rubella VACS particles which share several of the unique characteristics of the rod-shaped structures associated with the subacute spongiform virus encephalopathies. One of these, the scrapie agent, has been propagated in hamster brain, where the purified fractions containing high infectivity ($10^9 LD_{50}$/ml) have been found to be enriched for a 43 Kd protein which co-migrates with rabbit muscle actin on gel electrophoresis. Further, this infectivity was RNA nuclease sensitive under low salt conditions which are known to alter the conformation of F-actin as well as to disaggregate ribonucleoprotein complexes. These data suggest the intruiging possibility that scrapie could be the VACS derivative of a known ss RNA virus.

Q66 STUDIES INTO THE MECHANISM OF MHV-TRANSCRIPTION-Ralph S. Baric, Stephen A. Stohlman, and Michael M.C. Lai. Dept. of Microbiology, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033.

Analysis of MHV-infected cells has revealed the presence of discrete small RNAs which range between 70-800 nucleotides in length. The smaller leader-related RNAs range between 70-82 nucleotides in length and are thought to be involved in MHV transcription. The larger leader-containing RNAs are detected predominantly in the cytosol. By co-immunoprecipitation studies with sera from Lupus erythematosus patients, no small RNAs were associated with LA, RNP, SM or LA complexes in the cell. While the small leader-related RNAs were not precipitated with anti-pp60 sera, a portion of the larger leader-containing RNA population interacted with pp60. These data indicate that a pp60 binding signal is present on some leader-containing RNA but not leader RNA. Utilizing cDNA probes specific for different MHV genes, we have begun characterizing the structure and origin of the MHV leader-containing RNAs. These results indicate that the leader-containing RNAs originate from different portions of the viral genome. Analysis of the small E1 RNAs which contain leader sequences indicate that these RNAs terminiate at or very near to regions of secondary structure. These findings are reminiscent of QB or T7 RNA transcription in which the polymerase "pauses" at regions of 2^o structure. The presence of discrete leader-sized RNA intermediates suggests that MHV transcription might proceed in a discontinuous and nonprocessive manner, generating RNA intermediates as a result of transcriptional "pausing". If "pausing" occurs during MHV transcription, discrete leader-containing RNAs may reassort during mixed infection and explain the high frequency of RNA recombination in MHV-infected cells.

Q67 BIOCHEMICAL AND BIOLOGICAL ANALYSIS OF YELLOW FEVER VACCINE VIRUSES, Alan D.T. Barrett, Department of Microbiology, Surrey University, Guildford GU2 5XH. U.K.

Yellow fever 17D vaccine viruses manufactured in 10 different countries, the wild-type parent to 17D vaccines (Asibi virus) and the French Neurotropic vaccine virus were compared biochemically and biologically.
Analysis of intracellular virus-specified polypeptides of different vaccine viruses by PAGE showed that the vaccines had polypeptides of identical mobilities with the exception of the envelope glycoprotein. The mobility of the envelope glycoprotein varied depending upon which of the viruses was being examined. The molecular weights varied between 53,000 and 59,000. These differences appeared to be due to different extents of glycosylation of the envelope glycoprotein. Examination of the viruses in ELISA "signature analysis" tests using MAbs suggests that although epitopes of the envelope glycoprotein are shared by all vaccine viruses (as determined by indirect immunofluorescence) the epitopes are presented differently on the surface of the virus. Analysis of the viruses in mice following intracerebral, intranasal or intraperitoneal routes of inoculation showed that the time to death and pfu/LD_{50} varied between the viruses. Taken together, the above results would suggest that the YF vaccine viruses are biologically and antigenically distinct.

Q68 MOLECULAR ORGANIZATION OF THE BOVINE VIRAL DIARRHEA VIRUS GENOME, Marc S. Collett, Ruby Larson, and A.F. Purchio, Molecular Genetics, Inc., Minnetonka, MN 55343.

Bovine viral diarrhea virus (BVDV) is implicated as a major contributing factor in the bovine respiratory disease complex, as well as being the causative agent of virus-induced diarrhea-mucosa disease in cattle. As such, BVDV may be the single most important infectious agent, with respect to economic loss, in the cattle industry. We have initiated studies aimed at understanding the basic molecular characteristics of BVDV. BVDV has been classified as a member of the *Pestivirus* genus of the non-arthropod-borne togaviruses. The genome of BVDV is a highly structured, single-stranded RNA of approximately 9800 ± 200 nucleotides. We have molecularly cloned a major portion of this RNA. The cDNA clones have been oriented with respect to and positioned along the viral RNA. In an attempt to identify areas of the genome that encode specific gene products, select regions have been manipulated so as to express BVDV sequences as polypeptides in *E. coli*. The bacterially-produced proteins were then used to generate specific antisera in rabbits. These antisera were then employed in immunoprecipitation experiments with BVDV-infected cell lysates to identify the authentic BVDV proteins to which they were directed. Both the viral structural proteins and previously undescribed viral nonstructural polypeptides were identified and their coding regions localized along the BVDV genome. The results indicate that the genetic organization of the BVDV genome resembles quite closely that of members of Flaviviridae and suggest that BVDV (or the *Pestivirus* genus) might be placed in this virus family.

Q69 USE OF SYNTHETIC MUTANT RNAs IN STUDYING THE ROLE OF THE tRNA-LIKE STRUCTURE OF VIRAL RNA, Theo W. Dreher and Timothy C. Hall, Biology Department, Texas A&M University, College Station, TX 77843.

The three genomic RNAs of Brome Mosaic Virus share at their 3' end a segment about 200 bases long. This region contains a structure which loosely resembles tRNA, and which confers several tRNA-like properties on the viral RNA, notably specific tyrosylation. The same region of RNA is the site of template recognition, and of initiation of (-) strand synthesis, by BMV replicase. We have developed a system for producing *in vitro*, from cloned cDNA, 3' fragments of BMV RNA that can be aminoacylated and copied by BMV replicase (Nature (1984) 311, 171). In order to probe the functional role of aminoacylation, we have investigated the effect of several specific mutations on these two activities. These studies have demonstrated that the two functions have separate structural requirements; the regions most important to each function have been defined. We have also studied the effect of selected mutations on two other tRNA-associated *in vitro* activities, elongation factor binding and nucleotidyl transferase activity. Studies of the replicative fitness of these mutant 3' termini introduced on genomic RNA into barley protoplasts will provide an assessment of the relevance of tRNA-like activities to replication *in vivo*.

Q70 FUNCTIONAL DIFFERENCES BETWEEN THE PEPLOMERS OF TWO ANTIGENICALLY DISTINCT FELINE INFECTIOUS PERITONITIS VIRUS ISOLATES, Susan A. Fiscus and Yoshio A. Teramoto, Syngene Products and Research, Ft. Collins, CO 80524

We have previously reported that feline infectious peritonitis virus (FIPV) can be divided into at least two antigenic groups based on immunoreactivity to a library of monoclonal antibodies (mAb). Using plaque purified FIPV-DF2 and FIPV-UCD-2 as representatives of each of the antigenic groups, no major antigenic differences were found in the envelope (E1) and nucleocapsid (N) polypeptides. However, significant antigenic differences were found in the peplomer (E2) glycoproteins which are responsible for many of the biological functions of coronaviruses. No detectable structural differences were found between the peplomers as determined by molecular weight estimates using SDS-PAGE or by glycosylation patterns using biotinylated lectins. Structural differences were also examined by limited proteolytic digestion. A number of differences were found in assays designed to investigate E2 functions. FIPV-DF2 replicated in three cell lines and produced cytopathic effect (CPE) and large plaques in feline whole fetus cells (FCWF). FIPV-UCD-2 replicated and produced CPE and small plaques only in FCWF cells. FIPV-DF2 was stable over a wider pH range than UDC-2 at both 4C and 37C. MAb reactive with the peplomers of either FIPV-DF2 and UCD-2 neutralized the homologous virus but not the heterologous virus. Cats exposed to the UCD-2 antigenic group usually made detectable antibodies to all three structural polypeptides of FIPV. Cats exposed to the DF2 antigenic group usually made antibodies to E1 and N, but rarely to DF2 peplomer. In contrast, some cats exposed to the DF2 antigenic group made antibodies to the UCD-2 type of E2.

Q71 MOLECULAR DETERMINANTS OF ALPHAVIRUS NEUROVIRULENCE: NUCLEOTIDE AND DEDUCED PROTEIN SEQUENCE CHANGES DURING ATTENUATION AND REVERSION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS, Barbara J. B. Johnson, Richard M. Kinney, Joyce A. Grant, and Dennis W. Trent. Centers for Disease Control, Ft. Collins, CO 80522

The nucleotide and deduced amino acid sequences of the structural proteins of the virulent Trinidad donkey strain (TRD) of Venezuelan equine encephalitis virus and its avirulent vaccine derivative (TC-83) have been determined from cDNA clones containing the respective 26S mRNA coding regions. Comparison of the sequences of the TRD and TC-83 cDNA clones revealed 13 nucleotide differences. Neither the organization of the structural proteins nor the length of the open reading frame coding for the viral polyprotein precursor was altered during attenuation. Of the 13 nucleotide differences between the cDNA clones of TC-83 and TRD, 9 occurred in the dominant population of the respective genomic RNAs from plaque purified viruses. Six of the nine mutations were clustered in the E_2 surface glycoprotein, including all five of the nucleotide changes which produced non-conservative amino acid substitutions in the encoded proteins. Two mutations occurred in the E_1 glycoprotein gene; one was silent and the other did not alter the chemical character of the E_1. One nucleotide difference was found in the noncoding region immediately preceding the 5'-end of the 26S mRNA. The relative significance of each of the 9 nucleotide differences for neurovirulence was evaluated by sequencing a variety of phenotypic revertants of TC-83 vaccine virus isolated from human vaccinees and from mice and hamsters.

Q72 NUCLEIC ACID SEQUENCE ANALYSIS OF SINDBIS PATHOGENESIS AND PENETRATION MUTANTS, Robert E. Johnston, Nancy L. Davis, Susan Gidwitz and Frederick J. Fuller, North Carolina State University, Raleigh, North Carolina 27695

Attenuated mutants of Sindbis virus (SB) have been isolated. These differed from wild-type with respect to virulence in neonatal mice, penetration of BHK cells in culture, and neutralization sensitivity to monoclonal antibodies R6 and R13. These three phenotypes are genetically linked as shown by characterization of virulent revertants selected in mice, mutants selected for fast penetration, and antibody selected variants. The mutation responsible for these phenotypes most probably resides at amino acid position 114 of glycoprotein E2. A serine to arginine substitution at this position was the only coding change detected in the glycoprotein genes of the attenuated prototype. Reversion to serine at 114 was accompanied by reversion to the wild-type characteristics for each of the phenotypes. An antibody induced second site suppressor mutation at position 62 (asparagine to aspartic acid) also caused phenotypic reversion. These results suggest a major role for glycoprotein E2 in both virulence in animals and penetration in cultured cells.

Q73 CONSTRUCTION OF FULL-LENGTH cDNA CLONES OF TOBACCO MOSAIC VIRUS

D.A. Knorr, D.L. Beck, and W.O. Dawson
Dept. of Plant Pathology, University of California, Riverside, 92521

Genomic-length cDNA of tobacco mosaic virus (TMV) was assembled from a library of overlapping fragments. cDNA at the 5' and 3' ends of the viral genome were synthesized separately. A synthetic oligonucleotide primer was used to generate a PstI site (unique in the TMV genome) at the 5' terminus, while a different primer was used to generate an NdeI site (not unique in TMV) at the 3' terminus. Addition of these restriction sites permitted removal of non-TMV sequences from cloned cDNAs by restriction endonuclease cleavage followed by treatment with Exonuclease VII. To aid manipulation of the cloned genome, PstI linkers were added to 3' terminal cDNAs. Thus, full-genomic cDNAs ligated into plasmid cloning vectors could be cleanly excised as single fragments by treatment with PstI. Full-genomic TMV cDNA was ligated immediately downstream from the bacteriophage lambda PR promoter (from pPM1) and transcribed *in vitro* with *E. coli* RNA polymerase. RNA transcripts from three of four full-genomic cDNA constructions were infectious, even though they contained 6 non-TMV nucleotides at the 3' end. Transcripts of a construction containing 6 additional nucleotides at the 5' end also were infectious.

Q74 LEADER SEQUENCES OF MURINE CORONAVIRUS MRNAS CAN BE FREELY REASSORTED -EVIDENCE FOR THE TRANSCRIPTIONAL ROLE OF FREE LEADER RNA,

Shinji Makino, Stephen A. Stohlman, and Michael M.C. Lai, Department of Microbiology, University of Southern California, School of Medicine, Los Angeles, California 90033.

Mouse hepatitis virus (MHV), which replicates in cytoplasm, contains leader RNA sequences at the 5'-end of the virus-specific mRNAs. It has previously been shown that the leader RNA is joined to the mRNAs by a mechanism involving priming by a free leader RNA. If this is indeed true, then the leader sequences should behave like a free and separate transcriptional unit. Therefore, during a mixed infection by two different MHV strains, the leader sequences might be reassorted to the mRNAs of a co-infecting virus at a frequency as high as the reassortment of segmented RNAs. We have indeed succeeded in demonstrating such a reassortment of the leader sequences. This was achieved by separating mRNAs, which have same length, of the two co-infecting MHVs, using a novel RNase H digestion procedure. We synthesized oligodeoxyribonucleotides complementary to the most diverged region within the mRNA 7 of the two different MHV strains, and hybridized one of these oligomers to the mRNA 7 from cells doubly infected with the two MHVs. After RNase H digestion, only mRNA species from one of the viruses remained intact. We demonstrated by oligonucleotide fingerprinting that this mRNA contains leader sequences of both viruses. This finding suggests that the leader sequences can be freely reassorted. Thus, it was established that the free leader RNA is utilized for MHV mRNA transcription and possesses trans-acting property. This study provided the strongest evidence for the "leader-primed transcription" model of MHV transcription.

Q75 COMPARATIVE SEQUENCE ANALYSES OF WILD POLIOVIRUSES OF INDEPENDENT EPIDEMIOLOGIC ORIGIN,

Rebeca Rico-Hesse, Baldev Nottay and Olen Kew, Centers for Disease Control, Atlanta, GA 30333

Determination of the patterns of genomic variation of RNA viruses during natural replication has been a powerful method for establishing epidemiologic relationships among isolates. The technique most commonly used in such studies, RNase T1 oligonucleotide fingerprinting, is capable of detecting similarities only among very closely related viruses. Because the poliovirus genome evolves at a rate of about 2 base substitutions per week during epidemic transmission, alternate methods must be applied to identify more distant relationships among outbreak viruses. We have determined the genomic sequences encoding parts of the capsid protein VP1 for 40 wild polioviruses, obtained over a 40-year period from different geographic regions. Base sequence divergence among isolates of the same serotype was as high as 24%, compared with a maximum amino acid sequence divergence of 5%. Beyond revealing previously unrecognized links between outbreaks, these studies have provided a much broader view of the pathways of poliovirus transmission in nature.

Q76 CHARACTERISTICS OF THE TMV ENCAPSIDATION INITIATION SITE ON 18S RIBOSOMAL RNA, Chintamani D. Atreya, D'Ann Rochon and Albert Siegel Biology Dept., Wayne State University, Detroit, MI 48202

Preparations of tobacco mosaic virus contain pseudovirions, particles that contain host rather than viral RNA. The bulk of the pseudovirion RNA consists of a collection of chloroplast DNA transcripts with very little or no ribosomal RNA. Chloroplast DNA transcripts are also encapsidated *in vitro* when oligomers of TMV capsid protein are mixed with host RNA and incubated under viral reconstitution conditions. However, the major host component encapsidated *in vitro* is not found in pseudovirion RNA but proves to be a discrete 3/4 sized, truncated, 5' portion of 18S ribosomal RNA. The reaction is specific in that 25S RNA is not encapsidated. The 18S RNA encapsidation initiation site is evolutionarily conserved; bovine as well as tobacco 18S RNA is encapsidated. Most of the 18S RNA coding sequence of a cloned pumpkin ribosomal DNA repeat unit was subcloned into the plasmid pSP65 and synthetic 18S RNA generated with the aid of SP6 RNA polymerase. Synthetic 18S RNA differs from the native form in that all, rather than just a portion, is encapsidated. The encapsidation initiation site was located to a 70 base Xba I - Sal I sequence near (but not at) the 5' terminus of the 18S RNA. Its sequence is presented and compared with that of published viral encapsidation initiation sites.

Q77 MECHANISM OF CORONAVIRUS RNA TRANSCRIPTION--ANTI-SENSE LEADER RNA INHIBITS MRNA TRANSCRIPTION, Lisa Soe, Chien-Kou Shieh, Stephen A. Stohlman and Michael M.C. Lai Department of Microbiology, University of Southern California, School of Medicine. Los Angeles, CA 90033.

Mouse hepatitis virus (MHV), a member of Coronaviridae, synthesizes 7 mRNA species with a 72-nucleotide leader sequence at the 5' ends. Various biochemical studies have suggested that these leader RNAs are joined to mRNAs by a "leader-primed transcription" mechanism, in which a free leader RNA serves as the primer for mRNA transcription. To prove that the free leader RNA is indeed utilized as a primer, we have constructed vectors containing feline leukemia virus long-terminal repeat (LTR) with an insertion of the 72-nucleotide leader sequence. These vectors were transfected into L cells, and the vectors which express anti-sense leader RNA or sense leader RNA were selected by RNA dot blot analysis using (+) and (-) sense leader DNA, respectively, as probes. The vectors which express anti-sense leader RNA were transfected into L cells and superinfected with MHV. It was found that the kinetics of RNA synthesis and virus production were inhibited in the transfected cells. These studies suggest that the leader RNA is indeed required for the synthesis of MHV mRNAs. Preliminary data also showed that in the cells expressing (+) sense leader RNA, the RNA synthesis of the superinfecting MHV was stimulated.

To study the polymerase involved in RNA transcription, we have cloned almost the entire polymerase gene. The properties of the polymerase will be presented.

Q78 HOST RANGE DETERMINANTS OF AVIAN RETROVIRUS ENVELOPE GENES. Carol Bova and Ronald Swanstrom. Department of Biochemistry. University of North Carolina at Chapel Hill.

The *env* gene of avian retroviruses is allelic in the virus population allowing the virus to use different host cell receptors. We determined the nucleotide sequence of the region of the *env* gene that encodes the glycoprotein gp85 from virus subgroups A, B, and E, and compared these sequences to each other and to the previously published sequences of subgroup C and B *env* genes (1,2). Based on these comparisons we draw the following conclusions: i) Within the gp85 coding domain there are four variable regions ranging in sizes from 9 to 52 amino acids. ii) The variable regions appear in the same positions in each of the *env* gene alleles, and they are flanked by conserved domains that are 95% homologous. Preliminary sequence analysis of the subgroup D *env* gene shows a similar pattern of conserved and variable regions. iii) In pairwise comparisons the average homology within the variable regions ranges from 42% (A compared to B) to 57% (C compared to E). iv) Different virus isolates with the same subgroup *env* gene show a high level of conservation within the variable regions. Secondary structure predictions suggest that gp85 is composed mostly of beta sheets. Hydrophilic loops within the variable regions may define sites of receptor interaction and the binding sites of subgroup-specific neutralizing antibodies. We have made molecular recombinants between subgroup A, B, C, D, and E viruses. The host range of each recombinant is determined by the origin of the DNA that spans the variable regions. 1. Schwartz et al. (1983) Cell *32*: 853. 2. Dorner et al. (1985) J. Virol. *53*:32.

Virus-Host Interactions and Pathogenesis

Q79 CHARACTERIZATION OF HEART-SPECIFIC AUTOANTIBODIES INDUCED BY COXSACKIEVIRUS B_3 INFECTION, Kirk Beisel, Nicholas Neu, Floria Alvarez, Ahvie Herskowitz and Susan Craig. The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Immunochemical analyses of CB_3 post-infection sera demonstrated the presence of cardiac myosin specific autoantibodies. Several mouse strains which develop immunopathic myocarditis, A.CA/SnJ, A/J and A.SW/SnJ, were infected at two weeks of age with 10^5 $TCID_{50}$ CB_3 (NANCY). Animals were then bled three times a week for a period of 5 weeks. These sera were characterized by indirect immunofluorescence for their reactivity with various tissues using a goat anti-mouse IgM,G,A reagent. Heart-specific autoantibodies were found in sera that reacted with cardiac sarcolemmal membranes and myofibers. Immunochemical analyses of Western blotted, heart tissue preparations were done using serum pools containing these heart-specific antibodies and a HRP-labeled goat anti-mouse IgM,G,A reagent. The predominant reactivity observed was toward myosin. These myosin antibodies were characterized by ELISA for their reactivity with different myosin isoforms and for their isotype. Competitive inhibition assays suggested the presence of two populations of myosin antibodies. The first recognized an epitope(s) shared by myosins obtained from heart, skeletal muscle and brain. These antibodies were primarily IgM. The second antibody population was specific for cardiac myosin and was IgG. Eluted antibodies from cardiac myosin-Sepharose beads were analyzed for their reactivity with CB_3, myosin, various heart muscle preparations and with whole tissues. (This work was supported by a grant from the American Heart Association and PHS grant HL-30144.)

Q80 Receptors for mouse hepatitis virus. JOHN F. BOYLE, DAVID G. WEISMILLER, and KATHRYN V. HOLMES, USUHS, Bethesda, MD 20814.

Mouse hepatitis virus is a coronavirus which causes a wide spectrum of diseases in genetically susceptible mouse strains. We have used a solid phase assay to compare the MHV receptor activities of enterocytes and hepatocytes of 3 mouse strains: susceptible BALB/c, semi-susceptible C3H and resistant SJL. Brush border vesicles or liver plasma membranes were adsorbed to nitrocellulose, probed with virions and virus binding was detected immunologically. Membranes from adult and infant (8 day) susceptible BALB/c and semi-susceptible C3H enterocytes and hepatocytes showed strong affinity for MHV. Comparable preparations from resistant SJL mice had negligible MHV binding activity. The receptor activity was not inactivated by NP-40 or SDS, but was abolished by trypsin. Purified membranes were solubilized, resolved on SDS-PAGE and electroblotted to nitrocellulose and the solid phase MHV-binding assay performed. From BALB/c and C3H membranes, one broad protein band of 110K for enterocytes and 92K for hepatocytes bound virus. SJL membrane proteins did not bind virus. These experiments suggest that one molecular mechanism of genetic susceptibility of mice to MHV A59 may be the presence or absence of a virus tissue-specific receptor on the target cells.

Q81 Detection of Lactate-Dehydrogenase Elevating Virus (LDV)-Infected Cells in Mouse Tissues by In Situ Hybridization and Direct Antibody Staining.
C.H. Contag, S.P.K. Chan, S.W. Wietgrefe, A.T. Haase, and P.G.W. Plagemann. Dept. of Microbiology, Univ. of Minnesota, Minneapolis, MN.

Infection of mice with LDV invariably results in a lifelong persistent infection which is associated with continuous viremia but no overt clinical symptoms. The exceptions are old, immunosuppressed C58 mice, which rapidly develop a fatal paralytic disease after infection with LDV. LDV replication seems to be generally restricted to a subpopulation of macrophages. Using in situ hybridization and immunocytochemistry, we plan to investigate the tissue distribution of the LDV-permissive cells during the acute and persistent phases of infection as well as the extent of LDV replication in the CNS. For this purpose, DNA complimentary to the LDV genome has been cloned and fluorescein-conjugated and biotinylated mouse anti-LDV IgG prepared. Hybridization of the cloned DNA to sections of spleen from 1-day LDV-infected mice identified foci of infected cells in the red pulp of the spleen. No significant hybridization was observed in RNase-treated spleen sections or tissue sections from uninfected mice. The distribution of LDV-infected cells as detected by in situ hybridization correlated with that of cells specifically stained by the fluoresceinated or biotinylated anti-LDV IgG.

Q82 MODUIATION OF SEMLIKI FOREST VIRUS INFECTION OF MICE WITH DEFECTIVE INTERFERING VIRUS. Nigel J. Dimmock, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL.

The normally lethal infection of adult mice inoculated intranasally with Semliki Forest virus (SFV) can be modulated by defective interfering (DI) SFV to a subclinical infection which may be cleared or become persistent. The DI-modulated infections are accompanied by alterations in pathology, specific neurochemical changes and suppression of protective immunity. Different DI virus stocks modulate infection in different ways indicating that although grossly deleted, DI genomes are able to exert a variety of major biological effects on the normal course of infection.

Q83 SINDBIS VIRUS GENES AFFECTING HOST RANGE, Russell K. Durbin and Victor Stollar, Univ. of Medicine & Dent. of N. J.-Rutgers Medical School, Piscataway, N.J. 08854.

We have been exploring determinants of the host range of the alphavirus, Sindbis virus (SV) by characterizing mutants that deviate from the normal host range. Two such mutants have pointed to two aspects of metabolism critical to the ability of SV to replicate both in vertebrate and mosquito cells. The first area is glycoprotein glycosylation. The viral mutant, $SV_{ap15/21}$, replicates normally in mosquito cells, but is blocked at the level of virion assembly in vertebrate cells. Nucleotide sequence analysis has confirmed, as we had postulated earlier, that the mutant had acquired a new potential site for N-linked glycosylation in the structural glycoprotein E2. All revertants to the normal host range pattern also lost this glycosylation site. A second area of metabolism involved in host range determination is suggested by the host-specific response of SV replication to methionine (met) starvation: standard SV replicates normally in met-starved vertebrate cells deprived of met, but poorly in met-starved mosquito cells. We believe that this differential sensitivity reflects (1) the relatively rapid and severe decline in S-adenosylmethionine (ado met) pools in mosquito cells and (2) the requirement of a viral methyltransferase for high concentrations of ado met for RNA cap methylation. The viral mutant, SV_{LM21}, is able to overcome the sensitivity to met starvation. The assignment of this phenotypic alteration to changes in the viral methyltransferase is strengthened by our finding that SV_{LM21} is extraordinarily sensitive to a class of drugs known to interfere with the breakdown of S-adenosylhomocysteine. Further study of this mutant should facilitate the assignment of this enzyme function to one of the viral genes.

Q84 COMPARATIVE BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF THE FELINE CORONAVIRUSES: FELINE INFECTIOUS PERITONITIS VIRUS AND FELINE ENTERIC CORONAVIRUS, James F. Evermann, Alison J. McKeirnan, Geoffrey T. Tupper, Margaret E. Thouless, Robert Russel. Washington State University, Pullman, WA 99164 and University of Washington, Seattle, WA 98195.

Comparative studies were conducted with isolates of feline coronavirus to determine if there were some phenotypic and/or genotypic traits which allowed for the differentiation of the closely related strains of feline infectious peritonitis (FIP) and feline enteric coronavirus (FECV). The results indicated differences in plaque size for the various FIP strains in comparison with the FECV, of which the latter were smaller and more heterogenous in size. Physicochemical studies with trypsin indicated that the FIP strains were relatively resistant to proteolytic action while the infectivity of the FECV was decreased significantly. Rabbit polyclonal antibodies to purified FIP and FECV strains revealed differences in the neutralization index indicating distinct antigenic determinants of the peplomer (E2) proteins. The different antigenic properties were not associated with a difference in mw of the E2 protein by PAGE. Biochemical analysis of the two types of virus revealed differences in the mw of the nucleoprotein (N) which may serve as a useful marker to distinguish the closely related (antigenically) feline coronaviruses.

Q85 Pathogenesis of Theiler's viruses (TV): Sensitivity of central nervous system cells (CNS) to TV infection, and effect of anti I-A monoclonal antibodies on TV infection

Adam Friedmann[1], Gad Frankel[1], Abraham Shahar[2], Lawrence Steinman[3]
1 - Department of Genetics, Hebrew University Jerusalem, Israel
2 - Department of Cell Biology, The Biological Institute, Nes-Ziona, Israel
3 - Department of Neurology, Stanford University Medical Center, Stanford, California

The chronic demyelinating disease caused by TV serves as an important model for human demyelinating diseases. Immunohistochemical staining using monoclonal antibodies (MAbs) directed against specific CNS cell markers and hybridization with cloned cDNA probes to the viral genome, enabled us to show that oligodendrocytes ostrocytes and Schwann cells are infected by TV.

Anti I-A MAbs were shown to reduce, reverse but not prevent the chronic demyelinating disease induced in SJL mice by TV. These results are important for the development of therapeutical protocols with anti I-A MAbs for use in autoimmune demyelinating disease of man.

Q86 BIOCHEMICAL AND BIOLOGICAL ANALYSIS OF A YELLOW FEVER VACCINE VIRUS FROM A FATAL HUMAN ENCEPHALITIS CASE, Barry R. Miller, Alan D. T. Barrett, Carl J. Mitchell and Thomas P. Monath, Centers for Disease Control, Fort Collins, Colorado 80522

In 1965 a 3-year-old girl died from an acute encephalitis 12 days after inoculation of yellow fever vaccine. Because the vaccine virus had caused a human death, it was of interest to determine if a virulent variant had been selected which could be detected in biological assays (mosquito infectibility, mouse virulence) or a biochemical assay (T1 fingerprint analysis).

Oral infection rates in mosquitoes exposed to high titers of vaccine virus or virus from the human case were similar, as was the appearance of viral antigen in mosquito mesenteronal cells. Small amounts of infectious virus were recovered from these tissues, on the order of 10^2 PFU/mosquito. Intra-nasal inoculation of adult mice demonstrated that the 17D-204 vaccine virus did not kill mice, while the human isolate was highly virulent. RNA oligonucleotide fingerprints of the virus from the fatal infection and the current Connaught 17D vaccine were virtually identical, and shared 100% of their large T1-resistant oligonucleotides.

Q87 MOLECULAR AND ANTIGENIC EVOLUTION OF THE SABIN VACCINE STRAIN OF POLIOVIRUS TYPE 3 DURING THE PERIOD OF EXCRETION BY PRIMARY VACCINEE. Philip D. Minor, M. Ferguson, Ann John and Joseph P. Icenogle* NIBSC, Holly Hill, Hampstead, London NW3, UK and Scripps Institute, La Jolla, California.
A four month old child was immunised with a vaccine containing all three serotypes of the Sabin vaccine strains of poliovirus. Virus excretion occurred for 73 days. Novel strains of type 3 poliovirus arose at days 8, 42 and 52 days post vaccination, and were the product of intertypic recombination and point mutation. Antigenic variation was also observed, but was restricted to the two immunodominant sites which have been identified on type 3 poliovirus. The immunodominant site was completely conserved throughout the entire period of virus excretion.

Q88 PASSIVE IMMUNIZATION OF MICE WITH MONOCLONAL ANTIBODIES AGAINST TICK-BORNE ENCEPHALITIS VIRUS

Phillpotts, R.J., Stephenson, J.R., Porterfield, J.S., Centre for Applied Microbiology and Research, Porton Down, Wiltshire, UK. SP4 OJG.

Monoclonal antibodies (MAB) raised against the flavivirus Tick-borne encephalitis (TBEV) which react with either one of two infected cell polypeptides (51K or 58K), were tested for their ability to neutralise TBEV in vitro, and protect mice against peripheral challenge with TBEV. Protection correlated with neutralisation of virus, and not with the ability of MABs to fix complement, or mediate antibody-dependent cell cytotoxicity. Although most of the MABs tested are capable of enhancing TBEV infectivity in the mouse macrophage cell line $P388D_1$, there were no early deaths among passively immunized mice. The relationship between the 51K and 58K polypeptides is discussed.

Q89 NEUTRALIZATION EPITOPES OF HEPATITIS A VIRUS, Jack T. Stapleton and Stanley M. Lemon, University of North Carolina, Chapel Hill, N.C. 27514.

To topologically map the neutralization epitopes (N-eps) of hepatitis A virus (HAV), four neutralizing murine monoclonal antibodies (N-mcAbs) were examined for their ability to compete with each other for binding to purified HM-175 strain virus. These studies showed that K2-4F2, K3-4C8 and K3-2F2 were directed against slightly different but intimately overlapping N-eps, while that bound by B5-B3 was distinctly different. Nonetheless, minor inhibition of K3-4C8 binding, and reproducible enhancement of K2-4F2 binding, by B5-B3 suggested that these N-eps were closely spaced and perhaps even part of the same neutralization immunogenic site (N-Im). All four N-mcAbs partially blocked the binding of polyclonal human antibody to the virus. K2-4F2 and B5-B3 showed an additive effect and together completely blocked the binding of human antibody, indicating that the N-Im(s) represented by these N-eps are immunodominant in both man and the mouse. Twenty-nine spontaneous HAV variants that were resistant to neutralization mediated by these N-mcAbs were plaque purified and assessed for cross resistance to the panel of N-mcAbs. Each variant demonstrated significant resistance to each N-mcAb, as well as a ten-fold or greater reduction in neutralization mediated by polyclonal human antibody. Neutralization resistance was associated with a lack of N-mcAb binding. Together, these results support the existence of a single immunodominant N-Im on the HAV virion, and suggest that further analysis of neutralization resistant variants may be useful in fine structure mapping of HAV N-eps.

Q90 THREE UNIQUE NEUTRALIZATION EPITOPES OF FOOT-AND-MOUTH DISEASE VIRUS TYPE O_1 BRUGGE DEFINED BY MONOCLONAL ANTIBODIES, James W. Stave, Jay L. Card and Donald O. Morgan, Plum Island Animal Disease Center, USDA, ARS, Greenport, NY 11944

Purified foot-and-mouth disease virus (FMDV) type O_1 Brugge was used to infect mice and seven days later spleen cells were harvested for the production of monoclonal antibodies (MCABs). The resulting hybridomas were exclusively IgM producers. Twenty-two MCABs were adjusted to have equal concentrations of antibody protein and grouped according to their pattern of reactivity with purified FMDV antigens in radioimmunoassay (RIA) and capacity to neutralize infectious virus in suckling mouse protection assays. One group of neutralizing MCABs reacts specifically with the purified virion (140S) and not with the 12S subunit or the immunogenic capsid polypeptide VP1. A second group of neutralizing MCABs reacts with conformationally determined epitopes present on both 140S virions and 12S subunits, while a third group reacts with 140S virions, 12S subunits and isolated VP1. The most efficient neutralizing MCAB from each of the three neutralization groups was selected for further study. All three MCABs had similar capacities to neutralize infectious FMDV type O_1 Brugge while exhibiting unique patterns of neutralization against four additional strains of type O_1 FMDV.

Q91 CORRELATION BETWEEN REPLICATION OF FELINE CORONAVIRUSES IN FELINE MACROPHAGES IN VITRO AND THE PATHOGENESIS OF FELINE INFECTIOUS PERITONITIS, Cheryl A. Stoddart and Fredric W. Scott, Cornell University, Ithaca, NY 14853.

Feline infectious peritonitis (FIP) is a typically fatal immunologically-mediated disease caused by a coronavirus. Viremia and systemic virus replication occur after initial infection of mononuclear phagocytes, and destructive vasculitis is produced by type III (immune-complex mediated) hypersensitivity reactions involving virus antigen, coronavirus antibody, and complement. Cats with pre-existing antibody against certain coronavirus strains exhibit an accelerated form of the disease after experimental infection when compared with initially seronegative cats. Some feline coronavirus isolates (feline enteric coronavirus [FECV]) produce a mild localized infection of the intestinal tract and do not cause FIP. We report that feline infectious peritonitis virus (FIPV) strain 79–1146 causes FIP and replicates in cultured feline peritoneal macrophages whereas FECV strain 79–1683 causes little or no disease and cannot productively infect macrophages in vitro. Cats with pre-existing antibody to FIPV 79–1146 do not exhibit the accelerated disease after lethal infection, and likewise, pre–incubation of FIPV 79–1146 with feline anti–1146 antiserum does not enhance in vitro macrophage infection. The effects of pre–incubation of other FIPV strains (which do produce accelerated disease in sensitized cats) with polyclonal and monoclonal antibodies on virus replication kinetics will be described in the context of the putative immune–mediated pathogenesis of FIP.

Q92 ISOLATION AND CHARACTERIZATION OF A CELLULAR RECEPTOR INVOLVED IN ATTACHMENT OF HUMAN RHINOVIRUSES TO CELLS, Joanne E. Tomassini and Richard J. Colonno, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486

Human rhinoviruses (HRVs) can be classified into major and minor groups on the basis of receptor specificity. Recently, a mouse monoclonal antibody was isolated which selectively blocked the attachment of the major group of HRVs to cells. Solubilized receptor from detergent-treated HeLa cell membrane extracts eluted from gel filtration columns with an apparent molecular weight of 440 Kd. A cellular receptor protein, which had a molecular weight of 90,000 when analyzed on SDS polyacrylamide gels, was purified from solubilized extracts on an immunoaffinity column containing receptor antibody. Polyclonal rabbit antiserum, resulting from immunization with the isolated receptor protein, specifically blocked the attachment of the major group of HRVs and coxsackie A viruses to cells, but did not block the attachment of the minor group of HRVs or poliovirus. Neuraminidase digestion of the isolated receptor protein resulted in a shift in the mobility of the protein on SDS polyacrylamide gels to 80 Kd. This result demonstrated that the receptor protein is a glycoprotein containing sialic acid at the non-reducing ends of the carbohydrate. Further characterization of the carbohydrate moiety of the protein has suggested the presence of additional saccharide groups. The carbohydrate appears to be O-linked to the protein, since pre-treatment of cells with tunicamycin had no inhibitory effect on HRVs or receptor antibody binding. Isoelectric focusing of the purified protein indicated that it is an acidic protein.

Q93 CORONAVIRUS MHV-A59 CAUSES A PERSISTENT, PRODUCTIVE INFECTION OF GLIAL CELLS ALONG WITH INDUCTION OF SURFACE H-2 ANTIGENS, Susan R. Weiss, Ehud Lavi, Akio Suzumura, Mikio Hirayama, Maureen K. Highkin, and Donald Silberberg, University of Pennsylvania, Philadelphia, PA., 19104

MHV-A59 causes a chronic demyelinating disease in mice, associated with persistence of viral RNA in CNS white matter. To investigate the mechanism of demyelination, infection of glial cells, possible targets for chronic infection, was studied in vivo and in vitro. Primary mixed glial cultures were derived from newborn mouse brains and subsequently were fractionated into enriched oligodendrocyte and astrocyte cultures. About 10% of oligodendrocytes and 30% of astrocytes expressed viral antigens. All cultures had an initial burst of virus release followed by release of low titers of virus for up to 45 days. There was no cytopathic effect in any of the cultures. Cultures derived from previously infected mice were similar to those infected in vitro as far as antigen expression and virus release. Thus, MHV-A59 infects both oligodendrocytes and astrocytes in vitro and in vivo. These results support the hypothesis that MHV-A59 may reside in oligodendrocytes during chronic infection and may cause demyelination by direct infection of the myelin producing cells. Infection of astrocytes, but not oligodendrocytes, causes an induction of H-2, class I antigens, but not Ia antigens on the cell surface. Furthermore soluble factor(s) from the supernantants of these infected cells stimulate the appearance of surface H-2 antigens on oligodendrocytes as well as on astrocytes. Thus, the mechanism of MHV-A59- induced demyelination may involve an immune-mediated componant as well as direct infection.

Late Additions

Q94 Properties of infectious poliovirus RNA synthesized in vitro by T7 RNA polymerase. S.v.d.Werf,J.Bradley, F.W.Studier*, M.Girard, E.Wimmer** and J.J.Dunn*. Institut Pasteur, Paris, France. Brookhaven National Laboratory, Upton, N.Y. 11973,USA*.SUNY at Stony Brook, N.Y. 11794, USA**.

Full -length poliovirus cDNA was inserted in both orientations behind the Ø10 promoter of bacteriophage T7. Purified T7 RNA polymerase efficiently transcribes the entire poliovirus cDNA in vitro, to produce (+) or (-) strand full-length transcripts. The (+) RNA produced initially had an additional 60 nucleotides ahead of the poliovirus RNA sequence at the 5' end and an additional 626 nucleotides of pBR322 sequence beyond the poly(A) tract at the 3' end. Such RNA, while much more infectious than the plasmid DNA upon transfection of primate cells is only about 0.1% as infectious as virion RNA. Most of the excedentary sequences were removed by reconstructing the 5' region of the cDNA such as to place the viral sequences only 2 base pairs behind the T7 promoter. On the reconstructed template, T7 RNA polymerase synthesizes poliovirus RNA having only an additional pair of G residues at the 5' end, and no more than 7 nucleotides past the poly(A) tract at the 3' end. Such RNA had much higher specific infectivity , about 5% that of virion RNA. Analysis of the progeny virus RNA showed that the 5' excedentary sequences have been removed, most likely during the replication process. Preliminary data indicate that the T7 RNA polymerase is capable of initiating transcripts with synthetic cap sequences. The effect of capping on expression of the transcripts both in vivo and in vitro is currently being investigated

Q95 Molecular basis of antigenicity of poliovirus: P D Minor

D M A Evans, M Ferguson, G C Schild, J W Almond, G Stanway.
NIBSC, London, UK, University of Reading, Reading UK, University of Essex, UK.

The identification of antigenic sites involved in the neutralisation of polio- virus by murine monoclonal antibodies has been carried out by the isolation and characterization of antigenic variants. Three sites have been identified involving a continuous sequence in VP1 (site 1) a complex site composed of sequences from VP2 and VP1 (site 2) and a complex site composed of sequences from VP3 and VP1 (site 3). In mice and rats site 1 is strongly immunodominant for types 2 and 3, while barely immunogenic for type 1. Peptides based on the sequence of site 1 of type 3 are consistently able to raise broadly reactive type-specific neutralizing antisera. The contribution of antibodies to the three sites in the human immune response to natural infection is under study in recipients of vaccines and epidemic strains.